STATUS OF RESEARCH ON TWO PARASITIC ZOONOSES (TOXOPLASMOSIS AND TOXOCARIOSIS) IN SUB-SAHARAN AFRICA AND THEIR PREVALENCE IN SELECTED RURAL COMMUNITIES OF KWAZULU-NATAL PROVINCE OF SOUTH AFRICA USING FREE-RANGE CHICKENS AS A CASE STUDY

by

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As the candidate's supervisor I have approved this thesis for submission.

Signed: [redacted] Name: Prof. S. Mukaratirwa Date: 09th February 2022

PREFACE

The research contained in this thesis was completed by the candidate while based in the Discipline of Zoology, School of Life Sciences of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg, South Africa, under the supervision of Prof. S. Mukaratirwa. The research was financially supported by the National Research Foundation.

The contents of this work have not been submitted in any form to another University and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.

Signed: Prof. Mukaratirwa S.

Date: 09th February 2022

DECLARATION 1: PLAGIARISM

I, Adejumoke Oluwatosin Omonijo, declare that:

(i) the research reported in this dissertation, except where otherwise indicated or acknowledged, is my original work;

(ii) this dissertation has not been submitted in full or in part for any degree or examination to any other university;

(iii) this dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons;

(iv) this dissertation does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers.

Where other written sources have been quoted, then:

a) their words have been re-written, but the general information attributed to them has been referenced;

b) where their exact words have been used, their writing has been placed inside quotation marks, and referenced;

(v) where I have used material for which publications followed, I have indicated in detail my role in the work;

(vi) this dissertation is primarily a collection of material, prepared by me, published as journal articles or presented as a poster and oral presentations at conferences.

(vii) this dissertation does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the References sections.

Signed: Adejumoke Oluwatosin Omonijo

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DECLARATION 2: PUBLICATIONS

My role in each paper and presentation is indicated. The * indicates corresponding author.

1. Omonijo A.O.*, Kalinda C. and Mukaratirwa S. 2022. *Toxoplasma gondii* infections in animals and humans in southern Africa: A systematic review and meta-analysis. Pathogens, 11, 183. https://doi.org/10.3390/pathogens11020183.

   Omonijo A.O. conceptualized the study, did the review of the literature, and wrote the review paper.

2. Omonijo A.O.*, Kalinda C. and Mukaratirwa S. 2020. A systematic review and meta-analysis of canine, feline and human *Toxocara* infections in sub-Saharan Africa. Journal of Helminthology, 94, e96, https://doi.org/10.1017/S0022149X19000889.
   Omonijo A.O. conceptualized the study, did the review of the literature, and wrote the review paper.

3. Omonijo A.O.*, and Mukaratirwa S. 2021. Knowledge and practices on consumption of free-range in selected rural communities of KwaZulu-Natal, South Africa, with focus on zoonotic transmission of *Toxoplasma gondii* and *Toxocara* spp. Journal of Tropical Animal Health and Production. (submitted).
   Omonijo A.O. designed the study, conducted the research, and wrote the research paper.

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Conference Presentations

1. Zoonotic Gastrointestinal Parasites of Stray Dogs in Durban Metropolis, KwaZulu-Natal, South Africa. Oral presentation, International Conference on Promoting ecohealth Research in Africa: Towards the establishment of an African Chapter of ecohealth international, November 14/2018.

2. Knowledge and practices relating to viscera larva migrans from free-range chickens in KwaZulu-Natal Province. Poster presentation, College of Agriculture, Engineering & Science Postgraduate Research & Innovation Symposium, October 17/2019.

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Unto thee, O God, do we give thanks, unto thee do we give thanks, for that thy name is near thy wondrous work declare Psalms 75;1

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THESIS OUTLINE

The layout of the thesis is according to publication outputs. The publication outputs include those that have been published and submitted for publication in peer-reviewed journals. Each chapter is mostly self-contained, containing a brief introduction for the motivation of the study through literature review, materials and methods, results and discussion, and conclusions.

Chapter 1 focusses on the introduction, justification, aims, and objectives of the study.

Chapter 2 focusses on the literature review of the epidemiology of *Toxoplasma* infections in southern Africa. The review gives baseline information on the prevalence of *Toxoplasma gondii* infections in animals and humans including birds. It highlights the diverse routes of parasite transmission, the symptoms and diseases resulting from *Toxoplasma gondii* infections particularly the significant loss it causes in the animal production industry.

One peer reviewed publication has emanated from this work.

- **Omonijo A.O.**, Kalinda C., and Mukaratirwa S. 2022. *Toxoplasma gondii* infections in animals and humans in southern Africa: A systematic review and meta-analysis Pathogens, 11, 183, https://doi.org/10.3390/pathogens11020183

Chapter 3 features the epidemiology of *Toxocara* infections in animals and humans in sub-Saharan Africa. The review provides the background information on the prevalence of *Toxocara* spp infections in the environment and diverse definitive and paratenic hosts. It reviews the transmission routes of *Toxocara* spp, diagnosis, symptoms and diseases produced during infection by *Toxocara* spp.

The reviews reveal the limited studies that were available in southern Africa including the role of free-range chickens in the transmission of the zoonotic *Toxocara* spp. A peer reviewed publication emanated from this work.

- **Omonijo A.O.** Kalinda C., and Mukaratirwa S. 2020. A systematic review and meta-analysis of canine, feline and human *Toxocara* infections in sub-Saharan Africa. Journal of Helminthology, 94, e96, https://doi.org/10.1017/S0022149X19000889.

Chapter 4 describes the knowledge, attitude and perceptions ownership of free-range chickens in KZN. It shows the consumption pattern of free-range chickens' viscera in households in the study location. It also describes the level of awareness of zoonoses transmission from the consumption of raw or undercooked chicken viscera. Moreover, it reports the prevalence of *T. gondii* and *T. canis* in free-range chickens purchased from selected rural communities of KwaZulu-Natal, South Africa, using molecular approach. One peer reviewed publication has emanated from this work.

- **Omonijo A.O.** and Mukaratirwa S. 2022. Knowledge and practices on consumption of free-range in selected rural communities of KwaZulu-Natal, South Africa, with focus on zoonotic transmission of *Toxoplasma gondii* and *Toxocara* spp. Journal of Tropical Animal Health and production. (Under Review)

Chapter 5 summarizes the entire dissertation, the findings of study, conclusions, and recommendations for future work.

ABSTRACT

Free-range chickens are among the popular livestock that are owned by most households in rural communities. They constitute a major source of food security, financial income, and are used in socio-cultural practices. However, due to their habit of scavenging for food they are exposed to parasitic agents thereby making them host for several disease pathogens such as *Toxoplasma gondii* and *Toxocara* spp. *Toxoplasma gondii* and *Toxocara* spp are the etiological agents of human toxoplasmosis and human toxocariasis respectively. Humans become directly infected via accidental ingestion of sporulated oocysts of *T. gondii* from felids and tachizoites/bradyzoites of *T. gondii* from raw/undercooked meat or embryonated eggs with second stage larva of *Toxocara* spp via contact with contaminated faeces of definitive hosts (dogs and cats), or indirectly via ingestion of contaminated water or consumption of raw or undercooked chickens. Following infection, the parasites migrate through the human body causing varying degree of diseases known as toxoplasmosis and toxocariosis respectively.

Consumption of poultry meat viscera is an increasing dietary habit common in different communities worldwide and depending on socio-cultural preferences it can either be eaten raw, undercooked, or well cooked. However, the habit of eating raw/under cooked meat or viscera poses the risk of transmitting *T. gondii* and *Toxocara* spp from animals to humans.

Limited information exist on the epidemiology of *T. gondii* and *Toxocara* spp in sub-Saharan Africa and let alone the role of free-range chickens in the transmission of these zoonotic parasites, hence, this study was designed to:

- review the status of research on these two parasitic zoonoses in sub-Saharan Africa.
- determine prevalence of the parasites in free-range chickens from selected rural communities in KwaZulu-Natal province through molecular approach. determine the level of awareness of the zoonotic transmission of these parasites when the viscera or meat of Free-range chicken are consumed raw or undercooked. A sytematic review and meta-analysis was conducted following the Preferred Reporting items for systematic Reviews and Meta-Analysis (PRISMA) guidelines on the epidemiology of *T. gondii* in animals and humans in southern Africa and epidemiology of *Toxocara* spp in canine, feline, and humans in sub-Saharan Africa respectively. The reviews showed that there is paucity of information on *T. gondii* and *Toxocara* spp in food animals including free-range chickens.

Furthermore, to determine the prevalence of *T. gondii* and *Toxocara* spp in free-range chickens, free-range chickens were randomly purchased from selected rural communities namely,

Gingindlovu (GI), Ozwathini (O), uMzinto (MZ), and Shonwgweni (SH) in KwaZulu-Natal (KZN). The chickens were euthanized according to ethical guidelines. The brain tissue of each chicken was divided into two equal halves. One half was examined for the presence of parasites while the remaining half was preserved in 70% alcohol for molecular analysis. To detect the presence of *T. gondii*, the preserved brain tissues were subjected to molecular analysis based on analysis of DNA sequences of the nuclear ribosomal internal transcribed spacer (ITS-1 and ITS-2) region using TOX4 and TOX5 primers. To detect the presence of *Toxocara* spp, various parts of chickens such as brain, heart, liver, spleen, kidney, duodenum, pectoral, thigh, and breast were digested using the acid/pepsin; 1:1 method and the larvae were recovered with 20-µm sieve. Three (3) larvae were recovered from the right pectoral from a chicken collected in GI; two (2) from the lungs of a chicken from MZ; three (3) each in the liver and left thigh of two separate chickens from SH. The recovered larvae were subjected to molecular analysis using Nem_18S primers. *Toxoplasma gondii* was not detected in the tissue samples which were subjected to molecular analysis, however, *Toxocara canis* was identified in Gingindlovu (n=1), uMzinto (n=1), and Shongweni (n=2). The identified *T. canis* showed 100% homology with Genbank isolates from China, the United Kingdom, and the United State of America. The occurrence of *T. canis* in free-range chickens from KZN province reveals the possibility of human toxocariasis transmission in the province.

Moreover, we conducted a questionnaire survey to determine the knowledge and practices relating to consumption of free-range chicken viscera in selected rural communities of KwaZulu-Natal with respect to zoonotic transmission of *T. gondii* and *Toxocara* spp. There was low level of awareness of risk of zoonotic transmission of the parasites via ingestion of raw/undercooked free-range chicken meat/viscera and the majority of respondents consumed free-range chicken viscera. They preferred the viscera well cooked which reduces the risk of transmission of the the two parasites.

The study contributes new knowledge on the prevalence of zoonotic parasites in free-range chickens as well as the level of knowledge and awareness on zoonosis transmission via consumption raw/undercooked free-range chicken viscera or meat.

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LIST OF ABBREVIATIONS

Bp	Base pairs
DNA	Deoxyribonucleic acid
FRC	Free-range chicken
GI	Gingindlovu
HT	Human toxocariasis
ITS-1	Internal transcribed spacer 1
ITS-2	Internal transcribed spacer 2
KZN	KwaZulu-Natal
MZ	uMzinto
OZ	Ozwathini
PCR	Polymerase Chain Reaction
SH	Shongweni

CHAPTER 1: INTRODUCTION

1.1 Rationale for the research

Free-range poultry system is commonly practiced in developing countries where seventy five percent of the global estimated poultry census of 14.718 billion is reported to reside (Mukaratirwa and Khumalo, 2012). This system promotes freedom to foraging like eating grass and small invertebrates such as insects and earthworms (Giannenas, Nisianakis, Gavriil, Kontopidis, and Kyriazakis, 2009) and is associated with high prevalence of parasitism due to their exposure to intermediate hosts or other pathogens in contaminated environment (da Silva et al., 2018). Thus, contributing to increased risk of exposure to parasites of zoonotic and public health importance such as *Toxoplasma gondii,* and *Toxocara* spp. Studies have reported the natural infection of these parasites in chickens are probably acquired from pasture contaminated with faeces of definitive hosts or via the consumption of invertebrate paratenic hosts or intermediate hosts (Zibaei et al., 2017; Hamilton et al., 2019).

In South Africa and especially in KwaZulu-Natal (KZN) province, the greater population lives in rural areas and rear chickens following a free-range system (Naidoo, 2005; Mwale and Masika, 2009; Mukaratirwa and Khumalo, 2010a; Malatji et al., 2016a; Malatji et al., 2016b). The system contributes to improved livelihood of rural households through the provision of income, meat and eggs (Mwale and Masika, 2009). Besides the popular practice of free-range poultry system, abundance of stray dogs and feral cats which are definitive hosts of zoonotic parasites have been reported in KZN (Tannent et al., 2010, Mukaratirwa and Singh, 2010). Thus, the combinations of free-range poultry practice and poor pet ownership may be contributing to increased risk of parasitic infections in free-range chickens in the population.

Toxoplasma gondii is an apicomplexan parasite of economic and public health importance. *T. gondii* infects a wide range of warm-blooded animals including humans (Duarte et al., 2020). Felids are the definitive hosts of *T. gondii* while several other animals serve as the intermediate or reservoir hosts. Humans acquire *Toxoplasma* infection via the consumption of raw or undercooked meat contaminated with tissue cysts or water contaminated with *T. gondii* oocysts (Feng et al., 2016; dos Santos Silva et al., 2020; Duarte et al., 2020). Congenital transmission also occurs during pregnancy resulting in congenital toxoplasmosis (Hamilton et al., 2019).

Toxocara spp belong to phylum Nematoda and are major parasites of canids and felids but capable of utilizing a wide range of animals as paratenic hosts (Omonijo et al., 2020). Upon infections of free-range chickens with *Toxocara,* the larvae hatch from the egg shell and

migrate through various body viscera where they persist and remain infective to other paratenic or accidental (humans) hosts until when they are eaten by definitive host from raw or undercooked meat. Humans acquire infections via ingestion of water or vegetables contaminated with larvated eggs, or consumption of undercooked or raw meat or poultry viscera or via contact with contaminated environment (Loetsch, Vingerling, Spijker, and Grobusch, 2017; Oliveira, Rubinsky-Elefant, Merigueti, Batista, and Santarém, 2018). The larvae migrate continuously in a variety of body tissues and produce a disease known as human toxocariasis (Ma et al., 2018; Aghamolaie et al., 2019).

Studies have reported a significant association between the risk of zoonotic disease development from *T. gondii* and *Toxocara* spp, with consumption of raw or undercooked meat, raw or undercooked food, and contaminated water (Yoshida et al., 2016; Condoleo, Rinaldi, Sette, and Mezher, 2018; Belluco, Simonato, Mancin, Pietrobelli, and Ricci, 2018, Gaulin et al., 2020). This route of zoonotic transmission underscores the need to scale up awareness and implementation of disease control measures in meat and food industry.

Knowledge, attitude, and practices on the various transmission routes of these zoonotic parasites are therefore imperative in order to control and prevent the spread of the disease. Generally, there is lack of knowledge and awareness regarding zoonotic pathogens among livestock and poultry farmers, thereby contributing to disease prevalence (Singh et al., 2019). This limited awareness coupled with the lack of diagnostic capacity, unified and holistic approach to zoonoses control in most developing countries, tend to exacerbate disease transmission (Narrod et al., 2012). To enhance zoonoses control program, it is therefore important to scale up awareness among free-range poultry farmers and the general populace.

1.2 Problem Statement

Free-range chickens are popularly raised for their nutritional and economic importance most especially in developing countries (Mukaratirwa and Khumalo, 2010). This system affords chickens the opportunity to forage in the environment. However, this habit predisposes them to infective life cycle stages of zoonotic parasites thereby making them hosts to several zoonotic diseases (Rodrigues et al., 2019). The probability of free-range chickens to pick up zoonotic parasites increases with the abundance and availability of infected definitive hosts (dogs and cats). In South Africa, and particularly in KZN province, free-roaming or stray dogs and cats, which is suggestive of poor pet care have been reported (Mukaratirwa and Singh 2010; Tannent et al., 2010). This contributes to increased risk of environmental contamination with zoonotic parasites through indiscriminate defecation. Considering earlier reports of occurrence of *T. gondii*, *Toxocara* spp, abundance of definitive hosts, and the popular free-

range chicken farming practice in KZN (Mukaratirwa and Singh 2010; Tannent et al., 2010; Thekisoe, Mofokeng, Smit, and Taioe, 2020), it is likely that free-range chickens in KZN may be acting as reservoirs of parasites of public health importance infections such as *T. gondii* and *Toxocara* spp, most especially in rural areas where socio-economic factors are poor. In KZN and South Africa at large, there is dearth of information on the prevalence of zoonotic parasites in free-range chickens. Hence, there is the need to understand the prevalence of these zoonotic parasites in free-range chickens to gain more insight into parasite epidemiology in KZN.

Moreover, consumption of viscera of free-range chickens is a practice that is increasingly becoming a food security and public health concern due to its contribution to zoonosis transmission (Abduljaleel, 2014). For instance, a study in Brazil has identified *T. gondii* in free-range chickens intended for human consumption (dos Santos Silva et al., 2020). Similarly, human toxoplasmosis outbreak has been reported among individuals who consumed raw or undercooked meat (Dawson, 2005). Likewise, human toxocariasis has been reported in people after consuming raw chicken livers (Choi et al., 2012, Pinelli et al., 2011). Considering the increasing quest for free-range poultry due to a growing population as well as the habitual evolvement of consuming raw or undercooked food and poultry viscera, it is important to create awareness on zoonosis transmission via this route in order to enhance disease control measures. Hence, focused studies that address these gaps are imperative.

1.3 Research questions

The study was designed to address the following questions:

What is the state of research on two parasitic zoonoses (toxoplasmosis and toxocariosis in humans and domestic animals in sub-Saharan Africa?

Toxoplasmosis and toxocariosis are among the major parasitic zoonoses of humans and domestic animals worldwide. However, in sub-Saharan Africa, there is paucity of information in the literature on the prevalence of the causative pathogens in animals and humans. These reviews will provide insight on the state of research on these parasites and the diseases they cause.

***Are free-range chickens in rural localities in KwaZulu-Natal hosts to parasites of zoonotic importance (Toxoplasma gondii and Toxocara* spp)?**

Free-range chickens have been reported to be sentinel agents for several parasites due to their exposure to pathogenic agents in contaminated environment. However, there is paucity of information on the presence of *Toxoplasma gondii* and *Toxocara* spp, in free-range chickens in KwaZulu-Natal province. This research will provide insight into the prevalence of these parasites in free-range chickens in KwaZulu-Natal province.

What is the status of awareness regarding disease transmission via the consumption of raw or undercooked viscera of free-range chickens in KZN?

Consumption of raw or undercooked viscera of free-range chickens has been associated with disease development in Asian countries. In KZN, there is high demand for free-range poultry to meet nutritional, economic and socio-cultural purposes. However, despite available record of health risks associated with this consumption practice, there is generally paucity of information on awareness of this route thereby contributing to disease transmission among people with this socio-cultural practice. This research will provide baseline information on the level of awareness of this transmission route and the consumption pattern in KZN for the purpose of enhancing disease control in KZN.

1.4 The main objectives

The general objective of this research are to determine the status of research on two parasitic zoonoses (toxoplasmosis and toxocariosis) in sub-Saharan Africa and their prevalence in selected rural communities of KwaZulu-Natal of South Africa.

The specific objectives are as follows:

- *To assess the status of research on T. gondii and Toxocara spp in sub-Sharan Africa.*
- *To determine the prevalence of T. gondii and Toxocara spp in free-range chickens from selected rural communities of KZN province using molecular techniques.*
- *To assess the knowledge and practices relating to consumption of free-range chicken viscera in selected rural communities of KwaZulu-Natal.*

1.5 Ethical Consideration

This study was carried out in accordance with the National Guidelines for Experimental Animal Welfare of South Africa and Animal Research and Ethical Committee of the University of KwaZulu-Natal, South Africa under the protocol reference number (AREC/037/018D) and the Human and Social Sciences Research Ethics committee of the University of KwaZulu-Natal protocol reference number (HSS/1655/018D).

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CHAPTER 2: *TOXOPLASMA GONDII* INFECTIONS IN ANIMALS AND HUMANS IN SOUTHERN AFRICA: A SYSTEMATIC REVIEW AND META-ANALYSIS

A.O. Omonijo, C. Kalinda, S. Mukaratirwa

2.1 Abstract

Toxoplasma gondii is an apicomplexan parasite with zoonotic importance worldwide especially in pregnant women and immunocompromised people. This study is set to review the literature on *T. gondii* infections in humans and animals in southern Africa. We extracted data regarding *T. gondii* infections from published articles from southern Africa from 1955 to 2020 from four databases, namely Google Scholar, PubMed, EBSCO Host, and Science Direct. Forty articles from eight southern African countries were found eligible for the study. This review revealed a paucity of information on *T. gondii* infection in southern African countries, with an overall prevalence of 17% (95% CI: 7–29%). Domestic felids had a prevalence of 29% (95% CI: 7–54%), wild felids 79% (95% CI: 60–94), canids (domestic and wild) 69% (95% CI: 38–96%), cattle 20% (95% CI: 5–39%), pigs 13% (95% CI: 1–29%), small ruminants (goats and sheep) 11% (95% CI: 0–31%), chicken and birds 22% (95% CI: 0–84%), and humans 14% (95% CI: 5–25%). Enzyme-linked immunosorbent assay (ELISA) and immunofluorescence antibody test (IFAT) constituted the most frequently used diagnostic tests for *T. gondii*. We recommend more focused studies be conducted on the epidemiology of *T. gondii* in the environment and human population, most especially the at-risk populations.

Keywords: *Toxoplasma gondii*; infections; southern Africa; systematic review; animals; humans

2.2 Introduction

Toxoplasma gondii is an apicomplexan obligate parasite that infects animals and humans worldwide (Tonouhewa et al., 2017). The definitive hosts are felids although a recent study showed developmental success in mice subjected to certain enzymatic inhibition and diet modification (Attias et al., 2020). The intermediate hosts include terrestrial and aquatic mammals and birds (Tagwireyi et al., 2019; Attias et al., 2020). The pathways of *T. gondii* infection and transmission are multifaceted, involving the three developmental stages (tachyzoite, bradyzoite, and sporozoite) of the parasite's life cycle (Attias et al., 2020). Intermediate hosts, including humans, can acquire infection via (i) consumption of water, vegetables, and fruits contaminated with infective oocysts; (ii) consumption of raw or undercooked meat infected with tachyzoites or bradyzoites (Dubey et al., 2009); (iii) blood transfusion; (iv) organ transplant containing cysts or tachyzoites; and (v) congenital

transmission from the mother to fetus via the placenta. Feline definitive hosts acquire infections via the ingestion of sporulated oocysts or by carnivorism. However, rarely, consumption of non-pasteurized milk or milk products can serve as a potential source of *T. gondii* transmission (Chiari, C.A.; Neves 1989; Stelzer et al., 2019; Attias et al., 2020). Oysters and mussels can act as reservoir hosts for infective oocysts, which can later be transmitted to other animals upon consumption (Attias et al., 2020; Lindsay et al., 2004; Coupe et al., 2019; Monteiro et al., 2019). Parasites attain maturity in the intestine of felids and start releasing numerous oocysts into the environment within three to 18 days post-infection (Montazeri et al., 2020).

Furthermore, *Toxoplasma* infection in animals or humans causes toxoplasmosis which is prevalent worldwide. The infection rate varies according to geographic region and climatic conditions (Tonouhewa et al., 2017). Other risk factors of infection include age, gender, farm management, and geographic characteristics (Stelzer et al., 2019). Toxoplasmosis is accompanied by varying degrees of clinical symptoms depending on the inoculum size, virulence of parasite strain, and level of host immunity (Mose et al., 2020). *Toxoplasma* infections have been reported to alter reproductive parameters in hosts by having a negative impact on harming female reproductive functions (Abdoli et al., 2012), inducing apoptosis in spermatogonial cells directly or indirectly (Saki et al., 2020), thereby resulting in reduced quality of human sperm (Zhou et al., 2003) and decreased fertility in experimentally infected male rats (Terpsidis et al., 2009; Saki et al., 2020). A significant association has been reported between *T. gondii* seropositivity and abortion in small ruminants from certain districts of central Ethiopia (Gebremedhin et al., 2013). In sheep, an infection may cause early embryonic death and resorption, fetal death and mummification, abortion, and stillbirth, (Edwards and Dubey, 2013) thereby resulting in severe economic loss in the livestock industry (Tonouhewa et al., 2017; Tagwireyi et al., 2019). The economic impact of *T. gondii* infection in sheep and other livestock is abortions and increased lambing/kidding interval, culling of infected animals, reduced milk production, and reduced value of the breeding stock, hence, leading to major economic losses (Gebremedhin et al., 2013). The severity of infection is dependent on the stage of gestation the ewe acquires infections. Infection at the early gestational stage often results in fatal consequences (Dubey, 2009; Gebremedhin et al., 2013). In immunocompetent hosts, toxoplasmosis may be asymptomatic, whereas in immunocompromised humans, particularly AIDS patients, the disease has serious consequences (Frimpong et al., 2017; Tagwireyi et al., 2019). Similarly, infection in pregnant women is associated with congenital toxoplasmosis, and the severity and risk are dependent on the time of maternal infection and often accompanied

by developmental malformation, abortion, or reduced quality of life for the child (Frimpong et al., 2017; Tagwireyi et al., 2019; Mose et al., 2020).

While toxoplasmosis is a zoonosis that can be controlled or prevented in humans and animals worldwide, in sub-Saharan Africa, the control is hampered by various factors, including high poverty level, lack of diagnostic capacity, limited disease surveillance, and poor veterinary care (Hammond-Aryee et al., 2014). Since the fecal-oral route and consumption of raw or undercooked infected food or meat constitute the major transmission route in humans (Mose et al., 2020), effective control of toxoplasmosis requires adequate awareness of good veterinary practices, personal hygiene, improved culinary habits, dietary habits, and correct diagnosis.

Diagnosis involves direct methods, immunodiagnostic methods, and molecular techniques. The direct method involves isolation of parasite or bioassay, cellular culture, and histology. Immunodiagnostic methods include the Sabin–Feldman dye test (SFT), hemagglutination assay, immunofluorescent assay (IFA), modified agglutination test (MAT), avidity, western blot, enzyme-linked immunosorbent assay (ELISA), recombinant antigens, immunocytochemistry, and immunohistochemistry. Molecular techniques include Polymerase Chain Reaction (PCR), real-time PCR, PCR-restriction fragment length polymorphisms (PCR-RFLP), loop-mediated isothermal amplification (LAMP), and high-resolution melting (HRM) (Ramírez et al., 2017).

Toxoplasma gondii infection is accompanied by the emergence of IgM in the host, followed by the appearance of IgA and IgE at about two weeks post-infection (Montoya, 2002; Daka, 2015) while IgG spikes around four months post-infection and persists throughout lifetime (Daka, 2015). Toxoplasmosis in immunocompetent individuals resolves without treatment (Muhie and Keskes, 2004), but in immunocompromised individuals, clindamycin, sulfonamides, spiramycin, and pyrimethamine are used for treatment (EFSA et al., 2007; Vogel et al., 2010). Pyrimethamine and sulfadiazine drug combination is suitable for new-borns, infants, and pregnant women; however, to prevent transmission from mother to unborn fetus, an antibiotic (spiramycine) has been proven effective but not in latent infections, as antibiotics are unable to reach the bradyzoites in adequate concentrations (Overton and Bennet, 2010; Daka, 2015).Toxoplasmosis prevention is centered around avoidance of contact with sources of infection, such as cats, contaminated environment, consumption of raw or undercooked meat, personal hygiene, and and regular handwashing (Daka, 2015). The control of mechanical vectors of transmission, such as cockroaches, flies, or rodents in the surroundings, can also be adopted in disease control (Muhie and Keskes, 2014). This review

aims to analyze published literature on *Toxoplasma* infections in animals and humans in southern Africa and determine the epidemiological distribution of infection in various hosts in the region and identify gaps for future research.

2.3 Methods

2.4 Search Strategy

A systematic literature search was conducted in the following databases: Google Scholar, PubMed, EBSCO Host, and Science Direct using the following terms and Boolean operators (AND, OR): Toxoplasma AND Toxoplasmosis in southern Africa, Toxoplasma in cats AND southern Africa, Toxoplasma in livestock (sheep, goats, cattle) AND southern Africa, Toxoplasma in wildlife AND Southern Africa, Toxoplasma in felids, Toxoplasma in fowls AND Southern Africa, and Toxoplasma in humans AND southern Africa. The titles and abstracts of the search results were perused for the retrieval of relevant articles. References from selected articles were further used as a guide to other literature. The literature search was concluded in June 2021. Full-text articles were retrieved and managed in Endnote reference manager, version X7 (Clarivate Analytics, Philadelphia, PA, USA). This systematic review was performed following the PRISMA protocol (Reporting Items for Systematic Reviews and Meta-Analyses).

2.4.1 Inclusion and exclusion criteria

An article was included in this study if it was published between 1955 and 2020 in a peer-reviewed journal and reported on (1) prevalence of *T. gondii* in cats and/or other animals and (2) *Toxoplasma* seroprevalence in humans in southern Africa. Dead links, duplicates, and grey pieces of literature were excluded during the literature review. The Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) used in this review is shown in (Figure 2.1).

2.4.2 Data extraction and quality assessment

From each selected article, data on the study period, country of study, type of hosts, sample size, number of infected subjects/hosts, prevalence (%), and the diagnostic method(s) used were retrieved. Quality assessment of the identified articles was done as described by Munn et al. (Munn et al., 2015). Quality assessment of each article was based on the following information: (1) relevance of research objective(s) to *Toxoplasma*, (2) prevalence of *Toxoplasma* as the main objective of the study, (3) study design was appropriately defined (case reports, cross-sectional), (4) samples randomly selected, (5) study subjects categorized by age/sex were relevant, (6) use of valid diagnostic methods in the study, (7) reliability of

diagnostic methods, (8) representativeness of target sample to the general population, (9) description of the prevalence of *Toxoplasma* infection in the study community/animals, and (10) geographical location of *Toxoplasma* infection defined. The index score for each article was calculated by dividing the quality assessment of the study by ten.

2.5 Data Analysis

The extracted data from the search were entered in Microsoft Excel for analysis. The MetaXL (www.epigear.com) was used to carry out a meta-analysis. An Inverse Heterogeneity (IVhet) model was used to compute the prevalence estimates with their 95% confidence intervals (CIs). The inverse variance statistic (I^2 index) was used to quantify heterogeneity, and we tested for its significance using Cochran's Q test. The I^2 index was interpreted as no, low, moderate, or high heterogeneity if the value was 0%, ≤25%, 50%, or ≥75%, respectively. Forest plots were generated to show the prevalence of *Toxoplasma* among the study subjects. Furthermore, subgroup analysis was carried out to assess the mean pooled prevalence estimates according to host types and regions within southern Africa. The risk of publication bias was assessed using the Luis Furuya–Kanamori (LFK) index and funnel plot (Barendregt and Doi, 2022). The symmetry of the Doi plots was determined using the LFK index and a value within the range of ±1 was considered as symmetrical and classified as the absence of publication bias, while an LFK value within the range of ±2 was considered as minor asymmetry with slight publication bias, and an LFK value outside the range of ±2 was considered as major asymmetry and high publication bias (Barendregt and Doi, 2022).

2.6 Results

2.6.1 Systematic Review

A total of 3197 articles were identified from the following databases: Google Scholar, PubMed, EBSCO Host, and Science Direct. After duplicates (n = 2111) were removed, title and abstracts were perused for 1086 articles. An additional eight studies were identified from other sources. Overall, 1029 articles were excluded because they were not original articles, non-relevant to research objectives to the study, or abstracts. Of the 65 reviewed full-text articles, 40 were selected for inclusion in the systematic and meta-analysis. A flow diagram illustrating this selection process is presented in (Figure 2.1).

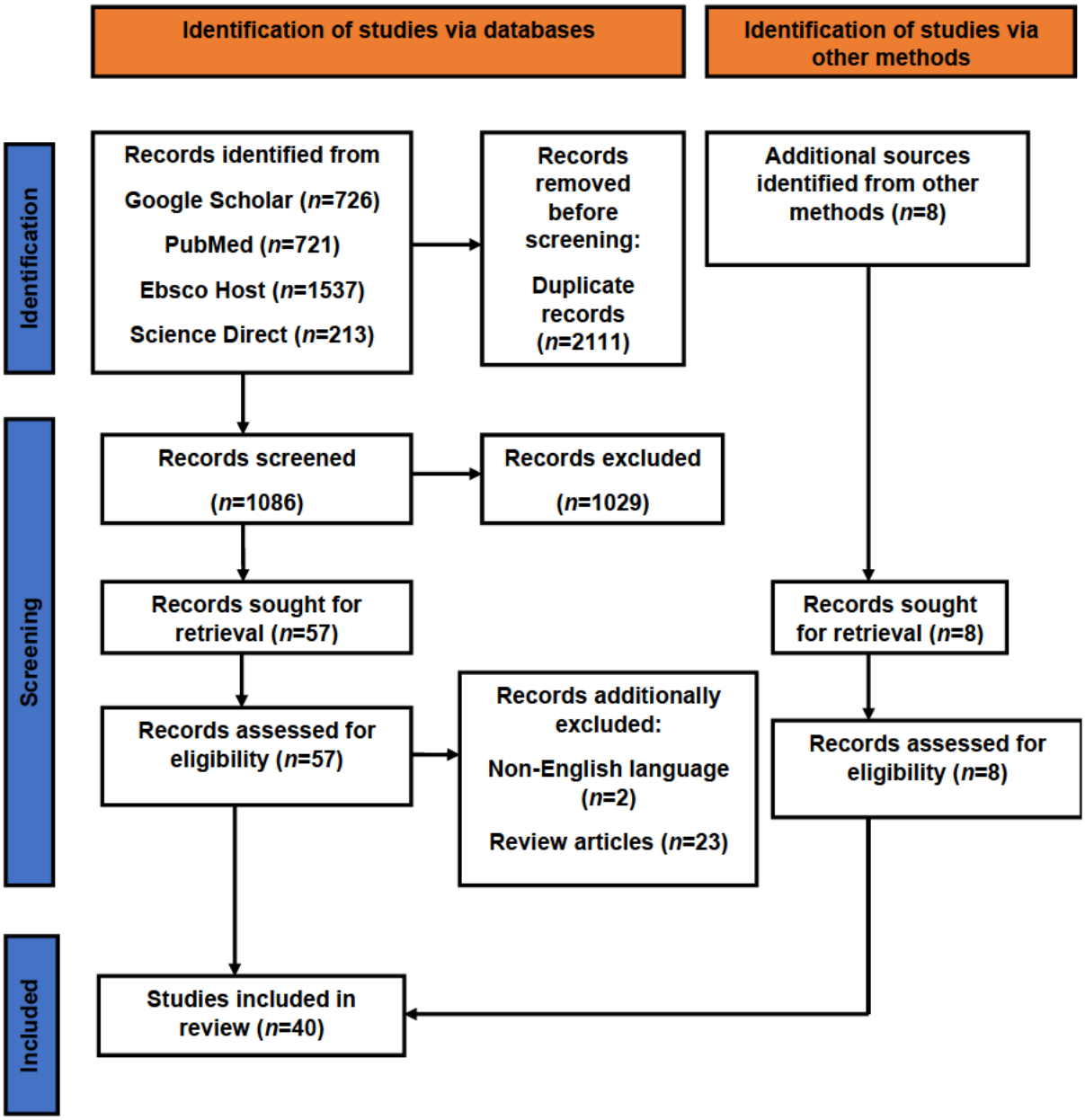

Figure 2.1 Reporting items for the systematic review of *Toxoplasma gondii* infections in animals and humans in southern Africa (adapted the Preferred Reporting Items for Systematic Reviews (PRISMA) statement.

2.6.2. Quality Assessment of Articles and Diagnostic Tests Used

The quality index of the reviewed articles ranged from 0.4 to 0.9. Diagnostic tests used in detecting the presence of *T. gondii* in the studies are shown in Tables 2.1.–2.5. Sample size ranged from 1–159 for domestic felids (Table 2.1), 1–250 for wild felids (Table 2.2), 4–39 for canids (Table 2.1), 109–184 for cattle (Table 2.3), 128–156 for goats (Table 2.3), 121–600 for sheep (Table 2.3), 70–311 pigs (Table 2. 3), 16–137 for chicken and birds (Table 2. 4), 20 for blue wildebeest (Table 2.2), 90 for baboons (Table 2.2), 20 for springbok (Table 2.2), and 1–3379 for humans (Table 2.5).

Table 2.1 Studies on the prevalence of *Toxoplasma gondii* in domestic canids and felids canids in southern African countries from 1961 to 2019.

Study Country	Host Species	*n*	Np	(%)	Diagnostic Test	Study Period	Quality Index Score	References
South Africa	Dogs	7	7	100	Histology	1955–1961	0.7	Smit 1961
South Africa	Cats	102	22	21.6	ELISA	2012	0.6	Lobetti and Lappin, 2012
South Africa	Cats	1	1	100	Histology and PCR	2012	0.9	Nagel, Williams, and Schoeman, 2013
South Africa	Cats	159	83	52.2	IFAT	2013–2014	0.8	Kenneth Hammond-Aryeea et al., 2015
Angola	Cats	102		3.9	MAT	2014–2016	0.7	Lopes et al., 2017
South Africa	Cats	109	35	32.1	LAT	2016	0.9	Tagwireyi et al., 2019

n, sample size*;* Np, number positive.

Table 2.2 Studies on the prevalence of *Toxoplasma gondii* in wildlife in southern African countries from 1966 to 2020.

Study Country	Host Species	*N*	Np	(%)	Diagnostic Test	Study Period	Quality Index Score	References
South Africa	Ferrets	7	4	42.9	Histology	1966	0.5	Bigalke et al., 1966
	Chinchilla	5	5	100				Du Plessis et al., 1967
	Baboons	90	30	11.7	IFAT, CF, Wolstenholme's modification, Sabin–Feldman dye test	1969–1971	0.8	Mc Connell et al., 1973

Namibia	Lions	66	65	98	Indirect Immunofluorescence Assay	1989–1991	0.6	Spencer 1993
					IFAT			
South Africa	Lions	18	18	100		1984–1996	0.8	Cheadle, Spencer, and Blagburn, 1999
	Leopard	2	2	100				
	Lions	5	5	100				
	Lions	3	3	100				
Bophuthatswana	Leopard	2	1	50				
	Cheetah	1	0	0				
Namibia	Lions	1	1	100				
	Cheetah	6	2	33.3				
South Africa	Cheetah	16	8	50				
	Lions	5	5	100				
	Lions	9	5	55.6				
Bophuthatswana	Lions	53	49	92		2002	0.5	Penzhorn et al., 2002
Zimbabwe	Lions	21	21	100				
South Africa	Lions	12	12	100				
	Lions	30	30	100				
Bophuthatswana	Leopard	1	1	100				
South Africa	Leopard	7	6	86				
	Caracal	29	24	3		2014–2017	0.9	Serieys et al., 2019
					ELISA			
Namibia	African Lion	59	55	93.2		2002–2015	0.6	Seltmann et al., 2020
	Brown hyena	19	12	92.3				
	Caracal	15	10	66.7				
	Cheetah	250	131	52.4				
	Leopard	58	47	81				
	Spotted hyena	11	10	90.9				
	African wild dog	7	5	71.4				
	Bat eared fox	4	1	25				
	Black backed jackal	39	26	66.7				
	Honey badger	10	7	70				
	Blue-wildebeest	20	2	10				

	Springbok	20	0	0

n, sample size; Np, number positive.

Table 2.3 Studies on the prevalence of *Toxoplasma gondii* in livestock in southern African countries from 1992 to 2020.

Study Country	Host Species	*N*	Np	(%)	Diagnostic Test	Study Period	Quality Index Score	References
Zimbabwe	Sheep	216	13	8.8	LAT and ELISA	1992	0.7	Pandey and Van Knapen, 1992
	Goats	156	7	7.1				
	Pigs	311	10	4.2				
		97	9	9.3	MAT	1995		Hove and Dubey 1999
		238	47	19.75	IFAT and ELISA	2000–2002	0.8	Hove et al., 2005a
		70	25	35.71				
South Africa	Sheep	600	26	4.3	ELISA	2007	0.9	Abu Samraa et al., 2007
	Cattle	178	37	20.8		2012	0.8	Ndou et al., 2013
	Sheep	292	23	7.9		2014	0.9	Hammond-Aryee et al., 2015
		121	78	64.5	LAT	2016		Tagwireyi et al., 2019
	Goats	128	69	53.9				
	Pigs	106	36	34				
	Cattle	184	60	32.6	ELISA	2013	0.8	Adesiyun et al., 2020
		109	5	4.6	PCR	2019		Mofokeng 2020

n, sample size; Np, number positive.

Table 2.4 Studies on the prevalence of *Toxoplasma gondii* in fowls (chicken and birds) in southern African countries from 2001 to 2019.

Study Country	Host Species	*N*	Np	(%)	Diagnostic Test	Study Period	Quality Index Score	References
Bophuthatswana	Pigeons	16	16	100	Indirect Haemaglutination Test (IHT)	2001	0.4	Mushi et al., 2001
South Africa	Birds	110	3	2.7	PCR	2014–2015	0.7	Lukášová et al., 2018
	Chickens	137	46	33.6	LAT	2016	0.9	Tagwireyi et al., 2019

n, sample size; Np, number positive.

Table 2.5 Studies on seroprevalence of toxoplasmosis reported in humans in southern African countries from 1974 to 2017.

Study Country	Human Description	*n*	Np	(%)	Diagnostic Test	Study Period	Quality Index Score	References
South Africa	People from different ethnic groups	806	296	37	IFAT	1974	0.8	Masons et al., 1974
	Reproductive age women	600	3	0.5		1975		Brink et al., 1975
Southern Africa	Blood donors from diverse ethnic groups	3379	665	20		1978		Jacobs and Mason 1978
Zambia	HIV-positive individuals	187	8	4.3	LAT and DT	1991		Zumla et al., 1991
	HIV-negative individuals	189	20	10.6		1991		
South Africa	HIV-positive individuals	307	25	8	ELISA	2007	0.9	Hari et al., 2007
Swaziland	Apparently healthy children	113	5	4.4	LAT	2009	0.8	Liao et al., 2009
South Africa	HIV-positive individuals	160	29	18.1	ELISA	2007–2008		Bessong and Mathomu 2010
Mozambique	HIV-positive patients	150	28	18.7		2010	0.7	Sitoe et al., 2010
South Africa	Immunocompetent individuals	497	32	6.4	Pastorex Toxo latex particle agglutination test and BioMèrieux ToxoScreen DA test	2011	0.8	Kistiah 2011
	HIV-negative patients	376	48	12.8				
	HIV-positive patients	376	37	9.8				
Mozambique	HIV-positive men	200	20	39.3	LAT	2010	0.7	Domingos et al., 2013
	HIV-positive women	200	25	50.9		2010		
Namibia	Blood donor	312	4	1.3	ELISA	2011–2012	0.8	van der Colf, Noden, Wilkinson, and Chipare, 2014
Zambia	Pregnant women	411	24	5.9	OnSite Toxo IgG/IgM Combo Rapid test	2015		Frimpong et al., 2017
South Africa	HIV-positive individuals	161	61	38	ELISA	2012–2013	0.7	Ngobeni and Samie, 2017

	HIV-negative individuals	161	27	16.7			
Namibia	Pregnant women	344		2.61	2016	0.9	Van der Colf et al., 2020

n, sample size; Np number positive.

2.6.3 Results from the Meta-Analysis

2.6.3.1 Pooled Prevalence and Heterogeneity

Toxoplasma gondii infection in southern African countries had an overall prevalence of 17% (95% confidence interval (CI): 7–29%). Angola had a prevalence of 4% (95% CI: 1–9%); Botswana, 92% (95% CI: 70–100%); Mozambique, 13% (95% CI: 9–18%); Namibia, 25% (95% CI: 0–69%); South Africa, 18% (95% CI: 6–33%); Swaziland, 4% (95% CI: 1–9); Zambia, 7% (95% CI: 4–10%); and Zimbabwe, 10% (95% CI: 0–24%) (Figure 2.2).

Based on animal groups, *T. gondii* infection in domestic felids in the region had an overall prevalence of 29% (95% CI: 7–54%) (Figure 2.3) and in wild felids, 79% (95% CI: 60–94%) (Figure 2.4). Canids (domestic and wild) had an overall prevalence of 69% (95% CI: 38–96%) (Figure 2.5); cattle, 20% (95% CI: 5–39%) (Figure 2.6); pigs, 13% (95% CI: 1–29%) (Figure 2.7); small ruminants (goats and sheep), 11% (95% CI: 0–31%) (Figure 2.8); and chicken and birds, 22% (95% CI: 0–84%) (Figure 2.9). The summary of studies on the prevalence of *T. gondii* in felids, canids, wildlife, livestock, and fowls in southern Africa are shown in Tables 2.1–2.4, respectively.

2.6.3.2 *Toxoplasma gondii* Infections in Humans in Southern African Countries

The pooled prevalence of *T. gondii* infection in humans was 14% (95% CI: 5–25%), with the highest prevalence of 17% (95% CI: 4–33%) recorded in South Africa and the least prevalence of 2% (95% CI: 1–3%) from Namibia (Figure 2.10). A summary of studies on *Toxoplasma* infections in humans in southern African countries is shown in Table 2.5. Out of a total of 8623 serum samples that were examined, 1342 were positive for *Toxoplasma* serology. Furthermore, an additional archaeological study on dead human remains was reportedly positive for *T. gondii*.

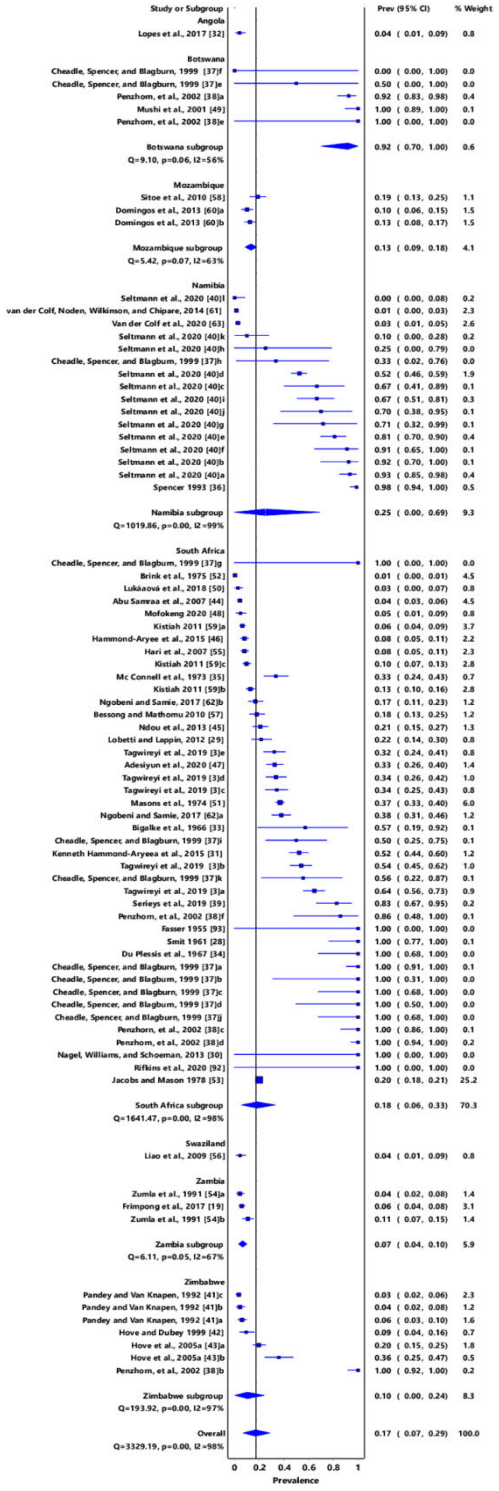

Figure 2.2 Forest plot of prevalence estimates of *Toxoplasma gondii* infections in Southern Africa. The confidence interval (CI) was 95%, and the diamond represents the pooled estimate (blue squares represent point estimation of the study weighted for population size).

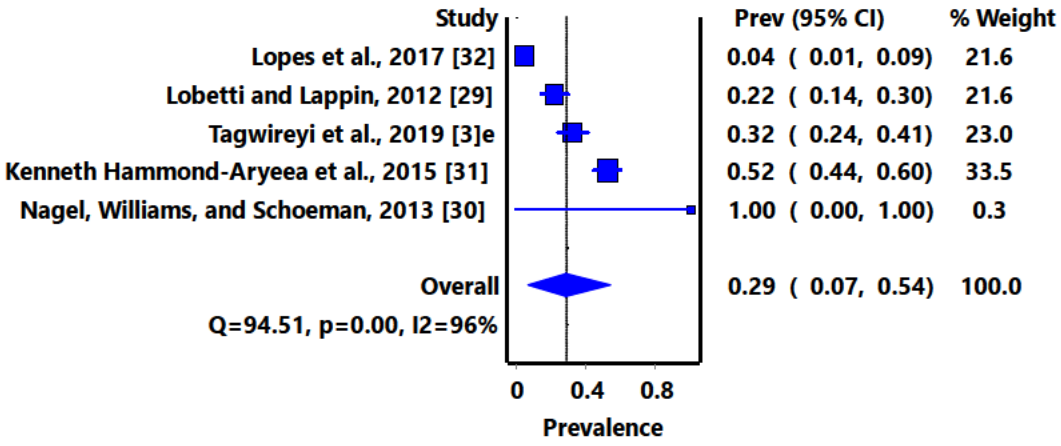

Figure 2.3 Forest plot of prevalence estimates of *Toxoplasma gondii* infections in domestic felids in southern Africa. The confidence interval (CI) was 95%, and the diamond represents the pooled estimate (blue squares represent point estimation of the study weighted for population size).

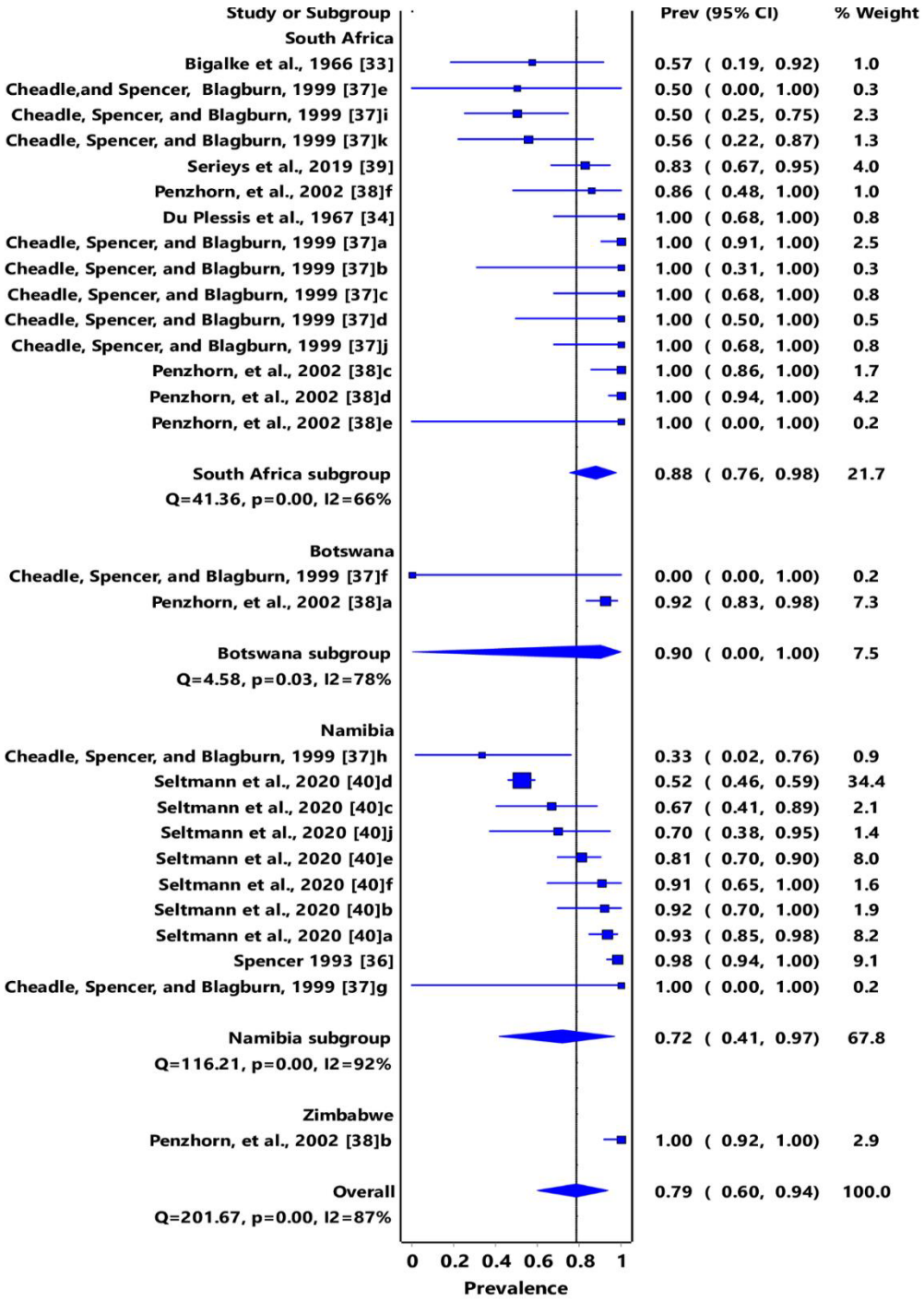

Figure 2.4 Forest plot of prevalence estimates of *Toxoplasma gondii* infections in wild felids in southern Africa. The confidence interval (CI) was 95%, and the diamond represents the pooled estimate (blue squares represent point estimation of the study weighted for population size).

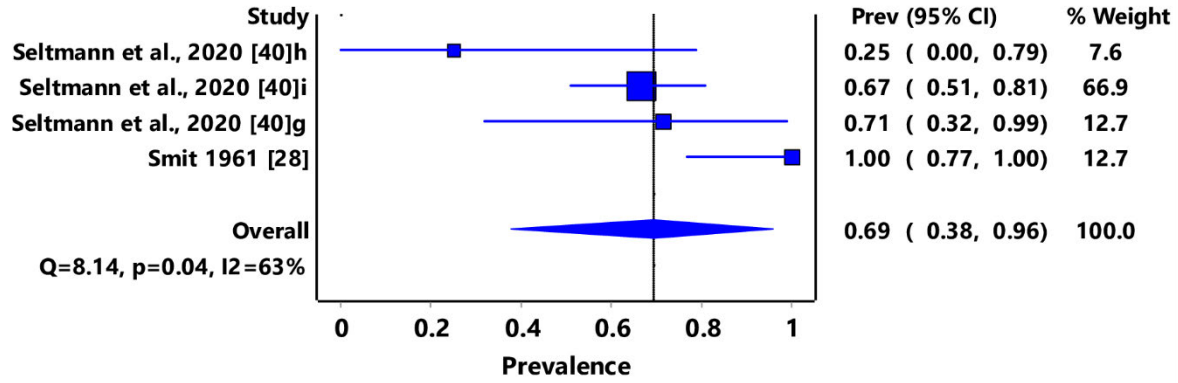

Figure 2.5 Forest plot of prevalence estimates of *Toxoplasma gondii* infections in canids in southern Africa. The confidence interval (CI) was 95%, and the diamond represents the pooled estimate (blue squares represent point estimation of the study weighted for population size).

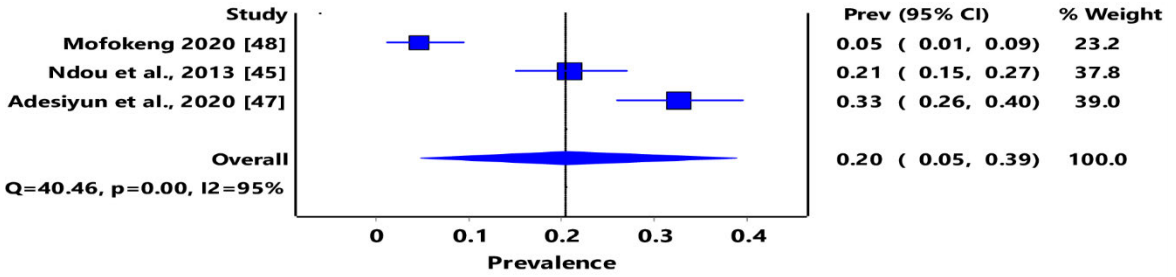

Figure 2.6 Forest plot of prevalence estimates of *Toxoplasma gondii* infections in cattle in southern Africa. The confidence interval (CI) was 95%, and the diamond represents the pooled estimate (blue squares represent point estimation of the study weighted for population size).

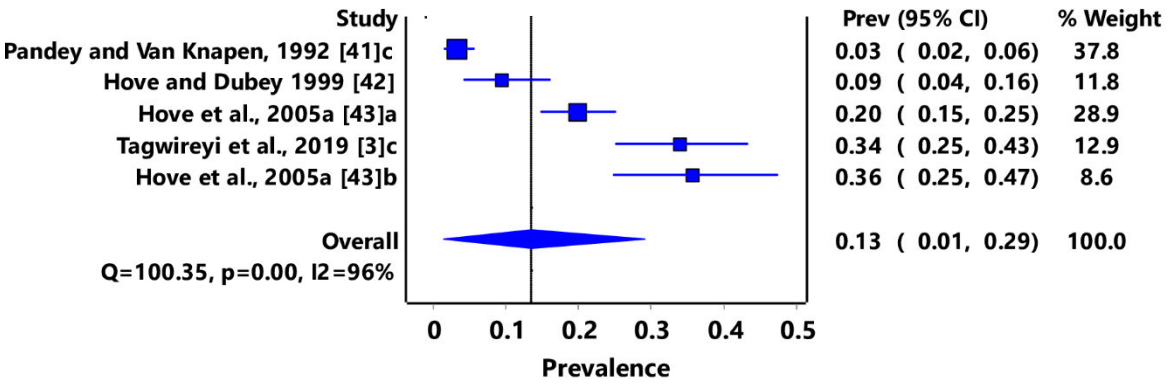

Figure 2.7 Forest plot of prevalence estimates of *Toxoplasma gondii* infections in pigs in southern Africa. The confidence interval (CI) was 95%, and the diamond represents the pooled estimate (blue squares represent point estimation of the study weighted for population size).

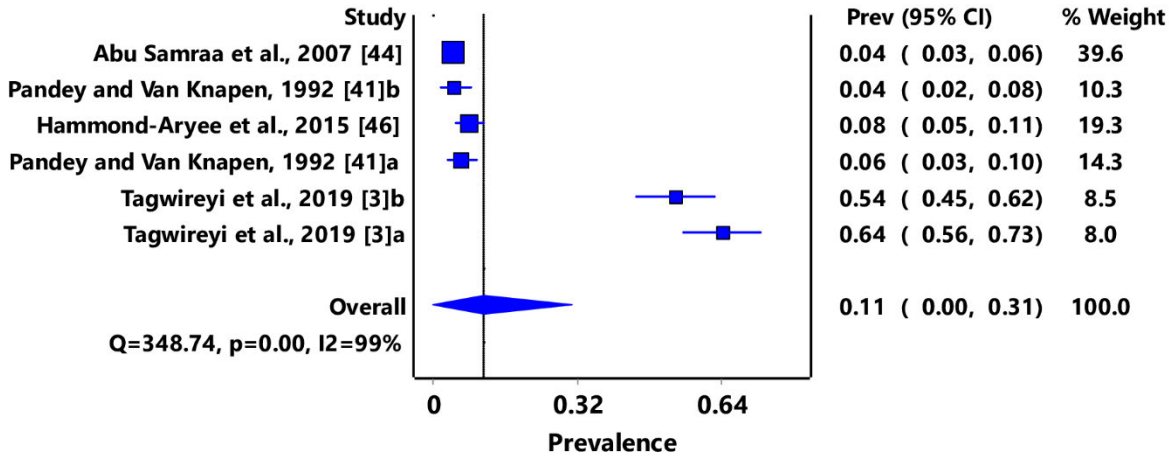

Figure 2.8 Forest plot of prevalence estimates of *Toxoplasma gondii* infections in small ruminants (sheep and goats) in southern Africa. The confidence interval (CI) was 95%, and the diamond represents the pooled estimate (blue squares represent point estimation of the study weighted fop r opulation size).

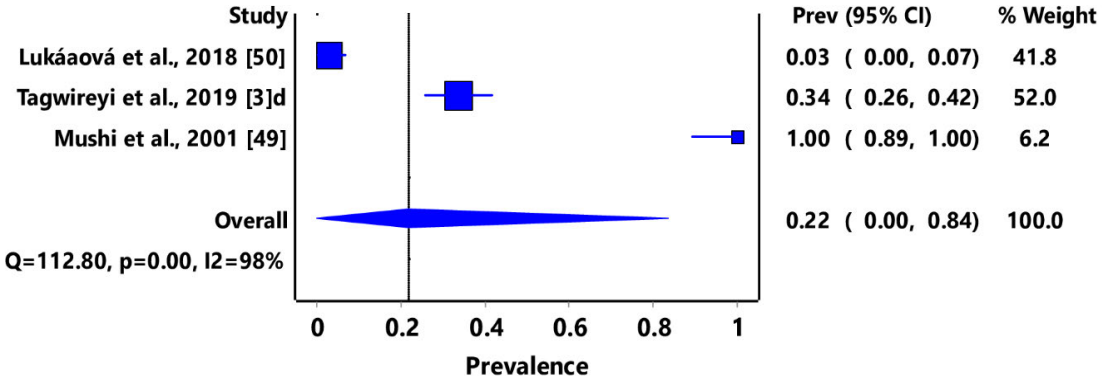

Figure 2.9 Forest plot of prevalence estimates of *Toxoplasma gondii* infections in birds in southern Africa. The confidence interval (CI) was 95%, and the diamond represents the pooled estimate (blue squares represent point estimation of the study weighted for population size).

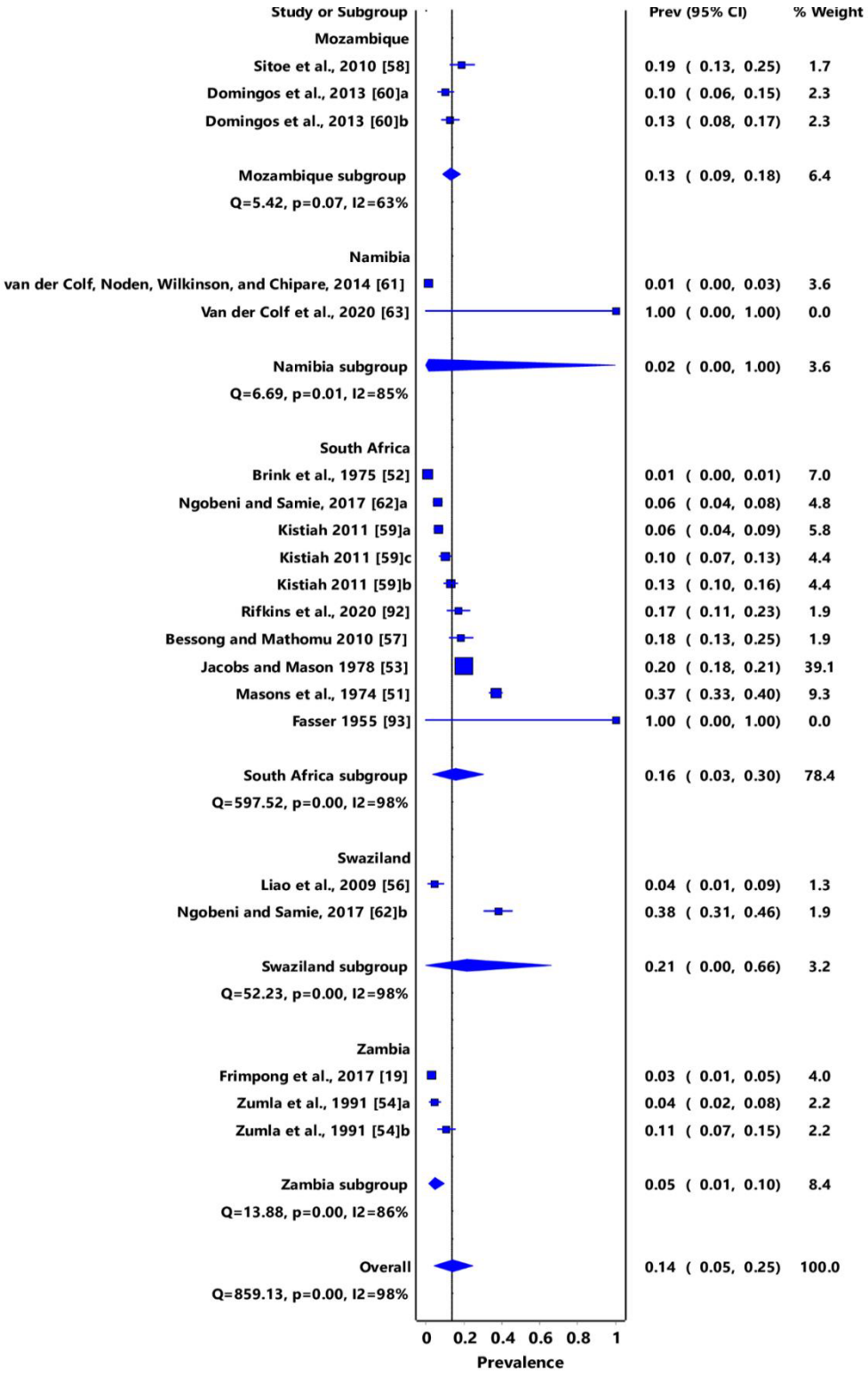

Figure 2.10 Forest plot of prevalence estimates of *Toxoplasma gondii* infections in humans in southern Africa. The confidence interval (CI) was 95%, and the diamond represents the pooled estimate (blue squares represent point estimation of the study weighted for population size).

2.6.3.3 Pooled Prevalence and Heterogeneity of Diagnostic Tests

Meta-analysis of the diagnostic methods used in detecting *T. gondii* infections in southern African countries had an overall pooled prevalence of 17% (95% CI: 7–29%). Molecular sub-group showed an estimated prevalence of 4% (95% CI: 0–11%); histology, 86% (95% CI: 55–100%); the latex agglutination test (LAT), 26% (95% CI: 11–42%); ELISA, 16% (95% CI: 5–28%); and IFAT, 22% (95% CI: 0–65%) (Figure 2.11). Diagnostics tests that were used less frequently, i.e., in less than three studies, were grouped separately and had a pooled prevalence of 9% (95% CI: 5–14%). These include MAT, LAT and ELISA; LAT and the Methylene blue dye test (DT); IFAT and ELISA; Pastorex Toxo latex particle agglutination test and BioMèrieux Toxo Screen DA test; and a combination of IFAT, CF (complement-fixation test), Wolstenholme's modification, and Sabin–Feldman dye test (Figure 2.11).

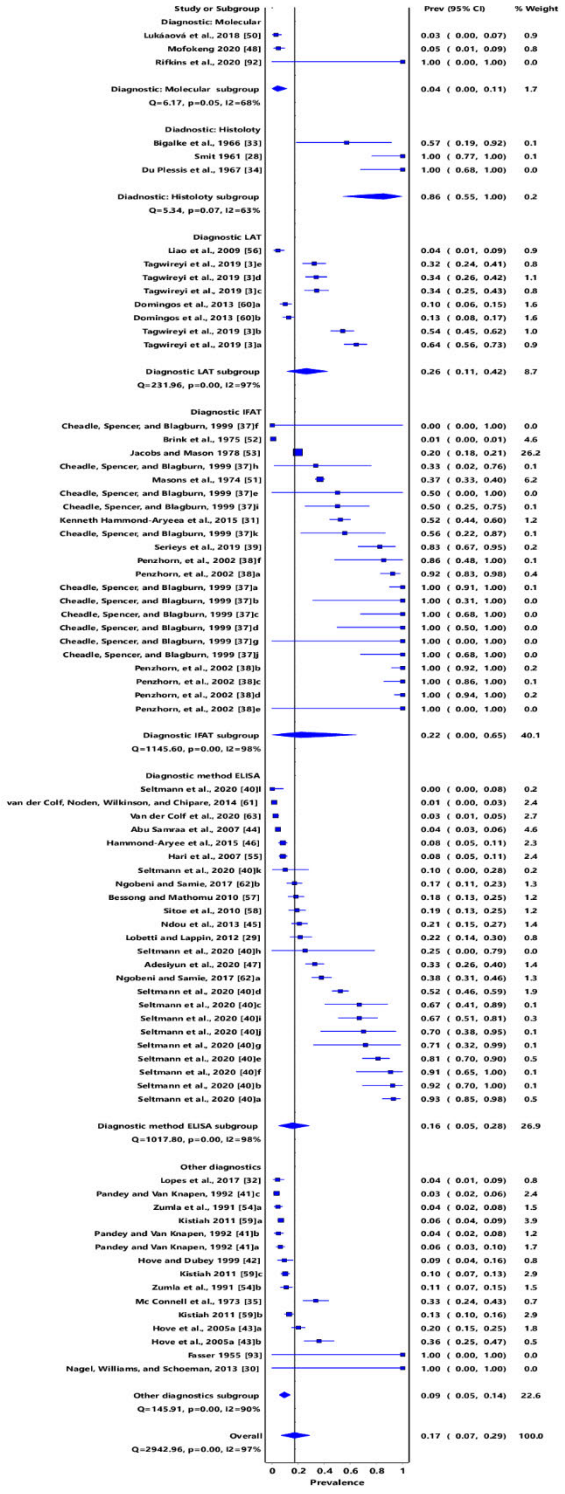

Figure 2.11 Forest plot of diagnostic methods of *Toxoplasma gondii* infections in southern Africa. The confidence interval (CI) was 95%, and the diamond represents the pooled estimate (blue squares represent point estimation of the study weighted for population size).

2.7 Discussion

Toxoplasma gondii is a coccidian cosmopolitan parasite of global economic and zoonotic importance. The importance of *T. gondii* in the meat industry and public health has been reported in a wide variety of hosts and humans, especially among immunocompromised individuals. This review revealed that there is limited information on the distribution of *T. gondii* in animals and humans in southern African countries. In this study, the overall pooled prevalence is estimated as 17% (95% CI: 7–29%).

The overall pooled prevalence of *T. gondii* infection 29% (95% CI: 7–54%) in domestic felids observed in this study is lower than the pooled seroprevalence of 51% (20–81%) reported in Africa, 52% (15–89%) in Australia (Montazeri, et al., 2020), and 30–40% global prevalence from previous studies (Dubey and Beattie, 2010; Webster and Dubey, 2010). However, the pooled prevalence of *T. gondii* infections observed in wild felids 79% (95% CI: 60–94%) in this study is higher than the pooled prevalence reported in Africa, Asia, Europe, and South America (Montazeri et al., 2020), while in north African countries, no data were available on wild felids (Rouatbi et al., 2019). The role of felids (domestic and wild) in *T. gondii* epidemiology has been documented in several reports (Elmore et al., 2010; Montazeri et al., 2020; Hatam-Nahavandi et al., 2021). In this review, seven (7) studies were on wild felids, while five (5) studies were on domestic cats. A single infected felid is capable of shedding millions of oocysts for 10–15 days, thereby contaminating the environment and posing infection risk to various intermediate hosts (Hatam-Nahavandi et al., 2021). Emphasis on the adequate veterinary care of animals, including frequent treatment of cats for toxoplasmosis and reduction in the population of stray cats in the environment, should be encouraged in southern African countries. Moreover, a surveillance system for *Toxoplasma* infection should be instituted at the wildlife-livestock interface areas in the region.

Limited studies exist on *T. gondii* infection in canids (domestic and wild), with an overall pooled prevalence of 69% (95% CI: 38–96%). This result is higher than the prevalence of 51.2.% reported in wild canids by (Dubey et al., 2020c) and the global prevalence of 39.6% reported in foxes (Wei et al., 2021). The studies in cattle were few and only done in South Africa and gave an overall pooled prevalence of 20% (95% CI: 5–39%), which is higher than the pooled prevalence of 16.3% (10.6–23.0%) from West Africa (Odeniran et al., 2020) and 12% (CI 8–17%) in the entire continent of Africa (Tonouhewa et al., 2017). The estimated prevalence is, however, lower than the reported seroprevalence from Brazil and Sudan (Costa et al., 2001; Khalil and Abdel Gadir, 2007). Studies have identified the consumption of raw or

undercooked beef as a possible risk of toxoplasmosis transmission in humans (Baril et al., 1999; Belluco et al., 2018).

Similarly, there is evidence of *T. gondii* infection in small ruminants (sheep and goats) (Belluco et al., 2018), and the pooled prevalence of 11% (95% CI: 0–31%) recorded in this study is lower than that of 29.1% (15.6–44.8) in sheep and 18.1% (4.0–38%) in goats in West Africa (Odeniran et al., 2020) and sheep 26.1% (95% CI: 17.0–37.0%) and goats 22.9% (95% CI: 12.3–36.0%) in Africa (Tonouhewa et al., 2017). Among livestock species, sheep constitutes an important source of animal protein as well as meat and milk from goats (Jones et al., 2009), whereas consumption of rare lamb and drinking of unpasteurized milk has been identified as risk factors in acute toxoplasmosis transmission in humans (Mancianti et al., 2013; Sadek et al., 2015; Sroka et al., 2017; Belluco et al., 2018).

Studies reporting the seroprevalence of *T. gondii* in pigs in southern Africa emanated from South Africa and Zimbabwe, with an overall pooled prevalence of 13% (95% CI: 1–29%). This is similar to the prevalence reported in pigs from Europe (Foroutan et al., 2019). but lower than the prevalence reported in pigs from North America, South America, Asia (Foroutan et al., 2019), West Africa (Odeniran et al., 2020), Africa (Tonouhewa et al., 2017), and globally (Foroutan et al., 2019). Pigs are among the popular food animals and have been reported as a source of human toxoplasmosis through ingestion of raw or undercooked pork (Dubey and Jones, 2008). *Toxoplasma gondii* infections in pigs are either acquired prenatally via transplacental transmission or postnatally via ingestion of oocysts from a contaminated environment (Tonouhewa et al., 2017). Hence, indoor rearing of pigs is important to reduce the exposure of pigs to *T. gondii* infections from the contaminated environment (Hove et al., 2005a; Bamba et al., 2016; Tonouhewa et al., 2017).

The overall pooled prevalence of 22% (95% CI: 0–84%) of *T. gondii* seroprevalence from chickens and birds in southern African countries is lower than the estimated prevalence of anti-*T. gondii* antibody 22% (95% CI: 0–84%) reported in chickens in West Africa (Odeniran et al., 2020) and 37.41% (95% CI: 29.20–46.00%) from chickens in Africa (Tonouhewa et al., 2017). Chicken meat is a key contributor to animal protein due to affordability and availability (Dubey et al., 2020a), however, it also plays a major role in human toxoplasmosis transmission when the meat is consumed raw or undercooked (Tonouhewa et al., 2017). The free-range chickens ingest *T. gondii* oocysts from the contaminated environment while foraging, thus acting as zoonotic agents of human toxoplasmosis. The role of birds, especially the birds of prey, in maintaining transmission between the sylvatic cycle and domestic cycle has also been documented (Dubey et al., 2021).

The pooled seroprevalence of anti-*T. gondii* antibody from humans came from studies that focused mainly on immunocompetent individuals, HIV+ patients, and pregnant women (Zumla et al., 1991; Bessong et al., 2010; Domingos et al., 2013; Ngobeni and Samie, 2017; Coupe et al., 2019, Van der Colf et al., 2020) as well as a few studies on blood donors and children (van der Colf et al., 2014). Overall, the pooled prevalence of 14% (95% CI: 5–25%) of *T. gondii* infection in humans from southern African countries was lower than the seroprevalence reported from a meta-analysis conducted on pregnant women in African regions, American regions, eastern Mediterranean regions, Europe, the South-East Asia region, globally (Bigna et al., 2020), and in some North African countries (Tunisia, Egypt, and Morocco) (Rouatbi et al., 2019). However, this prevalence is greater than the seroprevalence reported from Western pacific region and the World Health Organization (WHO) regions of the world, 1.1% (0.8–1.4) (Bigna et al., 2020). Humans acquire *T. gondii* infections either through ingestion of oocysts from the contaminated environment (Dubey, 2010; Rajendran et al., 2012), via tissue bradyzoites from consumption of raw or undercooked infected meat, transplacental transmission from mother to fetus (Mahmoud et al., 2015; Hammond-Aryee, and Van Helden, 2015), or organ transplants or blood transfusion (Robert-Gangeux, and Darde, et. al; 2012; Mose et al., 2020). Infections in immunocompetent individuals are not associated with critical symptoms compared to the immunosuppressed, particularly AIDS patients or newborns. Congenital transmission often results in clinical manifestations, such as encephalitis, pneumonia, and ophthalmologic disorders (Tonouhewa et al., 2017; Rouatbi, et al., 2019). The seropositivity of *T. gondii* prevalence in the subjects in the reviewed articles suggests an active transmission of human toxoplasmosis in the region and requires intervention to prevent infection. Control and prevention measures include environmental control of feral cats, provision of veterinary care of domestic animals, adoption of personal hygiene, creating awareness of the risk associated with consumption of raw or undercooked meat, adequate screening of blood or organ donors, and adopting a national toxoplasmosis treatment scheme for pregnant women in the region (Montazeri et al., 2020; Smit et al., 2021).

Diagnostic tools used in the reviewed articles varied widely and ranged from MAT, LAT, IFAT, ELISA, DT, CF, Wolstenholme's modification, and Sabin–Feldman dye test techniques to molecular approach. Studies have shown that different diagnostic techniques produce results that are heterogeneous (Rouatbi, et al., 2019). For instance, the diagnostic performance of the MAT technique has been reported to be higher than that of ELISA (Dubey, et al., 1995). In this study, the majority of articles adopted ELISA and IFAT to determine the seroprevalence of *T. gondii*. Although serological methods seem to lack sensitivity and

specificity, they remain a standard tool for the qualitative detection of antibodies (Rouatbi, et al., 2019). Studies that used LAT (Liao et al., 2009; Domingos et al., 2013; Tagwireyi et al., 2019), histology (Smit, 1961; Bigalke, et al., 1966; Du Plessis et al., 1967), and molecular techniques (Lukášová et al., 2018; Mofokeng et al., 2020; Rifkin et al., 2020) were few, while others used the combination of one or two of LAT, MAT, ELISA, IFAT, DT, CF, Wolstenholme's modification, and Sabin–Feldman dye test techniques (Fasser, et al., 1955; Hove et al., 2005b; Mc Connell et al., 1973; Zumla et al., 1991; Pandey, and Van Knapen 1992; Hove, T.; Dubey; Kistiah et al., 2011). A recent study comparing three serological diagnostic tools showed that ELISA and IFAT had relatively higher sensitivity and specificity than MAT (Sharma et al., 2019). Additionally, ELISA and IFAT are less laborious and time-consuming than MAT (Sharma et al., 2019). As much as molecular tools are reliable diagnostic tools, they were used in only three studies. Molecular tools are ideal for determining the distribution of *T. gondii* in the environment (soil and water samples), and the few studies might have been attributed to the non-availability of this diagnostic facility or the lack of competent individuals for such analysis. The adoption of molecular methods (both PCR and more discriminatory and advanced molecular tools, such as PCR-RFLP markers and DNA sequencing) will be imperative in identifying the *T. gondii* strains infecting various hosts.

Generally, substantial heterogeneity existed between the studies reviewed and subgroups. This may be due to a range of factors, such as people's varying hygiene practice levels, limited studies from some countries, varying diagnostic methods used, methods of rearing livestock animals, meat consumption pattern of studied individuals, or hostage.

2.8 Conclusions and Recommendation

This study showed that there are limited studies on *T. gondii* in humans and animals in southern Africa. Considering the limited information on the prevalence of *T. gondii* in southern African countries, more studies targeting the epidemiology of this parasite in the environment (soil and water), vegetable, food animals, wild animals, and humans (children, pregnant women, immunocompromised, and healthy people) must be conducted to better understand the transmission dynamics in the region. Additionally, there is a need to establish a surveillance system at the wild animals-livestock interface for monitoring transmission between livestock, wildlife, and humans. Furthermore, emphasis should be focused on health education and the preventive measures of toxoplasmosis, which include adequate cooking of meat, washing of fruits and vegetables before eating, and provision of potable water.

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CHAPTER 3: A SYSTEMATIC REVIEW AND META-ANALYSIS OF CANINE, FELINE AND HUMAN *TOXOCARA* INFECTIONS IN SUB-SAHARAN AFRICA

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3.1 Abstract

Toxocariasis is an emerging zoonotic disease caused by *Toxocara canis* and *T. cati*. Toxocariasis and its etiological agents are of global public health importance, whose burden appears underestimated, especially in sub-Saharan Africa (SSA). The diversity in the transmission routes of these parasites contributes to disease prevalence and often hinders disease control measures. This study aimed to review the epidemiological distribution of *Toxocara* infections in SSA region. A systematic review and meta-analysis were performed using PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis). We identified 94 relevant, peer-reviewed articles, out of which, 75 articles were found eligible based on *Toxocara* infections in dogs, cats, and humans. Overall, 27,102 samples were examined for *T. canis* in dogs, *T. cati* in cats and *Toxocara* serology in humans, out of which 6142 were positive for *Toxocara* infection: 3717 (13.7%) in dogs (faecal, 3487; necropsy, 180; hair, 50); 266 (1%) in cats (faecal, 101; necropsy, 165); and 2159 (8%) in humans (serology). Overall mean prevalences of 19% (95% confidence interval (CI): 14–23%), 9% (95% CI: 0–28%) and 36% (95% CI: 24–49%) were recorded in dogs, cats and humans, respectively. Substantial heterogeneity was observed between studies and subgroups (I^2 =99%, $P<0.01$). Findings from the review showed that studies on the epidemiology of *Toxocara* infections in the SSA region are limited. We strongly recommend focused, collaborative, and coordinated studies to determine *Toxocara* spp. prevalence in various hosts, including food animals and the environment, through a 'One Health' approach across SSA countries.

Key words: Prevalence; review; risk factors; sub-Saharan Africa; *Toxocara* infections; meta-analysis; zoonosis

3.2 Introduction

Toxocariasis is a zoonosis of global public health significance caused by *Toxocara canis* (canid roundworm) and *T. cati* (felid roundworm) (Luna et al., 2018). *Toxocara* infections have been reported globally, with prevalence ranging from 1.6–93% in humans and 3.7–87.1% in soil, to 1.2–100% in definitive canid and felid hosts (Magnaval et al., 1994; Mukaratirwa and Taruvinga, 1999; Quattrocchi et al., 2012; Okewole, 2016; Ma et al., 2018). The disease burden

is high in sub-Saharan African regions where poor hygiene practices and poor veterinary care of pets are prevalent (Liao et al., 2010; Ma et al., 2018). Toxocariasis burden is exacerbated due to the several transmission routes of the parasite via a wide range of invertebrates and vertebrate reservoir hosts, including earthworms, insects, land snails, rodents, birds and food animals (poultry, pigs, lamb, goats and cattle), which contribute to parasite maintenance in the environment (Pahari and Sasmal, 1991; Taira et al., 2004; Antolová et al., 2013; Fialho and Corrêa, 2016).

In humans, infection is acquired via waterborne or foodborne route via consumption of contaminated water, foods, and vegetables, and/or geophagia (Salem and Schantz, 1992; Yoshikawa et al., 2008; Macpherson, 2013; Fialho and Corrêa, 2016; Zibaei et al., 2017; McManus et al., 2018). There is substantial evidence of a strong association between human toxocariasis and the consumption of undercooked/raw meat viscera of food animals among people with such dietary habits in countries like Japan and Korea (Choi et al., 2012; Yoshida et al., 2016). In definitive hosts, *Toxocara* infections occur via several routes, including the accidental ingestion of infective eggs from a contaminated environment, the consumption of infective larvae via predator–prey interactions, mainly in felids, and transmission via lactogenic (canids and felids) and/or transplacental (in canids) transfer (Macpherson, 2013; Overgaauw and van Knapen, 2013).

In the definitive hosts, the larvae hatch in the small intestine and migrate via the liver, heart, and lungs through the circulatory system, during which the larvae enter the trachea from the lungs through the bronchus and reach the pharynx where they are re-ingested and reach the small intestine where maturity is attained (Selek et al., 2016). Un-embryonated eggs are voided with faeces in 4–5 weeks post infection in dogs (Schnieder et al., 2011) and in eight weeks post infection in cats (Sprent, 1956). If larvae are ingested from infected paratenic hosts where lifecycle migration has been satisfied, migration no longer holds in definitive hosts; rather, the larvae are released and develop to maturity in the small intestine (Overgaauw and van Knapen, 2013).

Both adults and migrating larvae of *Toxocara* spp. are pathogenic, causing a variety of pathological conditions in the definitive hosts (Fisher, 2014). *Toxocara cati* infections in cats are associated with increased eosinophils in blood and bronchoalveolar fluid and peribronchiolar opacity (Dillon et al., 2013), while *T. canis* causes eosinophilic gastroenteritis and focal lesions in variety tissues, including the liver and lungs (Fisher, 2014), as well as retinitis by migrating larvae (Schnieder et al., 2011; Cooper et al., 2014). In humans, only the migrating larvae are pathogenic, causing a varying degree of morbidities depending on the

affected organs, infection intensity, and duration, host age and immunity status of the infected host (Holland and Hamilton, 2013; Lee et al., 2014; Fan et al., 2015). Clinical manifestations include ocular larva migrans, visceral larva migrans, covert or common toxocariasis and neurotoxocariasis (González-García et al., 2017; Ma et al., 2018). Other toxocariasis-associated manifestations are, reduced cognitive function in children, poor academic performance, perpetuation of poverty in affected communities and health disparities in endemic areas (Walsh and Haseeb, 2012; Lee et al., 2014).

Diagnosis of *Toxocara* infection in non-definitive hosts includes histopathological examination for larvae in suspected tissues, and morphometric assessment of larvae, or the specific detection of larval DNA from tissue or body fluid samples (Smith and Noordin, 2006; Machado et al., 2017; Ma et al., 2018). In definitive hosts, infection is diagnosed by the morphological identification of eggs in faeces (Machado et al., 2017). Recent advancements in molecular technology and applications have enhanced the specific identification of *Toxocara* spp., which have contributed immensely to research in clinical toxocariasis and epidemiological studies (Smith et al., 2009; Strube et al., 2013; Poulsen et al., 2015; Ma et al., 2018).

Despite the availability of improved diagnostic tools and increasing global awareness of the public health importance of *Toxocara* spp., there is still limited information on the epidemiology of infections in sub-Saharan Africa (SSA). In order to recognize the public health importance and status of toxocariasis in SSA, it is important that the available information on epidemiology and incidence in animals, humans, and the environment within sub-Saharan regions be assessed and analysed. Therefore, this review focused on the systematic review and analysis of existing studies on *Toxocara* infection in SSA, to determine the epidemiological distribution of infection in various hosts in the region and identify research gaps for future research.

3.3 Methods

3.3.1 Search strategy

A systematic search was conducted in the search engines Google Scholar, PubMed, AJOL and EBSCO Host database using the following terms and Boolean operators (AND, OR): *Toxocara cati* AND *Toxocara canis*, *Toxocara* in dogs OR humans AND sub-Saharan Africa, *Toxocara* in cats AND sub-Saharan Africa, *Toxocara* in soil, visceral larva migrans AND ocular larva migrans. The titles and abstracts of the search results were perused for the selection of relevant articles. References within the identified articles were additionally used as a guide to other

literature. The literature search was concluded in May 2018. Full-text articles were retrieved and managed in Endnote reference manager, version X7 (Clarivate Analytics, Philadelphia, PA, USA).

3.3.2 Inclusion and exclusion criteria

An article was included in this study if it was published between 1979 and 2018 in a peer-reviewed journal and reported on (1) prevalence of *T. canis* in dogs and/or *T. cati* in cats, and/or *T. canis*/*T. cati* in soil in sub-Saharan African countries; and (2) *Toxocara* serology in humans. Grey literatures, dead links and duplicate results were excluded during the abstract review.

3.3.3 Quality assessment

A tool developed by Munn et al. (2015) was used to assess the quality of the selected articles for inclusion in the meta-analysis. The tool was modified to suit the kind of information that we desired to extract from the articles. Each article was assessed based on the following: (1) if the research objective(s) were relevant to the study; (2) if the prevalence of *Toxocara* was the main objective of the study; (3) if the study design was appropriately defined (observation, cross-sectional); (4) if the samples were randomly selected; (5) if the study subjects were categorized by age/sex where relevant; (6) if the study used valid diagnostic methods; (7) if the method(s) of diagnosis were conducted in a reliable and replicable way; (8) if the target sample was representative of the general population; (9) if the endemic status of *Toxocara* infection in the study community/animals was described; and (10) if the history of prior treatment of subjects for *Toxocara* infection was described. The quality index score for each article was calculated by dividing the study quality score by ten.

3.3.4 Data extraction

From each paper that had been selected for inclusion, we extracted data on the country the study was conducted, study period, the type of study subjects or hosts (dogs, cats, humans), the sample size, number of infected subjects/hosts, *Toxocara* spp, the percentage prevalence and the diagnostic method(s) used.

3.3.5 Data analysis

All the data extracted from the search were entered in Microsoft Excel for analysis. The MetaXL add-in for Microsoft Excel (www.epigear.com) was used to carry out a meta-analysis. Prevalence estimates with their 95% confidence intervals (CIs) and heterogeneity were computed using the quality-effect model. Heterogeneity was quantified using the inverse variance statistic (I^2 index) and we tested for its significance using Cochran's Q test. The I^2 index was interpreted as no, low, moderate or high heterogeneity if the value was 0%, ≤25%, 50% or ≥75%, respectively. Forest plots were generated to show the prevalence of *Toxocara*

among the study subjects. Furthermore, subgroup analysis was carried out to assess the mean pooled prevalence estimates according to host types and regions within SSA. The Luis Furuya–Kanamori (LFK) index and funnel plot were used to assess the risks of publication bias (Barendregt and Doi, 2016). We determined the symmetry of the Doi plots using the LFK index. An LFK value within the range of '±1' was considered as 'symmetrical' and classified as absence of publication bias, while an LFK value within the range of '±2' was considered as minor asymmetry with slight publication bias and an LFK value outside the range of '±2' was considered as major asymmetry and high publication bias (Barendregt and Doi, 2016).

3.4 Results

3.4.1 Search results

A total of 94 articles were obtained from the literature search, of which 11 articles were excluded after the title and abstract inspection. Articles were further read and assessed for inclusion. Eight studies were subsequently excluded due to lack of a valid diagnostic method to determine *Toxocara* species. Overall, 75 articles were included in the meta-analysis.

3.4.2 Study quality assessment

The quality index of the eligible articles selected ranged from 0.3 to 0.9. A variety of diagnostic methods used to detect *Toxocara* eggs in the studies are shown in Tables 3.1a–c, 3.2 and 3.3a, b). Different sampling methods for the study hosts were also used and sample sizes of the selected studies ranged from 200–384 (faecal) and 200–1502 (necropsy) in cats, 20–1647 (faecal) and 12–539 (necropsy) in dogs, to 40–601 (serology) in humans. Studies from the articles selected were conducted in Central Africa, East Africa, Southern Africa and West Africa within the SSA region. Data were derived from only 16 sub-Saharan countries, with most data from studies conducted in Nigeria (39). Other countries had less than ten studies (Tables 3.1a–c, 3.2 and 3.3a, b).

3.4.3. Meta-analysis

3.4.3.1. Prevalence of *T. canis*

A total of 18,463 samples were examined for *T. canis*, and, of these, 16,857 were obtained from faecal, 1189 from necropsy and 417 from hair. Summaries of studies on the prevalence of *T. canis* in dogs in the SSA region are shown in Table 3.1a–c). Out of the total samples examined, 3717 (20.1%) (faecal=3487; necropsy=180; hair=50) were positive for *T. canis* infection. *Toxocara canis* infection in the SSA region gave an overall prevalence of 19% (95% CI: 14–23%). Substantial heterogeneity was observed between studies (I^2 =98%, P<0.01). Among the regions within SSA, Central Africa had the highest prevalence of 35% (95% CI:

30–41%) for *T. canis*, followed by East Africa, which had a prevalence of 31% (95% CI: 20–43%). Southern Africa had the least prevalence of 11% (95% CI: 5–18%).

3.4.3.2. Prevalence of *T. cati*

Out of a total sample size of 2625 cases of *T. cati*, (faecal=584; necropsy=2041) that were examined, 266 (faecal=101; necropsy= 165) were positive, with an overall prevalence estimate of 9% (95% CI: 0.00–28%). A summary of studies on the prevalence of *T. cati* in the SSA region is shown in Table 3. 2). A high degree of heterogeneity was observed between studies (I^2=99%, P<0.01). Regional subgroup analysis was not carried out, as only three studies mentioned the species of *Toxocara*.

Table 3.1a Summary of studies on the prevalence of eggs of *Toxocara canis* in faeces of dogs in Eastern and Southern Africa from 1979-2018.

Study Period	Country	Objective	Sample Size (N)	Outcome (%)	Method of Diagnosis	References
Eastern Africa Region						
2005-2006	Ethiopia	Determination of gastrointestinal helminths in domestic dogs	100	21.0	Faecal flotation method	Yacob et al., 2007
2009	Tanzania	Determination of gastrointestinal parasites in non-descript dogs	241	13.7	Sedimentation and flotation technique	Swai et al., 2010
2007-2008	Ethiopia	Determination of prevalence of helminths parasites in dogs	70	70.0		Zewdu et al., 2010
2011		Determination of prevalence of helminths parasites in stray dogs	269	53.9	Kato-Katz technique	Jones et al., 2011
2011-2012		Determination of prevalence of helminth parasites in pet and stray dogs	328	93.7	Direct smear, sedimentation, and flotation technique	Abere et al., 2013
2010-2011		Determination of prevalence of helminths parasites in stray, semi-confined, and confined dogs	860	23.3		Mekbib et al., 2013
2009-2010		Determination of prevalence of helminths parasites in domestic and stray dogs	146	23.2	McMaster technique	Gugsa et al., 2015

2014-2015		Determination of prevalence of gastrointestinal parasites of domestic dogs	384	32.0	Faecal flotation method	Tamerat et al., 2015
		Southern Africa Region				
2008-2009	South Africa	Determination of prevalence of gastrointestinal parasites of stray dogs	240	7.9	Sedimentation technique	Mukaratirwa and Singh, 2010
1960-1977		Determination of prevalence of gastrointestinal parasites of domestic dogs	502	1.4	Faecal flotation method	Verster, 1979
1991	Bophuthatswana	Descriptive study of canine population	209	1.9	McMaster technique	Rautenbach et al., 1991
1998	Zimbabwe	Survey of environmental contamination of parks and playgrounds with canine helminths of zoonotic significance	161	5.6		Taruvinga and Mukaratirwa, 1999
1997-1998	South Africa	Determination of helminth parasites of dogs from resource-limited communities	31	19	Faecal flotation method	Minnaar et al., 1999
1997-1998		Determination of helminth parasites of dogs in an urban resource-limited community	69	36.2		Minnaar and Krecek, 2001
1998-1999		Determination of helminth parasites of dogs in a peri-urban resource-limited community	63	19		Minnaar et al., 2002

2006-2007		Survey of internal parasites in free-ranging African wild dogs	12	75		Flacke et al., 2010
2008-2009		Determination of prevalence of gastrointestinal parasites of stray dogs	240	7.9	Sedimentation technique	Mukaratirwa and Singh 2010
2010	Zambia	Determination of prevalence of gastrointestinal helminths of domestic dogs	452	22.1	Faecal flotation method	Bwalya et al., 2011
2005-2006		Determination of prevalence of gastrointestinal parasites of domestic dogs	540	7.6		Nonaka et al., 2011
2014	Malawi	Determination of parasites and antibodies against vector borne pathogens in domestic dogs	40	7.5	Faecal flotation method	Alvåsen et al., 2016

Table 3.1b Summary of studies on the prevalence of T. canis eggs in faeces of dogs in West Africa region from 1979-2018.

Study Period	Objective	Sample Size (N)	Outcome Percentage (%)	Method of Diagnosis	References
		Nigeria			
1979	Determination of prevalence of helminth ova in dogs	166	38.6	Faecal floatation technique	Dada and Belino, 1979
1985	Determination of prevalence of gastro-intestinal helminth of dogs	254	15		Ugochukwu and Ejimadu, 1985
1980	Canine toxocariasis and the associated environmental contamination	262	26.9	McMaster and Floatation technique	Chiejina and Ekwe, 1986
1996	Survey of intestinal parasites of dogs	197	31.5		Anene et al., 1996
2002	Determination of prevalence of gastrointestinal helminths of semi stray dogs	554	14.8	Direct and Concentration technique	Anosike et al., 2004
1983-1985, 1994-2002	Determination of prevalence of gastrointestinal parasites of domestic dogs	1647	21.4	Faecal floatation technique	Onyenwe and Ikpegbu, 2004
2005	Determination of prevalence of gastrointestinal parasites of domestic dogs	310	73.1		Ibidapo, 2005
		Cameroon			
2005	Determination of prevalence of gastrointestinal helminths of domestic dogs	116	34.5		Komtangi et al., 2005
		Nigeria			
2007	Determination of prevalence of helminth eggs in dogs	41	56.1		Omudu and Amuta, 2007

2004	Determination of prevalence and intensity of *T. canis* eggs in domestic dogs	269	33.5	Kato-Katz technique	Sowemimo 2007
1995-2005	Determination of prevalence of parasitic infection of dogs	651	17.4	Secondary data	Mbaya et al., 2008
2001-2002	Determination of epidemiology of helminths of domestic dogs	959	1.0	Kato-Katz technique	Sowemimo and Asaolu, 2008
2006-2007	Determination of prevalence of parasites of public health importance in dogs	271	41.7		Ugbomoiko et al., 2008
2009-2010	Determination of prevalence of gastrointestinal helminths in stray dogs	326	3.1	Faecal floatation and direct smear method	Awoke et al., 2011
2008-2010	Determination of parasitic causes of anemia in domestic dogs	84	11.9	Faecal floatation method	Kamani et al., 2011
2007-2008	Determination of epidemiology of gastrointestinal helminths in stray dogs	413	16.5	Kato-Katz technique	Okoye et al., 2011
2010	Determination of prevalence of ecto, endo, and haemoparasites in slaughtered dogs	543	5.0	McMaster technique	Adamu et al., 2012
2010	Determination of prevalence of helminths in domestic dogs	150	3.3	Faecal floatation method	Edosomwan and Chinweuba, 2012
2007	Survey of zoonotic gastro-intestinal parasites of dogs	52	10.8		Magaji et al., 2012
2012	Determination of prevalence of helminths in slaughtered dogs	40	7.5	Sedimentation and floatation technique	Mahmuda et al., 2012

2013	Determination of prevalence of helminths in domestic and stray dogs	150	18.0	Faecal floatation method	Alayande et al., 2013
2011-2012	Determination of prevalence of zoonotic gastrointestinal helminths in domestic dogs	104	3.85	Direct smear, and floatation technique	Odeniran and Ademola, 2013
2011-2012	Determination of prevalence and intensity of *T. canis* in domestic dogs	474	34.6	Kato-Katz technique	Akeredolu and Sowemimo, 2014
		Ghana			
2015	Determination of prevalence of helminths in clinic, domestic and hunting dogs	380	15.8	McMaster technique	Johnson et al., 2015
2015	Determination of prevalence of gastrointestinal helminths in domestic dogs	154	18.8	Faecal floatation method	Amissah-Reynolds et al., 2016
		Nigeria			
2015	Determination of prevalence of zoonotic gastrointestinal parasites in clinic, stray and slaughtered dogs	224	8.0	Sedimentation and floatation technique	Ogbaje et al., 2015
2016	Determination of prevalence of zoonotic gastrointestinal parasites in stray dogs	203	9.9	Faecal floatation method	Ayinmode et al., 2016
2015	Determination of prevalence of gastrointestinal helminths in stray dogs	400	23.0	Sedimentation and floatation technique	Matthew et al., 2016
2016	Determination of prevalence of gastrointestinal parasites in hunting dogs	250	1.6		Mustapha et al., 2016

2003	Study on pathogenesis, control and human toxocariasis	564	64.9	McMaster technique	Okewole, 2016
2011-2014	Determination of prevalence, pathogenesis, and control of canine toxocariasis	196	6.6	Secondary data	Okoh et al., 2016
2006-2013	Determination of prevalence of gastrointestinal helminths in domestic dogs	376	5.9		Idika et al., 2017
2013-2014		263	29.2	Sedimentation and floatation technique	
2018	Determination of diversity of gastrointestinal parasites in domestic animals (dogs)	88	50.0		Karaye et al., 2018
2014-2015	Determination of prevalence of gastrointestinal helminths in domestic and slaughtered dogs	250	23.6		Udoidung et al., 2018

Table 3.1c Summary of studies on the prevalence of *Toxocara canis* adult worms in dogs in sub-Saharan Africa from 1979-2018.

Study Period	Country	Objective	Sample Size (N)	Outcome (%)	References
		Southern Africa Region			
1960-1977	South Africa	Determination of prevalence of gastrointestinal helminths of domestic dogs	260	31.2	Verster, 1979
1960-1977		Determination of prevalence of gastrointestinal helminths of domestic dogs	502	1.4	Verster, 1979
1980-1982	Zambia	Determination of prevalence of helminths of domestic dogs	85	14	Islam and Chizyuka, 1983
		Western Africa Region			
1984	Nigeria	Determination of prevalence of canine toxocariasis	60	56.6	Arene, 1984
2009		Determination of prevalence of gastrointestinal helminths in slaughtered dogs	160	6.3	Umar, 2009
		Eastern Africa Region			
2005-2006	Ethiopia	Determination of prevalence of gastrointestinal helminths in stray dogs	20	45.0	Yacob *et al*., 2007
2007-2008		Determination of prevalence of helminth parasites in stray dogs	52	17.3	Zewdu *et al*., 2010
2011		Determination of prevalence of gastrointestinal parasites in stray dogs	13	61.5	Jones *et al*., 2011

Table 3.2 Summary of studies on the prevalence of *Toxocara cati* in cats in sub-Saharan Africa from 1979-2018.

Study Period	Country	Objective	Sample Size (N)	Method of Diagnosis	Outcome (%)	References
Southern Africa Region						
1980-1981	South Africa	Determination of prevalence of helminths of domestic cats	1502	Necropsy	11	Baker et al., 1989
Eastern Africa Region						
2014-2015	Ethiopia	Determination of prevalence of gastrointestinal parasites of domestic cats	384	Faecal flotation method	1.0	Tamerat et al., 2015
Western Africa Region						
1976-2001	Nigeria	Determination of prevalence of gastrointestinal helminths of domestic cats	537	Post-mortem examination	0.7	Oladele et al., 2006
2008		Determination of prevalence and intensity of gastrointestinal parasites of domestic cats	200	Kato-Katz technique	48.5	Sowemimo, 2012

Table 3.3a Summary of studies on *Toxocara* serology in humans in Central, Eastern, and Southern Africa from 1979-2018.

Study Period	Country	Objective	Sample Size (N)	Outcome (%)	Method of Diagnosis	References
			Eastern Africa			
1995	Kenya	Determination of toxocariasis serology	228	7.5	Positive ELISA (in-house ELISA)	Kenny et al., 1995
			Central Africa			
2001	Burundi	Determination of relationship between epilepsy and toxocariasis	119	59.7	Positive WB (*Toxocara* WB, IgG, LDBIO Diagnostics, Lyon, France	Nicoletti et al., 2007
		Determination of relationship between epilepsy and toxocariasis	119	50.8	Positive WB (*Toxocara* WB, IgG, LDBIO Diagnostics, Lyon, France	Nicoletti et al., 2007
			Eastern Africa			
2006	Tanzania	Determination of anticysticercal and antitoxocaral antibodies in people with epilepsy in rural population	40	67.5	ELISA and Western blot	Winkler et al., 2008
2012		Serological survey for human cysticercosis prevalence	544	14.2	Cysticercus IgG Western Blot Assay (LDBIO Diagnostics 69009 Lyon-France	Mwang'onde et al., 2012
2014		Study on the relationship between exposure to multiple	345 278	26.4 43.5	Positive ELISA Cypress (Diagnostics Belgium)	Kamuyu et al., 2014

parasites and prevalence of active convulsive epilepsy		

WB; Western Blots, IgG; Immunoglobulin G, ELISA; Enzyme linked immunosorbent assay.

Table 3.3b Summary of studies on *Toxocara* seroprevalence in humans in West Africa from 1979-2018.

Study Period	Country	Objective	Sample Size (N)	Outcome (%)	Method of Diagnosis	References
1996	Nigeria	Determination of frequency of human toxocariasis	104	29.8	ELISA	Ajayi et al., 2000
2004-2006	Cameroon	Serological studies of neurologic helminthic infections	168	36.3	Positive ELISA	Nkouawa et al., 2010
2014	Ghana	Study on the relationship between exposure to multiple parasites and prevalence of active convulsive epilepsy	292	17.8	Positive ELISA Cypress (Diagnostics Belgium)	Kamuyu et al., 2014
			173	22.0		
2012		Study on Seroepidemiology of *T. canis* infection in children	566	53.5	Positive ELISA (in-house ELISA)	Kyei et al., 2015
2013 – 2014	Nigeria	Determination of seroprevalence, disease awareness and risk factors associated to *T. canis* infection in children	366	86.1		Gyang et al., 2015
2003		Study on pathogenesis, control and human toxocariasis	128	57.5	Positive ELISA	Okewole, 2016
2016		Determination of seroepidemiology and risk factors	308	37.3	WB (LDBIO Diagnostics - *Toxocara*	Sowemimo et al., 2017

		associated with *T. canis* infection in preschool children			IgG, Lyon, France)	
2016	Gabon	Determination of seroprevalence of *Toxocara* spp in a rural population	332	59.9	Positive ELISA (Bordier affinity products Crissier Switzerland) WB (LDBIO Diagnostics - *Toxocara* IgG, Lyon, France)	Lötsch et al., 2016

3.4.3.3 *Toxocara* serology in humans

A summary of studies on *Toxocara* serology in humans in the SSA region is shown in Tables 3.3a,b. Out of a total of 6014 serum samples that were examined, in humans in the SSA region, 2159 were positive for *Toxocara* serology, with an overall prevalence of 36% (95% CI: 24–49%). Central Africa had the highest prevalence of 73% (95% CI: 41–99%), followed by West Africa with a prevalence of 49% (95% CI: 25–72%). South Africa had the least prevalence of 11% (95% CI: 1–24%). Substantial heterogeneity was observed between studies (I^2 =99%, P<0.01).

3.5 Discussion

Toxocariasis is a major public health challenge that poses a serious health concern in both developed and developing countries (Fakhri et al., 2018). The disease burden is high in SSA regions due to the existence of favourable transmission factors such as pet ownership with poor veterinary care, poor personal hygiene, socio-economic factors (poor sanitation level in disadvantaged areas), and favourable climatic conditions (humid and warm climate) for the embryonation and survival of *Toxocara* eggs (Liao et al., 2010; Gawor et al., 2015, Ma et al., 2018). This study revealed that only one-third of sub-Saharan countries have reported on the prevalence of *Toxocara* infections in dogs, cats and humans. The prevalence of *Toxocara* spp. in SSA has been associated with geographic regions with favourable climatic conditions (Fakhri et al., 2018), which probably explains the highest mean prevalence of *Toxocara* infections observed in Central Africa due to its location near the north of the

equator, with optimum environmental conditions (Nguyen and Duvel, 2008). In the environment, the development of eggs of *Toxocara* spp. directly correlates with humidity (Gamboa, 2005) and temperatures ranging between 23°C and 35°C, with limited development occurring at ≤17°C (Rocha et al., 2011).

Moreover, this review has revealed that *T. canis* is widely and more commonly reported in dogs than *T. cati* is in cats. Studies have established that dogs, mainly puppies in the close environment, are associated with the efficient transfer of *T. canis* (Glickman and Schantz, 1981; Barriga, 1988; Macpherson, 2013; Ferreira et al., 2016; Panova and Khrustalev, 2018). Puppies acquire infections via transplacental and transmammary routes, and are capable of shedding millions of eggs per day in less than three weeks after birth, thereby making them epidemiological reservoirs of *T. canis* in the environment (da Cunha Amaral et al., 2010; Macpherson, 2013; Ferreira et al., 2016; McManus et al., 2018). The efficient control of *Toxocara* infections, therefore, hinges upon control measures that target the elimination of the intestinal stage of *T. canis* populations in puppies and the sustained killing of the reservoir larvae in the tissues of bitches through the regular administration of effective anthelmintics (Barriga, 1988). However, the regular administration of anthelmintics may pose problems of cost to pet owners, making this a less feasible and efficient control measure; hence, an effective anti-*T. canis* vaccine has been proposed to become a reality in the foreseeable future (Barriga, 1988; Ma et al., 2018).

Also, dogs' hair, paws and their free-roaming habits (especially stray dogs) have been reported to largely contribute to environmental contamination with *T. canis* (Panova and Khrustalev, 2018; Sivajothi and Reddy, 2018). Although studies have reported the recovery of *T. canis* eggs in the hair of both owned and stray dogs (Wolfe and Wright, 2003; 2008; Aydenizöz-özkayhan et al., 2008; Roddie et al., 2008), these are probably acquired through the dogs' habits of scent rolling on diarrheal stools (Keegan and Holland, 2010; Lee et al., 2010; Merigueti et al., 2017). However, most recovered eggs were less viable due to desiccation by direct sunlight (Overgaauw et al., 2009). Low viability of *Toxocara* spp. eggs and their adhesive nature to dog or cat hair make them difficult to remove, which lessens the likelihood of them being accidentally swallowed by humans (Overgaauw et al., 2009; Keegan & Holland, 2010). Generally, the observed prevalence of *T. canis* eggs from dogs in SSA obtained in this study 19% (95% CI: 14-23%) was higher than the 1.8–2.0% prevalence reported in the US (Lucio-Forster et al., 2016). Thus, findings from this study underscore the role of dogs in the epidemiology of toxocariasis.

Similarly, studies have shown that cats are associated with a considerable level of environmental contamination with *T. cati* eggs (Fisher, 2003; Morgan et al., 2013; Nijsse et al., 2015). Unlike dogs, cats do not show age-resistance and are more prey eaters, which result in a direct development of egg-producing *Toxocara* worms without having a somatic migration (Overgaauw and van Knapen, 2013; Nijsse et al., 2016). However, this study showed that studies on the epidemiology of *T. cati* are limited in SSA regions. This is consistent with the assumption that studies on helminth infections in cats are scarce (Fisher, 2003; Mircean et al., 2010; Beugnet et al., 2014; Nijsse et al., 2016). The mean prevalence (9%) of *T. cati* observed in this study is lower than the mean prevalence (19.7%) reported in Europe (Beugnet et al., 2014); however, it is comparable to the observed prevalence in the US (Gates and Nolan, 2009). The limited data on cats from this region may not imply a less contributory role in toxocariasis; rather, observed heterogeneity may be attributed to inaccessibility to cat faeces due to their defecation habit of burying faeces, thus making *T. cati* appear as less prevalent than *T. canis* (Nijsse et al., 2016; Subrata et al., 2017). A recent study conducted in the US observed the prevalence of *T. cati* in faecal samples from cats to be approximately 3% higher than in faecal samples from dogs in the same region (Lucio-Forster et al., 2016). While this may not be generalized to all regions, it probably suggests that cats may be contributing more to toxocariasis than the available records in this review suggested.

There is poor understanding of the global impact and economic cost of human toxocariasis due to insufficient clinical awareness and unclear repository for the efficacy of clinical, laboratory, and treatment interventions (Smith et al., 2009). Most of the studies on *Toxocara* serology were associated with epilepsy and asthma and, as such, results were reported as odds ratios (Quattrocchi et al., 2012; Aghaei et al., 2018). The highest seroprevalence estimate observed in Central Africa could be explained as a statistical bias arising from limited studies from the region. The seroprevalence estimate of 49% observed in West Africa may, however, be attributed to risk factors such as geophagia, older age of study subjects, lack of potable water, consumption of raw vegetables, inadequate hygiene, contacts with pets and keeping pets (Kyei et al., 2015; Lötsch et al., 2017). Findings from this study are comparable with other results from the sub-Saharan region (Lötsch et al., 2017). A lack of focused studies on *Toxocara* serology in humans and the lack of a test to distinguish *T. canis* and *T. cati* infections indicate a public health underestimation of the *Toxocara* parasite in the SSA region. Such studies are needed to evaluate the relationship between exposure, seroprevalence and disease symptoms in human toxocariasis in SSA.

This analysis showed a substantial heterogeneity between studies and subgroups, which may be attributed to varying sampling techniques adopted by different studies, sociocultural beliefs, hygiene practices of people, level of awareness, degree of pet care, host age, human and animal bonding (Fakhri et al., 2018; Ma et al., 2018). The implication of this is that, despite the epidemiological information on *Toxocara* prevalence provided by this review, it may not be representing the true prevalence of *Toxocara* in the SSA region due to factors such as limited studies from some regions in SSA as well as unequal distribution of studies across the regions.

This study revealed a paucity of information on the prevalence of *Toxocara* spp. in a wide variety of hosts including food animals such as poultry, pigs, lamb, goats and cattle in SSA regions. Food animals are known to be zoonotic agents of several helminths of public health importance, including *Toxocara* spp. (Karshima, 2019). This transmission route is particularly of concern among communities with a cultural habit of consuming raw or undercooked meat viscera, thereby contributing significantly to the prevalence of human toxocariasis (Robertson et al., 2014). Therefore, understanding the epidemiology of *Toxocara* spp. in food animals and diverse hosts will be of importance in devising and implementing toxocariasis control measures.

3.6 Limitations

The major diagnostic methods employed in faecal analysis were mainly qualitative and, as a result, the intensity of infections could not be ascertained in the meta-analysis. Also, the lack of application of molecular techniques in identifying *Toxocara* spp. to species level resulted in the exclusion of some studies; thereby, the epidemiology of *Toxocara* spp. in the environment could not be determined.

3.7 Conclusions

Despite these limitations, this study revealed that the prevalence of *Toxocara* infections in the SSA region are under-reported and underscores the need to intensify awareness on and the need to practice good hygiene and adequate cooking of vegetables and meat, which are imperative in toxocariasis control. Also, there is the need to embark on more epidemiological studies in order to provide more insight into the transmission routes of *Toxocara* spp., understand disease prevalence and spatial distribution. Furthermore, policies that ensure proper care for pets are imperative to avoid stray animals contaminating the environment, as well as regular anthelmintic treatment of pets. The application of molecular techniques in species identification is imperative in order to understand the epidemiology of *Toxocara* spp. Lastly, there is a need for focused and collaborative studies in order to

measure exposure in the human population, disease states and reservoirs of infection in animals and the environment through a 'One Health' approach across SSA countries.

3.8 References

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CHAPTER 4: KNOWLEDGE AND PRACTICES ON CONSUMPTION OF FREE-RANGE IN SELECTED RURAL COMMUNITIES OF KWAZULU-NATAL, SOUTH AFRICA, WITH FOCUS ON ZOONOTIC TRANSMISSION OF *TOXOPLASMA GONDII* AND *TOXOCARA* SPP.

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4.1 Abstract

Chickens are a host to a variety of pathogens of zoonotic and economic importance and this depends more on the husbandry system practised and among these are *Toxoplasma gondii* and *Toxocara* spp which are more prevalent in free-range chickens (FRC). Humans may acquire infections from chickens via the ingestion of raw or undercooked meat (muscle) and/or viscera contaminated with infective stages of *T. gondii* and *Toxocara* spp. This study aimed to assess knowledge and practices on the household consumption of FRC meat and viscera by rural communities in KwaZulu-Natal (KZN) province, South Africa, as a risk factor in the transmission of zoonotic pathogens with special emphasis on *T. gondii* and *Toxocara* spp. A cross-sectional study was conducted on twenty (20) randomly selected households in four selected communities located on the northern coast (Gingindlovu and Ozwathini) and southern coast (uMzinto and Shongweni) of KZN province. An adult from each household was interviewed on FRC consumption practices using a semi-structured questionnaire. To determine the presence of selected zoonotic pathogens in FRC, birds were purchased from randomly selected households in the study localities for sacrifice. Tissues and organs such as brain, heart, liver, spleen, kidney, duodenum, pectoral, thigh, and breast were collected and subjected to molecular detection of *T. gondii* using TOX4 and TOX5 primers and of *Toxocara* spp using Nem 18S primers. Questionnaire data were analyzed using the statistical package for social sciences (SPSS) version 25.0. Descriptive, and Chi-square statistics were used to assess knowledge and practices related to FRC consumption and zoonotic pathogen transmission. Knowledge of zoonoses transmission related to consumption of chicken was estimated at 31.3% (25/80) in the four localities and a significant association was found between the educational level of respondents and study locations ($p<0.05$). Knowledge was highest among respondents with a high school education (13.75%, 11/80) and lowest (1.3%, 1/80) among respondents with no formal education. Overall, over three-quarters (76.3%, 61/80) of respondents reported consuming chicken viscera or meat, although the majority (96.7%, 59/80) preferred eating them ‘well-cooked’. *Toxoplasma gondii* was not detected in the FRC tissue samples which were subjected to molecular screening. However, four positive samples were recorded from Gingindlovu (n=1), uMzinto (n=1), and Shongweni (n=2) for *Toxocara* spp and all showed 100% homology with Genbank isolates of *T. canis* from China, United Kingdom,

and the United State of America. The occurrence of *T. canis* in FRC from KZN province revealed the risk of human toxocariasis transmission in the study localities should the meat or viscera of these chickens are eaten raw or undercooked. This result underscores the need to create awareness of health risks associated with the consumption of raw/undercooked viscera or meat from FRC with a future recommendation to screen for the presence of other zoonotic pathogens in this category of chickens.

Keywords *Toxoplasma gondii*. *Toxocara* spp. Knowledge. Practices, Free-range chickens. Zoonoses. KwaZulu-Natal. South Africa.

4.2 Introduction

Chickens are major agents in parasite transmission due to their exposure to infective stages of parasites existing in a contaminated environment (Javaregowda et al. 2016; De Vries et al. 2018). Commonly reported zoonotic parasites from chickens include but are not limited to *Toxoplasma gondii* and *Toxocara* spp (Zibaei et al. 2017; dos Santos Silva et al. 2020). *Toxoplasma gondii* is an apicomplexan parasite that causes toxoplasmosis in animals and humans globally (Dubey 2020). The main definitive hosts are felids, while birds and a wide range of animals serve as intermediate hosts (Gaulin et al. 2020). Although *Toxocara canis* and *T. cati* are helminth parasites of canids and felids, they also are responsible for human toxocariasis worldwide (Yoshida et al. 2016) and a variety of paratenic hosts are involved which include chickens and especially free-range chickens (FRC) due to the husbandry practices which allows them to have contact with contaminated environments (Zibaei et al. 2017). Although FRC contributes significantly as an affordable source of animal protein, reports on their role in the transmission of zoonotic pathogens are scanty (Rodrigues et al. 2019). They are normally infected during scavenging when they ingest the infective stage of various species of parasites from a contaminated environment (Sasse et al. 2020). Humans may acquire infection indirectly via the consumption of raw or undercooked infected chicken viscera or meat containing for example tissue cysts of *T. gondii* or larvae of *Toxocara* spp from canids and felids (Fan et al. 2015; Gaulin et al. 2020). Once ingested by humans, the parasites depending on the species migrate through the viscera and are deposited in various organs where they cause varying degrees of symptoms ranging from fever, headache, sore throat, arthralgia, myalgia, and blindness depending on the affected organs, infection intensity and duration, host age and immunity status of the infected host (Holland and Hamilton 2013; Fan et al. 2015; Gaulin et al. 2020). Consumption of FRC viscera or meat is a dietary habit common in different resource-poor rural communities worldwide and dependent on socio-cultural practices and

culinary habits where it can either be eaten raw or undercooked (Broglia and Kapel 2011). The practise of eating raw or undercooked viscera or meat is associated with zoonoses transmission (Trevisan et al. 2019), for instance, cases of toxocariasis transmission have been reported after ingesting raw chicken liver (Nagakura et al. 1989; Morimatsu et al. 2006; Campos-da-Silva et al. 2015). Similarly, human toxoplasmosis outbreaks have been reported among individuals who consumed raw or undercooked meat (Dawson, 2005; Choi et al. 2006). In South Africa, the greater population lives in rural areas and rears chickens following a free-range system (Mwale and Masika 2009; Mukaratirwa and Khumalo 2010a; Malatji et al. 2016). In KwaZulu-Natal (KZN) province of South Africa, the majority of the population are rural livestock farmers and rear FRC for consumption, marketing and socio-cultural purposes (Naidoo 2005). The farming practice, allows chickens to scavenge freely in the environment during the daytime and use trees for shelter at night or confined to rustic chicken runs (Naidoo 2005). Furthermore, there is an increased possibility of FRC ingesting infective oocysts and/or eggs of *T. gondii* and *Toxocara* spp respectively in the environment frequented by stray cats and dogs during scavenging in KwaZulu-Natal (KZN) province (Tannent et al. 2010; Mukaratirwa and Singh 2010b). Cats are the definitive hosts of *T. gondii,* while cats and dogs are definitive hosts of *Toxocara cati* and *Toxocara canis*. The occurrence of stray definitive hosts in the province may lead to persistent environmental contamination with these parasites (Mukaratirwa and Singh 2010b, Szwabe and Błaszkowska 2017). Moreover, change in globalization has led to the adoption of a variety of culinary and consumption patterns regarding raw or undercooked food as delicacies (Broglia and Kapel 2011). Besides, due to the high poverty level in rural areas of KZN, there is a high level of household food insecurity thereby leading to alternative foods and various ways of food preparation (Tarwireyi and Fanadzo 2013). Understanding the consumption pattern of the much available FRC viscera or meat is imperative in these communities as a basis for guaranteeing food security as well as identifying risks related to food-borne diseases such as toxoplasmosis and toxocariasis. Considering the poor socio-economic status and food insecurity of the rural communities in the KZN province of South Africa, this study was aimed to determine the presence of selected zoonotic parasites in FRC and the risk factors related to the transmission of zoonotic pathogens through household consumption patterns of FRC viscera or meat and preparation practices in the study areas.

4.3 Methodology

4.3.1 Study design and sample size determination

A cross-sectional study was conducted in four rural communities in KwaZulu-Natal province to assess knowledge and practices of consumption of FRC viscera or meat with a focus on *T. gondii and Toxocara* spp transmission from March to July 2019. Localities where the study was conducted, and their population sizes are as follows; Gingindlovu (GI) (1,109) and Ozwathini (OZ) (1,979) on the northern coast and uMzinto (MZ) (16,205) and Shongweni (SH) (427,613) on the southern coast of KZN (Fig 4.1) (http://www.durban.gov.za/). These localities have sugar-cane farming as their main livelihood followed by livestock farming which includes rearing of FRC. The study population comprised 80 participants selected using simple random sampling where all households in each locality were given numbers which were subjected to a random selection (lottery method) of 20 households per locality. The sampling frame consisted of the number of households in each study locality and each participant selected represented a household for each locality. The sample size was calculated using the following equation with a 95% confidence level and 11% error margin; $n=1.96^2\ pq/L^2$, where: n=sample size, p=expected prevalence (0.5), q=1-p, and L=limits of an error on the prevalence and the expected prevalence was set at 11%.

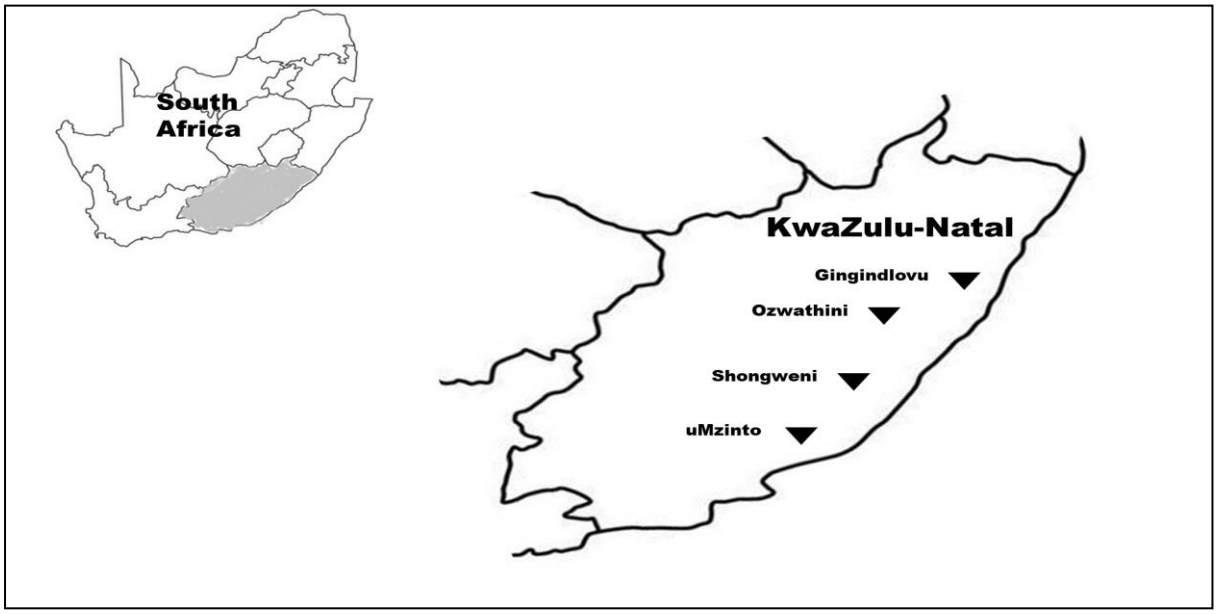

Figure 4.1 Map showing the study location sites in KwaZulu-Natal South Africa

4.3.2 Study procedure

After briefing the community leaders regarding the objectives of the study, 20 household representatives were randomly selected from each locality and consent was obtained regarding

their willingness to participate in the study. Questionnaires were translated from English to isiZulu, which is the local language in all the study localities, and were administered to the randomly selected participants following an interview-guided approach. Before administration, a pilot study was done to validate the tool. The questionnaire administration process took approximately 20 minutes for each participant. Before completion of the questionnaires, all participants gave their written informed consent to take part in the study. They were also reassured of the confidentiality of all disclosed information and that only anonymised findings will be disclosed during feedback and in written reports.

Data collected from the interview included socio-demographic information, knowledge, and practice of participants related to the preparation and consumption of FRC meat and viscera. Questions were asked specifically on habits related to consumption of chicken meat and viscera, the preferred method of preparation and designated members of the family who eat each type of viscera. The demographic information of the participants interviewed included age, gender, household size, educational qualifications, and occupation of respondents. Information on ownership of FRC including the number of FRC owned per household was also collected.

4.3.3 Collection of samples from free-range chickens

Forty-two FRC were randomly purchased from households owning chickens on a willing seller basis in four selected rural communities in the Northern [Gingindlovu (GI), Ozwathini (OZ)], and Southern Coasts [uMzinto (MZ), Shongweni (SH)], of KZN province. Chickens selected for the study were euthanized by decapitation according to guidelines approved by Animal ethics of the University of KwaZulu-Natal South Africa. Tissue from various parts of each chicken such as brain, heart, spleen, lungs, liver, kidney, crop, duodenum, intestines, thigh, breast, and pectoral) were collected, digested, and examined for *Toxocara* larvae using the modified acid/pepsin digestion (Zibaei et al., 2017). The digests were washed and filtered through a sieve with 200/125/20-µm apertures. Collected larvae were kept in 70% ethanol until DNA extraction. Brain samples were collected and preserved in 70% ethanol for detection of *T, gondii*.

4.3.4 Molecular identification of parasites

The retrieved nematode larvae from each of the chicken samples were subjected to molecular analysis using a QIAamp DNA Mini Kit (Qiagen Inc.) and used in subsequent PCR reactions. PCR reactions for the amplification of 18S rRNA were performed with nematode-specific primers Nem_18S_F (CGCGAATRGCTCATTACAACAGC (23 bases) and Nem_18S_R (GGGCGGTATCTGATCGCC (18 bases). A standard reaction volume was 20 µL,

comprising: NEB OneTaq 2X MasterMix with Standard Buffer at 10 µl, primers (10µM) at 1µl each, and nuclease-free water at 7µl was added. To each reaction, 1 µL of extracted nematode DNA template was added, typically containing around 10-30ng/µl of genomic DNA. The PCR conditions for the amplification of 18S rRNA were as follows: initial denaturation at 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 30 sec, primer annealing at 50 °C for 30 sec, and extension at 68 °C for 1 min. The final extension was at 68 °C for 10 min. The integrity of the PCR amplicons was visualized on a 1% agarose gel.

Thirty brain samples (halves) were tested for *Toxoplasma gondii* using TOX4 (5′-CGCTGCAGG-GAGGAAGACGAAAGTTG-3′) and TOX5 (5′-CGCTGCAGACACAGTGCATCTGGAT-T-3′) primers respectively. PCR was conducted according to a described protocol (Homan et al., 2000).

The cycling protocol for the *T. gondii* DNA amplification included an initial cycle of 94 ◦C for 7 min (initial denaturation), followed by 35 cycles of 94 ◦C for 1 min (denaturation), 60 ◦C for 1 min (annealing) and 72 ◦C for 1 min (extension), followed by a final extension 72 ◦C for 10 min. PCR products were analyzed on 2% agarose gel by electrophoresis for 25 min at 120V.

4.3.5 Statistical analysis

Data were processed and analyzed using the statistical package for social sciences (SPSS) version 25.0. Descriptive and Chi-square statistics were used to assess knowledge and practices related to the consumption of FRC meat and viscera and awareness of the zoonotic transmission of *T. gondii* and *Toxocara* spp. A p-value < 0.05 was considered statistically significant.

4.4 Results

4.4.1 Socio-demographic profile of participants

Table 1 shows the demographic characteristics of participants in all four study localities. Participants interviewed in the four localities ranged in the category of father, mother, and household member greater than 18 years. Overall, the mean age of the respondents was (47.11±18.02) (Table 4.1). Ozwathini (OZ) had the highest mean age (53.3±16.82), followed by Shongweni (SH) (50.25±17.12, GI (43±18.98), and uMzinto (MZ) (41.9±17.80). A significant difference was observed between the educational level of study respondents among study locations (p<0.05). Most respondents (47.5%, 38/80) had a high school education while only (20%, 16/80) had completed tertiary education. The percentage of respondents who had tertiary education was highest in OZ (40%, 8/20), followed by GI (30%, 6/20), and SH (10%, 2/20) while none of the respondents in MZ had tertiary education.

The percentage of respondents that were unemployed was highest (90%, 18/20) in OZ, (85%, 17/20) in GI and SH, followed by (70%, 14/20) in MZ. Overall, household sizes ranged from 1-16 with a mean of (6.40±3.26). Household size ranged from 2-11 with a mean of 7.05±2.65, 2-14 (6.65±3.79), 2-13 (5.70±2.89) and 1-16 (6.20±3.67) in GI, MZ, OZ, and SH respectively (Table 4.1).

Table 4.1 Socio-demographic characteristics of study respondents from four localities of KwaZulu-Natal province of South Africa (GI = Gingindlovu; OZ = Ozwathini; MZ = uMzinto; SH = Shongweni)

Variable	**Localities**										
	GI (n = 20)		**OZ (n = 20)**		**MZ (n = 20)**		**SH (n = 20)**		**Total (n = 80)**		***p*-Value**
	Freq	**%**	**Freq**	**%**	**Freq**	**%**	**Freq**	**%**	**Freq**	**%**	
Age groups (years)											
<21	1	5	1	5	0	0	1	5	3	3.75	0.733
21-30	7	35	6	30	3	15	2	10	18	22.50	
31-40	1	5	4	20	1	5	2	10	8	10.00	
41-50	5	25	4	20	5	25	5	25	19	23.75	
51-60	2	10	2	10	4	20	4	20	12	15.00	
61+	4	20	3	15	7	35	6	30	20	25.00	
Education level											
None	2	10	2	10	1	5	1	5	6	7.50	0.020
Primary	1	5	3	15	8	40	8	40	20	25.00	
High School	11	55	7	35	11	55	9	45	38	47.50	
Tertiary	6	30	8	40	0	0	2	10	16	20.00	
Occupation status											
Employed	0	0	4	20	1	5	2	10	7	8.75	0.310
Unemployed	17	85	14	70	18	90	17	85	66	82.50	
Self employed	3	15	2	10	1	5	1	5	7	8.75	

Respondents' categories											
Father	1	5	2	10	1	5	5	25	9	11.25	0.088
Mother	11	55	10	50	16	80	12	60	49	61.25	
Family members ≥ 18 years	8	40	8	40	3	15	3	15	22	27.50	
Household size											
1-5	6	30	10	50	8	40	11	55	35	43.75	0.340
6-10	13	65	8	40	8	40	7	35	36	45.00	
11-15	1	5	2	10	4	20	1	5	8	10.00	
16-20	0	0	0	0	0	0	1	5	1	1.25	

4.4.2 Knowledge of zoonoses transmission associated with the consumption of FRC viscera

Overall, knowledge of zoonotic disease (mainly related to toxoplasmosis and toxocariasis) transmission associated with consumption of raw or undercooked FRC meat and viscera in the study localities was estimated at 31.3%. There were no significant associations found between knowledge and considered variables. Knowledge did not vary among the localities (35%, 7/20) in MZ and SH followed by GI (30%, 6/20) and OZ (25%, 5/20) (p>0.05) (Table 4.2). The proportion of respondents knowing zoonoses transmission through consumption of raw/undercooked chicken viscera was high in the age group 41-50 years and highest in GI (20%, 4/20) followed by SH (15%, 3/20) and OZ (10%, 2/20) while in MZ, it was highest in the age group ≥61 (20%, 4/20). This difference was however, not statistically significant (p>0.05).

Based on the education level of participants, knowledge of zoonoses transmission (toxoplasmosis and toxocariasis) was highest among respondents with high school education (13.8%, 11/80), followed by tertiary education (8.8%, 7/80), primary education (7.5%, 6/80) and (1.3%, 1/80) among respondents with no formal education although, this difference was not statistically significant (p>0.05) (Table 4.2). The knowledge of zoonoses (toxoplasmosis and toxocariasis) due to consuming undercooked/raw FRC did not significantly vary among the localities (35%, 7/20) in MZ and OZ, followed by (30%, 6/20), and (25%, 5/20) in GI and OZ respectively (p>0.05) (Table 4.2). Regarding occupation, although knowledge was highest among the unemployed, (26.3%, 21/80), followed by (3.8%, 3/80) and (2.5%, 2/80) among the

employed and self-employed participants respectively, the observed difference was not statistically significant ($p>0.05$). Furthermore, based on the household size, knowledge was highest (13.8%, 11/80) in household sizes 1-5 and 6-10, and decreased in household sizes 11-15 (2.5%, 2/80) and 16-20 (1.3%, 1/80), however, the difference was not statistically significant, ($p>0.05$) (Table 4.2).

4.4.3 Ownership of FRC in study localities

Overall, 65% (52/80) of the interviewed households in the study population owned FRC ranging from 1-51 (17.2±1.4). There was no significant association recorded based on FRC ownership ($p>0.05$). Twenty per cent (16/80) of the households had FRC greater than 20, while (10%, 8/80) of the study population have ≤5. FRC ownership was highest in MZ (80%, 16/20), followed by OZ (70%, 14/20), SH (60%, 12/20), and GI (50%, 10/20) ($p>0.05$) (Table 4.3).

4.4.4 Chicken viscera consumption

Overall, 76.3% (61/80) of respondents reported consumption of chicken viscera (Table 4.4). The proportion of respondents consuming chicken viscera was highest in SH (90%, 18/20), followed by OZ (80%, 16/20), MZ (75%, 15/20), and GI (60%, 12/20). The proportion and category of respondents consuming the combination of all chicken viscera are shown in Table 4.4 and Fig. 4.2a-c.

Table 4. 2 Responses on knowledge of zoonoses transmission from free range chickens in four localities in the KwaZulu-Natal province of South Africa (GI = Gingindlovu; OZ = Ozwathini; MZ = uMzinto; SH = Shongweni).

Variable	**Localities**							
	GI (n=20)		**OZ (n=20)**		**MZ (n=20)**		**SH (n=20)**	
	Yes (%)	**No (%)**	**Yes (%)**	**No (%)**	**Yes (%)**	**No (%)**	**Yes (%)**	**No (%)**
Age								
<21	0	1 (100)	0	1 (100)	0	0	0	1 (100)
21-30	2 (29)	5 (71)	1 (17)	5 (83)	1 (33)	2 (67)	2 (100)	0
31-40	0	1 (100)	1 (25)	3 (75)	0	1 (100)	0	0
41-50	4 (80)	1 (20)	2 (50)	2 (50)	0	5 (100)	3 (60)	2 (40)
51-60	0	2 (100)	1 (50)	1 (50)	2 (50)	2 (50)	0	4 (100)
61+	0	4 (100)	0	3 (100)	4 (57)	3 (43)	2 (33)	4 (67)
Total	6 (30)	14 (70)	5 (25)	15 (75)	7 (35)	13 (65)	7 (35)	13 (65)
X^2, *p*-Value	(X^2 = 9.388, *p* = 0.095)		(X^2 = 3.556, *p* = 0.615)		(X^2 = 5.139, *p* = 0.273)		(X^2 = 8.864, *p* = 0.115)	
Educational level								
None	0	2 (100)	0	2 (100)	0	1 (100)	1 (100)	0
Primary	0	1 (100)	0	3 (100)	4 (50)	4 (50)	2 (25)	6 (75)
High school	2 (18)	9 (82)	3 (43)	4 (57)	`3 (27)	8 (73)	3 (33)	6 (67)
Tertiary	4 (67)	2 (33)	2 (25)	6 (75)	0	0	1 (50)	1 (50)

	6 (30)	14 (70)	5 (25)	15 (75)	7 (35)	13 (65)	7 (35)	13 (65)
X^2, p-Value	(X^2 = 5.859, p = 0.119)		(X^2 = 2.857, p = 0.414)		(X^2 = 1.618, p = 0.445)		(X^2 = 2.418, p = 0.490)	
Occupation								
Employed	0	0	1 (25)	3 (75)	0	1 (100)	1 (50)	1 (50)
Unemployed	4 (24)	13 (76)	4 (29)	10 (71)	7 (39)	11 (61)	6 (35)	11 (65)
Self employed	2 (67)	1 (33)	0	2 (100)	0	1 (100)	0	1 (100)
Total	6 (30)	14 (70)	5 (25)	15 (75)	7 (35)	13 (65)	7 (35)	13 (65)
X^2, p-Value	(X^2 = 2.260, p = 0.133)		(X^2 = 0.762, p = 0.683)		(X^2 = 1.197, p = 0.550)		(X^2 = 0.737, p = 0.692)	
Participant's ID								
Father	0	1 (100)	0	2 (100)	0	1 (100)	1 (20)	4 (80)
Mother	3 (27)	8 (73)	3 (30)	7 (70)	6 (38)	10 (62)	4 (33)	8 (67)
Children >18yrs	3 (38)	5 (62)	2 (25)	6 (75)	1 (33)	2 (67)	2 (67)	1 (33)
Total	6 (30)	14 (70)	5 (25)	15 (75)	7 (35)	13 (65)	7 (35)	13 (65)
X^2, p-Value	(X^2 = 0.682, p = 0.711)		(X^2 = 0.800, p = 0.670)		(X^2 = 0.586, p = 0.746)		(X^2 = 1.832, p = 0.400)	
Household Size								
1-5	1 (17)	5 (83)	3 (30)	7 (70)	2 (25)	6 (75)	5 (45)	6 (55)
6-10	4 (31)	9 (69)	2 (25)	6 (75)	4 (50)	4 (50)	1 (14)	6 (86)
11-15	1 (100)	0	0	2 (100)	1 (25)	3 (75)	0	1 (100)
16-20	0	0	0	0	0	0	1 (100)	0

Total	6 (30)	14 (70)	5 (25)	15 (75)	7 (35)	13 (65)	7 (35)	13 (65)
X^2, p-Value	(X^2 = 2.845, p = 0.241)		(X^2 = 0.800, p = 0.670)		(X^2 = 1.319, p = 0.517)		(X^2 = 4.244, p = 0.236)	

Table 4.3 Ownership of free-range chicken in four localities in the KwaZulu-Natal province of South Africa (GI = Gingindlovu; OZ = Ozwathini; MZ = uMzinto; SH = Shongweni)

Variable	**Localities**										
	GI (n=20)		**OZ (n=20)**		**MZ (n=20)**		**SH (n=20)**		**Total (n=80)**		***p*-Value**
	Freq	**(%)**	**Freq**	**(%)**	**Freq**	**(%)**	**Freq**	**(%)**	**Freq**	**(%)**	
Ownership of free-range chickens											
Yes	10	50	14	70	16	80	12	60	52	65.00	0.222
No	10	50	6	30	4	20	8	40	28	35.00	
Number of free-range chickens in the household											
1 – 5	1	5	4	20	1	5	11	55	8	10.00	0.416
6 – 10	1	5	5	25	2	10	7	35	10	12.50	
11 – 15	2	10	2	10	7	35	1	5	14	17.50	
16 – 20	1	5	0	0	1	5	1	5	4	5.00	
20+	5	25	3	15	5	25	0	0	16	20.00	

Table 4.4 Responses from participants on consumption and type of viscera free range chickens in four localities in KwaZulu-Natal province of South Africa

Respondents Category	Viscera type consumed in Gingindlovu (n = 12)							
	1, 3	**1, 2, 3**	**1, 3, 4, 6**	**1, 2, 3, 6**	**1, 2, 3, 4, 5**	**1, 3, 4, 5, 6**	**1, 2, 3, 5, 6**	**1, 2, 3, 4, 5, 6**
Father	0	0	0	0	0	0	0	1
Mother	0	0	0	0	0	1	2	4
Family members ≥ 18 years	0	0	0	0	0	0	2	2
Total	0	0	0	0	0	1	4	7
Respondents Category	Viscera type consumed in Ozwathini (n=16)							
Father	0	0	0	0	0	0	0	1
Mother	0	1	0	1	0	0	3	2
Family members ≥ 18 years	1	0	1	0	0	1	2	3
Total	1	1	1	1	0	1	5	6
Respondents Category	Viscera type consumed in uMzinto (n=15)							
Father	0	0	0	0	0	0	0	1
Mother	0	0	0	0	7	0	0	5
Family members ≥ 18 years	0	0	0	0	2	0	0	0
Total	0	0	0	0	9	0	0	6
Respondents Category	Viscera type consumed in Shongweni (n=18)							
Father	0	1	0	0	1	0	0	2
Mother	0	0	0	3	3	0	0	5
Family members ≥ 18 years	0	0	0	0	1	0	0	2
Total	0	1	0	3	5	0	0	9
Overall Total	1	2	1	4	14	2	9	28

Chicken viscera types are denoted as follows; 1 = Gizzard, 2 = Heart, 3 = Liver, 4=Lungs, 5 = Kidney, 6 = Intestines

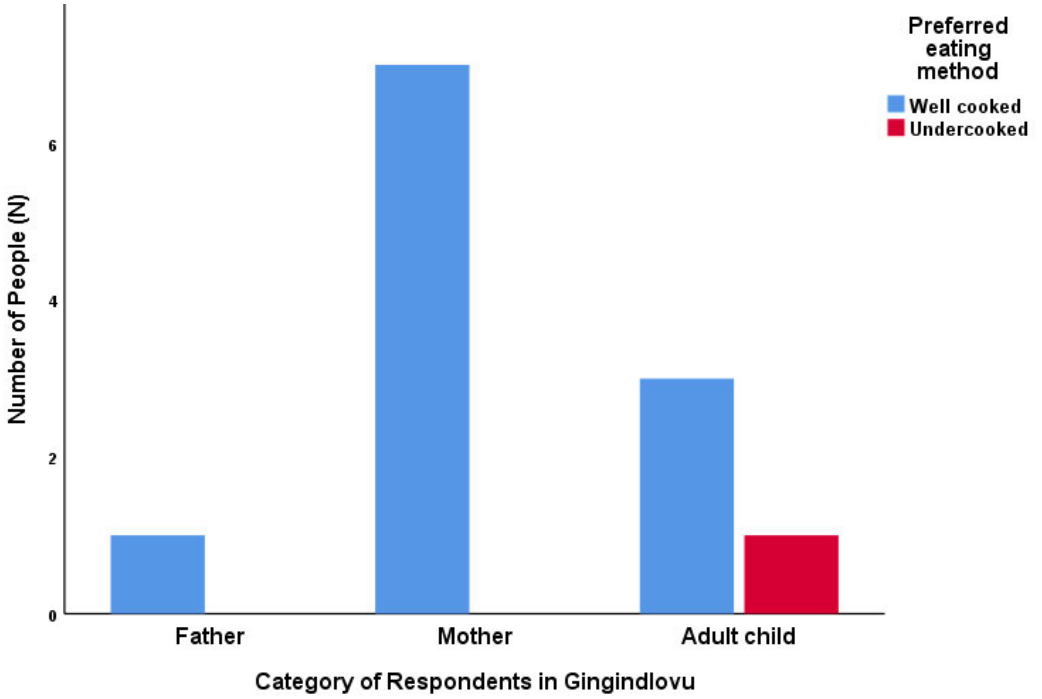

Figure 4.2a Clustered bar charts showing the preferred method of consumption of free-range chicken among respondents in Gingindlovu.

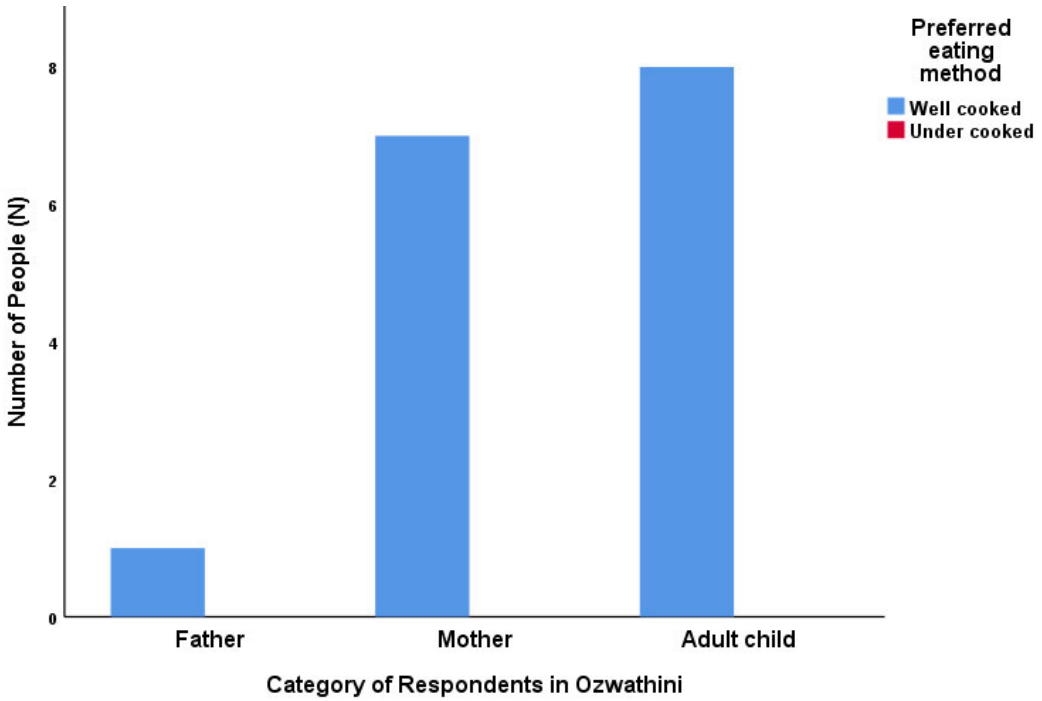

Figure 4.2b Clustered bar charts showing the preferred method of consumption of free-range chicken among respondents in Ozwathini.

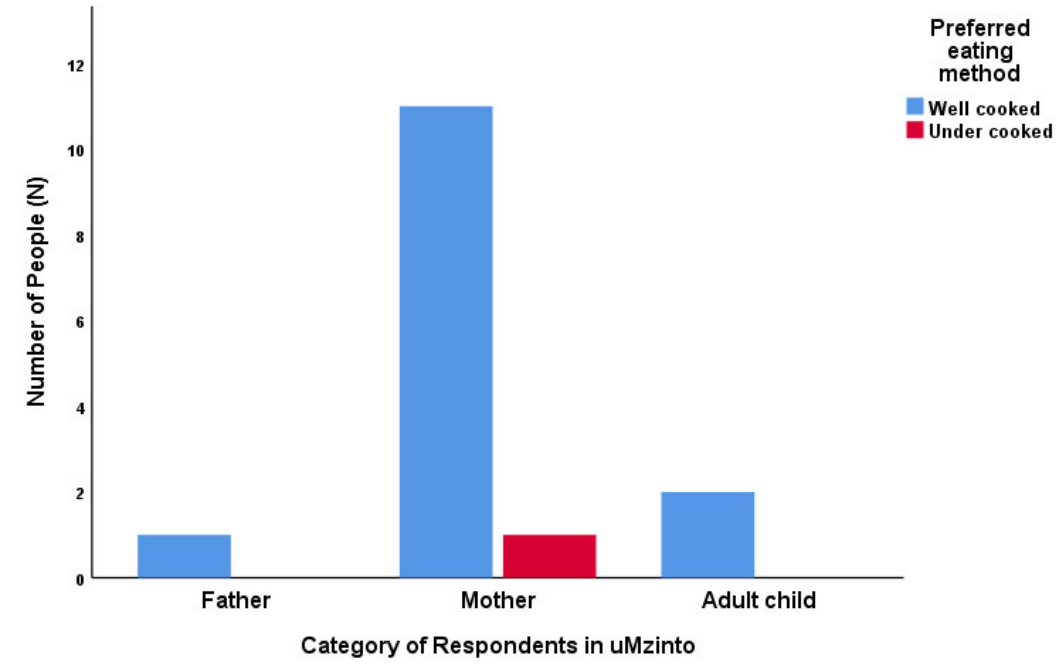

Figure 4.2c Clustered bar charts showing the preferred method of consumption of free-range chicken among respondents in uMzinto.

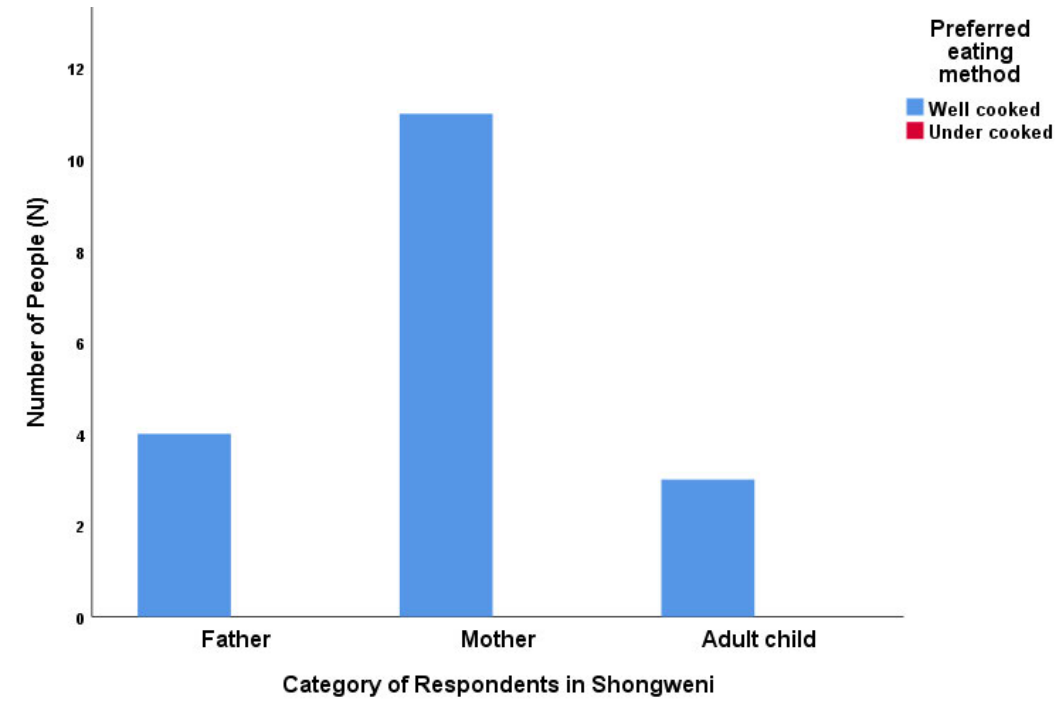

Figure 4.2d Clustered bar charts showing the preferred method of consumption of free-range chicken among respondents in Shongweni.

Table 4.5 The sample ID, sequence length, accession number, and homology of sequences with those of GenBank for *Toxocara canis* from dogs all showing 100% query coverage.

Sample ID	Sequence length (bp)	Accession #	% Similarity	Country
C(GI)	761	JN256976.1	99.74	China
	761	U94382.1	99.74	USA
	761	AF036608.1	99.74	UK
C(SH)	740	JN256976.1	99.46	China
	740	U94382.1	99.46	USA
	740	AF036608.1	99.46	UK
C(SH)	786	JN256976.1	94.66	China
	786	U94382.1	94.66	USA
C(MZ)	773	JN256976.1	99.09	China
	773	U94382.1	99.09	USA
	773	AF036608.1	99.09	UK

4.4.5 Molecular results

All the 30 brain samples were negative for *T. gondii*. *Toxocara canis* larvae were detected and confirmed through sequencing in the right pectoral muscle, liver, left thigh, and lungs of FRC from Gingindlovu (GI); (n=1), Mzinto (MZ); (n=1), and Shongweni (SH); (n=2). There were no *Toxocara* larvae found in FRC from Ozwathini (OZ). The summary of molecular results is shown in Table 4.5. The 761-bp 18S rRNA sequences of *T. canis* from GI showed 99.74% identity, with *T. canis* JN256976 from China, with *T. canis* U94382.1 from USA, and with *T. canis* AF036608.1 from UK with 100% homology (Table 4.5). The 773-bp long 18S rRNA sequences of *T. canis* from MZ showed 99.09% identity, with *T. canis* JN256976 from China, with *T. canis* U94382.1 from the USA, and with *T. canis* AF036608.1 from the UK with 100% homology (Table 4.5). The 740- and 786 bp 18S rRNA sequences of *T. canis* from SH showed 99.46% and 94.66% identity respectively with *T. canis* JN256976.1 from China, U94382.1 from the USA, and AF036608.1 from the UK with 100% homology (Table 4.5).

4.5 Discussion

Several studies have reported the vulnerability of resource-poor communities who keep FRC to zoonotic diseases such as toxoplasmosis and toxocariasis (Neghina 2010; Santarém et al. 2011; Mirza and Rathore 2019). Socio-demographic factors have been reported to influence the food and meat gathering practices of people in a way that predisposes them to parasitic

infections (Simeone 2008; Drescher et al. 2012; Goyette et al. 2014). Our study showed that a quarter of the respondents in the study areas had only completed primary education. This is consistent with reports from other rural regions of South Africa where the low levels of education have been reported (Mwale and Masika, 2009; Spaull 2015).

Also, this study showed that the overall percentage of zoonoses transmission awareness was comparable to 30.1% recorded from cattle farmers in Senegal (Tebug et al. 2015), but higher than the awareness level of the 19.1% of zoonotic risk associated with livestock in Ibadan Nigeria (Awosanya and Akande 2015). However, it was lower than 69% and 79.74% that were reported in Cambodia and Punjab respectively (Osbjer et al. 2015; Singh et al. 2019). Although, no significant associations were found between knowledge of zoonoses transmission and considered variables in all the localities, the high level of knowledge obtained among respondents with high school education disagrees with the report from Western Ethiopia where KAP scores were higher among people with tertiary education (Tamiru et al. 2022). This can be attributed to the fact that the majority (45%) of the respondents in this study have a high school education. Similarly, regarding occupation, the higher level of knowledge observed among unemployed respondents disagrees with the report from Western Ethiopia where good KAP scores were recorded among people with good job types (Tamiru et al. 2022). This can be attributed to the large percentage of unemployed (82.5%) constituting the respondents. A similar explanation is responsible for the high knowledge of zoonoses transmission observed among women in the study locations where most of the respondents (61.3%) were women.

Furthermore, this study revealed that ownership of FRC in the study locations was 65% (52/80), which is higher than the 57.7% (41/71) reported in Ethiopia (Sambo et al. 2015) but lower than the 93.5% and 84% poultry (duck and chicken) ownership observed in Eastern Cape province of South Africa and Cambodia respectively (Mwale and Masika 2009; Osbjer et al. 2015). Also, the average flock size (17.2±1.4) observed in this study is higher than (16±2.1), reported in the Eastern Cape province of South Africa (Mwale and Masika 2009), but lower than (22.03±2.85) in Limpopo province and (28.40±2.57) earlier reported in KwaZulu-Natal province respectively (Malatji et al. 2016).

Regarding consumption patterns, the majority (76.3%, 61/80) of respondents reported the practice of consumption of FRC viscera in their households. The reason for the high demand for chicken viscera in the study area is however unknown. Studies have identified the role of poor socioeconomic factors as well as globalization as important factors in meat consumption patterns (Tambi 2001; Simeone 2008; Goyette et al. 2014; Robertson et al. 2014). Additionally,

the viscera of chicken and other avian animals have been reported to be rich in essential nutrients for humans (Schönfeldt and Gibson 2008).

Chickens are used as sentinel agents in monitoring the prevalence of infections in the environment due to their ground-feeding habits (Dubey et al., 2005). Molecular techniques employing non-coding 529 base pairs DNA fragment and the internal transcribed spacer 1 (ITS-1) of the rRNA gene have proved effective in the identification of *T. gondii* and several organisms to species level due to the variations of the (ITS-1) of the rDNA (Santos et al., 2010), resulting in a higher detection rate (Chemoh et al., 2016). Also, PCR has been used unequivocally for the detection of animal and human toxocariasis (Dewair and Bessat 2020).

This study is the first to consider the prevalence of *T. gondii* in free-range chickens from KZN province, South Africa, using a molecular approach. The absence of *T. gondii* observed in the brain tissues of chicken in this study might be an indication that the FRC sampled in our study may have not been exposed to *T. gondii* oocysts. This is consistent with a report from a study conducted on retail turkey meat products where *T. gondii* DNA was not detectable using magnetic-capture PCR (Koethe et al., 2015). Another study reported a low prevalence of *T. gondii* infection in feral rodents and insectivores (Meerburg et al., 2010). The absence of *T. gondii* in this study may be attributed to non-survival of *T. gondii* oocysts in the environment due to hot and dry temperatures (Lukášová et al., 2017). *T*oxoplasma *gondii* infections thrive in mild temperature climates than in a hot and dry environment (Dubey, 1998; Gilot-Fromont et al., 2012) or in dry and very cold winter (Smallbone, 2012). For instance, *T. gondii* oocysts survived for 32 days at 35 °C, 9 days at 40 °C, and only 1 day at 45 °C (Dubey, 1998). The chickens used in this study were obtained between March and July which is usually characterized by the highest temperature and lowest temperature respectively (Masemola et al., 2020). The mean daily minimum temperature in KZN being around 35 °C in March and 16.76 °C in July (Dzikiti, et al., 2022).

On the other hand, the presence of *T. canis* observed in this study agrees with Zibae et al. (2017) who isolated *Toxocara canis* larvae from the liver, skeletal muscles, duodenum and brain of broiler chickens and Okada et al. (2021) who reported the occurrence of *T. cati* and *T. tanuki* from the thigh and breast meat from chicken, respectively. Similarly, Davidson et al. (2012) reported *T. cati* from pigs at a slaughterhouse in Norway. Also, *T. vitulorum* was detected in bovine milk samples using a molecular approach (Dewair and Bessat 2020). The prevalence of *Toxocara* infection observed in this study (9.5%: 4/42) is consistent with reports from other studies where low prevalence has been reported. Zibae et al. (2017) reported 15.2%

(5/33) from broiler chickens while Okada et al. (2021) reported 4% (2/50) from culled chickens from a commercial farm and Davidson et al. (2012) reported a prevalence of 1% (1/100) from a pig in a slaughterhouse from Norway. The occurrence of *T. canis* in FRC observed in this study indicates that the chickens are being exposed to environments contaminated with *T. canis* eggs from dogs in the localities studied. Considering the high rate of consumption of chicken viscera practised by communities in this study, although most preferred "well-cooked", it is important to create awareness of the role of FRC as paratenic hosts of important zoonotic parasites of dogs and cats such as *T. gondii* and *Toxocara* spp. Furthermore, the application of the PCR technique used in this study could be employed in routine detection methods for *Toxocara* larvae in organs of suspected infected animals, especially in toxocariasis endemic areas.

We also recommend the participation of all stakeholders through a One Health approach in designing control and prevention strategies of zoonotic pathogens affecting these communities using the findings from this study as a basis.

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Author contributions. Adejumoke O. Omonijo and Samson Mukaratirwa conceived and designed the study. Adejumoke O. Omonijo wrote the article and Samson Mukaratirwa reviewed the article. Both authors read and approved the final manuscript.

Data availability. The data used to support the findings of this study are available from the corresponding author upon request.

Declarations.

Ethics approval. The study was conducted under the Human and Social Sciences Research Ethics committee of the University of KwaZulu-Natal protocol reference number HSS/1655/018D.

Consent to participate.

• We confirm the confidentiality of each participant's answer, and the data will be treated with complete confidentiality, and only for research and statistical purposes only.

• We confirm that their participation in the study was voluntary, with no financial compensation.

• We confirm the participants' right to not answer any question they do not want to, and their right to withdraw from the study at any time they wish without giving reasons without any negative consequences being applied to them.

• Written consent was obtained from each participant before their participation in the study.

Consent for publication. The corresponding author confirms that the manuscript has been read and approved for submission by all the co-authors.

Conflict of interest. The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

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CHAPTER 5: SUMMARY AND CONCLUSIONS

5.1 Introduction

The study aimed at determining the status of research on two parasitic zoonoses (toxoplasmosis and toxocariosis) in sub-Saharan Africa and their prevalence in selected rural communities of KwaZulu-Natal of South Africa. Toxoplasmosis is caused by *Toxoplasma gondii*- an apicomplexan parasite, while toxocariasis is caused by a nematode known as *Toxocara* spp.

The background information in chapter one described the popular practice of free-range poultry system in sub-Saharan Africa and their importance as sentinel livestocks due to their exposure to pathogenic organisms in the environment (da Silva et al., 2018). The abundance of stray dogs and feral cats which are definitive hosts of zoonotic parasites have been reported in KZN (Tannent et al., 2010, Mukaratirwa and Singh, 2010). Some of the pathogenic organisms harboured by free-range chickens are of zoonotic importance and produce diseases such as toxoplasmosis and toxocarosis when the infective stages of these parasites are consumed with chicken meat or viscera either as raw or undercooked thereby making free range chickens a possible route of these zoonotic transmissions. There is the need to understand the prevalence of these zoonotic parasites in free-range chickens to gain more insight into parasite epidemiology in KZN. It is important to create awareness on zoonosis transmission via this route to enhance disease control measures.

This study reviewed literature on the status of research on *T. gondii* in southern Africa and *Toxocara* infections in sub-Saharan Africa. This was achieved by reviewing and extracting data from forty published articles regarding *T. gondii* infections in humans and animals in southern Africa over a period of sixty-five years in Chapter 2. Chapter 3 reviewed the epidemiological distribution of *Toxocara* infections in dogs, cats and humans in SSA region from 75 articles based on *Toxocara* infections. The review of both *Toxoplasma* and *Toxocara* infections showed paucity of information in southern African countries and the SSA region respectively. Chapter four reviewed the knowledge and practices on the household consumption of Free-range chickens (FRC) meat and viscera by rural communities in KwaZulu-Natal (KZN), South Africa as risk factors in the transmission of zoonotic pathogens. The chapter also used ITS-1 and ITS-2 primers to determine the prevalence of *T. gondii* from free-range chicken from four selected rural communities in KwaZulu-Natal (KZN) province of South Africa. Detection of *Toxocara* spp infection in chicken samples from the selected rural areas in KZN was based on the amplification of the 18S rRNA gene using the Nem 18S-F1 with Nem 18S-R1 primers. The sections below discuss the findings from the four manuscripts included in the thesis.

5.2 T*oxoplasma gondii* infections in animals and humans in southern Africa: a systematic review and meta-analysis

Chapter 2 analyzed published literature on *Toxoplasma* infections in animals and humans in southern Africa. It determined the epidemiological distribution of infection in various hosts in the region and identified gaps for future research.

This review revealed that there is limited information on the distribution of *T. gondii* in animals and humans in southern African countries. In this study, the overall pooled prevalence is estimated at 17% (95% CI: 7–29%), 29% (95% CI: 7–54%) in domestic felids and 79% (95% CI: 60–94%) in wild felids. The role of felids (domestic and wild) in *T. gondii* epidemiology has been documented in several reports (Elmore et al., 2010; Montazeri et al., 2020; Hatam-Nahavandi et al., 2021). A single infected felid is capable of shedding millions of oocysts for 10–15 days, thereby contaminating the environment and posing infection risk to various intermediate hosts (Hatam-Nahavandi et al., 2021). The overall pooled prevalence of *T. gondii* infection in canids (domestic and wild), cattle and small ruminants (sheep and goats) were 69%, 20% and 11% respectively. Also, the overall pooled prevalence of *T. gondii* infection in pigs, chickens and birds showed a seroprevalence of 13% and 22% respectively.

The pooled seroprevalence of anti-*T. gondii* antibody from humans was 14% (95% CI: 5–25%) and it came from studies that focused mainly on immunocompetent individuals, HIV+ patients, and pregnant women (Zumla et al., 1991; Bessong et al., 2010; Domingos et al., 2013; Ngobeni and Samie, 2017; Coupe et al., 2019, Van der Colf et al., 2020) as well as a few studies on blood donors and children (van der Colf et al., 2014).

Diagnostic tools used in the reviewed articles varied widely and ranged from MAT, LAT, IFAT, ELISA, DT, CF, Wolstenholme's modification, and Sabin–Feldman dye test techniques to molecular approach. In this study, the majority of articles adopted ELISA and IFAT to determine the seroprevalence of *T. gondii*. Although serological methods seem to lack sensitivity and specificity, they remain a standard tool for the qualitative detection of antibodies (Rouatbi, et al., 2019). A comparation of three serological diagnostic tools showed that ELISA and IFAT had relatively higher sensitivity and specificity than MAT (Sharma et al., 2019). Additionally, ELISA and IFAT are less laborious and time-consuming than MAT (Sharma et al., 2019).

Generally, substantial heterogeneity existed between the studies reviewed and subgroups. This may be due to a range of factors, such as people's varying hygiene practice levels, limited studies from some countries, varying diagnostic methods used, methods of rearing livestock animals, meat consumption pattern of studied individuals, or hostage.

5.3 A systematic review and meta-analysis of canine, feline, and human *Toxocara* infections in sub-saharan Africa

Chapter 3 focused on the systematic review and analysis of existing studies on *Toxocara* infection in SSA. It determined the epidemiological distribution of infection in various hosts in the region and identified research gaps for future research.

This study revealed that only one-third of sub-Saharan countries have reported on the prevalence of *Toxocara* infections in dogs, cats and humans. In the environment, the development of eggs of *Toxocara* spp. directly correlates with humidity (Gamboa, 2005) and temperatures ranging between 23°C and 35°C, with limited development occurring at ≤17°C (Rocha et al., 2011).

The observed prevalence of *T. canis* eggs from dogs and *T. cati eggs from cats* in SSA obtained in this study was 19% and 9% respectively. This study showed that studies on the epidemiology of *T. cati* are limited in SSA regions. Moreover, this review has revealed that *T. canis* is widely and more commonly reported in dogs than *T. cati* is in cats. The limited data on cats from this region may not imply a less contributory role in toxocariasis; rather, observed heterogeneity may be attributed to inaccessibility to cat faeces due to their defecation habit of burying faeces, thus making *T. cati* appear as less prevalent than *T. canis* (Nijsse et al., 2016; Subrata et al., 2017).

In dogs, puppies are epidemiological reservoirs of *T. canis* and they acquire infections via transplacental and transmammary routes and are capable of shedding millions of eggs per day in less than three weeks after birth. (da Cunha Amaral et al., 2010; Macpherson, 2013; Ferreira et al., 2016; McManus et al., 2018). Unlike dogs, cats do not show age-resistance and are more prey eaters, which results in a direct development of egg-producing *Toxocara* worms without having a somatic migration (Overgaauw and van Knapen, 2013; Nijsse et al., 2016).

This study revealed a paucity of information on the prevalence of *Toxocara* spp. in a wide variety of hosts including food animals such as poultry, pigs, lamb, goats and cattle in SSA regions. (Karshima, 2019). Food animals are known to be zoonotic agents of several helminths of public health importance, including *Toxocara* spp. (Karshima, 2019). This transmission route is particularly of concern among communities with a cultural habit of consuming raw or undercooked meat viscera, thereby contributing significantly to the prevalence of human toxocariasis (Robertson et al., 2014).

5.4 Knowledge and practices on consumption of free-range in selected rural communities of KwaZulu-Natal, South Africa, with focus on zoonotic transmission of *Toxoplasma gondii* and *Toxocara* spp.

Chapter 4 highlighted the knowledge and practices on the household consumption of FRC meat and viscera by rural communities in KwaZulu-Natal (KZN), South Africa as risk factors in the transmission of zoonotic pathogens.

The socio-demographic parameters obtained in this study revealed resource-limited settings, low level of education and high unemployment rate. Limited employment and socio-demographic factors influence the food and meat gathering practices of people in a way that predisposes them to parasitic infections (Simeone 2008; Drescher et al., 2012; Goyette et al., 2014). This study showed that knowledge of zoonotic disease transmission was highest in the southern localities (MZ and SH) than in the northern (GI and OZ) of KZN respectively. The percentage of zoonoses transmission awareness was 30.1%. Although, no significant associations were found between knowledge of zoonoses transmission and considered variables in all the localities, the high level of knowledge obtained among respondents with high school education, unemployed respondents, and women can be attributed to the large percentage of these categories constituting the respondents.

Furthermore, this study revealed that ownership of free-range chickens in the study locations was 65% (52/80). This is higher than the 57.7% (41/71) reported in Ethiopia (Sambo et al. 2015), but lower than the 93.5% and 84% poultry (duck and chicken) ownership observed in Eastern Cape province of South Africa and Cambodia respectively (Mwale and Masika 2009; Osbjer et al. 2015). Also, the average flock size (17.2±1.4) observed in this study is higher than (16±2.1), reported in the Eastern Cape province of South Africa (Mwale and Masika 2009), but lower than (22.03±2.85) in Limpopo province and (28.40±2.57) earlier reported in KwaZulu-Natal province respectively (Malatji et al. 2016).

Regarding consumption patterns, the majority (76.3%, 61/80) of respondents that reported the practice of consumption of FRC viscera in the households preferred them well cooked. The reason for the high demand for chicken viscera in the study area is however unknown. Studies have identified the role of poor socioeconomic factors as well as globalization as important factors in meat consumption patterns (Tambi 2001; Simeone 2008; Goyette et al. 2014; Robertson et al. 2014). Additionally, the viscera of chicken and other avian

animals have been reported to be rich in essential nutrients for humans (Schönfeldt and Gibson 2008).

With regard to the parasites screened from the sacrificed birds, *T. gondii* was screened for using ITS-1 and ITS-2, however, *T. gondii* was not detected in the brain tissues of FRC in this study and this might be an indication that the FRC sampled in our study may have not been exposed to *T. gondii* oocysts and several factors might have influenced the results including the non-survival of *T. gondii* oocysts in the environment due to hot and dry temperatures in the localities where the chickens were collected (Lukášová et al., 2017). Previous studies have shown low or non-prevalence of *T.* gondii oocysts in turkey meat products and feral rodents (Koethe et al., 2015; Meerburg et al., 2010). On the other hand, detection of *T. canis* infection was based on amplification of the 18S rRNA gene using the Nem 18S-F1 with Nem 18S-R1 primers. This is consistent with other studies where PCR techniques have been used to detect *Toxocara* spp. in animals (Davidson et al. 2012; Zibae et al. 2017; Dewair and Bessat 2020; Okada et al. 2021).

The prevalence of *Toxocara* infection observed in this study was low- 9.5% (4/42) and this is in agreement with previous studies (Zibae et al. (2017); Okada et al. (2021); Davidson et al. (2012). The occurrence of *T. canis* in FRC observed in this study indicates that the chickens are being exposed to environments contaminated with *T. canis* eggs from dogs in the localities studied. Among parts of FRC where *T. canis* were found are liver and lungs which constituted parts of FRC that were consumed by some respondents in this study. The occurrence of *T. canis* in free range chickens observed in this study reveals the possibility of toxocariasis transmission in the province most especially when the meat or viscera are eaten raw or undercooked. Considering the high rate of consumption of parts of FRC practised by communities in this study, although most preferring "well-cooked", it is important to create awareness on the role of FRC as paratenic hosts of *T. gondii* and *Toxocara* spp. Furthermore, the application of PCR technique used in this study could be employed in routine detection methods for *Toxocara* larvae in organs of suspected infected animals, especially in toxocariasis endemic areas. We also recommend participation of all stakeholders through a One Health approach in designing control and prevention strategies of zoonotic pathogens affecting these communities using the findings from this study as basis.

5.5 General conclusion and recommendation for future research

I recommend further studies where larger samples sizes are used for the detection of both *T. gondii* and *Toxocara* spp in FRCs combined with sero-epidemiology of human toxoplasmosis

and toxocariasis, in the rural communities of KZN in order to understand disease prevalence in the province.

5.6 References

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APPENDICES

1. Link to publication1: https://doi.org/10.1017/S0022149X19000889

2. Link to publication 2: https://doi.org/10.3390/pathogens11020183