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Epidemiology of *Toxoplasma gondii* infections in humans and selected wildlife and domestic animal species in an interface area in the Bushbuckridge Municipality, Mpumalanga, South Africa

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List of Abbreviations

Abbreviations	Full Scientific Names
CSF	Cerebrospinal fluid
CLIA	Chemiluminescence assays
DNA	Deoxyribonucleic acid
DAT	Direct agglutination test
DALLRD	Department of Agriculture, Land Reform and Rural Development
EIA	Enzyme immunoassay
ELFA	Enzyme-linked fluorescence assay
ELISA	Enzyme linked immunosorbent assay
ICT	Immunochromatographic assay
ISAGA	Immunosorbent agglutination assay
IFAT	Indirect fluorescent antibody test
IHA	Indirect hemagglutination test
LAT	Latex agglutination test
MC	Microscopy
MT	Microtiter Test
MAT	Modified agglutination test
McMaster	Modified McMaster technique
mnPCR	Multiplex nested polymerase chain reaction
PCR	Polymerase chain reaction
PM	Postmortem assessment
PK1	Pyruvate Kinase 1
rt-PCR	Real-time polymerase chain reaction

rflp pcr	Restriction Fragment Length Polymorphism polymerase chain reaction
SAG1 and SAG3	Surface antigen 1/2/3
SFDT	Sabin Feldman Dye Test
T. gondii	*Toxoplasma gondii*

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Declaration

To my knowledge the work contained in this thesis is original and was undertaken by myself (Refilwe Philadelphia Bokaba) with occasional assistance as indicated in the acknowledgements. The interpretation and analysis of the data were also my primary responsibilities.

Thesis Summary

Epidemiology of toxoplasmosis in humans and animals in an interface area in the Bushbuckridge Municipality, Mpumalanga, South Africa

By

Refilwe Philadelphia Bokaba

Supervisor: Prof. L. Neves

Co-supervisors: Prof. P. Dorny and Dr D. Morar-Leather

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Degree: PhD (Veterinary Science)

Toxoplasma gondii (*T. gondii)* is an obligate protozoan parasite in warm-blooded animals of global importance and affecting approximately 30-50 % of the human population (approximately more than two billion people) worldwide. This parasite is able to infect a wide range of mammals and birds including livestock, wildlife, domestic animals, and humans. Its dissemination within the different animal species is highly dependent on the interspecies interactions and the environment. In the agricultural sector *T. gondii* was seen to cause economical losses in goats and sheep due to the increase of abortions.

Toxoplasma gondii has a noticeable effect globally on food safety and environmental health, which are the main issues of concern to reducing and preventing its spread. In most African countries including South Africa (SA), however, information on the national seroprevalence of *T. gondii* is limited in people and animals and has not yet been fully investigated.

This study investigated the epidemiology and dynamics surrounding *T. gondii* in the Bushbuckridge Municipality (Mpumalanga, South Africa). Both serological (latex agglutination commercial kit) and molecular techniques (multiplex nested PCR detecting genetic markers SAG1, SAG2b, SAG3, PK1 and C22-8) were used to determine the prevalence in humans, livestock from informal farms, domestic animals and feral rodents (captured rodents). A questionnaire survey targeting the community members was also conducted to get a better understanding of the socio-economical, environmental and husbandry conditions in the area. The seroprevalence in humans, cats, chickens, goats and rodents was 8.8%, 0.0%, 4.2%, 11.2% and 18.0%, respectively. Seroprevalence in impalas, kudus, wild dogs, wildebeests, warthogs and zebras was estimated at 5.2%, 7.3%, 100.0%, 20.9%, 13.4% and 9.1%, respectively. The results reveal a low prevalence of *T. gondii* in the human, domestic cycle and most of the wildlife species. The highest seroprevalence was detected in wild dogs possibly indicating that the dominating route of transmission could be through the ingestion of contaminated meat in wildlife. Rodents captured in different habitats showed a relatively high seroprevalence of *T. gondii* in human dwellings (19.0%), croplands (15.3%) and nature conservation (20.2%) habitats, which suggests that rodents could be the possible bridges between the wildlife cycle and the surrounding human and domestic cycles in the interface area. The questionnaire revealed that 63.0% of the household owners are informal farmers and 35.9% are pet owners. A high female participation was found at 75.3% when compared to males at 24.7%, which also indicates that the women are possibly the ones that care and manage the livestock and pets in the households. Strains from lineages Type II and Type III were detected circulating in the studied area with variation detected in satellite markers SAG1 and SAG3, possibly suggesting an atypical *T. gondii* circulating in the area. This study is important and beneficial because it displays current epidemiological information relating to the distribution, the possible transmission and incidence of *T. gondii* in the Bushbuckridge Municipality (Mpumalanga, South Africa).

CHAPTER 1

General Introduction, Literature Review, Justification, Study Aim & Objectives

1.1 Introduction

Toxoplasmosis is a disease caused by an obligate protozoan parasite known as *Toxoplasma gondii* (*T. gondii*). It is a zoonotic parasite reported across the globe, infectious to a spectrum of species that can be divided into to two main types of hosts namely the definitive and intermediate hosts (Torrey and Yolken 2013). The Felidae family is known as the definitive hosts while the remaining species of mammals and birds can act as the intermediate hosts. *Toxoplasma gondii*-infected hosts (definitive and intermediate) may elicit mild to severe clinical symptoms such as, mild flu like symptoms, encephalitis, retinitis, and abortions (Schlüter, Däubener et al. 2014). The main modes of transmission are through the ingestion of infected meat sources, the ingestion/inhalation of sporulated oocysts from the environment (including soil, water and vegetation) and through transplacental infections in some mammal species (including humans) (Figure 1.1). Other less frequent routes are infections during organ transplants and laboratory contamination through the handling and testing of collected specimens during laboratory analysis (Herwaldt 2001, Wendte, Gibson et al. 2011).

Toxoplasma gondii has been known to science for over 100 years since it was first mentioned in 1908. Researchers, Nicolle and Manceaux discovered a new species of microorganisms while investigating leishmaniosis in rodents (*Ctenodactylus gondi*) in Tunisia (Nicolle and Manceaux 1908). Another confirmed case in a mammalian species was detected in a rabbit (*Oryctolagus cuniculus*) in Brazil (Splendore 1908, Dubey 2008, Dubey 2009). The earliest description of the parasite's zoonotic nature was presented by researchers Sabin and Olitsky (1937) who isolated *T. gondii* for the first time and showed that it was identical to the human isolates, using the cross-protection technique (Sabin and Olitsky 1937, Dubey 2008).

Toxoplasma gondii was first described and confirmed in humans in 1938 in the USA with the earliest probable congenital toxoplasmosis case. A hospital reported a newborn girl who experienced severe symptoms such as lesions on the maculae of her eyes and convulsive seizures which led to her death within the first month of birth. Free living and intracellular parasites in the tissue cells of the spinal cord, brain and eye tissue were found (Wolf, Cowen et al. 1939). After this case, further research was done using intracerebral inoculation in mice and rabbits from the infected cerebral cortex and spinal cord from the newborn. Parasites from the tissue were inoculated in rabbits and mice using a bioassay technique resulting in the earliest evidence of

cerebral toxoplasmosis (Wolf, Cowen et al. 1939). Further research also resulted in the detection of congenital toxoplasmosis in rodents and sheep (Hartley and Marshall 1957).

The interspecies transmission route of *T. gondii* through tissue cysts was first proposed in 1954 by Weinman and Chandler (Weinman and Chandler 1954). Their assumption was further proven by Jacob et al. (1960) who exposed tissue cysts to proteolytic enzymes and explained that the cyst wall was easily degenerated by the enzymes but the bradyzoite walls survived long enough to infect tissue cells of the host (Jacobs, Remington et al. 1960). The second route of transmission is through the inhalation or ingestion of sporulated oocysts from vegetation, soil and water in the environment (Dubey 2008). In 1965 Hutchison proved that sporulated oocysts are infective (Hutchison 1965).

1.2 Literature Review

1.2.1 Life cycle

Toxoplasma gondii has three life cycle stages, namely the bradyzoites that develop into tissue cysts, the tachyzoites that multiply in various cell types in the acute phase of an infection and lastly, oocysts, which are produced and excreted by the felid definitive hosts (Tenter, Heckeroth et al. 2000). The definitive hosts shed unsporulated oocysts (resultant of the sexual cycle) in their faecal matter, dispersing millions of oocysts into the environment. Sporulation occurs outside the definitive host and oocysts become infective 1 to 5 days after excretion (Halonen and Weiss 2013, Schlüter, Däubener et al. 2014). After oral infection by ingesting tissue cyst-infected meat, the tissue cysts rupture, releasing bradyzoites that enter intestinal epithelial cells and convert to tachyzoites. The tachyzoites are the fast-replicating stage of the parasite, able to infect and destroy host cells resulting in tissue damage. Tachyzoites can also be transmitted via unpasteurised milk, organ transplants and accidental laboratory contaminations but these pathways are considered rare (Wendte, Gibson et al. 2011).

A similar path is followed when hosts are infected by sporulated oocysts. As the oocysts rupture, they release sporozoites which convert into tachyzoites and further

infect cells of peripheral organs. When the effector response of the host's immune system is activated, the tachyzoites in both pathways convert to bradyzoites in tissues cysts resulting in a chronic infection in the affected host, which is able to persist throughout the life span of the host (Halonen and Weiss 2013). The tissue cysts mostly remain in muscle and brain tissue in a dormant state, ready to be re-activated when the host immune system is weakened (Tenter, Heckeroth et al. 2000, Khan, Taylor et al. 2005). In immune-competent individuals the symptoms experienced by a patient can mimic a common flu but in immune-compromised patients the symptoms can vary from general to serious clinical manifestations (Hill and Dubey 2002).

1.2.2 T. gondii detection techniques

In earlier years researchers relied on microscopic and bioassay techniques to detect and identify *T. gondii* parasites (Sabin and Olitsky 1937). In 1948 researchers Sabin and Feldman (1948) further advanced to a serological test referred to as the Sabin Feldman Dye Test (SFDT) which became the gold standard technique for detecting *T. gondii* (Sabin and Feldman 1948). In the following years, more techniques were developed and improved, having increased sensitivity and specificity. These tests included both serological and molecular assays. With numerous studies showing that *T. gondii* elicits a strong and long lasting immunological response in hosts there are a number of serological techniques used to investigate its prevalence and its potential environmental impact by detecting anti-*T. gondii* antibodies such as IgG for latent/chronic infection and IgA and IgM for acute infections (Liu, Wang et al. 2015, Rostami, Karanis et al. 2018, Khan and Noordin 2020). The most documented serological techniques used for anti-*T. gondii* antibody or *T. gondii* surface antigen detection include, enzyme linked immunosorbent assays (ELISAs), indirect fluorescent antibody test (IFAT), the modified agglutination test (MAT), the SFDT, the latex agglutination test (LAT) and the indirect hemagglutination test (IHA) (Villard, Cimon et al. 2016, Rostami, Karanis et al. 2018). More advanced assays are being introduced which are automated (not labour intensive), are not affected by human error, provide quick reproducible results, are considered inexpensive, provide mass rapid testing, detect the immunoglobulins of interest accurately (for serological tests) and are quicker to process (Pomares, Zhang et al. 2017, Rostami, Karanis et al. 2018). Assays like the chemiluminescence assays (CLIA), enzyme-linked fluorescence assay

(ELFA), immunochromatographic test (ICT), serum IgG avidity test and immunosorbent agglutination assays (ISAGA) were created to improve the quality and experience of diagnostics (Rostami, Karanis et al. 2018). Although serological techniques are effective in diagnosis and epidemiological studies, molecular techniques were developed for diagnosis in specific cases and for genotyping and molecular epidemiology. The first molecular technique was developed by Burg et al. (1989). The aim of their study was to identify and detect the B1 gene sequences in *T. gondii* DNA (Burg, Grover et al. 1989). Their research encouraged the further development of molecular techniques such as genetic sequencing and quantitative, nested and reverse fragment length polymorphism (RFLP) polymerase chain reaction (PCR). These assays have also been used for genotyping and genetic characterization of *T. gondii*, the diagnosis of toxoplasmosis in host tissues and body fluids and to further distinguish between cerebral, congenital and ocular toxoplasmosis (Jauregui, Higgins et al. 2001, Wyrosdick and Schaefer 2015, Poulle, Forin-Wiart et al. 2016, Mahami-Oskouei, Moradi et al. 2017).

1.2.3 Clinical signs and epidemiology

Cats

Felidae play two main important roles in the life cycle of the parasite, genetic recombination and oocyst production. As mentioned above domestic and wild felids are the only definitive hosts that can shed environmentally resistant oocysts that are able to survive for approximately a year in moist soil (Marchiondo, Duszynski et al. 1976, Boughattas, Behnke et al. 2016, Liu, He et al. 2017). Although felines generally experience an asymptomatic infection, feral and domesticated cats are good indicators of possible environmental contamination because it can be assumed that a high prevalence of *T. gondii* found in cats surveyed in a specific area corresponds to an increased oocyst shedding in the area (Tenter, Heckeroth et al. 2000, Boughattas, Behnke et al. 2016). The period from ingestion of parasite cells to the time of oocyst excretion can differ depending on which infective stage of the parasite the cat is infected with. When infected with bradyzoites the cats start producing oocysts within a period of about 3-10 days from the day of ingestion, while when they are infected with oocysts or tachyzoites this takes approximately 18 days or longer (Dubey, Miller et al. 1970). It is alleged that cats mainly become infected through the consumption of infected meat when for example feeding on rodents. The presence of cats in

communities can impact the public health in those communities. A number of preventative measures that can be implicated in the communities to reduce parasite transmission through cats are the frequent disposal of cat litter, restricting cats indoors, reducing/preventing interactions between pregnant women or immune-suppressed individuals or children with cats and cat litter (Dubey, Weigel et al. 1995, Elmore, Jones et al. 2010, Wang, Zhou et al. 2017). The seroprevalence of *T. gondii* detected in cats in China, Qatar, Poland was 21.3% (47/221, MAT), 82% (406/495, MAT) and 68.8% (143/208, IFAT), respectively (Wu, Zhu et al. 2011, Boughattas, Behnke et al. 2016, Sroka, Karamon et al. 2018). The studies explained the high seroprevalence in cats to be caused by the cats having more access to the outdoors and therefore being more exposed to pathogens than indoor cats (Boughattas, Behnke et al. 2016, Sroka, Karamon et al. 2018). Two studies done on cats in South Africa found a seroprevalence of 31.62% (n=109, latex agglutination test), 37.1%, (IgG, enzyme immunoassay) and 8.8% (IgM, EIA) (Hammond-Aryee, Esser et al. 2015, Tagwireyi, Etter et al. 2019).

Humans

In humans, *T. gondii* can cause signs and symptoms such as, fever, malaise, lymphadenopathy, muscle weakness, encephalitis, pulmonitis, retinochoroiditis, anterior uveitis, deafness, seizures and mental retardation in infected foetuses (through congenital toxoplasmosis), immune-suppressed and immune-competent patients (Torrey and Yolken 2013). Foetuses from mothers that are infected in the first trimester are seen to experience a more severe clinical toxoplasmosis when compared to mothers infected in second or third trimesters (Li, Wei et al. 2014). Researchers have therefore indicated the necessity of compulsory routine *T. gondii* screening in pregnant women at specific intervals of their pregnancy for early diagnosis and treatment (Wang et al, 2017). Patients with acquired immunodeficiency syndrome (AIDS) commonly experience severe central nervous system conditions resulting in debilitating morbidity and possible mortality. A study in Myanmar detected a seroprevalence of 11.5% (29/251) in reproductive-aged women (age range 15-30 years) using an ELISA (Aye, Nagayasu et al. 2020). A survey done on 856 human sera in Indonesia found a seroprevalence of 58.5% (501/856) using a latex agglutination test (LAT) (Tuda, Adiani et al. 2017). The researchers explained that the

high prevalence could have been due to high cat population in the area (Tuda, Adiani et al. 2017). In a study done in South Africa a prevalence of 9.8% was found in HIV-positive patients, 12.8% in HIV-negative patients and 6.4% in the biobank serum samples using an enzyme immunoassay (EIA) (Kistiah, Winiecka-Krusnell et al. 2011). Although the seroprevalence detected in Indonesia is high, the general global seroprevalence in humans seems to be at a low rate.

Dogs

Although intermediate hosts are unable to produce oocysts, dogs are able to disperse oocysts into the environment (Lopes, Granada et al. 2014). Dogs have high olfactory capabilities and are habitually known to be seekers and frequently roll about everywhere in their surroundings. This can therefore result in them contracting and trapping oocysts in their fur and mechanically disseminating the parasite in the environment and their human counter parts (Lopes, Granada et al. 2014). Additionally, it has been discovered that dogs can ingest oocysts which pass intact through their gastrointestinal tract (GIT) and also release them in their faecal material although they are unable to create the oocyst themselves (Lindsay, Dubey et al. 1997, Schares, Pantchev et al. 2005, Wu, Huang et al. 2011, Lopes, Granada et al. 2014). In some countries like China, dog meat is consumed which could be another mode of transmission in the human population (Jiang, Li et al. 2015). Typically, infected dogs seldom experience clinical toxoplasmosis but may endure an adverse infection when their immune system is compromised (Calero-Bernal and Gennari 2019). Clinical infections in dogs have also been linked to the lack of canine distemper virus vaccination in *T. gondii* infected dogs. The clinical manifestations include conditions affecting the nervous system leading to seizures, ataxia, tremors (Calero-Bernal and Gennari 2019).

A study done in Ethiopia found a seroprevalence of *T. gondii* of 82.86% (319/385, using the direct agglutination test (DAT)) in both stray and owned dogs (Gebremedhin, Sarba et al. 2021). In China, the seroprevalence in owned dogs was 10.81% (28/259) with a MAT (Wu, Huang et al. 2011). The seroprevalence in hunting, pet and watchdogs in Spain was 30.6% (235/769) also using a MAT (Cano-Terriza, Puig-Ribas

et al. 2016). In Brazil the seroprevalence in owned dogs was found to be 16.32% (119/729) with a higher prevalence found in their human owners at 41.54% (248/597) (Benitez, Martins et al. 2017). The seroprevalence data documented seem to vary but most of the data showing *T. gondii* being highly prevalent in dogs globally. To our knowledge there is not data on the seroprevalence of *T. gondii* in dogs in South Africa.

T. gondii in Livestock – chickens, goats, pigs, sheep and cattle

Livestock such as cattle, goats, sheep, pigs and chickens are considered as a global source of food and nutrients, further transmitting the parasite in humans through the consumption of contaminated meat and possibly through goat milk (Paulino and Vitor 1999, OECD 2020). According to the statistics published by the Organisation for Economic Co-operation and Development (OECD), in South Africa an estimate of 2.9 million tons of poultry, beef and pork are consumed by the population annually with 60 % of meat consumed being poultry (OECD 2020). The frequent consumption of meat from livestock by the human population has made it one of the most frequent modes of *T. gondii* transmission in humans, thus having a negative impact on public health as a whole (Cook, Gilbert et al. 2000, Feitosa, Vilela et al. 2014, Onyiche and Ademola 2015). Since *T. gondii* can be transmitted by ingestion of undercooked and raw meat, it is considered a foodborne disease, thus raising concerns in food safety. There has been an increased focus on factors such as husbandry practices and informal and formal farms to reduce the rate of transmission within communities. Infections particularly in sheep, goats and pigs can lead to abortions with these species and can therefore cause economic losses for commercial farmers (Buxton, Maley et al. 2007, Innes, Bartley et al. 2009, Feitosa, Vilela et al. 2014, Hammond-Aryee, van Helden et al. 2015). A significant foetal loss of 2% in sheep due to toxoplasmosis was experienced in the United Kingdom (Buxton, Maley et al. 2007, Innes, Bartley et al. 2009, Hammond-Aryee, Esser et al. 2015).

In Ghana, the seroprevalence using an ELISA was found to be 33.2% (243/732) and 26.8% (141/526) in sheep and goats, respectively (van der Puije, Bosompem et al. 2000). In Nigeria, the seroprevalence in sheep was 1.4% (3/215), 53.6% (82/153) in goats and 29.14% (88/302) in pigs using ELISA techniques (Onyiche and Ademola 2015, Tonouhewa, Akpo et al. 2019). A study done in Indonesia detected a seroprevalence of 2.3% (7/310) in pigs with LAT (Tuda, Adiani et al. 2017). In South Africa, Tagwireyi et al (2019), detected a seroprevalence of 33.23% in chickens

(46/137), 55.63% in goats (69/128), 33.65% in pigs (36/106) and 67.25% in sheep (78/121) using the LAT (Tagwireyi, Etter et al. 2019). Although the data documented in the above studies show a wide seroprevalence range in livestock, *T. gondii* infection seems to be more prevalent globally in goats and sheep (van der Puije, Bosompem et al. 2000, Onyiche and Ademola 2015, Tagwireyi, Etter et al. 2019).

Researchers have indicated a high genetic resistance of *T. gondii* in cattle with cattle experiencing non-clinical toxoplasmosis and a low seroprevalence (Dubey 1986, Onyiche and Ademola 2015). There is a difficulty in determining the influence *T. gondii* has on cattle and its involvement in further transmitting the parasite. The difficulty comes with some cases presenting as seropositive for *T. gondii* with no tissue cyst detection which results in some speculating that this could be due to the assays used or that cattle are able to eliminate the tissue cysts effectively when infected by *T. gondii* (Opsteegh, Teunis et al. 2011). The observed seroprevalence in cattle in Estonia was 18.62 % (743/3991), 4.4% (13/295) in Algeria, 10.7% in Ethopia and 13.81 % (29/210) in Nigeria (Onyiche and Ademola 2015, Jokelainen, Tagel et al. 2017, Khames, Yekkour et al. 2018, Tilahun, Tolossa et al. 2018). The prevalence data show a lower prevalence rate in cattle when compared to other livestock species.

Wildlife species

Toxoplasma gondii is prevalent in wildlife species with publications documenting numerous studies detecting *T. gondii* in diverse land and aquatic herbivorous, omnivorous and carnivorous wildlife species globally (Dorny and Fransen 1989, Smith, Fisher et al. 1995, Hove, Lind et al. 2005, Ferroglio, Bosio et al. 2014, Lukášová, Kobédová et al. 2018, Uzelac, Klun et al. 2019, Seltmann, Schares et al. 2020). When infected most wildlife species do not experience clinical signs but researchers have documented cases of severe infections where some have led to death or euthanasia. Documented cases of clinical toxoplasmosis are for instance, two lions in Nigeria, marsupials in Australia and bobcats and wild turkeys from the US (Quist, Dubey et al. 1995, Smith, Fisher et al. 1995, Hillman, Lymbery et al. 2016).

The prevalence rates in wildlife detected across the world seem to vary. A seroprevalence of 59% (43/73) in American mink, and 77% (10/13) in otters was detected in a study done in the south of Chile using MAT (Barros, Cabezon et al. 2018). In Australia, the seroprevalence in kangaroos was 15.5% (34/219) (Parameswaran, O'Handley et al. 2009). Researchers in the north of Iran found a

seroprevalence of 24.42% (31/127) in wild rats using an immunochromatographic assay (ICT) (Mosallanejad, Avizeh et al. 2012). A seroprevalence of 58.8% (261/444) was detected in white-tailed deer from the USA using MAT. In Namibia researchers found a prevalence of 52.4% (131/250) in cheetah using an ELISA (Ballash, Dubey et al. 2015, Seltmann, Schares et al. 2020). The prevalence data in wildlife clearly indicate an active parasite cycle in wildlife which is possibly impactful to its surrounding habitats were humans, domestic animals and wildlife may intercept.

It is probable that the wildlife species can further transmit *T. gondii* in neighbouring habitats where human settlements and domestic animals cohabit near wildlife. Like domestic felids, it has also been proven that wild felids worldwide, are able to excrete unsporulated oocysts into their environment (Jewell, Frenkel et al. 1972, Miller, Frenkel et al. 1972, Marchiondo, Duszynski et al. 1976, Dorny and Fransen 1989, Lukešová and Literák 1998, Basso, Edelhofer et al. 2005). This is one the most probable ways that the wildlife cycle can also contribute to the dissemination of *T. gondii* in areas/habitats shared by humans, livestock and domestic animals (Figure 1.1). Other methods of transmission could be through human consumption of meat from wildlife and the rodent population found in the area (Ferroglio, Bosio et al. 2014, Jiang, Li et al. 2015, Almeria, Cabezon et al. 2018). Rodents are known as carriers of pathogens and can easily create a bridge of pathogen dissemination between habitats (Mosallanejad, Avizeh et al. 2012).

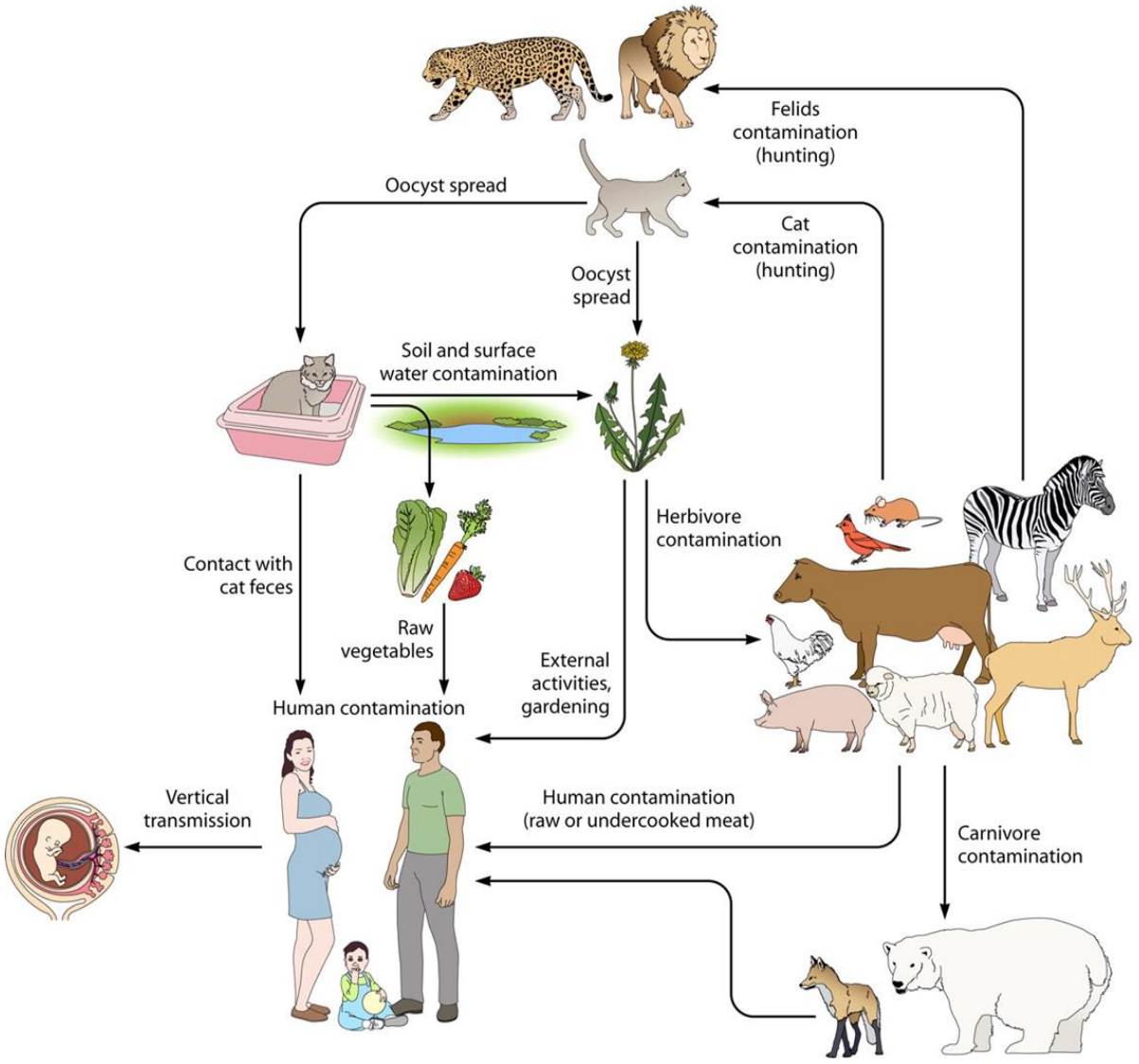

Figure 1.1: Diagram showing the cycle of T. gondii in humans, domestic animals and wildlife (Robert-Gangneux and Darde 2012).

Detection of *Toxoplasma gondii* in tissues and distribution of strains

As indicated earlier, in most hosts *T. gondii* infections causes sub-clinical infection. In some cases, toxoplasmosis may cause severe and even fatal disease. The disease severity is possibly linked to the route of infection of the host, the genotype of the *T. gondii* strain, the immune response of the host and possibly the dose of infection (Dupont, Christian et al. 2012). In mice, strains from clonal lineages II and III are considered avirulent while strains from clonal lineage I, such as the RH strain, are considered highly virulent and may cause severe clinical manifestations and death (Filisetti and Candolfi 2004).

Although the majority of *T. gondii* strains originate from the three main lineages (type I, II and III) which are prominent in Europe and North America (Filisetti and Candolfi 2004, Berger-Schoch, Herrmann et al. 2011, Dupont, Christian et al. 2012, Wang, Chen et al. 2013), recent research shows a more genetically diverse *T. gondii* population globally. The detected diversity disproves a notion that was previously documented that the parasite propagated clonally with strains found originating from only three lineages, which dominate in North America and Europe (Howe and Sibley 1995, Filisetti and Candolfi 2004, Berger-Schoch, Herrmann et al. 2011, Dupont, Christian et al. 2012, Su, Khan et al. 2012, Wang, Chen et al. 2013)

Recent research has found atypical strains in Brazil, Africa (mainly Egypt), the UK, Central, North and South America (Su, Khan et al. 2012, Shwab, Zhu et al. 2014, Witter, Pena et al. 2022). A fourth clonal lineage was found and described in North America referred to as Type 12. Furthermore, a rare genotype, Type X, was also identified in the US. Many of the clinical cases documented have been associated with atypical strains, likely indicating a positive association of clinical cases in immune-competent hosts with an increase of more virulent atypical strains in the environment (Khan, Dubey et al. 2011, Su, Khan et al. 2012).

Global Impact of climate and weather

Increased prevalence rates of *T. gondii* have been positively associated with areas having lower altitudes, and warmer and more humid climates (Hammond-Aryee, Esser et al. 2015). For example, Boughattas et al. (2017) found a significantly higher prevalence of *T. gondii* in feral cats tested in summer when compared to winter showing the impact of seasonal change on the transmission of the parasite (Boughattas, Behnke et al. 2016). Although *T. gondii* has been successful in maintaining its existence, environmental factors such as climate change, temperature and humidity can significantly affect its dissemination (Patz, Graczyk et al. 2000). This includes anthropogenic factors such as urbanisation or industrialisation of communities, water supply systems and irrigation systems. In industrialised countries the intensification of the livestock sector (controlled housing conditions) has drastically reduced the transmission of the parasite (e.g. in pigs and poultry) and consequently also in the human population. The increase in organic farms again increases the chances of *T. gondii* transmission. Changes in the mentioned factors can affect the stability of the ecosystem and in turn affect the soil, which then impacts the survival

and dissemination of oocysts and therefore affects the transmission rate of the parasite in an area. Changes in temperature, rainfall and the pH in the soil play a significant role in the sporulation and survival of oocysts in the environment which show how impactful climate change may be on the transmission of *T. gondii*. These conditions may result in wide ranges of *T. gondii* prevalence detected globally in the different species studied (Meerburg and Kijlstra 2009, Yan, Liang et al. 2016, Tonouhewa, Akpo et al. 2019).

Overall aim of study

There are limited studies on *Toxoplasma gondii* in South Africa and Africa as whole especially in interface areas were more than one habitat intercept. In areas like these, public health, veterinary health, and wildlife conservation are collectively important. The following chapters will elucidate the epidemiology of *T. gondii* in the Bushbuckridge Municipality, Mpumalanga, in South Africa. Documenting the review of the prevalence of *T. gondii* in African wildlife, the seroprevalence in humans, livestock, domestic animals and wildlife species, and genetic characterization on rodent tissue in Mpumalanga, South Africa.

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CHAPTER 2

Toxoplasma gondii in African wildlife: a systematic review

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Toxoplasma gondii in African wildlife: a systematic review

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Abstract

Toxoplasma gondii (*T. gondii*) is a protozoan parasite, which infects a wide variety of mammals and bird species globally. In large parts of the world, this parasite is relatively well documented in wildlife species, however this topic is poorly documented in Africa. The current review systematically explores the presence and distribution of *T. gondii* in African wildlife species through a key word search in PubMed, Web of Science and CAB Direct. A total of 66 records were identified and included in the qualitative analysis, of which 19 records were retained for the quantitative synthesis. The presence of *T. gondii* was reported in a wide range of wildlife species, found in twelve countries, spread over the African continent. The retained records reported a prevalence range of 6-100 % in herbivores, 8-100% in omnivores and 14-100 % in carnivores. In wild felines (cheetahs, leopards and lions) a prevalence range of 33-100 % was found. Reports from South Africa, and on the presence of *T. gondii* in lion were most common. Overall, the results indicate the scarcity of information on *T. gondii* in Africa and its circulation in wildlife. The lack of knowledge on the parasite in Africa, especially in areas at the human-livestock-wildlife interface, prevents us from understanding how prevalent it is on the continent, what strains are circulating in wildlife and what the most common routes of transmission are in the different habitats in Africa.

Keywords: *Toxoplasma gondii*, wildlife, prevalence, Africa

2.1 Introduction

Toxoplasma gondii (*T. gondii)* is an apicomplexan protozoan parasite that causes a zoonotic infection known as toxoplasmosis. This parasite is one of the most resilient and persistent parasites in existence able to infect a large diversity of homeotherms worldwide (Aguirre, Longcore et al. 2019). Domestic and wild species of felines act as the definitive hosts of the parasite, in which it undergoes both the sexual (gametogony) and asexual cycle (schizogony) (Schlüter, Däubener et al. 2014). Homeotherm species other than felids are known to act as the intermediate hosts in which the parasite can only undergo the asexual cycle (Tenter, Heckeroth et al. 2000, Ferroglio, Bosio et al. 2014).

There are two common routes of transmission between species. The first is through the ingestion of meat contaminated with tissue cysts, containing bradyzoites (Weinman and Chandler 1954, Jacobs, Remington et al. 1960, Tenter, Heckeroth et al. 2000). The second is through the ingestion of sporulated oocysts from vegetation, soil and water in the environment (Dubey 2008). Vertical transmission, in which tachyzoites cross the placenta infecting the foetus and causing congenital toxoplasmosis, is a less frequent transmission route (Dubey 2009, Berger-Schoch, Herrmann et al. 2011).

In sheep and goats, toxoplasmosis can cause abortions, resulting in economic losses for farmers (Buxton, Maley et al. 2007, Torrey and Yolken 2013, Hammond-Aryee 2014). In immune-competent hosts, *T. gondii* generally causes an asymptomatic infection, while hosts with a suppressed immune system are more susceptible to experiencing severe clinical manifestations from this parasite (Khademvatan, Foroutan et al. 2017). Recent publications however have indicated that immune-competent individuals are also able to experience a severe clinical disease and that the severity is possibly linked to the type of strain infecting the host (Dupont, Christian et al. 2012). Genetic analysis of isolates from around the world has revealed more diverse strains including a fourth clonal lineage, which can also possibly be linked to the severity of the infection (Dubey, Sundar et al. 2008, Khan, Dubey et al. 2011, Su, Khan et al. 2012). Particularly in Central and South America, an abundance of atypical (non-clonal) strain types have been found, which may be due to a greater diversity and number of wild animal hosts occurring in these (sub-) tropical zones, each of which

might favour the selection of different *T. gondii* genotypes, enabling a wider variety of strains to proliferate (Shwab, Zhu et al. 2014).

Research done on toxoplasmosis in Africa is limited with the majority of what is published focusing on its incidence and prevalence in humans and livestock and little on wildlife. Until now, sampling in tropical regions has been done mainly on the American continent which is therefore necessary to understand the *T. gondii* population structures in other tropical regions such as Africa. The impact of *T. gondii* in wildlife species in Africa is poorly elucidated including its clinical implications in wildlife species and especially in documenting the possible role it plays in the bridges found between human-livestock-wildlife interface areas. The excretion of oocysts from both domestic and wild felines dispersed in the environment possibly affect both herbivorous and omnivorous species found in an interface area. In many countries wildlife species are also a source of protein for many humans. These pathways are possible routes of transmission and a possible bridge that can be further investigated in interface areas. This is surprising as Africa contains a number of human-livestock-wildlife interface areas that co-exist and possibly affect each other.

Therefore, the current review will systematically explore the past and current occurrence, prevalence and distribution of *T. gondii* in wildlife in Africa from its earliest mention to date. Additionally, the article will investigate the most commonly reported transmission routes for the different dietary wildlife types (herbivores, omnivores and carnivores).

2.2 Results

2.2.1 Search Results

A total of 188 records were retrieved, 145 were found through the database search, whereas 43 additional records were identified through snowballing of reference lists of relevant reviews and research articles (PRISMA Flow diagram: Appendix B). After duplicate removal, the title/abstracts of 103 records were screened for relevance. Next, the full text articles of 86 remaining records were further evaluated against the inclusion and exclusion criteria. Twenty records were removed for not being in line with the objectives of this review. Finally, a total of 66 publications were retained and

included in the qualitative synthesis. The quantitative synthesis included 20 articles with the majority of the records reporting data from South Africa (n = 7), followed by Zimbabwe (n = 3), Kenya (n = 2), Tanzania (n = 2), Botswana (n = 2), Namibia (n = 2), Uganda (n = 1), Zambia (n = 1), Nigeria (n = 1), Madagascar (n = 1), Senegal (n = 2) and Tunisia (n = 2) (Figure 2.1).

2.2.2 Figures, Tables and Schemes

Figure 2.1 The African continent summarizing the prevalence ranges (%) and the number of studies done (n) in wildlife species in each documented country from the records included in the study. Only the African countries with published studies on T. gondii in wildlife species are highlighted in grey.

Table 2.1 T. gondii detection in wildlife species in Africa.

Country	Common animal species name	Scientific name	Prevalence % (Positive/n)	Methods of detection	Reference
Tunisia	Common gundi[H]	*Ctenodactylys gundi*	100 (3/3)	PM, MC	Nicolle and Manceaux, 1908
South Africa	African wild dog [C]	*Lycaon pictus*	50(1/2)	MC	Hofmeyr, 1956
Kenya	Lion [C]	*Panthera leo*	100(1/1)	IHA	Riemann et al. 1975
Uganda	Defassa waterbuck [C]	*Kobus ellipsiprymnus*	100(2/2)	IHA	Riemann et al. 1975
Tanzania	Rock Hyrax [O]	*Procavia capensis*	100(1/1)	IHA	Riemann et al. 1975
Tanzania	Burchells Zebra [H]	*Equus quagga burchellii*	28(8/29)	IHA	Riemann et al. 1975
Zambia	African elephant [H]	*Loxodonta africana*	6(4/63)	IHA	Riemann et al. 1975
Zambia	Hippopotamus [H]	*Hippopotamus amphibius*	8(4/49)	IHA	Riemann et al. 1975
Kenya	Silver-backed jackal [C]	*Canis mesomelas*	67(4/6)	SFTD	Bakal et al. 1980
Kenya	White tailed mongoose [C]	*Ichneumia albicauda*	50(1/2)	SFTD	Bakal et al. 1980
Kenya	Spotted hyena [C]	*Crocuta crocuta*	100(6/6)	SFTD	Bakal et al. 1980
Kenya	Zebra [H]	*Equus quagga burchellii*	90(9/10)	SFTD	Bakal et al. 1980
Kenya	Warthog [O]	*Phacochoerus africanus*	100(2/2)	SFTD	Bakal et al. 1980
Kenya	Giraffe [H]	*Giraffa camelopardalis*	50(5/10)	SFTD	Bakal et al. 1980
Kenya	Eland [H]	*Taurotragus oryx*	100(10/10)	SFTD	Bakal et al. 1980
Kenya	Bushbuck [H]	*Tragelaphus scriptus*	80(8/10)	SFTD	Bakal et al. 1980
Kenya	Fringe-eared oryx [H]	*Oryx beisa callotis*	50(2/4)	SFTD	Bakal et al. 1980
Kenya	Waterbuck [H]	*Kobus ellipsiprymnus*	27(5/11)	SFTD	Bakal et al. 1980
Kenya	Hartebeest [H]	*Alcelaphus buselaphus*	83(10/12)	SFTD	Bakal et al. 1980
Kenya	Topi [H]	*Damaliscus lunatus jimela*	82(9/11)	SFTD	Bakal et al. 1980
Kenya	Wildebeest [H]	*Connochaetes taurinus*	90(9/10)	SFTD	Bakal et al. 1980
Kenya	Impala [H]	*Aepyceros melampus*	80(8/10)	SFTD	Bakal et al. 1980
Kenya	Grant's gazelle [H]	*Nanger granti*	80(8/10)	SFTD	Bakal et al. 1980
Kenya	Thomson's gazelle [H]	*Eudorcas thomsonii*	90(9/10)	SFTD	Bakal et al. 1980
Kenya	Steenbok [H]	*Raphicerus campestris*	50(1/2)	SFTD	Bakal et al. 1980
Kenya	Dikdik [H]	*Rhynchotragus spp.*	100(5/5)	SFTD	Bakal et al. 1980
Kenya	African buffalo [H]	*Syncerus caffer*	100(10/10)	SFTD	Bakal et al. 1980
Nigeria	Lion [C]	*Panthera leo*	40(2/5)	SFTD, PM, MT, MC oocysts in feces	Ocholi et al. 1989
South Africa	Lion [C]	*Panthera leo*	90(36/40)	IFAT	Cheadle et al. 1999
South Africa	Leopard [C]	*Panthera pardus*	100(2/2)	IFAT	Cheadle et al. 1999
Botswana	Leopard [C]	*Panthera pardus*	50(1/2)	IFAT	Cheadle et al. 1999
Namibia	Lion [C]	*Panthera leo*	100(1/1)	IFAT	Cheadle et al. 1999
Namibia	Cheetah [C]	*Acinonyx jubatus*	33(2/6)	IFAT	Cheadle et al. 1999
South Africa	Cheetah [C]	*Acinonyx jubatus*	50(8/16)	IFAT	Cheadle et al. 1999
South Africa	African wild dog [C]	*Lycaon pictus*	100(16/16)	IFAT	Van Heerden et al. 1993
Botswana	Lion [C]	*Panthera leo*	92(49/53)	IFAT	Penzhorn et al. 2002
Botswana	Leopard [C]	*Panthera pardus*	100(1/1)	IFAT	Penzhorn et al. 2002
South Africa	Lion [C]	*Panthera leo*	100(42/42)	IFAT	Penzhorn et al. 2002
South Africa	Leopard [C]	*Panthera pardus*	86(6/7)	IFAT	Penzhorn et al. 2002
Zimbabwe	Lion [C]	*Panthera leo*	100(21/21)	IFAT	Penzhorn et al. 2002
Zimbabwe	Giraffe [H]	*Giraffa camelopardalis*	10(1/10)	MAT	Hove and Mukaratirwa, 2005

Zimbabwe	Greater kudu [H]	*Tragelaphus strepsiceros*	20(2/10)	MAT	Hove and Mukaratirwa, 2005
Zimbabwe	Nyala [H]	*Tragelaphus angasii*	90(9/10)	MAT	Hove and Mukaratirwa, 2005
Zimbabwe	Bushbuck [H]	*Tragelaphus scriptus*	57(8/14)	MAT	Hove and Mukaratirwa, 2005
Zimbabwe	Black rhino [H]	*Diceros bicornis*	27(3/11)	MAT	Hove and Mukaratirwa, 2005
Zimbabwe	African elephant [H]	*Loxodonta africana*	10(2/20)	MAT	Hove and Mukaratirwa, 2005
Zimbabwe	Lion [C]	*Panthera leo*	92(24/26)	MAT	Hove and Mukaratirwa, 2005
Zimbabwe	Ostrich [H]	*Struthio camelus*	48(24/50)	MAT	Hove and Mukaratirwa, 2005
Madagascar	Black lemur [H]	*Eulemur macaco macaco*	10(1/10)	Serum biochemical profile (IgG and IgM)	Junge et al. 2007
Senegal	Lion [C]	*Panthera leo*	43(3/7)	ELISA	Kamga-Waladjo et al. 2009
Zimbabwe	Lion [C]	*Panthera leo*	17(5/30)	McMaster (feces)	Makarati et al. 2013
Madagascar	Fossa [C]	*Cryptoprocta ferox*	93(42/25)	ELISA	Pomerantz et al. 2016
South Africa	Gerbil [H]	*Gerbilliscus sp.*	1(1/122)	ELISA	Lukasova et al. 2018
South Africa	Kudu [H]	*Tragelaphus strepsiceros*	8(1/13)	ELISA	Lukasova et al. 2018
South Africa	Honey badger [C]	*Mellivora capensis*	25(1/4)	ELISA	Lukasova et al. 2018
South Africa	White tailed mongoose [C]	*Ichneumia albicauda*	14(1/7)	ELISA	Lukasova et al. 2018
South Africa	Southern Yellow-billed Hornbill (bird) [O]	*Tockus leucomelas*	25(1/4)	PCR (brain)	Lukasova et al. 2018
South Africa	Laughing Dove (bird) [O]	*Spilopelia senegalensis*	25(1/4)	PCR (brain)	Lukasova et al. 2018
South Africa	Red-eyed Dove (bird) [O]	*Streptopelia semitorquata*	20(1/5)	PCR (brain)	Lukasova et al. 2018
Tanzania	Spotted hyena [C]	*Crocuta crocuta*	75(45/60)	ELISA	Ferreira et al. 2018
Senegal	Rodents [O]	*Mus musculus domesticus*	4.8(32/671) and 13.1(88/671)	MAT and PCR	Galal et al. 2019
Senegal	Rodents [O]	*Rattus rattus*	2.6(2/78) and 3.8(3/78)	MAT and PCR	Galal et al. 2019
Senegal	Rodents [O]	*Cricetomys gambianus*	31.9(15/47) and 27.7(13/47)	MAT and PCR	Galal et al. 2019
Senegal	Shrew [O]	*Crocidura olivieri*	37.5(12/32) and 15.6(5/32)	MAT and PCR	Galal et al. 2019
Tunisia	Yellow-legged gull [O]	*Larus michahellis*	3(30 nests, Sfax), 11(37 nest, Djerba)	ELISA	Gamble et al. 2019
South Africa	Caracal [C]	*Caracal caracal*	83(24/29),	IFAT	Serleys et al. 2019
Namibia	Blue wildebeest [H]	*Connochaetes taurinus*	10 (2/20) and	ELISA and IB	Seltmann et al.2020
Namibia	Honey badger [C]	*Mellivora capensis*	70 (7/10) and 60 (6/10)	ELISA and IB	Seltmann et al.2020
Namibia	Lion [C]	*Panthera leo*	93 (55/59) and 93 (55/59)	ELISA and IB	Seltmann et al.2020

Namibia	Brown Hyena [C]	*Hyaena brunnea*	92 (12/13) and 92 (12/13)	ELISA and IB	Seltmann et al.2020
Namibia	Caracal [C]	*Caracal caracal*	67 (10/15) and 67 (10/15)	ELISA and IB	Seltmann et al.2020
Namibia	Cheetah [C]	*Acinonyx jubatus*	52 (131/250) and 52 (131/250)	ELISA and IB	Seltmann et al.2020
Namibia	Leopard [C]	*Panthera pardus*	81 (47/58) and 81 (47/58)	ELISA and IB	Seltmann et al.2020
Namibia	Spotted hyena [C]	*Crocuta crocuta*	91 (10/11) and 91 (10/11)	ELISA and IB	Seltmann et al.2020
Namibia	Wild dog [C]	*Lycaon pictus*	71 (5/7) and 57 (4/7)	ELISA and IB	Seltmann et al.2020
Namibia	Bat-eared fox [O]	*Otocyon megalotis*	25 (1/4) and 0 (0/4)	ELISA and IB	Seltmann et al.2020
Namibia	Black-backed jackal [C]	*Canis mesomelas*	67 (26/39) and 67 (26/39)	ELISA and IB	Seltmann et al.2020

[1] Detection methods; IHA: Indirect Haemagglutination test; IB: Immunoblot; SFDT: Sabin Feldman Dye Test; IFAT: Indirect Fluorescent Antibody Technique; ELISA: Enzyme Linked Immunosorbent Assay; PCR: Polymerase Chain Reaction; MC: Microscopy; PM: Postmortem assessment; MT: Microtiter Test; McMaster: Modified McMaster technique. Dietary types; [H]: Herbivores; [O]: Omnivores; [C]: Carnivore

2.2.3 Historical overview of T. gondii in African wildlife

All prevalence data of *T. gondii* in African wildlife as extracted from the retained records are summarised and presented chronologically in Table 2.1. A summary that visually distinguishes between the countries with studies focusing on *T. gondii* in wildlife species (including the prevalence ranges) and the African countries that still need to be investigated are presented in Figure 2.1.

The earliest mention of *T. gondii* in wildlife in Africa, was in a rodent (*Ctenodactylys gundi*) published by Nicolle and Manceaux in 1908 in Tunisia, northern Africa. The researchers were initially investigating leishmaniosis but instead detected tachyzoites and discovered *T. gondii* (Nicolle and Manceaux 1908). A few decades later, *T. gondii* was detected for the first time in a carnivore in Africa. In the Zoological Gardens in South Africa, Hofmeyr (1956) performed a necropsy on a cape hunting dog (*Lycaon pictus*), also known as African wild dog and microscopically detected parasites that were identified as *T. gondii* (Hofmeyr 1956) (Table 2.1). This finding raised the awareness of the possible dissemination of *T. gondii* in wildlife species in Africa and therefore promoted more surveys to be done in a wider diversity of wildlife species. In

1975 the University of California, USA, noticed that a number of their imported African exotic animals tested positive for *T. gondii*. This led to an investigation to determine the seroprevalence in free-living wild animals from Tanzania, Uganda, Zambia and Kenya using an indirect hemagglutination test (IHA). Seropositivity was found in four African elephants (*Loxodonta Africana*, n=63, 6 %), one lion (*Panthera leo*, n=1, 100 %), two defessa waterboks (*Kobus ellipsiprymnus,* n=2,100 %), eight Burchell zebras (*Equus quagga burchelli*, n=29, 28 %), one rock hyrax (*Procavia capensis*, n=1, 100 %) and four hippopotamuses (*Hippopotamus amphibious,* n=49, 8 %). This study documented the earliest serological detection of *T. gondii* specifically in a wild felid and in a variety of wild herbivores, omnivores and carnivores in Africa (Riemann, Burridge et al. 1975).

Subsequently, in 1980 a serological survey in Kenya detected *T. gondii* infection in free ranging herbivorous and carnivorous captive wild mammals. The Sabin Feldman Dye test (SFDT) was conducted, which resulted in seroprevalence ranges of 50-100 % in the investigated carnivore and 27-100 % in the included herbivore species, indicated in Table 2.1 (Bakal, Karstad et al. 1980).

In Nigeria, severe acute toxoplasmosis was documented in two lions (*P. leo*) from the Jos Zoological Gardens (Ocholi, Kalejaiye et al. 1989). The two lions were kept in a cage with three other lions. The two lions had been experiencing severe symptoms such as, a depression of the respiratory system, dyspnoea and excessive diarrhoea. One lion was treated with a combination of neomycin and methscopolamine while the other lion was treated with oxytetracycline. The condition of the two lions (*P. leo*) did not improve after treatment, and lead to one dying after seven days of treatment and the other being euthanised after symptoms worsened (Ocholi, Kalejaiye et al. 1989). A SFDT done on their blood samples before their death, was positive for *T. gondii*. The researchers found necrosis in the tissues and identified tachyzoites in the tissue cells. *Toxoplasma gondii*-like oocysts were also detected from their faecal material and this is possibly the earliest identification of oocysts in wild felids in Africa but the researchers never confirmed whether the oocysts were *T. gondii* oocysts (Ocholi, Kalejaiye et al. 1989). Another study was also done on lions (*P. leo*) from the Etosha National Park in Namibia. Serology was performed on 63 serum samples from free-

ranging lions *(P. leo*) using the indirect fluorescent assay (IFA). The researchers found a high seroprevalence (96%) (Spencer and Markel 1993).

A high seroprevalence of 100 % (n=16) was also detected in African wild dogs (*Lycaon pictus*) in South Africa using an IFAT (Van Heerden, Mills et al. 1995). The researchers indicated that there was significant decline in the wild dog population and although this was attributed to a combination of factors such as loss of habitat, a decline in the availability of prey and inter- and intra-species competition, the burden of diseases was possibly another vital factor in the fall in population numbers (Van Heerden, Mills et al. 1995). Researchers from Auburn University (USA) also found a high seroprevalence of *T. gondii* in wild felids from the southern part of Africa (Cheadle, Spencer et al. 1999). The survey was done on a variety of free-ranging and captive felids in South Africa, Botswana and Namibia using an IFAT. The researchers were investigating the seroprevalence of both *Neospora caninum* and *T. gondii* in felids and found that *T. gondii* was more prevalent. The seroprevalence ranged from 56-100 % in lions (*P. leo*), 33-50 % in cheetahs (*Acinonyx jubatus*) and 50-100 % in leopards (*Panthera pardus*) (Cheadle, Spencer et al. 1999).

Penzhorn et al (2002) also determined the seroprevalence of *T. gondii* in a variety of wild felids from South Africa, Botswana and Zimbabwe. The seroprevalence in lions was 100 % (n=42) in South Africa, 92 % (n=53) in Botswana and 100 % (n=21) in Zimbabwe. In leopards, the seroprevalence was 100 % (n=1) and 86 % (n=7) in Botswana and South Africa, respectively (Penzhorn, Stylianides et al. 2002). Using a Modified Agglutination Test (MAT) on diverse carnivorous and herbivorous species, Hove and Mukaratirwa (2005) detected a seroprevalence of 92 % (n=26) in lions (*P. leo*), 10 % (n=10) in giraffe (*Giraffa camelopardalis*), 20 % (n=10) in Greater kudu (*Tragelaphus strepsiceros*), 90 % (n=10) in nyala (*Tragelaphus angasii*), 57 % (n=14) in bushbucks (*Tragelaphus criptus*), 27 % (n=11) in black rhinos (*Diceros bicornis*) and 10 % (n=20) in African elephants (*Loxodonta africana*) (Hove and Mukaratirwa 2005). Another study investigating the presence of *T. gondii* in a predominantly herbivorous species was conducted in Madagascar in black lemurs (*Eulemur macaco macaco*) (Junge and Louis 2007). By using a serum biological profile technique that detected both IgG and IgM antibodies against *T. gondii*, the researchers detected a prevalence of 10 % (n=10) (Junge and Louis 2007).

A seroprevalence of 43 % (n=7) was also detected in captive lions (*P. leo*) in a zoo (Hann Dakar-Senegal) in Senegal using an ELISA (Kamga-Waladjo, Gbati et al. 2009). Another study also focussing on captive lions, detected *Toxoplasma*-like oocysts using a modified McMaster technique in 17% (n=30) (Mukarati, Vassilev et al. 2013). The researchers did stress the uncertainty regarding their identification of the parasite and therefore only reported their findings as *Toxoplasma*-like (Mukarati, Vassilev et al. 2013).

One of the endangered wildlife species native to Madagascar, the fossa (*Cryptoprocta ferox*) had a high seroprevalence of *T. gondii* at 93 % (n=45). The researchers indicated that the extinction of most of their carnivorous native species is among other reasons due to the burden of diseases and this could possibly be due to the increased infiltration of cats and dogs in the area which also affect the wildlife habitats (Pomerantz, Rasambainarivo et al. 2016).

A study was done in South Africa on brain samples from 106 free-ranging birds and four chickens, mostly from Limpopo and a few from the Free State, KwaZulu-Natal, Mpumalanga, Northern Cape and North West provinces (Lukášová, Kobédová et al. 2018). The brains were collected opportunistically from birds found deceased due to roadkill, turbulent weather, treatment failure, infections and unknown circumstances. Using a polymerase chain reaction assay (PCR), *T. gondii* was detected in three bird species with a prevalence of 25 % (n=4) in southern yellow-billed hornbills (*Tockus leucomelas*), 25 % (n=4) in laughing doves (*Spilopelia senegalensis*) and 20 % (n=5) in red-eyed doves (*Streptopelia semitorquata*), which were all from the Limpopo province (Lukášová, Kobédová et al. 2018). The researchers further characterised the DNA extracted from positive birds using a single multiplex PCR with 15 genetic markers and only detected 8 markers from the red-eyed doves which were specific to a type II strain. To the authors' knowledge this is the only study that specifically focused on the detection of *T. gondii* in wild bird species in South Africa and on the genotyping in the whole of Africa in wildlife species (Lukášová, Kobédová et al. 2018).

Another study also done by Lukasova et al (2018) investigated the seroprevalence of *T. gondii* in a variety of herbivorous, omnivorous and carnivorous wildlife species in South Africa. An Enzyme Linked Immunosorbent Assay (ELISA) was used to determine the seroprevalence which was 1 % (n=122), 8 % (n=13), 25 % (n=4) and

14% (n=7) in gerbils (*Gerbilliscus* sp.), kudus (*Tragelaphus strepsiceros*), honey badgers (*Mellivora capensis*) and white-tailed mongooses (*Ichneumia albicauda*), respectively (Lukášová, Halajian et al. 2018). Antibodies to *T. gondii* were also detected in 60 of 80 (75 %) spotted hyenas (*Crocuta crocuta*) in Tanzania using an ELISA (Ferreira, Torelli et al. 2018). A prevalence study was done on captured wild rodents and shrew in Senegal using serology (MAT) and a molecular analysis (PCR). Seroprevalence results were 4.8% (n=671), 2.6% (n=78), 31.9% (n=47) and 37.5% (n=32) in *Mus musculus domesticus* (rodent species), *Rattus rattus*, *Cricetomys gambianus* (rodent species) and *Crocidura olivieri* (shrew), respectively (Galal, Schares et al. 2019). The PCR results of each of the rodent species are presented in Table 2.1 (Galal, Schares et al. 2019).

Another rare survey was done on yellow-legged gulls (*Larus michahellis*) in Tunisia. The researchers performed serology on sampled eggs to avoid the logistical challenges of capturing wild birds (Gamble, Ramos et al. 2019). The researchers further indicated that by sampling the eggs this could be a more efficient way to measure the exposure of the females breeding in the area (Gamble, Ramos et al. 2019). The eggs were collected from two locations in namely Sfax and Djerba with 3 % and 11 % of seroprevalence determined, respectively, using an ELISA (Gamble, Ramos et al. 2019).

A high overall seroprevalence of 83 % (n=29) was detected in caracals (*Caracal caracal*) in South Africa using an IFAT that serologically detected both IgM and IgG anti-*T. gondii* antibodies (Serieys, Hammond-Aryee et al. 2019). A seroprevalence of 79 % (n=29) and 38 % (n=29) was also determined using an IFAT that separately detected IgG and IgM, respectively (Serieys, Hammond-Aryee et al. 2019).

Lastly, free-ranging wildlife species were surveyed in Namibia using an ELISA and further confirmed using a immunoblot technique (IB). The carnivore species investigated were African lions (*P. leo*), brown hyenas (*Hyaena brunnea*), caracals (*Caracal caracal*), cheetahs (*A. jubatus*), leopards (*P. pardus*), spotted hyena (*Crocuta crocuta*), African wild dogs (*L. pictus*), bat eared foxes (*Otocyon megalotis*) and black-backed jackals (*Canis mesomelas*) with seroprevalence estimates ranging between 25 and 93 % (Seltmann, Schares et al. 2020). In blue wildebeests (*Connochaetes taurinus*), the seroprevalence of *T. gondii* was 10 % (Seltmann, Schares et al. 2020).

2.3 Discussion

The information obtained from this systematic review indicates that *T. gondii* is prevalent and widespread in wildlife in Africa. Overall, however, the number of studies published on the topic is quite limited, and the majority of records focused on samples from countries with well-developed wildlife reserves, with only few reported data from samples obtained in areas at the human-livestock-wildlife interface. Also, data were only available for twelve countries on the African continent, leaving many blind spots on the map for the distribution of *T. gondii* in wildlife in Africa.

The prevalence estimates, obtained by a wide range of techniques, were highly variable, ranging between 0 and 100%. Aside from the records with a positive detection of *T. gondii* identified in the current systematic review, few other studies have investigated the presence of *T. gondii* in wildlife in Africa but failed to detect the parasite or only indicated protozoan parasite detection but with no *T. gondii* confirmation in predominantly herbivorous and omnivorous wild species from Madagascar, Kenya, Tanzania and Uganda (Riemann, Burridge et al. 1975, Bakal, Karstad et al. 1980, Junge and Louis 2005, Miller, Sauther et al. 2007, Dutton, Junge et al. 2008, Alexander, Poirotte et al. 2016).

Overall, the prevalence estimates reported in the records identified in the current systematic review, suggest that *T. gondii* seems to be more prevalent in carnivores compared to herbivores (Cheadle, Spencer et al. 1999, Penzhorn, Stylianides et al. 2002, Seltmann, Schares et al. 2020), which is consistent with several studies in wildlife in USA and Europe. For instance, Marchiondo et al. (1976) in the USA found a higher seroprevalence in carnivores (45 %) compared to omnivores (28 %) and herbivores (21 %) (Marchiondo, Duszynski et al. 1976). Another study in the USA also found a higher prevalence in carnivores (66 %) when compared to omnivores (11 %) and herbivores (15 %)) (Smith and Frenkel 1995). Similarly, in Europe, researchers also found a higher seroprevalence in carnivores (20.21 %) and omnivores (16.91 %) when compared to herbivores (0 - 2.48 %) (Ferroglio, Bosio et al. 2014). From these data we can assume that the most common route of transmission in wild carnivores is through the ingestion of infected meat (Smith and Frenkel 1995). This could also be due to the fact that carnivores are higher on the food pyramid. Unfortunately, information on the prevalence of *T. gondii* in potential prey like herbivores and omnivores is even more limited in Africa, hampering more accurate deductions.

Further research targeted at wildlife with association to the different feeding types is required (Smith, Fisher et al. 1995, Cheadle, Spencer et al. 1999, Penzhorn, Stylianides et al. 2002, Hammond-Aryee 2014).

Severe toxoplasmosis has been described in a few studies, similar to the two cases mentioned in the two lions and the cape hunting dog (Hofmeyr 1956, Ocholi, Kalejaiye et al. 1989). In the USA, Dubey (1987) and Smith et al. (1995) described severe clinical toxoplasmosis in captive bobcats (*Lynx rufus*). Smith et al. (1995) found necrosis in the liver, renal pelvis, heart and skeletal muscle tissue of a sick bobcat *(L. rufus*) that was serologically positive for *T. gondii* (Smith, Fisher et al. 1995). The bobcat documented by Dubey (1987) had died only one week after birth. In another clinical case reported in the USA, a sick wild turkey (*Meleagris gallopavo*) died shortly after being captured. Necrosis was detected on the kidneys, liver, spleen and pulmonary interstitium and toxoplasmosis was confirmed using an avidin-biotin immunohistochemical technique in liver sections (Quist, Dubey et al. 1995). Data on the clinical impact of toxoplasmosis in wildlife species is limited in Africa and should further be investigated.

In the two cases of the severely infected lions, Ocholi et al. (1989) further stated that the possible reason why the remaining lions living in the same cage did not experience a clinical disease even though they were serologically positive for *T. gondii*, could have been due to a number of factors such as the type of strain of the parasite, the age of the animals, the organs specifically infected and the animals' immunological response (Ocholi, Kalejaiye et al. 1989). Due to the evidence of strain diversity of *T. gondii*, researchers have indicated concerns regarding the discovery of more diverse strains resulting in possible severe infections in hosts (Khan, Dubey et al. 2011, Su, Khan et al. 2012). A study done in the United Kingdom screened wildlife species including, ferrets (*Mustela putorius furo*), red foxes (*Vulpes vulpes*), polecats (*Mustela putorius*), minks (*Neovison vison*), badgers (*Meles meles*) and stoats (*Mustela erminea*) using PCR-RFLP (polymerase chain reaction - restriction fragment length polymorphism) with satellite markers. The researchers found all three clonal genotypes I, II and III with genotype II being dominant (Miller, Miller et al. 2008). In a study in the USA in otters (*Enhydra lutris*), mountain lions (*Puma concolor*) and foxes (*Vulpes vulpes*), the common three clonal lineages were also found by PCR and DNA sequencing, but

additionally a diverse strain referred to as Genotype X was identified (Miller, Miller et al. 2008). In Brazil, a high diversity of non-clonal strains was found by PCR-RFLP in wild Felidae, including, jaguarundi (*Puma yagouaroundi*), Geoffroy's cat (*Leopardus geoffroyi*), oncilla *(L. tigrinus*), margay (*L. wiedii*), ocelot (*L. pardalis*) and pampas cat (*L. colocolo*). Two new genotypes, Lw#31Tn and Py#21Sm and one previously described genotype Py#56Br were identified [52] Currently, there is very limited data on the *T. gondii* strains circulating in African wildlife (Shwab, Zhu et al. 2014, Galal, Schares et al. 2019).

The case documented by Dubey (1987) in bobcat kittens, of which two died within the first week after birth indicates the possibility of congenital infection [53]. Similar cases of congenital toxoplasmosis have been documented in white-tailed deers (*Odocoileus virginianus*) and pallas cats (*Otocolobus manul*) from USA and Austria, respectively (Basso, Edelhofer et al. 2005, Dubey, Velmurugan et al. 2008). These cases suggest the possible occurrence of congenital toxoplasmosis in wildlife species in Africa (Dubey, Quinn et al. 1987). Congenital toxoplasmosis is rarely documented in wildlife worldwide including the possibility of *T. gondii* related abortions in wildlife species.

A high prevalence of *T. gondii* in humans and livestock is assumed to be associated with the presence of cats. *Toxoplasma gondii* only occurs where felids are present (Spencer and Markel 1993, Cheadle, Spencer et al. 1999). Publications have mentioned that a high prevalence of this parasite in wild herbivores indicates the most common mode of transmission which is through contamination of the environment with sporulated oocysts, wild felids being the shedders (Dubey, Kramer et al. 1985, Smith, Fisher et al. 1995, Ferreira, Vidal et al. 2008).To the authors' knowledge there has only been one publication in Africa which documents the detection of oocysts in an African lion (*P. Leo*). However, in that case no techniques were used to differentiate *T. gondii* oocysts from those of *Hammondia hammondi*, a non-zoonotic coccidian protozoa of felids of which the oocysts bear a close morphological resemblance to those of *T. gondii* (Ocholi, Kalejaiye et al. 1989, Dubey and Sreekumar 2003). In contrast to Africa, there are numerous studies in countries such as the Czech Republic, Belgium and the USA showing oocyst production in wild felids. Oocyst identification has been done in a variety of wild felids found globally which are confirmed definitive hosts such as, bobcat (*L. rufus*), cheetah (*A. jubatus*), mountain

lion (*Puma concolor*), wild cat (*Felis silvestris*), Siberian tiger (*Panthera tigris altaica*), amur leopard cat (Panthera pardus orientalis), Geoffroy's cat (L geoffroyi), cougar (*Felis concolor*), pallas cat (*F. manul*), jaguarundi (*Puma yagouaroundi*) and ocelot (*Felis pardalis*) (Jewell, Frenkel et al. 1972, Miller, Frenkel et al. 1972, Marchiondo, Duszynski et al. 1976, Dorny and Fransen 1989, Lukešová and Literák 1998, Basso, Edelhofer et al. 2005). There are still a number of wild felid species in Africa that have not yet been surveyed for *T. gondii* prevalence or oocyst shedding such as, serval (*Leptailurus serval*), caracal (*Caracal caracal*), African wildcat (*Felis silvestris lybica*), African golden cat (*Caracal aurata*), jungle cat (*Felis chaus*) and the black-footed cat (*Felis nigripes*). Although this is the case, the confirmed reports of oocyst shedding from wild felids found worldwide can lead to speculation that the unconfirmed wild felids found in Africa play a similar role as definitive hosts and are possibly essential in the dissemination and preservation of the parasite in the different ecological niches forming a possible bridge where the human dwellings, livestock and wildlife interface.

Strong winds and rainfall can disperse oocysts produced by both domestic and wild felids and can cause contamination of the environment across habitats whether fenced or unfenced (Yan, Liang et al. 2016). Smaller animals like rodents can also act as reservoirs of the parasite, due to their size they can easily move through habitats possibly disseminating the parasite within the different communities. Lastly, in numerous countries humans are known to also hunt wild mammals and birds for consumption and this increases the probability of infecting humans (Ferroglio, Bosio et al. 2014, Jiang, Li et al. 2015, Almeria, Cabezon et al. 2018). This shows that the circulation of *T. gondii* in wildlife can possibly have an effect on human and livestock populations living in close proximity and vice versa with the transfer of pathogens between habits especially zoonotic pathogens possibly being a main concern (Schlüter, Däubener et al. 2014, Pomerantz, Rasambainarivo et al. 2016).

The reviewed publications included in the study used a variety of direct and indirect *T. gondii* detection methods. The direct methods included in the studies are microscopy and PCR techniques (Liyanage, Wiethoelter et al. 2021). Microscopic detection includes the examination of faeces, water and environmental samples for oocysts and tissue samples for tachyzoites and tissue cysts. Although microscopy is commonly used it lacks specificity, it is a subjective technique (misinterpretation of results) and

therefore be unreliable. It also lacks sensitivity especially when using light microscopy, but can be improved by staining (Giemsa, Haematoxylin and Eosin, and the Periodic acid Schiff) or immunofluorescent or immunohistological staining (Liu, Wang et al. 2015, Liyanage, Wiethoelter et al. 2021).

Molecular techniques (PCR) such nested-PCR (nPCR), multiplex PCR and quantitative-PCR (qPCR) can be highly sensitive and specific as they directly target the parasite DNA (Liu, Wang et al. 2015, Rostami, Karanis et al. 2018). The analytical sensitivity of nPCR techniques range from 96-99% and the specificity ranges between 95-100%, both in blood, urine and foetal tissues (Rostami, Karanis et al. 2018, Santoro, Viscardi et al. 2019). These techniques use DNA extracted from various samples and can be further utilised for DNA characterization and genotyping (excluding qPCR) (Liu, Wang et al. 2015, Liyanage, Wiethoelter et al. 2021).

Serological techniques are commonly used when determining the prevalence in hosts and this is because *T. gondii* evokes a very strong and long lasting immunological response in hosts (Rostami, Karanis et al. 2018). The assays use blood or constituents of blood (serum and plasma) for the detection of anti-*T. gondii* antibodies (IgG, IgM and IgA), which can also help in distinguishing between acute and chronic infections. The most frequently used serological tests in the reviewed articles are the Sabin Feldman Dye Test (SFDT), Enzyme-Linked Immunosorbent Assay (ELISA) and Modified Agglutination Test (MAT). The SFDT is considered the gold standard serological technique. It was reported to be both highly sensitive and specific. It however requires to be adapted to the animal species tested because of differences in the complement system and it also requires the culturing of live parasites in mice or tissue cultures making it very technical and hazardous (Reiter-Owona, Petersen et al. 1999, Liu, Wang et al. 2015). In sows, the sensitivity of ELISA, Indirect Haemagglutination Assay (IHA), MAT, Latex Agglutination Test (LAT) and SFDT was estimated at 72.9%, 29.4%, 82.9%, 45.9%, and 54.4%, respectively, while the specificities were estimated at 85.9%, 98.3%, 90.3%, 96.9% and 90.8%, respectively (Dubey, Thulliez et al. 1995). Another serological study done in sheep found high sensitivities of 96%, 90.1% and 80.4% using MAT, ELISA and Indirect Fluorescent Assay (IFAT), respectively. The detected specificities were 88.9% using MAT, 85.9% using ELISA and 91.4% using IFAT. Due to the possibility of false negative reactions

on serological assays, it is advisable to perform more than one detection assay, as is reported in some studies (Ocholi, Kalejaiye et al. 1989, Galal, Schares et al. 2019, Seltmann, Schares et al. 2020).

Serological prevalence studies are more common than molecular studies on *T. gondii* in wildlife species. Only few studies have investigated the specificity and sensitivity of the different detection techniques and validated them for use in wildlife species due to the difficulty in accessing wildlife samples (Bachand, Ravel et al. 2019). Thus, highlighting another gap in *Toxoplasma* research in wildlife.

The present study has some limitations. Most records retrieved in this systematic review reported the findings of studies applying convenience sampling, at small sizes. Therefore, the reported prevalence estimates might not be representative of the entire population of each of the investigated wildlife species due to selection bias. For instance, in domestic animals (and humans), it is known that the seroprevalence increases with age as a result of longer exposure (Boughattas, Behnke et al. 2016). Moreover, the small sample sizes will inherently lead to imprecise estimates. Finally, the identified records used a wide range of, mostly serological, techniques to detect the presence of *T. gondii* in wildlife, many of them which have not been validated for use in the investigated wildlife species. Despite the limitations the review's findings are important in providing the latest epidemiological data on *T. gondii* in South Africa, a country where the lack of research focused on this topic is evident.

2.4 Materials and Methods

2.4.1 Search strategy

The aim of the study was to summarize existing knowledge on the occurrence, prevalence, distribution and history of *T. gondii* in wildlife on the African continent. PRISMA guidelines were used for reporting the review process (Moher, Liberati et al. 2009) (PRISMA checklist: Appendix B). Relevant records, published between 1st January 1900 and 31st December 2020, were searched by means of three international bibliographic databases: PubMed (https://pubmed.ncbi.nlm.nih.gov/), Web of Science (https://webofknowledge.com) and CAB Direct (https://cabdirect.org) (Protocol: Appendix A). A search phrase was developed for use in the bibliographic

databases, based in part on the phrase developed by Pienaar et al. (Pienaar, Grobler et al. 2011): (*Toxoplasma gondii* OR Toxoplasmosis OR *T. gondii*) AND (zoo OR wildlife OR wild) AND ((Africa OR African continent OR Africa OR Algeria OR Angola OR Benin OR Botswana OR Burkina Faso OR Burundi OR Cameroon OR Canary Islands OR Cape Verde OR Central African Republic OR Chad OR Comoros OR Congo OR Democratic Republic of Congo OR Djibouti OR Egypt OR Equatorial Guinea OR Eritrea OR Ethiopia OR Gabon OR Gambia OR Ghana OR Guinea OR Guinea Bissau OR Ivory Coast OR Cote d'Ivoire OR Jamahiriya OR Jamahiryia OR Kenya OR Lesotho OR Liberia OR Libya OR Libia OR Madagascar OR Malawi OR Mali OR Mauritania OR Mauritius OR Mayote OR Morocco OR Mozambique OR Mocambique OR Namibia OR Niger OR Nigeria OR Principe OR Reunion OR Rwanda OR Sao Tome OR Senegal OR Seychelles OR Sierra Leone OR Somalia OR South Africa OR St Helena OR Sudan OR Swaziland OR Tanzania OR Togo OR Tunisia OR Uganda OR Western Sahara OR Zaire OR Zambia OR Zimbabwe OR Central Africa OR Central African OR West Africa OR West African OR Western Africa OR Western African OR East Africa OR East African OR Eastern Africa OR Eastern African OR North Africa OR North African OR Northern Africa OR Northern African OR South African OR Southern Africa OR Southern African OR sub Saharan Africa OR sub Saharan African OR subSaharan Africa OR subSaharan African) NOT (guinea pig OR guinea pigs OR aspergillus niger)). Furthermore, reference lists of retained records and/or review articles were snowballed for relevant sources.

2.4.2 Selection criteria

After extracting the records from the three databases, duplicate records were removed, and the titles and abstracts were screened for relevance. The inclusion criteria included studies reporting data on *T. gondii* from the African continent in both free ranging and captive wild species. The exclusion criteria were: i) publications on parasites other than *T. gondii*, ii) records documenting the detection of *T. gondii* in domestic species rather than wildlife species, iii) studies reporting/using data older than 1900 or published after December 31st 2020, v) studies that were conducted in countries outside the African continent, vi) publications with information not in line with the review question (prevalence, detection and history of *T. gondii* in African wildlife), and

lastly, vi) duplicate studies. Subsequent to the screening process, full texts were evaluated using the same criteria described above.

2.4.3 Data extraction and analysis

The following variables were extracted from the articles and entered into Microsoft Excel worksheets: author name and publication year, country, species name, reported prevalence, method of detection.

2.5 Conclusions

The current review highlights a substantial gap on the research done on *T. gondii* in wildlife in Africa. The lack of knowledge in Africa, particularly in areas where humans, livestock and wildlife overlap prevent the determination of its impact and distribution in the different habitats. The specific role played by the wild cycle and possibly the direct or indirect implications it might have on the public health of the surrounding habitats and the occupants affected for better disease control, diagnosis and treatment. It is also important to investigate the common circulating genotypes, whether there is evidence of genetic variation and the possibility of congenital toxoplasmosis to better understand the parasite and the severity of the clinical infection experienced by the hosts. Further research to fill the identified knowledge gaps is therefore recommended.

2.6 Supplementary Materials

Data is contained within the article, Table 2.1 and the supplementary material in appendices A and B.

2.7 Author Contributions

All the authors contributed to the conceptualization of the study. The methodology, formal analysis, investigation, resources, data curation was done by the first author, Refilwe Bokaba and the second author, Veronique Dermauw. The writing of the original draft preparation including the writing-review and editing was done by the first author, Refilwe Bokaba. All the authors commented and contributed to previous versions of the manuscript. All authors have read and agreed to the published version of the manuscript.

2.8 Funding

This research received no external funding.

2.9 Institutional Review Board Statement

Not applicable.

2.10 Data Availability Statement

The data generated and analysed during the current study are available in the manuscript as supplementary material in appendices A and B.

2.11 Conflicts of Interest

The authors declare no conflict of interest.

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Supplementary Files

Appendix A

Systematic Review Protocol

Aim: The aim was to summarize knowledge on the presence of *Toxoplasma gondii* in wildlife in Africa.

Research questions:

- Which African countries have reported the presence of *T. gondii* in wildlife?
- What is the reported prevalence range of *T. gondii* in wildlife in Africa?
- What is the history of *T. gondii* in relation to its presence in wildlife in Africa?

Methods: The systematic review followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines for reporting systematic reviews (Moher et al. 2009). Briefly, records were extracted from the different sources, duplicates were removed, and title/abstract was screened for fitting the topic of the review. Next, the full texts of the retained

articles were evaluated for eligibility and data were extracted for the final set of included records.

Sources:

- Bibliographic databases: PubMed (https://pubmed.ncbi.nlm.nih.gov/), Web of Science (https://webofknowledge.com) and CAB Direct (https://cabdirect.org).
- Additional sources: reference lists of retained records and/or review articles were snowballed for relevant sources.

Search phrase:

(*Toxoplasma gondii* OR Toxoplasmosis OR *T. gondii*) AND (zoo OR wildlife OR wild) AND ((Africa OR African continent OR Africa OR Algeria OR Angola OR Benin OR Botswana OR Burkina Faso OR Burundi OR Cameroon OR Canary Islands OR Cape Verde OR Central African Republic OR Chad OR Comoros OR Congo OR Democratic Republic of Congo OR Djibouti OR Egypt OR Equatorial Guinea OR Eritrea OR Ethiopia OR Gabon OR Gambia OR Ghana OR Guinea OR Guinea Bissau OR Ivory Coast OR Cote d'Ivoire OR Jamahiriya OR Jamahiryia OR Kenya OR Lesotho OR Liberia OR Libya OR Libia OR Madagascar OR Malawi OR Mali OR Mauritania OR Mauritius OR Mayote OR Morocco OR Mozambique OR Mocambique OR Namibia OR Niger OR Nigeria OR Principe OR Reunion OR Rwanda OR Sao Tome OR Senegal OR Seychelles OR Sierra Leone OR Somalia OR South Africa OR St Helena OR Sudan OR Swaziland OR Tanzania OR Togo OR Tunisia OR Uganda OR Western Sahara OR Zaire OR Zambia OR Zimbabwe OR Central Africa OR Central African OR West Africa OR West African OR Western Africa OR Western African OR East Africa OR East African OR Eastern Africa OR Eastern African OR North Africa OR North African OR Northern Africa OR Northern African OR South African OR Southern Africa OR Southern African OR sub Saharan Africa OR sub Saharan African OR subSaharan Africa OR subSaharan African) NOT (guinea pig OR guinea pigs OR aspergillus niger))

Note: for the African countries, we used the search phrase developed by Pienaar et al. (2011)

Search phrase translated for use in PubMed: ("*Toxoplasma gondii*" OR Toxoplasmosis OR "*T. gondii*") AND ("animals, zoo"[MeSH] OR "animals, wild"[MeSH] OR Wildlife OR wild) AND ((Africa OR "African continent" OR "Africa"[MeSH] OR Africa*[tw] OR Algeria[tw] OR Angola[tw] OR Benin[tw] OR Botswana[tw] OR "Burkina Faso"[tw] OR Burundi[tw] OR Cameroon[tw] OR "Canary Islands"[tw] OR "Cape Verde"[tw] OR "Central African Republic"[tw] OR Chad[tw] OR Comoros[tw] OR Congo[tw] OR "Democratic Republic of Congo"[tw] OR Djibouti[tw] OR Egypt[tw] OR "Equatorial Guinea"[tw] OR Eritrea[tw] OR Ethiopia[tw] OR Gabon[tw] OR Gambia[tw] OR Ghana[tw] OR Guinea[tw] OR "Guinea Bissau"[tw] OR "Ivory Coast"[tw] OR "Cote d'Ivoire"[tw] OR Jamahiriya[tw] OR Jamahiryia[tw] OR Kenya[tw] OR Lesotho[tw] OR Liberia[tw] OR Libya[tw] OR Libia[tw] OR Madagascar[tw] OR Malawi[tw] OR Mali[tw] OR Mauritania[tw] OR Mauritius[tw] OR Mayote[tw] OR Morocco[tw] OR Mozambique[tw] OR Mocambique[tw] OR Namibia[tw] OR Niger[tw] OR Nigeria[tw] OR Principe[tw] OR Reunion[tw] OR Rwanda[tw] OR "Sao Tome"[tw] OR Senegal[tw] OR Seychelles[tw] OR "Sierra Leone"[tw] OR Somalia[tw] OR "South Africa"[tw] OR "St Helena"[tw] OR Sudan[tw] OR Swaziland[tw] OR Tanzania[tw] OR Togo[tw] OR Tunisia[tw] OR Uganda[tw] OR "Western Sahara"[tw] OR Zaire[tw] OR Zambia[tw] OR Zimbabwe[tw] OR "Central Africa"[tw] OR "Central African"[tw] OR "West Africa"[tw] OR "West African"[tw] OR "Western Africa"[tw] OR "Western African"[tw] OR "East Africa"[tw] OR "East African"[tw] OR "Eastern Africa"[tw] OR "Eastern African"[tw] OR "North Africa"[tw] OR "North African"[tw] OR "Northern Africa"[tw] OR "Northern African"[tw] OR "South African"[tw] OR "Southern Africa"[tw] OR "Southern African"[tw] OR "sub Saharan Africa"[tw] OR "sub Saharan African"[tw] OR "subSaharan Africa"[tw] OR "subSaharan African"[tw]) NOT ("guinea pig"[tw] OR "guinea pigs"[tw] OR "aspergillus niger"[tw]))

Search phrase translated for use in Web of Science and CAB Direct: ("*Toxoplasma gondii*" OR Toxoplasmosis OR "*T. gondii*") AND (zoo OR wildlife OR wild) AND ((Africa OR "African continent" OR Africa* OR Algeria OR Angola OR Benin OR Botswana OR "Burkina Faso" OR Burundi OR Cameroon OR "Canary Islands" OR "Cape Verde" OR "Central African Republic" OR Chad OR Comoros OR Congo OR "Democratic Republic of Congo" OR Djibouti OR Egypt

OR “Equatorial Guinea” OR Eritrea OR Ethiopia OR Gabon OR Gambia OR Ghana OR Guinea OR “Guinea Bissau” OR “Ivory Coast” OR “Cote d’Ivoire” OR Jamahiriya OR Jamahiryia OR Kenya OR Lesotho OR Liberia OR Libya OR Libia OR Madagascar OR Malawi OR Mali OR Mauritania OR Mauritius OR Mayote OR Morocco OR Mozambique OR Mocambique OR Namibia OR Niger OR Nigeria OR Principe OR Reunion OR Rwanda OR Sao Tome OR Senegal OR Seychelles OR “Sierra Leone” OR Somalia OR South Africa OR St Helena OR Sudan OR Swaziland OR Tanzania OR Togo OR Tunisia OR Uganda OR “Western Sahara” OR Zaire OR Zambia OR Zimbabwe OR “Central Africa” OR “Central African” OR “West Africa” OR “West African” OR “Western Africa” OR “Western African” OR “East Africa” OR “East African” OR “Eastern Africa” OR “Eastern African” OR “North Africa” OR “North African” OR “Northern Africa” OR “Northern African” OR “South African” OR “Southern Africa” OR “Southern African” OR “sub Saharan Africa” OR “sub Saharan African” OR “subSaharan Africa” OR “subSaharan African”) NOT (“guinea pig” OR “guinea pigs” OR “aspergillus niger”))

Inclusion/exclusion criteria:

- Exclusion criteria
- Studies concerning a different parasite than *T. gondii*.
- Studies on *T. gondii* in domestic animal species.
- Studies reporting/using data older than 1900 or published after December 31st 2020
- Studies reporting results from outside the study area
- Studies reporting results out of the scope of the review question.
- Duplicate records.

- Inclusion criteria
- Studies reporting data on *T. gondii* from the African continent in both free ranging or captive wild species.

Variables extracted: Authors, year of publication, country, animal species, number of animals sampled, number of animals positive and detection method. Data were entered in Excel sheets.

Languages: English articles.

Study period: 1st January 1990 – 31st December 2020

Geographical range: All countries within the African continent.

References

Moher, D., A. Liberati, J. Tetzlaff, D. G. Altman and P. G. The (2009). "Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement (Reprinted from Annals of Internal Medicine)." PLoS Med 89(7): 873-880.

Pienaar, E., L. Grobler, K. Busgeeth, A. Eisinga and N. Siegfried (2011). "Developing a geographic search filter to identify randomised controlled trials in Africa: finding the optimal balance between sensitivity and precision." Health Info Libr J 28(3): 210-215.

Appendix B

PRISMA checklist

Table 2a Prisma checklist.

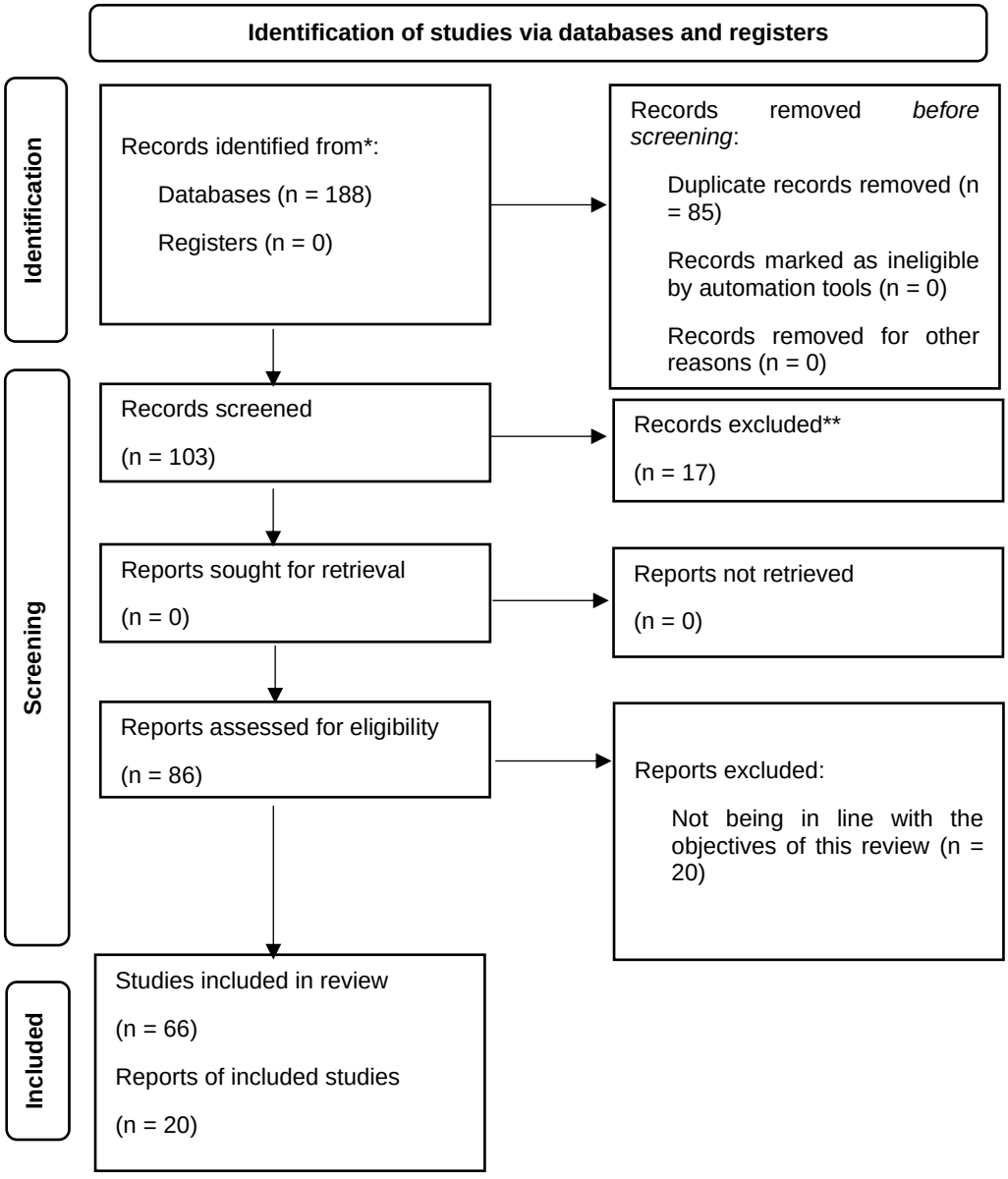
Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Page 23 and 24
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	Page 24
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Pages 25-16
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	Pages 40-41
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Pages 41
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Pages 40-42
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Pages 40-42
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Pages 40-42
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Pages 40-42
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Pages 40-42
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Pages 40-42
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	Pages 26-27

Section and Topic	Item #	Checklist item	Location where item is reported
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	Pages 26-27
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	Pages 40-42
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	Pages 40-42
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Pages 40-42
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	Not applicable
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	Not applicable
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	Not applicable
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	Pages 26-27
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	Pages 26-27
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Pages 26-34
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Pages 40-42
Study characteristics	17	Cite each included study and present its characteristics.	Pages 26-34
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Pages 26-34
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Page 27-30
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	Pages 35-40
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	Pages 26-34

Section and Topic	Item #	Checklist item	Location where item is reported
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	Pages 26-34
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	Pages 26-34
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	Page 34
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	Pages 26-34
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	Pages 35-42
	23b	Discuss any limitations of the evidence included in the review.	Page 34
	23c	Discuss any limitations of the review processes used.	Page 34
	23d	Discuss implications of the results for practice, policy, and future research.	Pages 35-42
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	Not applicable
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	Page 51-55
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	Pages 51-55
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	Page 43
Competing interests	26	Declare any competing interests of review authors.	Page 43
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Page 24

PRISMA 2020 Flow diagram

Figure 2a PRISMA 2020 Flow diagram



From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

CHAPTER 3

Toxoplasma gondii at the human-livestock-wildlife interface in the Bushbuckridge Municipality, South Africa: a serosurvey and investigation of knowledge and practices

Toxoplasma gondii at the human-livestock-wildlife interface in the Bushbuckridge Municipality, South Africa: a serosurvey and investigation of knowledge and practices

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Abstract

Interface areas, areas shared by humans, domestic and wild animals, may serve as high transmission contexts for *Toxoplasma gondii*. Up to now, however, not much is known about the epidemiology of *T. gondii* in such areas. A cross-sectional study was therefore conducted to determine the seroprevalence of *T. gondii* in different hosts residing in an interface area, and to investigate the local knowledge about this parasite and related practices by administering a questionnaire survey in the community. In the Bushbuckridge Municipality, Mpumalanga, South Africa, blood samples were obtained from humans (n=160), cats (n=9), chickens (n=336) and goats (n=358), while serum samples from wildlife such as, impalas (n=97), kudus (n=55), warthogs (n=97), wild dogs (n=54), wildebeests (n=43) and zebras (n=68) were obtained from private nature reserves bordering the community. The samples were analysed using a commercial latex agglutination kit. The seroprevalence detected in humans, cats, chickens and goats was 8.8%, 0.0%, 4.2% and 11.2%, respectively. Seroprevalence in impalas, kudus, wild dogs, wildebeests, warthogs and zebras was estimated at 5.2%, 7.3%, 100.0%, 20.9%, 13.4% and 9.1%, respectively. The questionnaire revealed that 63.0% of the household owners were informal farmers and 35.9% were pet owners. A high female participation was found at 75.3% when compared to males at 24.7% which also indicated that the women were possibly the ones that care and manage the livestock and pets in the households. The results show a low circulation of *T. gondii* in the human and domestic cycle. The study suggests the presence of possible bridges between the wildlife cycle and the surrounding human and domestic cycles in this interface area which requires further investigation.

Keywords: *Toxoplasma gondii*, seroprevalence, humans, domestic animals, wildlife, interface area, South Africa

3.1 Introduction

Toxoplasma gondii is a zoonotic protozoan parasite with a global distribution infecting almost all mammals and birds (Halonen and Weiss 2013). The consumption of infected meat and the ingestion or inhalation of infective oocysts from the environment are the most frequent and most documented sources of infection for intermediate hosts, including man (Brouat, Diagne et al. 2018). While most infections in humans are asymptomatic or cause mild clinical signs, toxoplasmosis can result in a severe and even fatal disease in congenitally infected foetuses and in immunocompromised hosts (Hill and Dubey 2002). The transmission of *T. gondii* between species has been studied mainly in the domestic life cycle, although circulation of the parasite in wildlife

has also been documented (Pomerantz, Rasambainarivo et al. 2016, Serieys, Hammond-Aryee et al. 2019, Seltmann, Schares et al. 2020). In the sylvatic cycle of the parasite, herbivores and omnivores most commonly acquire the infection through the ingestion of oocysts from the environment while carnivores get infected via the consumption of infected prey.

Interface areas are spaces which are shared by humans, wildlife and domestic animals, thereby allowing interactions between the different species and their pathogens, including *T. gondii*. In such areas, both domestic and wildlife feline populations (definitive hosts) have the capability of disseminating oocysts into their respective environments and can thus contribute to the dissemination of the parasite in areas shared by livestock and herbivorous or omnivorous wild species (Halonen and Weiss 2013, Boughattas, Behnke et al. 2016). Furthermore, human-livestock-wildlife interactions can occur, for instance, rodent populations can act as bridges between the different ecological systems in the interface area. Lastly, the consumption of bushmeat is a possible pathway through which humans can become infected with *T. gondii* (Ferroglio, Bosio et al. 2014, Jiang, Li et al. 2015, Almeria, Cabezon et al. 2018).

In South Africa, there are many areas where wildlife, domestic animals and humans converge and share resources. In previous studies, the *T. gondii* seroprevalence has been determined in different hosts in the country (Penzhorn, Stylianides et al. 2002, Hammond-Aryee 2014, Hammond-Aryee, Esser et al. 2015, Hammond-Aryee, van Helden et al. 2015, Lukášová, Halajian et al. 2018, Lukášová, Kobédová et al. 2018). However, there are no studies reporting on the *T. gondii* seroprevalence in hosts residing in areas where resources are shared. Since overall information about *T. gondii* transmission dynamics at the interface is insufficient, data from such studies would contribute to the understanding of the epidemiology of *T. gondii* in these wildlife-human-domestic interface areas. Thus, the current study determines the seroprevalence of *T. gondii* in humans, livestock, domestic animals, wild animals and furthermore assesses the knowledge and associated practices of the human population in an interface area in South Africa.

3.2 Materials & methods

3.2.1 Study area & study population

The study area consists of the Bushbuckridge Municipality, Mpumalanga, South Africa (Figure 3.1). The Bushbuckridge Municipality is an interface area inhabited by humans, livestock and wildlife (Berrian, van Rooyen et al. 2016). The area has a human population of approximately 500,000 people surrounded by five national parks. The majority of domestic animals are chickens, goats, cattle and dogs, while there is also a small cat population (Berrian, van Rooyen et al. 2016). Human and domestic animal habitats co-exist and are separated from the wildlife habitats by a fence. The Mnisi community is situated in the north-eastern corner of the Bushbuckridge Municipality. The study population consisted of humans, cats, chickens, goats, and wildlife species present in the Bushbuckridge Municipality interface area.

3.2.2 Study design

This was a cross-sectional study consisting of three parts. Part I was the testing of human serum samples obtained during routine surveillance activities at the Hluvukani Community Health Centre. Part II was a community study performed in four villages (Athol, Gottenburg, Thlavekisa and Utah) in the Mnisi Community, collecting household data via questionnaire and aiming to prospectively collect cat, chicken and goat blood samples. Part III consisted of the use of banked wildlife serum samples collected in conservation areas associated with the study area.

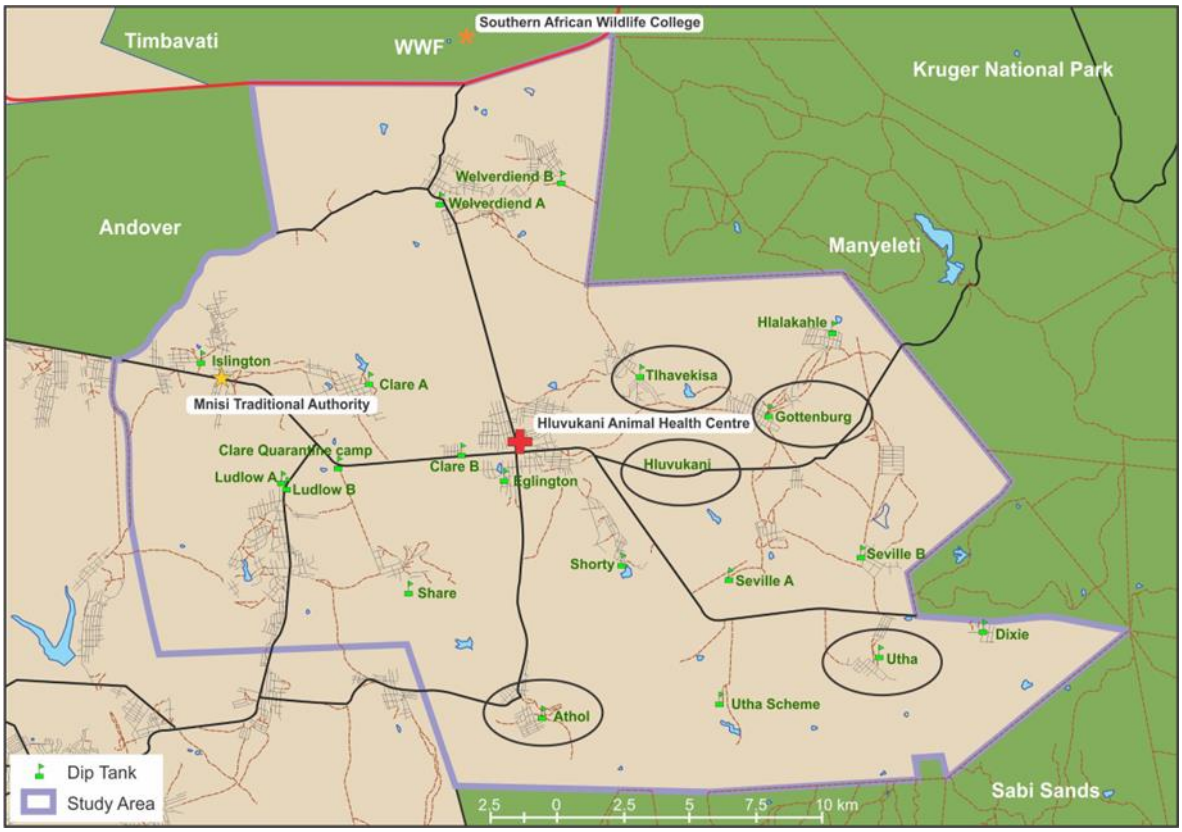

Figure 3.1 Map of the Mnisi study area (outlined in purple), Mpumalanga Province, Republic of South Africa, adapted from Berrian et al, 2016. The circled locations highlight the location of the four selected villages and the village were the Hluvukani Community Health Centre is situated (circles). Image courtesy of Mnisi Community Programme, University of Pretoria (Berrian, van Rooyen et al. 2016).

3.2.3 Study population & sampling procedure

Part I: Human surveillance sampling

Serum samples were collected by the National Institute for Communicable Diseases (NICD). The NICD has a clinical-based surveillance team stationed at the Hluvukani Community Health Centre in Hluvukani. The samples were collected in the context of on-going investigations for emerging human pathogens, causing acute febrile illness (e.g., *Brucella*, *Anaplasma phagocytophilum, Leptospira, Toxoplasma*). Blood samples were collected from patients meeting the following inclusion criteria: having a body temperature equal or above 37^{o}C or a history of fever in the last 48 hours prior to visiting the clinic, being 18 years old and above. Patients with proven malaria were excluded from the study.

Part II: Community survey: chickens, goats and cats sampling & questionnaire

Blood samples from chickens, goats and cats were collected from four villages in the area, namely, Athol, Gottenburg, Tlhavekisa and Utah. Initially, systematic sampling was envisaged by sampling households in every second street of these villages. However, due to the lack of availability of informal farmers (household owners), pet owners (household owners) and the targeted livestock and pets in the villages, opportunistic sampling was performed from eligible households per street. All household owners who had informal farms or were pet owners with the targeted animal species were invited to participate in the study. In participating households, a blood sample was collected from targeted animal species. Blood from goats was drawn from the jugular vein in 5 ml red top vacutainer tubes. In chickens, blood was collected from the brachial vein from the underside of a stretched wing. The cephalic vein was used to draw blood in cats. Blood samples from cat and chickens were collected in 2 ml red top vacutainer tubes.

Furthermore, questionnaires (n =384) were conducted in households in the four villages. The questionnaire focused on obtaining information regarding the socio-environmental and husbandry conditions per household as well as knowledge about *Toxoplasma.* An opportunistic sampling method was used as the surveys were highly dependent on the availability of community members. The inclusion criteria were as follows: i) members surveyed should be residents in the villages of interest, ii) members should give formal written consent agreeing to participate in the questionnaire and acknowledge that the information attained will be used in a research study, iii) members participating should be 18 years and older and iv) with no gender preferences.

Part III: Wildlife biobank samples: impalas, kudus, wild dogs, wildebeests, warthogs and zebras

Serum samples of selected wild species, i.e. impalas (*Aepyceros melampus*), greater kudus (*Tragelaphus strepsiceros*), warthogs (*Phacochoerus africanus*), blue wildebeest (*Connochaetes taurinus*), African wild dogs (*Lycaon pictus)* and Burchell's zebras (*Equus quagga*) were obtained from the South African National Parks (SANParks) biobank from samples collected in areas in and bordering the Kruger National Park: Afsaal, Barnard Grave, Biyamiti weir, Crocodile Bridge, De Cuiper, Doispane firebreak, Eco Training Camp, Greater Kruger National Park, Mashisiti, Hapi

pan, Ingala, Kingfisherspruit,, Klopperfontein, Levuvu Highwater Bridge South, Lindondard Metsi Metsi, Lindondard, Nwamariwa, Lower Sabie, Malelane, Maleteni, Mangala, Manyeleti, Mapungubwe, Marloth, Mashikiri, Matsisisi, Mayingani pan, Metsi Metsi, Mphongolo, Nhlangaluwe pan, Nwapi pan, Nyala pan, Orpen gate, Pafuri, Paradys Windmill, Phabeni gate, Punda, Return Africa Camp, S1/S4, Sabi Sands, Sabie Park, Satara, Shireni, Skukuza, South of Hapi Pan, Tamboti, Tshokwane, Tulamela and Xiphampana. The focus was to investigate the most common species found in the area that included all the main feeding types (herbivores, omnivores and carnivores) and these samples were also subject to the availability of serum samples in the biobank.

Sample sizes

The required sample sizes for the different groups were calculated using the following equation: $n_0 = \frac{z^2 P_{exp}(1-P_{exp})}{d^2}$, with n_0 the sample size of each species of interest, z, the z-score for the desired confidence interval (CI), d, the desired absolute precision and P_{exp} , the expected prevalence (Cochran 1977). For non-wildlife samples, the desired CI was set at 95%, d at 5%, while for wildlife samples, CI was set at 90%, and d at 10%, with for both P_{exp} set at 50 % (as the expected prevalence is unknown in South Africa). Afterwards, a correction was made for the finite population size: $n = \frac{n_0 N}{n_0+(N-1)}$, with n = the finite-population corrected sample size, n_0 , the sample size calculated using the base formula, and N, the estimated population size in the area (from census data).

Eventually, serum samples (n=1275) from humans (n=160), chickens (n=336), goats (n=358), cats (n=9), impalas (n=97), greater kudus (n=55), warthogs (n=97), African wild dogs (n=54), blue wildebeest (n=43) and Burchell's zebras (n=66) were serologically tested. Also, 384 questionnaires were conducted in the households.

3.3.4 Laboratory analysis

Subsequent to collection, all blood samples were stored overnight in the fridge. The next day, samples were centrifuged at 3500 g for 10 minutes, the sera collected and stored at −20°C until used in the serological assay. To detect anti-*T. gondii* antibodies, the MAST® Toxoreagent™ ID rapid latex agglutination commercial kit (Mast Group

Ltd., United Kingdom) was used following the manufacturer's instructions. The latex agglutination test (LAT) detects both IgM and IgG and it therefore cannot distinguish between chronic and acute *T. gondii* infections, but it is ideal for routine screening or seroprevalence studies (especially in epidemiological studies). The LAT is non-species specific and can therefore be used for screening in a variety of species (Lappin and Powell 1991). The LAT has a reported sensitivity and specificity of 94% and 84% when compared to IFA (immunofluorescence assay) and enzyme linked immunosorbent assay (ELISA), respectively, in humans (Mazumder, Chuang et al. 1988). In pigs, a lower sensitivity range of 45.9 - 47.2% and a high specificity range of 91.4 – 96.9% was detected (Dubey et al. 1995, Sroka et al. 2008). A titer of 1/32 was used as the cut-off for human samples, while this was 1/64 in animal samples.

3.3.5 Statistical analysis

A descriptive statistical analysis was conducted. Seroprevalence and questionnaire data are presented in percentages with 95% confidence intervals (95%CI). In case of low cell counts, exact confidence intervals were calculated. The χ^2-test of independence was used to investigate the association between presence of infection and other variables (e.g. age group, village). The significance level was set at 0.05. All statistical procedures were run using SPSS Statistics Version 21 (IBM Corporation).

3.4 Results

3.4.1 Seroprevalence

Part I: Human samples

The seroprevalence in the human samples collected from the Hluvukani Community Health Clinic was estimated at 8.8 % (n=160 95% CI: 4.9 - 14.2%) (Table 3.1). The highest positive titre detected was 1/32. Most samples originated from subjects between the ages of 18 - 35. No significant association was detected between the presence of infection and the categorized age groups (Table 3a, Supplementary files, page 96).

Table 3.1 Seroprevalence of T. gondii in humans, domestic and wildlife species.

Study	Species	n+/n (%)	95%CI
Part I: Clinic	Human	14/160 (8.8%)	4.9 – 14.2%
Part II: Community	Cat	0/9 (0.0%)	0.0 - 33.6 %
	Chicken	14/336 (4.2%)	2.3 - 6.9%
	Goat	40/358 (11.2%)	8.1 - 14.9%
Part III: Conservation area	Impala	5/97 (5.2%)	1.7 - 11.6%
	Kudu	4/55 (7.3%)	2 - 17.6%
	Warthog	13/97 (13.4%)	7.3 - 21.8%
	Wildebeest	9/43 (20.9%)	10 - 36%
	Wild dog	54/54 (100%)	93.4 - 100%
	Zebra	6/66 (9.1%)	3.4 - 18.7%

95%CI: 95% confidence interval

Part II: Community sampling

Cats, goats and chicken were sampled within the community study. A seroprevalence of 11.2% (n=358, 95% CI: 8.1 - 14.9%) was detected in goats with the highest titre of 1/128 observed. No significant association could be detected between the presence of infection, and village, age groups and sex of the animals (Table 3a, Supplementary files, page 96).

A seroprevalence of 4.2% (n=336, 95% CI: 2.3 - 6.9%) was detected in chickens, with the highest positive titre of 1/64. The highest seroprevalence was observed in the village, Athol, which was significant (p<0.01) when compared to the other villages. No statistical association was found for presence of infection and age group or gender (Table 3a, Supplementary files, page 96).

None of the sampled cats were seropositive (CI: 0.0 - 33.6 %) for *T. gondii*. All nine cats sampled were female, with eight of them from Thlavekisa and only one cat from Athol. Two of the cats were in the age range of 6 -11 months and the remaining seven cats were between 1-2 years old (Table 3a, Supplementary files, page 96).

Part II: Wildlife sampling

The seroprevalence detected in the selected wildlife species was 5.2% (95%CI 1.7 – 11.6%) in impalas (*Aepyceros melampus*, n=97), 7.3% (95%CI: 2 – 17.6%) in kudus (*Tragelaphus strepsiceros*, n=55), 13.4% (95%CI: 7.3 – 21.8%) in warthogs (*Phacochoerus africanus*, n=97), 100.0% (95%CI: 93.4 - 100%) in wild dogs (Lycaon pictus, n=54) , 20.9% (95%CI: 10 - 36%) in wildebeests (Connochaetes taurinus, n=43) and 9.1% (95%CI: 3.4-18.7%) in zebras (Equus quagga, n=66) (Table 3.1).

3.4.2 Questionnaire results

Most participants of the questionnaire were female (289/384, 75.3%), between the ages of 18-40 years (199/384, 51.8%). One hundred and thirty-nine of the participants completed secondary school education (36.2%) (Table 3.2). Only 2.1% (8/384) of the household owners knew or had heard about toxoplasmosis. Among the household owners 63.0% (242/384) were informal farmers and 35.9% (138/384) owned pets. Both pets and livestock resided indoors and outdoors with percentages of 79.7% (110/384) and 84.7% (205/242), respectively (Table 3b, Supplementary files, page 99). Around one third (48/138, 34.8%) of the pet owners remove their pet's faeces by cleaning the yard and enclosures three times a week. The majority of the pet owners and informal farmers dispose of their deceased companion animal (67/138, 48.6%) or livestock carcasses (141/242, 58.3%) by throwing them away in the bush/river. Nearly all informal farmers interviewed, practiced slaughtering (233/242, 96.3%), with 81.4% (196/242) of these cases slaughtering inside their yards. All the community members surveyed have access to water, but 67.7% (260/384) of them have to fetch water from boreholes in the villages and only 33.1% (127/384) of them are able to grow fruits and vegetables (Table 3b, Supplementary files, page 99). When it comes to the consumption of meat, most household owners prefer their meat well cooked (340/384, 81.8%).

Table 3.2 Socio-demographic variables to questionnaire from participants (n=384).

Socio-demographics	
Variables	Frequency, x/n(%)
Village	

Athol	80/384 (20.8%)
Gottenburg	121/384 (31.5%)
Thlavekisa	98/384 (25.5%)
Utah	85/384 (22.1%)
Gender	
Female	289/384 (75.3%)
Male	95/384 (24.7%)
Age	
Age range 18 - 30	110/384 (28.6%)
Age range 31 - 40	89/384 (23.2%)
Age range 41 - 50	73/384 (19.0%)
Age range 51 - 60	51/384 (13.3%)
Age range 61 and older	61/384 (15.9%)
Education	
No education	99/384 (25.8%)
Grade 4 - 7	44/384 (11.5%)
Grade 8 - 11	80/384 (20.8%)
Matric	139/384 (36.2%)
Higher certificate	7/384 (1.8%)
Diploma	12/384 (3.1%)
Degree	3/384 (0.8%)
Postgraduate	0/384 (0.0%)

3.5 Discussion

The data obtained in this study reveals a prevalence range of 0 - 100% *T. gondii* antibodies in humans, domestic animals and wildlife in the interface area of the

Bushbuckridge Municipality in Mpumalanga. Knowledge about this parasite within the community is very low, since only 2.1% of the members had heard or had some knowledge about toxoplasmosis.

In humans, a seroprevalence of 8.8% (95% CI: 4.9% - 40.2%) was detected. A similar low prevalence was found in Gauteng province in 2011 (Kistiah, Winiecka-Krusnell et al. 2011). In that study the seroprevalence was determined using the LAT in three groups namely, 9.8% (95% CI: 7.1% - 13.4%) in HIV-positive patients (n=376), 12.8% (95 CI: 8.9% - 15.8%) in HIV-negative patients (n=376) and 6.4% (95% CI: 4.5% - 9%) in serum samples from a residual serum bank (n=497) from a previous rubella prevalence study (Kistiah, Winiecka-Krusnell et al. 2011). Earlier studies in South Africa detected higher seroprevalence rates in humans, suggesting a possible decrease in prevalence as time progressed. For instance, a study done in 1974 in the Transvaal area in South Africa detected a seroprevalence of *T. gondii* of 37% (n=806) using IFA (Mason, Jacobs et al. 1974). Another study done in 1978 in humans also detected a higher prevalence of 18% (n=698), 24% (n=973) and 30% (n=645) in Cape Town, Port Elizabeth and Durban, respectively (Jacobs and Mason 1978).

Similar to humans, the seroprevalence obtained in chickens and goats was relatively low at 4.2% (95% CI: 2.3% - 6.9%) and 11.2% (95% CI: 8.1% - 40.9%), respectively. Antibodies against *T. gondii* were not detected in the nine cats sampled. Previous studies in domestic animals in South Africa detected a higher seroprevalence when compared to the current study. A study in Eastern Cape, also using a LAT, obtained a seroprevalence of 33.2% (95% CI: 25.3 - 41.1%) in chickens (n=137), 55.6% (95% CI: 47.0 - 64.2%) in goats (n=128) and 31.62% (95% CI: 22.9 - 40.4%) in cats (n=109) (Tagwireyi, Etter et al. 2019). Hammond-Aryee et al. (2015) found a seroprevalence of *T. gondii* in feral cats in the Western Cape province of South Africa of 37.1%, (95% Ci: 29.6 – 44.6%) and 8.8% (95% CI: 4.4 - 13.2%) of IgG and IgM, respectively, and of 6.3% for both IgG and IgM antibodies (Hammond-Aryee, Esser et al. 2015).

Similar to the prevalence obtained in humans, and domestic animals, the seroprevalence in herbivorous and omnivorous wildlife species was also relatively low, from 5.2% in impalas to 20.9% in blue wildebeests. Grazing species such as blue wildebeests and zebras had a higher seroprevalence than browsing species including impalas and kudus, which is likely due to the higher exposure of grazers to oocysts on

the soil. Warthogs also had a higher prevalence as they are omnivores that can become infected both through grazing and by consumption of infected meat. A low seroprevalence was detected by ELISA in blue wildebeests in Namibia at 10% (n=20) and in kudus from South Africa at 8% (n=13) (Lukášová, Halajian et al. 2018, Seltmann, Schares et al. 2020). In earlier studies a higher seroprevalence was found in wildebeests at 90% (n=10), 90% in zebras (n=10) and 100% in warthogs (n=2) from Kenya using the Sabin Feldman dye test (Bakal, Karstad et al. 1980). Zebras (n=29) from Tanzania were shown to have a seroprevalence of 28% using an IHA (Riemann, Burridge et al. 1975). Although a higher prevalence of *T. gondii* antibodies was detected in those studies, they are dated, and the sample sizes were smaller.

A 100% seroprevalence was found in African wild dogs (carnivorous), which is in agreement with two other studies that also surveyed wild dogs in South Africa and found a prevalence of 50% and 100% using microscopy and IFAT, respectively (Hofmeyr 1956, Van Heerden, Mills et al. 1995). Unfortunately, those studies are also dated and consisted of small sample sizes. A more recent study done on African wild dogs in Namibia also found a high seroprevalence of 71% using a commercial ELISA (Seltmann, Schares et al. 2020). The African wild dog mainly feeds on antelope species which were found to be infected with *T.* gondii in this study. Studies done on a variety of wild felid species such as, lions, cheetahs, leopards and caracals done in South Africa found seroprevalences that ranged from 50-100% using IFAT. From those studies it appears that *T. gondii* infection is more prevalent in carnivores than in herbivores and omnivores. However, results have to be interpreted with caution as only few studies were done on wildlife and the sample sizes were often small and not always representative for the populations (Cheadle, Spencer et al. 1999, Penzhorn, Stylianides et al. 2002, Serieys, Hammond-Aryee et al. 2019). A study on honey badgers and white-tailed mongooses found a lower seroprevalence of 25% and 14%, respectively, but the results could be affected by the predator-prey relationship and the limited sample sizes (Lukášová, Halajian et al. 2018).

The low seroprevalence of *T. gondii* in humans and domestic animals detected in this study could be due the low population of domestic cats in the sampled villages. Indeed it is known that the presence of cats are indicators of the presence of *T. gondii* in surrounding communities (Spencer and Markel 1993, Cheadle, Spencer et al. 1999). Munday (1972) found a significantly higher seroprevalence of toxoplasmosis in sheep

on islands with a cat population compared to islands with no cats. In the current study, observations during the period of sampling and the information provided by environmental monitors, veterinarians, and animal technicians in the villages indicated the existence of a small cat population in the area. This also explains the small cat sample size obtained in the study. Furthermore, observations by the researcher during sampling and when the questionnaire study was conducted indicated that the cats in the area were feral. These cats did not seem to have close interactions with their owners and therefore would be easily frightened and run when approached, which could have also contributed to the limited number of samples.

Besides the presence of cats, differences in prevalence between studies can have several other causes, such as climate and humidity in the area, the type of farm and husbandry practices, the feeding habits of the animals, water sources, and the presence of rodent populations in the area (Tonouhewa, Akpo et al. 2017). In addition, the test used for serological analysis of the serum samples can affect the apparent seroprevalence. In this study a commercial Latex Agglutination Test was chosen because of its ease of use and applicability on a variety of species (Lappin and Powell 1991). The LAT detects both IgG and IgM antibodies and therefore cannot differentiate acute from latent infection. It is therefore intended to be used as a screening technique that should be followed by other serological techniques or other detection methods to confirm the results. Although the performance of the LAT in humans is quite satisfactory (Mazumder, Chuang et al. 1988), its sensitivity in pigs was found to be rather low (Dubey et al. 1995, Sroka et al. 2008). In addition, the LAT hasn't been validated in all animal species, and its application on dog serum samples was not always successful (Ohshima, Tsubota et al. 1981). Therefore, the results of this study should be interpreted with caution, especially those in the African wild dog.

However, in comparison with earlier studies in humans and domestic and wild animals in South Africa, a decreasing trend in seroprevalence was observed in this study. Tenter et al. (2000) indicated that possible causes of a decrease in the prevalence of *T. gondii* in humans observed in recent years, particularly in developed countries could be due to: the implementation of more advanced farming systems, the awareness of proper hygienic practices and the proper management of meat (how the carcasses of slaughtered or dead animals are handled and disposed) (Tenter, Heckeroth et al. 2000). From the questionnaire survey it appeared that some risk factors for

transmission of the parasite exist in the area such as, free grazing practices and the disposal of carcasses of dead pets and livestock in the bush or rivers. However, most respondents (81.8%) in the survey area indicated that they prefer to consume their meat well cooked, which is likely to reduce the risk of *T. gondii* infection. Cooking meat at a temperature above 66°C is known to kill *T. gondii* tissue cysts (Dubey, Kotula et al. 1990).

3.6 Conclusion

In conclusion, we were able to provide baseline data on the seroprevalence of *T. gondii* in humans, domestic animals and multiple wildlife species in an interface area. Nevertheless, the possible impact of the geographical proximity of these different groups on the epidemiology of *T.* gondii could not be assessed. The low prevalence found in humans and most animal species could be due to a low population of domestic cats in the area, the current culinary practice of cooking meat well, the level of hygiene in households and farms and the dry weather conditions in the area, which may affect the survival of oocysts in the environment. However, this study showed that *T. gondii* is present in the wildlife cycle, which requires further investigation focusing on, prevalence, strain identification, the impact of the wildlife cycle on the neighbouring habitats and whether there are overlapping bridges within interface areas. This will help in better understanding the most frequent routes of transmission, reveal the most current data and determine the impact of toxoplasmosis on public health provincially and nationally for better disease control.

3.6 Acknowledgements

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3.8 Conflict of interest

The authors declare that they have no conflict of interest.

3.9 Availability of data and material

The data generated during and analysed during the current study are available from the corresponding author on reasonable request.

3.10 Authors' contributions

All the authors contributed to the conception and design of the study. Material preparation, data collection and analysis were performed by author one, Refilwe P. Bokaba (corresponding author) and data was further statistically analysed by author two, Veronique Dermauw. The first draft of the manuscript was written by author one, Refilwe P. Bokaba and all the authors commented and contributed to previous versions of the manuscript. All authors read and approved the final manuscript.

3.11 Ethics Approval

The human samples obtained from the NICD have Human Research Ethics Committee (Medical, R14/49) approval from the University of Witwatersrand with an additional Health Sciences Ethics Committee approval obtained from the University of Pretoria. The participation of the animal donors was subject to approval from the Research Ethics Committee (Faculty of Veterinary, University of Pretoria, REC036-19), and written consent from the owners of the animals from the Mnisi community, Bushbuckridge Municipality, Mpumalanga, was required. The Animal Ethics Committee (AEC, V064-18) approval was obtained for the study from the University of Pretoria. Participation and information of human participants involved in the questionnaire in the Mnisi study area were also subject to ethics approval from the Research Ethics Committee and the Faculty of Humanities (HUM015/0120), University of Pretoria. Approval was also obtained from the South African National Parks (SANParks) biobank for the use of wildlife species serum samples stored at their facilities. Approval for the Department of Agriculture, Land Reform and Rural

Development (DARRLD) Section 20 (12/11/1/1/6) biosafety and security was obtained for the samples used for the domestic and wildlife samples.

3.12 Consent to participate

All the authors consent to the participation in the manuscript.

3.13 Consent for publication

All the authors consent to the publication of the manuscript.

3.14 References

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Supplementary files

Appendix A

Supplementary Data

Table 3a Seroprevalence of T. gondii between age, sex and village categories in humans and the different domestic and wildlife species.

Categories		Value	n+/n (%)
Part I: Clinic			
Humans (n=160)			
	Age	18-35 years	5/93 (5.4%)
		36-45 years	5/24 (20.8%)
		46-55 years	0/20 (0.0%)
		56-65 years	3/14 (21.4%)
		>65 years	1/9 (11.1%)
Part II: Community			
Cats (n=9):			
	Age	6 -11 months	0/2 (0.0%)
		1-2 years old	0/7 (0.0%)
	Sex	Female	0/9 (0.0%)
		Male	0/0 (0.0%)
	Village	Athol	0/1 (0.0%)
		Gottenburg	0/0 (0.0%)
		Thlavekisa	0/8 (0.0%)
		Utha	0/0 (0.0%)
Chickens (n=336):			
	Age	3 -6 months	8/112 (7.1%)
		7 -11 months	1/39 (2.6%)
		1-3 years old	5/165 (3.0%)
		>3 years old	0/20 (0.0%)

Sex	Female	13/263 (4.9%)
	Male	1/73 (1.4%)
Village	Athol	12/72 (16.7%)
	Gottenburg	0/90 (0.0%)
	Thlavekisa	0/93 (0.0%)
	Utha	2/81 (2.5%)

Goats (n=358):

Age	3 - 1year	12/148 (8.1%)
	2-5 years old	22/175 (12.6%)
	6-10 years old	5/33 (15.2%)
	>10 years old	1/2 (50.0%)
Sex	Female	32/258 (12.4%)
	Male	8/100 (8.0%)
Village	Athol	5/75 (6.7%)
	Gottenburg	12/123 (9.8%)
	Thlavekisa	10/79 (12.7%)
	Utha	13/81 (16.1%)

Part III: Conservation area

Impala (n=97):

Age	Juvenile	0/10 (0.0%)
	Young adult	0/5 (0.0%)
	Sub-adult	1/22 (4.5%)
	Old	4/60 (6.7%)
Sex	Female	2/41 (4.9%)
	Male	3/56 (5.4%)

Wild dogs (n=54):

Age	Juvenile	2/2 (3.7%)

	Young adult	8/8 (100.0%)
	Prime Adult	1/1 (100.0%)
	Adult	27/27 (100.0%)
	Old	2/2 (100.0%)
	Unknown	14/14 (100.0%)
Sex	Female	16/16 (100.0%)
	Male	25/25 (100.0%)
	Unknown	13/13 (100.0%)

Wildebeest (n=43):

Age	Sub-adult	0/1 (0.0%)
	Old	9/35 (25.7%)
	Unknown	0/7 (0.0%)
Sex	Female	9/33 (27.27%)
	Male	0/3 (0.0%)
	Unknown	0/7 (0.0%)

Warthog (n=97):

Age	Piglet	0/1 (0.0%)
	Juvenile	0/15 (0.0%)
	Young adult	2/23 (8.7%)
	Sub-adult	1/3 (33.3%)
	Prime adult	4/13 (30.8%)
	Old	6/42 (14.3%)
Sex	Female	6/58 (10.3%)
	Male	7/39 (17.9%)

Zebra (n=66):

Age	Young adult	0/5 (0.0%)
	Sub-adult	1/5 (20.0%)

	Prime adult	0/1 (0.0%)
	Old	5/53 (9.4%)
	Unknown	0/2 (0.0%)
Sex	Female	4/37 (10.8%)
	Male	2/29 (6.9%)

Table 3b Knowledge and practices related to Toxoplasma transmission in questionnaire participants (n=384).

Knowledge and animal practices	
Variables	Frequency (%)
Knowledge on toxoplasmosis (n=384)	
Yes	8/384 (2.1%)
No	376/384 (97.9%)
Accessibility to water (n=384)	
Yes	384/384 (100.0%)
No	0/384 (0.0%)
Source of water supply (n=384)	
Fetch water at main tap and borehole	260/384 (67.7%)
Have their own tap and borehole	124/384 (32.3%)
Plant fruits and vegetables (n=384)	
No	257/384 (66.9%)
Yes	127/384 (33.1%)
Reason for growing fruits and vegetables (n=127)	
Consumption in the household	115/127 (90.6%)
Selling in the communities	0/127 (0.0%)
Both consumption and selling	12/127 (9.4%)
How do they prefer their meat to be cooked (n=384)	
Rare (cooked outside, bloody on the inside)	16/384 (4.2%)
Medium done (bit of blood on the inside)	54/384 (40.1%)
Well done (fully cooked)	340/384 (81.8%)
Pet owner or farmer or both (n=384)	
Pet owner (cats and dogs)	138/384 (35.9%)
Informal farmer (livestock)	242/384 (63.0%)

Both pet owner and farmer	110/384 (28.6%)
None of the above	114/384 (29.7%)
Do you clean after your companion animals (n=138)	
No	3/138 (2.2%)
Yes	135/138 (97.8%)
How frequent do the household owners clean after their companion animals (n=138)	
Once a week	11/138 (8.0%)
Twice a week	37/138 (26.8%)
Three times a week	48/138 (34.8%)
Four times a week	7/138 (5.1%)
Five times a week	1/138 (0.7%)
Everyday	26/138 (19.6%)
Less than once a week	5/138 (3.6%)
Never	3/138 (2.2%)
Where do the companion animals reside (n=138)	
Indoors/in the yard	13/138 (9.4%)
Outdoors/free roaming	15/384 (10.9%)
Both indoors and outdoors	110/384 (79.7%)
Practice of carcass disposal of companion animals (n=138)	
Bury in the yard	35/138 (25.4%)
Bury in the bush/river	30/138 (21.7%)
Burn the carcass in the yard	1/138 (0.7%)
Throw it away in the bush/river	67/138 (48.6%)
No experience	5/138 (3.6%)
Do you clean after your farm animals (n=242)	

No	17/242 (7.0%)
Yes	225/242 (93.0%)
How frequent do the household owners clean after their farm animals (n=242)	
Once a week	30/242 (12.4%)
Twice a week	64/242 (26.5%)
Three times a week	70/242 (28.9%)
Four times a week	15/242 (6.2%)
Everyday	26/242 (10.7%)
Less than once a week	20/242 (8.3%)
Never	17/242 (7.0%)
Where do your farm animals reside (n=242)	
Indoors/in the yard	15/242 (6.2%)
Outdoors/free roaming	22/242 (9.1%)
Both indoors and outdoors	205/242 (84.7%)
Practice slaughtering of livestock (n=242)	
No	(9/242) 3.7%
Yes	(233/242) 96.3%
Where is the slaughtering done (n=242)	
Inside the yard	196/242 (81.0%)
Outside the yard	15/242 (6.2%)
Both inside and outside the yard	22/242 (9.1%)
No slaughtering	9/242 (3.7%)
Practice of carcass disposal of livestock (n=242)	
Bury in the yard	28/242 (11.6%)
Burn the carcass in the yard	3/242 (1.2%)
Bury in the bush/river	11/242 (4.6%)

Throw it away in the bush/river	141/242 (58.3%)
Consume/Eat	46/242 (19.0%)
Feed the dogs	3/242 (1.2%)
No experience	10/242 (4.1%)

Chapter 4

The seroprevalence and molecular analysis of *Toxoplasma gondii* in rodents at the human-livestock-wildlife interface in the Bushbuckridge Municipality, South Africa

The seroprevalence and molecular analysis of *Toxoplasma gondii* in rodents in the human-livestock-wildlife interface in the Bushbuckridge Municipality, South Africa

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Abstract

Rodent populations in interface areas may play a pivotal role in the transmission of zoonotic infections with the ability to become carriers of over 60 zoonotic diseases affecting public health in human communities and veterinary health as a whole. Research on the role that rodents play as the intermediate host of *Toxoplasma gondii* in surrounding communities requires more investigation. The current study was an epidemiological study focusing on the seroprevalence and genetic characterization of *T. gondii* in captured rodents in the Bushbuckridge Municipality, Mpumalanga, South Africa. The latex agglutination test was used to survey 384 rodents (n) from three types of habitats. The combined seroprevalence was found to be 18.0%. The seroprevalences in the human dwellings, the croplands and wildlife conservation habitats were 19.0% (n =158), 15.3% (n =137) and 20.2% (n =89), respectively. Strains exhibiting lineages Type II (cropland) and Type III (human dwelling) alleles were detected. The results of this study show a relatively high seroprevalence in the rodent population with no statistical significance on the habitats and their effect on seropositivity, possibly indicating the rodent population being the bridge between the shared habitats. The strains detected possibly indicate an atypical avirulent genetic cycle of *T. gondii* in Mpumalanga.

Keywords: *Toxoplasma gondii*, seroprevalence, genotyping, rodents, interface area, human-domestic-wildlife interface

4.1 Introduction

Toxoplasma gondii is a common protozoan parasite with a global presence. Its low host specificity, the various infection paths and the resistance of the infective stages (oocysts, tissue cysts) has made it one of the most successful parasites to date. Infected hosts can experience symptoms that range from a non-symptomatic or mild infection to a severe clinical disease that can possibly lead to death. Numerous studies have used rodent species to investigate different topics of *T. gondii* including congenital toxoplasmosis, cerebral toxoplasmosis, genetic characterization and its epidemiology (Nicolle and Manceaux 1908, Splendore 1908, Paulino and Vitor 1999, Tenter, Heckeroth et al. 2000, Jauregui, Higgins et al. 2001, Mosallanejad, Avizeh et al. 2012, Dubey, Ferreira et al. 2016, Khademvatan, Foroutan et al. 2017, Galal, Stragier et al. 2020).

Rodents are animals that can populate and inhabit diverse ecological niches with an advantageous ability to adapt and exist through challenging and unexpected environmental changes (Khademvatan, Foroutan et al. 2017). In interface areas there is always a risk that rodent populations could contribute to the distribution of infectious agents to different host species from different habitats as they are known to be carriers of over 60 zoonotic pathogens that can lead to severe clinical infections in humans (Morand, Jittapalapong et al. 2015, Khademvatan, Foroutan et al. 2017). There are around 50 rodent species that can be found in South Africa; some of the species reside in human communities and become invasive by harming and causing destruction to vegetation and areas where edibles are stored and managed (Bastos, Chimimba et al. 2005). Rodents are prey for a variety of domestic (birds, pigs, dogs and cats) and wild species (wild felids) easily contracting *T. gondii* through ingestion of infected rodents which show that rodents play a substantial role in the dispersion of *T. gondii* (Dubey and Frenkel 1998). Rodents generally become infected through environmental contamination by ingesting oocysts excreted by domestic and wild felids (DeFeo, Dubey et al. 2002, Dabritz, Miller et al. 2008, Mercier, Garba et al. 2013). In some countries humans hunt and eat rodents as a source of nutrition, which is another possible route of transmission in humans (Ferroglio, Bosio et al. 2014, Jiang, Li et al. 2015, Almeria, Cabezon et al. 2018).

Research on how impactful the rodent populations are in the surrounding communities specifically focusing on toxoplasmosis are scarce in South Africa. In general *T. gondii* research as a topic in South Africa has been poorly investigated especially in areas where a variety of connected animal (domestic and wildlife) and human communities reside (Kistiah, Winiecka-Krusnell et al. 2011, Hammond-Aryee 2014, Hammond-Aryee, Esser et al. 2015, Bokaba, Dermauw et al. 2022).

Thus, the current study aimed to determine the seroprevalence of *T. gondii* in rodent species captured from different habitats in an interface area and further analyse the molecular structure of *T. gondii* strains isolated from rodents in Mpumalanga, South Africa.

4.2 Materials & methods

4.2.1 Study area & study population

The study area consists of the Bushbuckridge Municipality, Mpumalanga, South Africa, which is an interface area with three habitat types that co-exist, namely; the human dwellings (villages), the agricultural pasture lands (croplands) and wildlife (nature reserve). The study sites include four villages (human dwellings), namely; Athol, Gottenburg, Thlavekisa and Utah, their corresponding agricultural pasture lands (croplands) and a wildlife nature reserve referred to as the Manyeleti Nature Reserve (wildlife) bordering the Mnisi Community.

4.2.2 Study design

The current cross-sectional study consisted of two parts. Part I was capturing, sampling and serologically surveying the rodents for the detection of *T. gondii*. Part II was the molecular analyses of DNA from dissected tissue of seropositive rodents.

4.2.3 Study population & sampling procedure

Part I: Rodent capturing and surveillance sampling

Sherman and tomahawk traps were used for the capturing of rodents with a duration of four nights of trapping per habitat. A combination of peanut butter and oats was used as bait and during trapping days traps were checked twice daily. In the human dwellings, 20 households were selected randomly in every second street of each village. In each of the selected households four to five traps were strategically placed both inside the house (in the kitchen and areas where the owners might have spotted rodents) and outside in the yard (away from direct sunshine). In the croplands baited traps were placed every ten steps (10 m) in two perpendicular trap lines, which amounted to 25-30 traps per cropland in each village. In the wildlife conservation habitat starting points of' four trap lines were placed near the fence separating the community areas with the conservation area. The trapping points were approximately 1-2 km's apart. Each of the four trap lines consisted of 40 traps which were positioned every ten steps moving from the starting point to the conservation area in roughly a straight line. Information such as, trap location and rodent species was collected. Euthanasia and laboratory procedures were done at the Hans Hoheisen Wildlife Research Station. Captured rodents were euthanised with isoflurane. Cardiac blood was drawn immediately after euthanasia. Serology was done and only brain tissue were collected in seropositive rodents for further molecular analysis.

Collected rodent blood samples were first left overnight for clotting and the following day were processed for serum by centrifugation at 3500g for 10 minutes and stored at −20°C until use in the serological assay. The MAST® Toxoreagent™ ID rapid latex agglutination commercial kit (Mast Group Ltd., United Kingdom) was used to serologically detect anti-*T. gondii* antibodies by following the described manufacturer's instructions. A titer of 1/64 was used as the cut-off for rodent species (Jittapalapong, Sarataphan et al. 2011).

The following standard prevalence equation: $n_0 = \frac{z^2 P_{exp}(1-P_{exp})}{d^2}$, was used to determine the sample size for calculation of the prevalence in rodents with n_0, the sample size of interest, z, the z-score for the desired confidence interval (CI), d, the desired absolute precision and P_{exp} , the expected prevalence (Cochran 1977). A 50% expected prevalence was used as it was unknown. A 95% desired CI was used with a d at 5%. The samples size for rodent capturing was n=384 rodents.

Part II: DNA extraction and molecular analysis of seropositive rodent brain tissue

A commercial kit, the High Pure PCR Template Preparation Kit (Roche Diagnostics GmbHG, Germany), was utilized in the extraction of DNA from the dissected rodent brain tissue, according to the instructions of the manufacturer.

A multiplex nested polymerase chain reaction technique (mnPCR) was used to genotype the *T. gondii* samples collected from rodents. The mnPCR reaction was conducted using five genetic markers SAG1, SAG2b, SAG3, C22-8 and PK1. The KAPA 2G fast multiplex master mix was used for the primary multiplex PCR which consisted of the forward and reverse external primers, prepared separately. The master mix consisted of 4.5 µl PCR grade water, 12.5 µl 2G fast multiplex mix, 1.5 µl forward primer mix of each marker (2.5 µM), 1.5 µl forward external primer mix (2.5 µM) and 1.5 µl of the reverse external primer mix (2.5 µM) combined in one mixture to make a final volume of 25 µl for each rodent DNA sample (Kahn et al, 2005; Rather et al 2017; Su et al, 2010). The amplification process was done using a Thermal cycler 2400 (Applied Biosystems, Foster City, CA). The forward and reverse mixtures were denatured at 95 °C for 3 minutes. Subsequent to denaturation, 35 cycles of the following parameters were applied: 95 °C for 15 seconds, 58 °C for 15 seconds, 72 °C for 30 seconds and finally 72 °C for 10 minutes. The product amplicons from the primary PCR were further diluted in 1:1 ratio with molecular grade water. The secondary PCR master mix consisted of 12 µl of KAPA 2G robust master mix, 8 µl of PCR graded water, 1.5 µl of each forward and reverse internal primer (2.5 µM) for each marker per genetic marker separately and 1.5 µl of the diluted DNA template from the primary PCR to also make a final volume of 25 µl (Kahn et al, 2005; Rather

et al 2017; Su et al, 2010). The forward and reverse mixtures were denatured at 95 °C for 3 minutes. After denaturation, 35 cycles of the following parameters were applied; 95 °C for 15 seconds, 60 °C for 15 seconds, 72 °C for 30 seconds and finally 72 °C for 10 minutes. The amplification process for both the primary and secondary PCR were done using a Thermal cycler 2400 (Applied Biosystems, Foster City, CA). Subsequent to the PCR technique end products were analysed with gel electrophoresis. The protocol used for the multiplex nested PCR was adapted from previous studies (Khan, Su et al. 2005, Su, Zhang et al. 2006).

The *T. gondii Gangji* strain was used as a positive control. This strain was cultured and provided by Dr. Bavo Verhaegen from Sciensano, the Belgian Public Health Institute. Amplicons from the final PCR where further analysed with electrophoresis. The amplicons that showed a positive band were sent for sequencing at the University of Stellenbosch Central DNA Sequencing Facility. After receiving the nucleotide sequences, they were analysed using Bioedit and Chromas software. References were obtained from GenBank (NCBI website, www.ncbi.nlm.nih.gov). For comparison the CLC Genomics Workbench 8 was used to align the sequences.

4.2.4 Statistical analysis

The χ^2-test of independence was used to investigate the association between presence of infection and other variables (e.g., habitat type and observed species) including a z-test to compare the independent proportions. The significance level was set at 0.05. The SPSS Statistics Version 21 (IBM Corporation) was used for the statistical analysis.

4.2.4 Ethical clearance

The participation of the animal donors was subject to approval from the Research Ethics Committee (REC036-19) and the Animal Ethics Committee (AEC, V064-18) from the Faculty of Veterinary Sciences, University of Pretoria. Written consent was required from volunteering homeowners of households where rodents were captured in the villages of interest in the Mnisi community, Bushbuckridge Municipality, Mpumalanga. Approval was also obtained from the South African National Parks (SANParks) for the capturing of rodents in the Manyeleti Nature Reserve. Approval for

the DARRLD Section 20 (12/11/1/1/6) biosafety and security was also obtained for the rodent samples used.

4.3 Results

4.3.1 Seroprevalence

Part I: Rodent surveillance sampling

The total seroprevalence in rodents was found to be 18.0% (n=384 95% CI: 14.3 - 22.2%). The seroprevalence of *T. gondii* in the different habitats was 19.0% (n =158) in the human dwellings, 15.3% (n =137) in the crop lands and 20.2% (n =89) in the wildlife conservation area, with no significant association or dependence between the prevalence and habitat type (p= 0.587) indicated in Table 4.1. The seroprevalence was significantly higher in Athol, and the lowest in Utah, both p-values were lower than 0.001.

Table 4.1 Seroprevalence of T. gondii *in captured rodents in different locations and habitat types of the Bushbuckridge Municipality, Mpumalanga, South Africa.*

Categories	n+/n (%)
Habitat type	
Human dwelling	30/158(19.0%)
Croplands	21/137(15.3%)
Nature Reserve	18/89(20.2%)
Locations	
Athol	26/107(24.3%)*
Gottenburg	6/49(12.2%)
Thlavekisa	15/77(19.5%)
Utah	4/62(6.5%)*
Manyeleti Nature Reserve	18/89(20.2%)
Total:	**69/384 (18.0%)**

*=p value lower than 0.05

The seroprevalence obtained in the different observed species ranged from 0.0% - 44.7%. The highest seroprevalence of 44.7% (n =47) was found in the Black rat (*Rattus rattus)* and the lowest of 2.6 % (n =38) in the South African pouched mouse (*Saccostomus campestris)*. The data show a significant association between prevalence and observed species at a p-value lower than 0.001, indicated in Table 4.2.

Table 4.2 Seroprevalence of T. gondii *in captured rodents in different locations and habitats of the Bushbuckridge Municipality, Mpumalanga, South Africa.*

Observed Rodent Species (n=384)	**n+/n (%)**
Aethomys sp	2/8 (25.0%)
Gerbilliscus leucogaster	16/91(17.6%)
Lemniscomys rosalia	1/13(7.7%)
Mastomys sp	27/176(15.3%)
Mus minutoides	0/1(0.0%)
Rattus rattus	21/47(44.7%)*
Rattus tanezumi	1/10(10.0%)
Saccostomus campestris	1/38(2.6%)*

*=p value lower than 0.05

4.3.2 Molecular analysis

Part II: Molecular analysis of seropositive rodent brain tissues

From the seropositive rodents (n =69) that had undergone a molecular analysis only 23 samples (one sample for SAG1, three samples for SAG2b, six sample for SAG3 and thirteen samples for C22-8) showed a band during gel electrophoresis analysis and were sent for sequencing. Unfortunately, only seven (SAG1 marker and six SAG3 markers were detected from the samples tested) of the samples produced results after the analysis of the sequences. The genetic marker PK1 was not detected from any of

the seropositive samples that were tested. Figure 1 displays the gel electrophoresis images of 10-15 rodent DNA samples for some of the markers that were analysed.

The one sample that detected marker SAG1 was from a *Mastomys* sp. captured in the human dwellings in Thlavekisa. Sequencing results revealed that the nucleotide sequences showed an 84.58% identity to strain VEG indicating a high probability that the SAG1 gene marker expressed an allele from a Type III lineage (Accession number, MN958072.1). A closely related sequence (99.7% homology) to the VEG strain was also isolated in an adult female Eurasian otter (*Lutra lutra*) in Italy (Howe and Sibley 1995, Viscardi, Santoro et al. 2022).

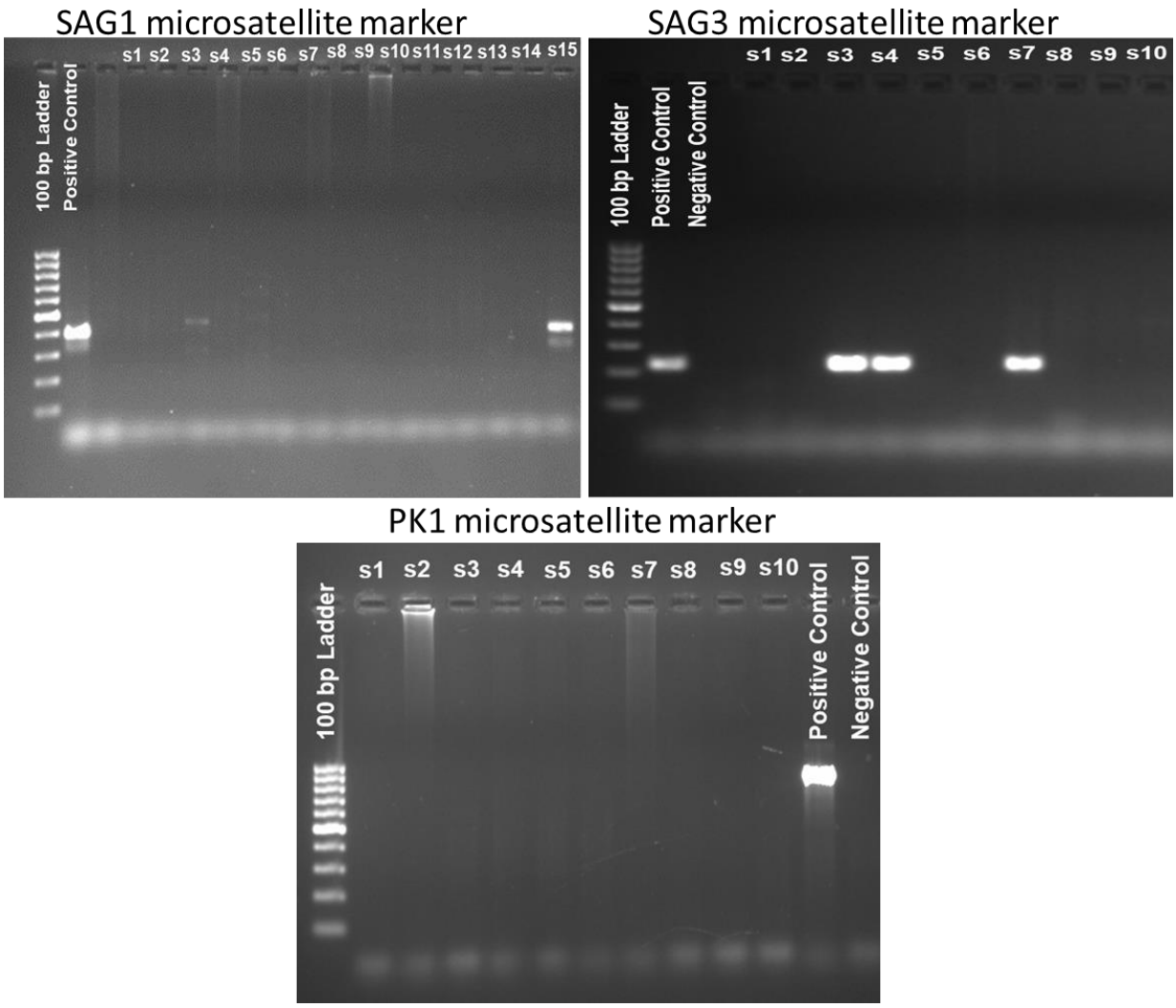

Figure 4.1 Gel electrophoresis image of end products of the nested multiplex-PCR amplification of markers SAG1, SAG3 and PK1 genetic markers.

Table 4.3 Results obtained from blasted (NCBI GenBank) sequences for Satellite markers SAG1 and SAG3.

Sample ID	Genetic Marker	Description	Original Tissue Sampled	Genotype	E value	Homology (%)	Country Detected (reference)	Accession (reference)
TgRd1SA	SAG1	*Toxoplasma gondii* isolate ID60322 brain marker SAG1 genomic sequence	Eurasian otter (*Lutra lutra*)	Type III	1e-51	84.58%	Italy	MN958072.1
TgRd2SA	SAG3	*Toxoplasma gondii* isolate CSe surface antigen (SAG3) gene, partial sequence	Human blood and cerebrospinal fluid (CSF)	Type II	2e-30	89.57%	Brazil	EU053940.1
TgRd3SA	SAG3	*Toxoplasma gondii* isolate 165 surface antigen (SAG3) gene, partial cds	Black bear (*Ursus americanus*) serum	Type II (halogroup 12)	1e-93	94.78%	USA	MH744789.1
TgRd4SA	SAG3	*Toxoplasma gondii* voucher ID_17-4_2 surface antigen 3 (SAG3) gene, partial cds	Sheep fetal brain	Type II	5e-83	97.86%	Spain	MT361125.1
TgRd5SA	SAG3	*Toxoplasma gondii* strain ME49 surface antigen (SAG3) gene, partial sequence	Human blood or cerebrospinal fluid (CSF)	Type II	8e-55	92.02%	Brazil	EU053937.1
TgRd6SA	SAG3	*Toxoplasma gondii* voucher ID_17-4_2 surface antigen 3 (SAG3) gene, partial cds	Sheep fetal brain	Type II	3e-75	90.83%	Spain	MT361125.1
TgRd7SA	SAG3	*Toxoplasma gondii* voucher ID_17-4_2 surface antigen 3 (SAG3) gene, partial cds	Sheep fetal brain	Type II	2e-91	97.54%	Spain	MT361125.1

Furthermore, the six samples in which marker SAG3 was detected, were from *G. leucogaster* (captured in the croplands in Athol), *Rattus rattus* (captured in the human dwelling in Gottenburg and Thlavekisa), *Mastomys sp* (captured in the conservation area in the Manyeleti Nature Reserve) and *Aethomys sp* (captured in the croplands in Thlavekisa) indicated on Table 4.4.

The nucleotide sequences showed a percent identity that ranged between 89-98%, indicating a high probability that the detected strains expressed Type II alleles for the SAG3 gene marker. When blasted against the NCBI GenBank database the sequences showed similarities to isolates detected in Brazil, the United States (US) and Spain represented in Table 4.3, which also includes the tissues analysed (Ferreira, Vidal et al. 2008, Scimeca, Perez et al. 2020, Viscardi, Santoro et al. 2022).

Table 4.4 Observed species, location and habitat of genetically analysed seropositive samples from the Bushbuckridge Municipality, Mpumalanga, South Africa.

Sample ID	Observed species	Location (Habitat)	Habitat	Expressed Allele	Reference
TgRd1SA	*Mastomys sp*	Thlavekisa	Human dwelling	Type III	(Viscardi, Santoro et al. 2022)
TgRd2SA	*Gerbilliscus leucogaster*	Athol	Croplands	Type II	(Ferreira, Vidal et al. 2008)
TgRd3SA	*Rattus rattus*	Athol	Human dwelling	Type II (haplogroup 12)	(Scimeca, Perez et al. 2020)
TgRd4SA	*Mastomys sp*	Manyeleti Nature Reserve	Nature Conservation	Type II	(Fernández-Escobar, Calero-Bernal et al. 2020)
TgRd5SA	*Aethomys sp*	Thlavekisa	Croplands	Type II	(Ferreira, Vidal et al. 2008)
TgRd6SA	*Rattus rattus*	Gottenburg	Human dwelling	Type II	(Fernández-Escobar, Calero-Bernal et al. 2020)
TgRd7SA	*Rattus rattus*	Gottenburg	Human dwelling	Type II	(Fernández-Escobar, Calero-Bernal et al. 2020)

4.4 Discussion

The data presented in this study displays a relatively high combined *T. gondii* seroprevalence in rodents of 18.0% (n=384 95% CI: 14.3 - 22.2%) in the Mpumalanga Province. The prevalence in the three habitats studied was not significantly different. This may indicate the possibility of a similar *T. gondii* transmission cycle in the rodent populations caused by a rotation of similar rodent species that have accessibility to all the habitats in short, bridging the habitats together. The best represented rodent species in the traps were *Mastomys* sp. (176 captured (45.8%)), followed by *G.*

leucogaster (91 captured (23.7%)) and *R. rattus* (47 captured (12.2%)). *Rattus rattus* showed a significantly higher seroprevalence (44.7%, 21/47). *Rattus tanezumi* was the least captured rodent (10 captured). *Saccostomus campestris* showed the lowest significant seroprevalence at 2.6% (1/38). The relatively high seroprevalence obtained in rodents in the current study is in contrast to two other studies done in Durban, South Africa. Those studies, also using the LAT, showed a seroprevalence of 4.1% (9/217) (Taylor, Arntzen et al. 2008) and 11.3% (Archer, Appleton et al. 2017), respectively. The commonest rodent species in the first study was the brown rat (*Rattus norvegicus)* (91.2%) (Taylor, Arntzen et al. 2008). With the relatively high seroprevalence of *T. gondii* obtained in the captured rodents in the study we could further speculate that the wild felids are responsible for oocyst contamination in the environment therefore infecting the rodents through the ingestion of sporulated oocysts (Dubey, Kramer et al. 1985, Smith, Fisher et al. 1995, Ferreira, Vidal et al. 2008). Otherwise, the seroprevalence obtained could also be due to the prey-predator interactions within the rodent population.

Other studies done within the African continent also showed a low seroprevalence when compared to the current study. A survey done on native and invasive captured rodents in Niger using a modified agglutination test (MAT) found an overall seroprevalence of 1.96% (15/766). The most common species captured in that study included *Mastomys natalensis* (*n*=502) and *R. rattus* (*n*=122) (Mercier, Garba et al. 2013). The highest prevalence was found in *Arvicanthis niloticus* (*n*=9.1% (6/66)). In that study rodents were also captured in different habitats such as an industrial area, gardens, houses and market habitats; however, in contrast to our study the habitats had a significant effect on seropositivity (Mercier, Garba et al. 2013). Another study done on captured rodents from Senegal found a low seroprevalence of 3.7% (44/1205) also using MAT (Brouat, Diagne et al. 2018). The commonest species captured were *Mus musculus domesticus* (*n*=689), *Mastomys erythroleucus* (*n*=376) and *R. rattus* (*n*=33). The highest prevalence was found in *M. m. domesticus* at 5% (25/689) (Brouat, Diagne et al. 2018). In other parts of the world other seroprevalence studies also show similar and contradicting prevalence rates when comparing them with the current study. A serological survey done on rodents in Poland found an overall prevalence of 5.5% (32/577) using an ELISA kit (Grzybek, Antolová et al. 2021). Another study in Malaysia also found a lower prevalence of 5.9% (31/526) using an

IFAT (Normaznah, Azizah et al. 2015). A concordant study was performed in Iran, where the researchers detected a seroprevalence of 24.41% (31/127) in wild rats (*R. rattus*) using immunochromatography (Mosallanejad, Avizeh et al. 2012). The differences in seroprevalence ranges could be attributed to numerous variables including the climate, environmental changes, the cat population in the different areas and the serological test used.

In our study, in seven of the 69 seropositive rodents, *Toxoplasma* DNA could be detected. The results obtained from the molecular analysis showed that the *T. gondii* isolates from the rodents, expressed alleles from lineages Type III (SAG3 gene marker) in the human dwellings and Type II (SAG1 gene marker) from all the habitats (human dwelling, cropland and conservation area) with the percentage identity ranging from 84 – 98 %. This shows that the strains circulating in the Bushbuckridge Municipality are possibly atypical. Although the isolates expressed Type II and III alleles, the strains circulating in the Bushbuckridge Municipality may have variation due to the slightly lower identity percentages obtained. We cannot conclusively confirm whether the two strains are naturally found combined or are two distinct strains circulating in the rodent population in the studied rodents. Variation in the SAG3 marker was also found in a study, examining the variation of *T. gondii* in sheep flock in Spain (Fernández-Escobar, Calero-Bernal et al. 2020). Strains of Type II and III lineages are considered avirulent in mice, but further research is required for confirmation (Filisetti and Candolfi 2004). In Europe most of the strains isolated belong to Type I, II, III lineages, showing clonality in the circulation of *T. gondii* in that continent (Filisetti and Candolfi 2004, Berger-Schoch, Herrmann et al. 2011, Dupont, Christian et al. 2012, Su, Khan et al. 2012). Research in South America and especially in Brazil has shown that atypical strains are common, and the occurrence of those strains has been associated with their circulation in wildlife. In addition, atypical strains seem to display a higher virulence in their hosts, including in humans (Su, Khan et al. 2012, Shwab, Zhu et al. 2014). Atypical strains have also been detected in Egypt; therefore, further research is required in order to reveal the molecular epidemiology of *T. gondii* in the interface areas of Africa (Shwab, Zhu et al. 2014, Galal, Ajzenberg et al. 2018, Galal, Schares et al. 2019).

Several limitations were experienced in the study, more so in the molecular analysis. What is noticeable, is the inconsistency in the sample sizes between the different

habitats and the observed rodent species which should be considered when examining the results. Serological tests can result in false positives and the LAT used in the study is unable to distinguish between acute and chronic infection; therefore, a supporting detection method should be used for further validation of the results (Liu, Wang et al. 2015, Rostami, Karanis et al. 2018). In the molecular part of the study, 23 out of the 69 seropositive samples showed positive bands for four (no positive band was detected for the PK1 marker) of the genetic markers used (one sample for SAG1, three samples for SAG2b, six sample for SAG3 and thirteen samples for C22-8). Unfortunately, some of the end sequences had background disturbances and some samples amplified a *Sarcocystis* species which is a protozoan parasite closely related to *T. gondii* (Dubey, Zarnke et al. 2003). Only seven samples were able to undergo sequence analysis. Only about 10% of the seropositive rodents gave a positive result in the PCR and this disagreement could be a consequence of the uneven distribution of *T. gondii* cysts in tissues of chronically infected animals. Only a small portion of tissue is used for extraction. That is why some researchers have developed techniques to increase the sample volume for DNA extraction (Gisbert Algaba, Geerts et al. 2017). Another reason could also be due to the differences in specificity and sensitivity between serological tests and molecular techniques (Herwaldt 2001, Liu, Wang et al. 2015, Khan and Noordin 2020). There are ways to overcome the challenges for example using techniques that can help increase the sample volume for DNA extraction as mentioned above and including a purification step for the amplicons in the primary mnPCR not only in the final amplicons from the secondary PCR before sequencing. Using an alternative molecular procedure like the restriction fragment length polymorphism PCR (rflp PCR) which ideally requires positives *T. gondii* strains from the three types for accuracy. Overall, further research is needed to overcome the limitations experienced in the study.

In conclusion, this study has provided some preliminary data on the epidemiology and genetic variation of *T. gondii* in an interface area in South Africa. To the authors' knowledge the present study is the first that has investigated the genetic variation of *T. gondii* in South Africa. The two seroprevalence studies in Durban are the only other studies that could be found on the detection of *T. gondii* in the rodent population in South Africa. From the analysed data obtained it can be presumed that the rodent population in the studied interface area are the bridge between the cycle of *T. gondii*

in the wildlife and domestic habitats. It can further be presumed that the cycle of *T. gondii* in the three habitats (human dwelling, cropland and nature conservation) are similar due the rodent population and because of the similar prevalence rates obtained. It can also be speculated that the strains from lineages Type II, and Type III are circulating in the area but they have variation or may be atypical. More research is required not only to improve the limitations experienced in the current study but to further advance knowledge of toxoplasmosis in South Africa thus preserving and maintaining public health and veterinary health.

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4.6 Conflict of interest

The authors declare that they have no conflict of interest.

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Chapter 5

General Discussion and conclusion

General Discussion

Sub-Saharan Africa is a unique area that contains areas where human-livestock and wildlife coexist (De Garine-Wichatitsky, Caron et al. 2013). In many of these areas the habitats included may completely overlap with no mechanical barriers or at most are separated/divided by a fence (De Garine-Wichatitsky, Caron et al. 2013, Berrian, van Rooyen et al. 2016).

The systematic review presented in Chapter 2 (page 23) showed scarcity of research on *T. gondii* in wildlife particularly in African wildlife species. The review further highlighted the lack in confirmation of definitive hosts in some felid species in Africa (serval (*Leptailurus serval*), caracal (*Caracal caracal*), African wildcat (*Felis silvestris lybica*), African golden cat (*Caracal aurata*), jungle cat (*Felis chaus*) and the black-footed cat (*Felis nigripes*)) (Jewell, Frenkel et al. 1972, Miller, Frenkel et al. 1972, Dorny and Fransen 1989, Lukešová and Literák 1998, Bokaba, Dermauw et al. 2022). The review also raised questions of whether congenital toxoplasmosis occurs in wildlife species, whether wildlife species experience severe infection which is important to determine the disease's impact in wildlife for conservation biology and survival (relating of mortality and morbidity). Furthermore, it raised the question of whether there is direct transmission from wildlife to humans that consume meat from wildlife (Ferroglio, Bosio et al. 2014, Jiang, Li et al. 2015, Almeria, Cabezon et al. 2018).

The above-mentioned questions led to the research conducted in the seroprevalence study (page 60), focusing on determining the seroprevalence of *T. gondii* in humans, domestic animals and wildlife species, running in parallel to investigate some of the gaps. The survey showed a low seroprevalence range in humans, the domestic animals and most of the wildlife species. A slightly higher seroprevalence was detected in grazing species (wildebeests and zebras) and an omnivorous species (warthog). The highest detection was in a carnivorous species, with a 100% detection rate. The differences and similarities in the obtained prevalence rates in the study even when compared to others within the continent and globally show very dynamic and complex biological phenomena affected by numerous variables including environmental conditions, social practices, husbandry practices, the dominating transmission route/s, rodent populations, cat populations, the habitat types included in

the area, the dietary types of the different populations included and types of strains of *T. gondii* that might impact the severity of the infection in an area (Spencer and Markel 1993, Cheadle, Spencer et al. 1999, Patz, Graczyk et al. 2000, Tonouhewa, Akpo et al. 2017).

Chapter 4 (page 87) mainly focused on determining the seroprevalence within the rodent population from different habitat types in an interface area and to determine the types of *T. gondii* strains that maybe circulating in the area and how essential they are in creating a bridge between the surrounding habitats in terms of the parasite's cycle in the different habitats. The results showed a relatively high seroprevalence (approximately 20%) of *T. gondii* in the rodent population which was consistent in rodent populations across the three habitats. Suggesting a similar *T. gondii* and rodent population within the habitats. Although the detected *T. gondii* strains are from Type II and Type III lineages, due to the challenges experienced mainly in the molecular technique and the possible contamination by a *Sarcocystis sp.*, additional testing and investigation is required to confirm these findings.

General Conclusion

In conclusion the wild felids in the study maybe the main environmental contaminators in the studied interface area. High prevalence rates might be found in the omnivorous and carnivorous wildlife populations, atypical strains might be circulating in the area which might result in severe infections and lastly, the rodent population is a key player in the transmission of *T. gondii*. The topics addressed in the current study show how little focus is directed to investigating the dynamics of *T. gondii* in South Africa and in the whole of Africa. The topics reveal noticeable gaps and research opportunities such as: investigating congenital toxoplasmosis in domestic and wildlife species, genetic variation in the different species, prevalence studies focusing on the main dietary types (herbivores, omnivores and carnivores) to determine the dominant transmission route/s of *T. gondii*, investigating abattoirs/slaughterhouses for *T. gondii* detection, cell culturing of native *T. gondii* strains for more sophisticated genetic research and lastly investigating *T. gondii* in interface areas. Therefore, further research is required for

more accurate deductions in understanding the epidemiology of *T. gondii* in the continent which will be beneficial in understanding its cycle in Africa.

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