Seroprevalence and associated risk factors of *Toxoplasma gondii* infection in domestic animals in the O.R Tambo District, South Africa

By

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DEDICATION

- Through God who strengthens me and to the love of my life, my dear wife for providing me with the necessary love and support.

DECLARATION

I Whatmore Munetsi Tagwireyi hereby declare that this dissertation, which I hereby submit for the Master of Science degree in the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, is my own work and has not been previously submitted by me for degree purposes at another tertiary institution.

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LIST OF ABBREVIATIONS

O.R Tambo	Oliver Reginald Tambo
T. gondii	*Toxoplasma gondii*
DAT	Direct agglutination test
ELISA	Enzyme-linked immunosorbent assay.
IFAT	Indirect immunofluorescence antibody test
IHAT	Indirect haemagglutination test
LAT	Latex agglutination test
MAT	Modified agglutination test
HIV	Human immunodeficiency virus
AIDS	Acquired immune deficiency syndrome
DT	Sabin – Feldman dye test
PCR	Polymerase chain reaction

SUMMARY

Title :

SEROPREVALENCE AND ASSOCIATED RISK FACTORS OF *TOXOPLASMA GONDII* INFECTION IN DOMESTIC ANIMALS IN THE O.R TAMBO DISTRICT, SOUTH AFRICA.

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Key words: *Toxoplasma gondii*, latex agglutination test, seroprevalence, Oliver Reginald Tambo District, domestic animals, human immune deficiency virus.

ABSTRACT:

Toxoplasma gondii is a single-celled parasite that has a wide range of hosts including humans. A cross-sectional survey was conducted to investigate *T. gondii* seroprevalence and associated risk factors in small ruminants, pigs, poultry and cats in the Oliver Reginald Tambo District in the Eastern Cape in South Africa between June 2016 and October 2016. Household-level and animal-level data were collected using a close-ended questionnaire. One sample of each present species was collected in each household. The Toxoreagent®, Mast Group, United Kingdom, latex agglutination test, was used for *T. gondii* antibody detection. Positive samples had agglutination patterns at dilutions of 1:64 or greater, except for chickens, whose cut off titre was 1:32. A household was classified as *T. gondii* seropositive if at least one species tested positive. The study revealed that 78 out of 121 sheep (64.46%), 69 out of 128 goats (53.91%), 36 out of 106 pigs (33.96%), 35 out of 109 cats (32.11%) and 46 out of 137 chickens (33.58%) were seropositive for the parasite. Seropositivity was assessed for association with potential risk factors. Age, location, climate, animal production system, rodent control, cat-feed access and cat faecal disposal were found to be significantly associated with seropositivity using the Chi-Squared test or odds ratio confirmed by the

Fisher's exact test. The relatively high seroprevalence of *T. gondii* detected in this study suggests that the infection *T. gondii* poses a substantial public health risk through the consumption of infected raw or undercooked meat infected with *T.gondii* cysts as well as contact with cat faeces infected with *T. gondii* oocysts.

CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

Toxoplasma gondii is the most successful and unrestricted parasitic pathogen with a wide range of mammalian hosts (Torrey & Yolken 2013). Toxoplasmosis is zoonotic and has a worldwide distribution. It is an intracellular parasite *i*, which causes reproductive failure in animals, particularly in sheep, goats and pigs, resulting in huge economic losses (Dubey *et al*. 1986, Abu Samraa *et al.* 2007, Gebremedhin *et al.* 2015a).Both domestic and wild felids are the definitive hosts and have a central role in the epidemiology of this parasite, as they are the only animal that sheds environmentally resistant *T. gondii* oocysts (Dubey 2010b). Animals and humans primarily acquire infection through ingestion of feed or food and water contaminated with oocysts, undercooked or raw meat with tissue cysts and exceptionally from drinking unpasteurised milk with circulating tachyzoites (Hill & Dubey 2002, Jones *et al.* 2009, Kijlstra & Jongert 2009). Molecular genotyping has classified *T. gondii* into three clonal lineages, namely types I, II, and III and these are related to the pathogenicity and susceptibility of host species (Howe & Sibley 1995, Howe *et al.* 1997).

Apart from causing production losses in animals, toxoplasmosis is a significant zoonotic concern and can cause fatal diseases in humans such as encephalitis in immunocompromised people, abortions in pregnant women and hydrocephalus in infants (Dubey 2010b, Asgari *et al.* 2013). Toxoplasmosis-related illnesses have led to the surge in interest in the parasite, particularly with the onset of the current human immunodeficiency virus (HIV) epidemic (Hammond-Aryee, Esser & Van Helden 2014). Serological surveys of *T. gondii* have been conducted in both humans and animals in various parts of the world. *Toxoplasma gondii* infection seroprevalences vary widely at a local, regional and international scale. However, in South Africa there is paucity of data, of which is either outdated or scant (Bigalke 1966, Du Plessis, Bigalke & Grunell 1967, Brink, De Wet & Van Rensburg 1975, Van Rensburg & Silkstone 1984, Abu Samraa *et al.* 2007).Food-producing animals, such as sheep, goats, pigs and chickens are susceptible and are considered to be the main sources of human infection. Hence, more information on the seroprevalence and associated risk factors across a wide array of domestic animals as well as on the potential role each species plays in human infection

is required. Various factors within the O.R Tambo district contribute to the need to assess the risk of toxoplasmosis, namely:

- the type of farming practised in the region,
- the climatic conditions of the region,
- informal slaughter of animals and consumption of meat without inspection,
- the high HIV/AIDS prevalence in the region,
- the level of poverty of the region.

Many patients infected with HIV can die of toxoplasmosis-related complications, and this is of great concern in South Africa, particularly the Eastern Cape, which has a high prevalence of HIV (Shisana & Simbayi 2002, Dubey *et al.* 2012). Quantifying the seroprevalence of *T. gondii* infection in food-producing animals and cats in the district will assist in further understanding the risk factors associated with infection in animals. Hence, a study was embarked so as to determine the seroprevalence of *T. gondii* infection in cats, chickens, goats, pigs and sheep using a commercial latex agglutination test (LAT) and to evaluate the risk factors associated with *T. gondii* infection in these animals through the use of a questionnaire survey.

1.2. Historical aspect

The protozoan parasite, *Toxoplasma gondii* was discovered in 1908 (Dubey & Jones 2008). In 1939, its medical importance became known when it was found in a congenitally-infected infant in the United States and much later in 1957, its veterinary significance became known when it caused abortion storms in Australian sheep (Wolf, Cowen & Paige 1939, Hartley & Marshall 1957). The Sabin-Feldman dye test was first described in 1948 and it allowed for the diagnosis of *T. gondii* as a common parasite of animals and people (Sabin & Feldman 1948). Sulphonamides, spiramycin and clindamycin were found to be effective against *T. gondii* and have since been used for treatment (Sabin & Warren 1942, Garin & Eyles 1958, McMaster *et al.* 1973). Frenkel and Dubey (1972) advocated for measures of hygiene to reduce human exposure to oocysts. Measures to reduce *T. gondii* infection in farm animals were only developed much later, in 1995 (Dubey, Lappin & Thulliez 1995a, Weigel *et al.* 1995).

1.3. Epidemiology and life cycle of parasite

Toxoplasma gondii can infect almost all mammals and birds and its transmission is foodborne either by ingestion of oocyst-contaminated feed, water and/or tissue cysts in undercooked or raw meat (Onyiche & Ademola 2015). People may get infected after contact with oocyst-contaminated soil, cat litter, water and vegetables (Cook *et al.* 2000). Its life cycle includes definitive hosts and intermediate hosts which were well defined by 1970 (Frenkel, Dubey & Miller 1970, Jewell *et al.* 1972, Miller, Frenkel & Dubey 1972). *Toxoplasma gondii* life cycle is divided into two phases: an sexual phase which occurs in the definitive host (felids) and the asexual phase which occurs in intermediate hosts, of all warm-blooded mammals and birds, (Weiss & Kim 2011). During the asexual phase, tachyzoites invade epithelial cells and multiply rapidly targeting macrophages, which reach neural tissue throughout the body (Carruthers & Suzuki 2007). The second stage of the asexual phase results in the formation of bradyzoites contained within tissue cysts. This end stage of the life cycle in intermediate hosts has an affinity for muscle and neural tissues (Evans 1992, Dubey, Lindsay & Speer 1998). Cats play an important role in the *T. gondii* life cycle and are infected by ingesting infected prey, particularly rodents, which are easily infected by the parasite (Dubey 2010b). Cats excrete millions of oocysts in their faeces for 5 - 12 days (Frenkel *et al.* 1970, Al-Kappany *et al.* 2010, Elmore *et al.* 2010). Under favourable climatic conditions, these may survive in the environment and remain infective in soil for several years (Frenkel *et al.* 1970, Yilmaz & Hopkins 1972). Wild felids also shed oocysts and in environments that have no domestic cats, their role in parasite transmission becomes important. The wildlife, livestock and human interface provides a perfect platform for the maintenance of *T. gondii* (Tenter *et al*. 2000).

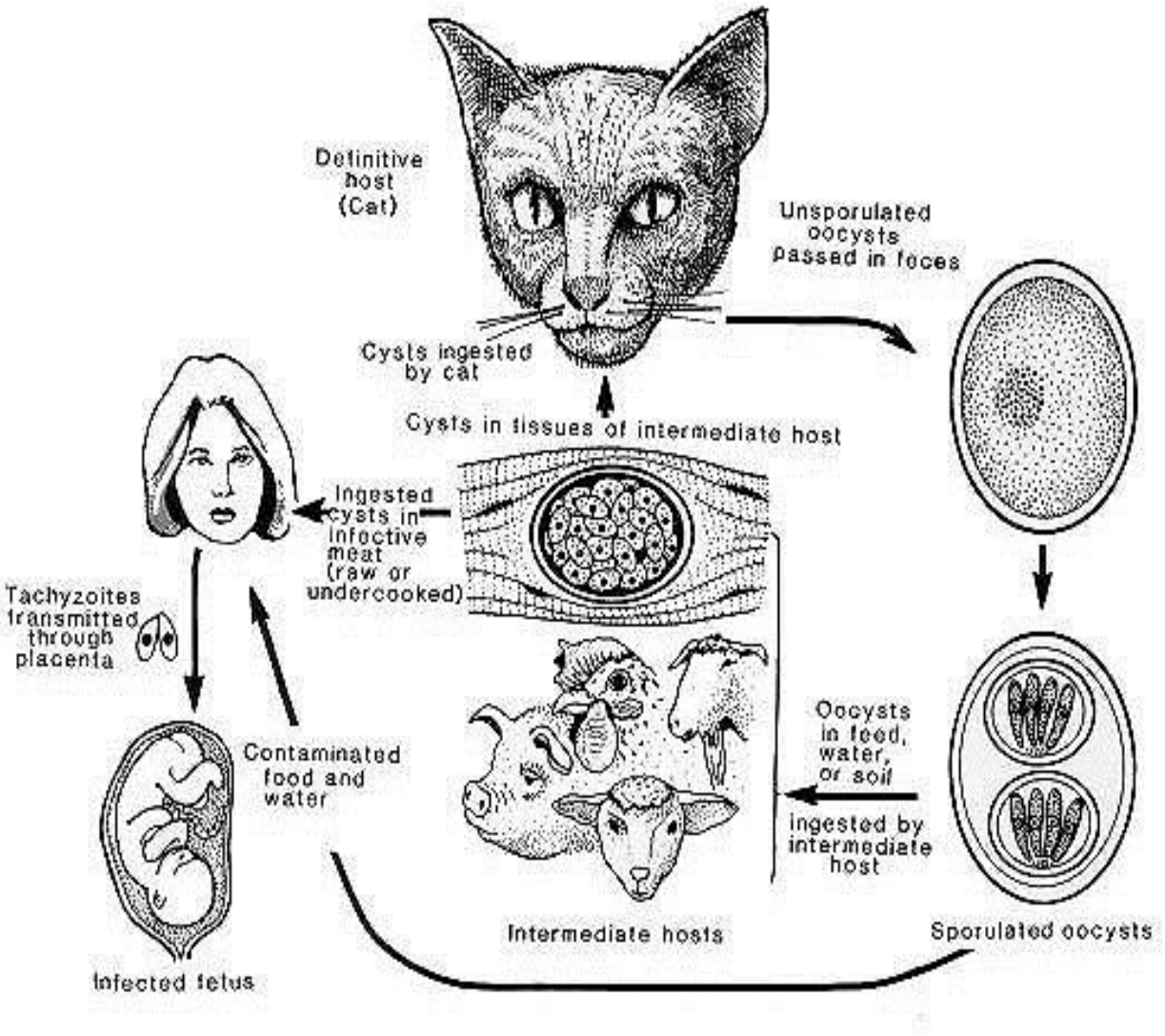

Figure 1.1: *Toxoplasma gondii* life cycle (Dubey 2004).

1.4. Risk factors and prevalence of toxoplasmosis in different animal species

1.4.1. Risk factors and prevalence of toxoplasmosis in cats

Stray cats generally fend for themselves through hunting, although humans sometimes feed them (Lepczyk *et al*. 2015). Such animals are at greater risk of acquiring *T. gondii* than domestic cats that are kept indoors. Prevalence of *T. gondii* increases with age in cats (Dubey *et al*. 1995a, Dubey & Jones 2008, Al-Kappany *et al.* 2010). Since oocysts are hardly found in cat faeces, seroprevalence data is the most reliable method of determining the epidemiological significance of *T. gondii* infection in cats (Dubey *et al*. 1995a). Serological surveys conducted in Thailand and Egypt detected the presence of

T. gondii antibodies in 15.4% and 97.4% of tested subjects respectively, indicating a wide seroprevalence range in cats (Thiangtum *et al.* 2006, Al-Kappany *et al.* 2010).The prevalence of *T. gondii* as shown in Table 1.1, differs from one country to another and this has been partly attributed to the serologic test and the screening dilution used (Dubey *et al.* 2009). Little information is available concerning *T. gondii* infection in cats from Africa and studies have been done in Nigeria, Egypt and South Africa (Al-Kappany *et al.* 2010, Kamani *et al.* 2010, Hammond-Aryee *et al*. 2015a).

Table 1.1: *Toxoplasma gondii* seroprevalence studies in cats

Reference	Country	Method	Samples size	Seroprevalence (%)	Year
Hammond-Aryee *et al*	South Africa	IFA	159	37.1	2015
Can *et al*	Turkey	IFA & ELISA	1121	33.4 & 34.4	2014
Al-Kappany *et al*	Egypt	MAT	158	97.4	2010
Kamani *et al*	Nigeria	LAT	105	36.2	2010
Thiangtum *et al*	Thailand	LAT	136	15.4	2006

1.4.2. Risk factors and prevalence of toxoplasmosis in chickens

A comparison of indoor raised chickens and free range chickens shows a higher seroprevalence of *T. gondii* in the latter (Dubey 2010a). Free ranging chickens can acquire infection via ingesting tissue cysts after ingesting infected rodents and eating leftover food infected with oocysts (Sousa *et al.* 2016). Studies in Ethiopia and Nigeria have shown that *T. gondii* seropositivity in chickens from rural areas and midland altitudes, in the presence of cats, increased with age, extensive management and were higher in cross and exotic chicken breeds (Ayinmode & Olaosebikan 2014, Gebremedhin *et al.* 2015b). Serological surveys in different countries have shown that *T. gondii* seroprevalences in chickens can vary considerably as shown in Table 1.2.

Table 1.2: *Toxoplasma gondii* seroprevalence studies in free ranging chickens (*Gallus domesticus*)

Reference	Country	Method	Samples size	Seroprevalence (%)	Year
Sousa *et al*	Brazil	IFAT	60	25	2016
Hussien *et al*	Sudan	LAT	58	100	2016
Gebremedhin *et al*	Ethiopia	MAT	601	30.5	2015b
Ayinmode and Olaosebikan	Nigeria	MAT	225	40.4	2014
Xu *et al*	China	MAT	160	18.8	2012
Dubey *et al*	Israel	MAT	96	46.6	2004

1.4.3. Risk factors and prevalence of toxoplasmosis in small ruminants

The estimated global seroprevalence of *T. gondii* in goats is 15% (Dubey 2004). Table 1.3 shows studies in goats with a very wide range of seroprevalence. A study by Zewdu *et al.* (2013) in Ethiopia reported a higher *T. gondii* seropositivity value in smaller flocks, semi-intensively managed goats, females and goats kept under sedentary farming systems with agro-pastoral components. Another study in dairy goats in Tanzania showed an increasing *T. gondii* seropositivity with age and in females as compared to males, supporting some of the findings by the previous author (Swai & Kaaya 2012).

Dubey (2004) reported a global seroprevalence of 30% of *T. gondii* in sheep and prevalence reported in different countries varies widely (Table 1.4). In Zimbabwe, sheep that had access to surface drinking water and sheep from communal areas, especially overgrazed ones, were found to have higher seroprevalences than those from commercial farms (Hove, Lind & Mukaratirwa 2005b). Seroprevalence in sheep is thought to increase with age (Dubey *et al.* 1986, Dumètre *et al.* 2006, Gebremedhin *et al.* 2013). An increased seropositivity was seen in sheep from farms that had cats present, that were non-commercial as compared to commercial and that had extensive as compared to semi–intensive or intensive farming set ups. However unlike goats, larger sheep flock sizes contributed to higher *T. gondii* seroprevalences (Hove *et al*. 2005b, Andrade *et al.* 2013).

Table 1.3: Toxoplasma *gondii* seroprevalence studies in goats

Reference	Country	Method	Sample size	Seroprevalence (%)	Year
Davoust *et al*	Senegal	DAT	52	15	2014
Rahman *et al*	Bangladesh	LAT	146	61	2014
Zewdu *et al*	Ethiopia	ELISA	927	19.7	2013
Swai and Kaaya	Tanzania	LAT	337	19.3	2012
Kyan *et al*	Okinawa	LAT	24	75	2012
Hove *et al*	Zimbabwe	IFA	335	68.6	2005b

Table 1.4: *Toxoplasma gondii* seroprevalence studies in sheep

Reference	Country	Method	Samples size	Seroprevalence (%)	Year
Hutchinson & Smith	England and Wales	LAT	4 354	54.2	2015
Hammond-Aryee *et al*	South Africa	ELISA	292	8	2015b
Davoust *et al*	Senegal	DAT	43	16	2014
Gebremedhin *et al*	Ethiopia	ELISA	1 130	31.59	2013
Abu Samraa *et al*	South Africa	IFAT	600	5.6	2007
Hove *et al*	Zimbabwe	IFA	335	47.8	2005b

1.4.4. Risk factors and prevalence of toxoplasmosis in pigs

Various levels of *T. gondii* seroprevalence have been reported in pigs and wild boars in African countries like Ethiopia, Tanzania, Senegal and Zimbabwe, allowing for comparison (Table 1.5). A common trend was observed in four of these five countries; low seroprevalence levels in intensively bred pigs, medium seroprevalence levels in organic or outdoor pigs and high seroprevalence levels observed in wild suids. This trend is coherent with the hypothesis that wild boar and organically/free ranged pigs get infected mainly from an oocyst-contaminated environment by rooting and/or by consumption of infected rodents, whereas intensively bred pigs are not exposed to an oocyst-contaminated environment or to infected rodents and appear to be less seropositive (Djokic *et al*. 2016). Risk factors associated with high *T. gondii* seropositivity in pigs are the presence of cats, unhygienic living conditions and poor management and maintenance of farms (Djokic *et al*. 2016, Ortega-Pacheco *et al*. 2013).It was also observed that where rodent baits were used, *T. gondii* seroprevalence was lower (Herrero *et al.* 2016).

Table 1.5: *Toxoplasma gondii* seroprevalence studies in pigs

Reference	Country	Method	Samples size	Seroprevalence (%)	Year
Gebremedhin *et al*	Ethiopia	DAT	402	32.1	2015a
Onyiche and Ademola	Nigeria	ELISA	302	24.19	2015
Tsai *et al*	Taiwan	LAT	395	10.1	2007
Hove *et al*	Zimbabwe	IFA	474	35.71	2005a
Arko-Mensah *et al*	Ghana	ELISA	641	39	2000
Hove and Dubey	Zimbabwe	MAT	97	9.3	1999

1.5. Public Health Importance of toxoplasmosis in humans

1.5.1. Toxoplasmosis in humans

Toxoplasma gondii is of public health importance in terms of food safety, human and animal health. In people with intact immune systems, *T. gondii,* remains dormant, but in

immunocompromised individuals with AIDS or organ transplantations, it can cause life threatening illness. *Toxoplasma gondii* infection causes abortion in pregnant women as well as mental retardation and blindness in congenitally-infected children (Dubey & Jones 2008). It can be acquired after birth or congenitally and is associated with foodborne illness and deaths (Dubey 2010b, Scallan *et al.* 2011). Undercooked meat particularly lamb, pork, and venison, containing tissue cysts and vegetables, fruits, water and soil contaminated with oocysts are the main sources of foodborne transmission to humans (Dubey 2010b, Tenter *et al*. 2000). Unpasteurized raw goat milk and goat cheeses could also be a sources of *T. gondii* infection (Jones *et al.* 2009, Dubey 2010b). Toxoplasmosis is one of the most reported parasitic zoonoses in humans in the European Union, however, there is little known about it in Africa and its overall burden is severely underestimated (Olivier *et al.* 2007).

1.5.2. Prevalence of toxoplasmosis in humans in South Africa

There is scarcity of data on the prevalence of *T. gondii* in humans in South Africa. Historically, in the Eastern Cape, formerly the Transvaal, a human seroprevalence of 37% was reported in 1974 by Mason *et al,* while Jacobs & Mason (1978) reported a nationwide seroprevalence of 20%. The seroprevalence of *T. gondii* in pregnant women, HIV positive and negative people was found to be 6.4%, 9.8% and 12.8% respectively in a study conducted in South Africa (Kistiah *et al.* 2012). *T. gondii*-related complications may occur in immunocompromised individuals resulting in death from encephalitis, pneumonitis and myocarditis (Eza & Lucas 2006, Saadatnia & Golkar 2012).

1.6. Diagnostic tests and diagnosis of toxoplasmosis

The diagnosis of *T. gondii* infection is important for the control and prevention of toxoplasmosis in both humans and animals and can also be used to aid in surveillance programs. Laboratory diagnosis has been performed using various approaches namely, serological, immunological, etiological, imaging and molecular techniques (Liu *et al.* 2015). Serological methods have been used worldwide to determine *T. gondii* prevalence and they are generally highly sensitive, simple and convenient (Tenter, Heckeroth & Weiss 2000). Toxoplasmosis can be diagnosed serologically by several tests namely, the Sabin-Feldman dye test (Sabin & Feldman 1948), latex agglutination test (Beverley, Freeman & Watson 1973), indirect latex agglutination test (Tsubota *et al.*

1977), hemagglutination test (Jacobs & Lunde 1957), enzyme linked immunosorbent assay (Hughes 1985) and fluorescence particle immunoassay tests (Arthur & Blewett 1988). In response to *T. gondii* infection, IgA antibodies are produced first, followed by IgM antibodies both of which are detectable for several months. IgG antibodies are used to indicate occurrence of infection, while IgE antibodies are used in the timing of a current infection (Liu *et al.* 2015).

2.6.1. Sabin–Feldman dye test (DT)

Albert Sabin and Harry Feldman (1948) developed the dye test, which constitutes one of the greatest advancements in the diagnosis of toxoplasmosis. This serological test is used in humans as the gold standard for the detection of anti-*T. gondii* antibodies and the test is highly specific and sensitive(Sabin & Feldman 1948, Reiter-Owona *et al.* 1999). The dye test is based on the cytolysis of live *T. gondii* tachyzoites coated with antibody via complement (Sabin & Feldman 1948). The dye test was the first to be used to detect low levels of specific antibodies to *T. gondii* and to differentiate the timing of infection. It has been used as a screening test for *T. gondii* infection in the veterinary field. However, it has proved to be unreliable in avian and cattle samples (Tsubota *et al.* 1977, Dubey *et al.* 1985, Dubey *et al.* 1993). The major disadvantage of this test is that it requires a high level of technical expertise, live parasites and can only be performed in reference laboratories (Ashburn *et al.* 2001).

1.6.2. Indirect fluorescent antibody test (IFAT)

This serological test is used for the detection of IgM and IgG antibodies in humans and animals. However, species-specific conjugates are necessary (Shaapan, El-Nawawi & Tawfik 2008). Fluorescent anti-antibodies are added after incubation of acetone fixed *T. gondii* tachyzoites with sera from test samples. IFAT shows sensitivities between 80.4% and 100 % and specificities ranging from 91.4% to 95.8 % (Shaapan *et al.* 2008, dos Santos *et al.* 2010). The test has numerous advantages as it is relatively affordable and multi-species fluorescent-labelled antibodies are commercially available (Liu *et al.* 2015). Its disadvantages are that a skilled individual is required to read and interpret the results with the aid of a fluorescence microscope and there is also a possibility of cross-

reactivity with anti-nuclear antibodies and rheumatoid factor (Balfour *et al.* 1982, Filice *et al.* 1983).

1.6.3. Enzyme-linked immunoabsorbent assay (ELISA)

This test method can detect *T. gondii* specific immunoglobulins and antigens. It uses the reaction between antigen and antibody, which is amplified by adding a secondary enzyme-linked antibody and the resultant colour absorbance, is measured by a reader (Sudan, Jaiswal & Shanker 2013). The procedure can be used in the field since it is economical and simple to perform (Anthony, Christensen & Johnson 1980, Liu *et al.* 2015). The ELISA methods have been used successfully for screening programs since they are highly sensitive. This test has shown a sensitivity of 90.1% and specificity of 85.9% in sheep while in pigs, it showed a sensitivity and specificity of 98% and 88.6% respectively (Gamble, Dubey & Lambillotte 2005, Shaapan *et al.* 2008).

1.6.4. Modified agglutination test (MAT)

Fulton and Turk (1959) first described this test, which uses formalin fixed *T. gondii* tachyzoites to detect antibodies. The test has a low sensitivity and specificity due to the binding of non-specific IgM to the surface of *T. gondii* cells (Macrì *et al.* 2009, Zhu, Cui & Zhang 2012). Since the test does not require species-specific reagents, it can be used in different animal species (Dubey & Jones 2008). In addition to this, MAT can be used in the screening of slaughtered sheep by testing cardiac fluids (Villena *et al.* 2012). In a study in pigs in the United States, the test showed a sensitivity of 82.9% and a specificity of 90.29% for the detection of *T. gondii* IgG antibodies (Dubey *et al.* 1995b). Other studies in sheep in Egypt showed a higher sensitivity and specificity for this test; 96% and 88.9%, respectively (Shaapan *et al.* 2008).

1.6.5. Latex Agglutination Test (LAT)

The latex agglutination test is simple and easy to use and detects IgG antibodies against *T. gondii*. Agglutination occurs in the presence of positive test sera when latex particles coated with antigen are added. The LAT has a sensitivity of 91.4% - 95.8 % and specificity of 96.2% - 97.2% in humans (Kobayashi *et al.* 1977, Balfour *et al.* 1982, Holliman 1990). The test does not require species-specific conjugates and has been used in different animal species. The test has been used in epidemiologic surveys as a screening test in a variety of animal species and has been widely used, especially in developing countries, because of its high specificity and sensitivity in humans and also due to the fact that it is simple and convenient to use (Murata 1989, Holliman 1990, Shahiduzzaman *et al.* 2011, Kyan *et al.* 2012, Giangaspero *et al.* 2013). Studies on test sensitivity and specificity in sheep are scarce. However, one study in sheep in Turkey reported a low sensitivity and specificity of 78.6 % and of 61.9 % respectively (Öncel *et al.* 2005). In pigs, it showed varied sensitivities of 45.9% to 47.2% and specificities of 91.4% to 96.9% respectively (Dubey *et al.* 1995b, Sroka *et al*. 2008). As a screening test in a study on humans by Balfour (1982) the Toxoreagent (Eiken) latex agglutination test showed 96.6% agreement with the Sabin-Feldman dye test**.** Kobayashi *et al.* (1977) reported a comparable level of agreement of 94.4% between the same tests. A commercially available latex agglutination diagnostic kit for use in animals and humans, Toxoreagent, has been used extensively for fundamental research in animals as shown in Table 1.6 (Samad 1992, Thiangtum *et al.* 2006). Though LAT requires a long incubation period of at least 12 hours, it provides qualitative and semi quantitative results (Tsubota *et al.* 1977).

Table 1.6: Studies in which LAT was used for detection of *T. gondii* in various animals

Reference	Country	Animals	Year
Hussien *et al*	Sudan	Chickens	2016
Oi *et al*	Japan	Dogs and cats	2015
Saqib *et al*	Pakistan	Horses	2015
Swai and Kaaya	Tanzania	Goats	2012
Shahiduzzaman *et al*	Bangladesh	Goats and sheep	2011
Tsai *et al*	Taiwan	Pigs	2007

1.6.6. Indirect hemagglutination test (IHAT)

The principle of the indirect hemagglutination test (IHAT) is agglutination of *T. gondii* sensitized red blood cells by positive sera (Dubey 2010b). An IHAT that used sensitized sheep red blood cells was both sensitive and specific as described by different authors (Jacobs & Lunde 1957, Jennis 1966, Welch *et al.* 1980). Dubey *et al*. (1995b) however, reported a very low sensitivity of 29.4% and high specificity of 98.3% in sows. This rapid test is simple to use and advocated for use in mass screening in epidemiologic studies (Caruana 1980). The test has a few disadvantages, as it can fail to detect acute and congenital infections and is nonspecific in the detection of lower titre antibodies (Eissa *et al.* 1990, Dubey 2010b).

1.6.7. Detection of *Toxoplasma gondii* DNA

Development of polymerase chain reaction (PCR)-based tests for the detection of *T. gondii* DNA from several tissues such as skeletal muscle, heart muscle, neural tissue and placenta has been done. The main gene targets used in the tests are the 18S ribosomal DNA, B1 repetitive sequence and P30 gene (Pelloux *et al.* 1996). PCR-based tests are good diagnostic aids when used in conjunction with other tests (Sudan, Jaiswal & Shanker 2013). These PCR-based tests have been used in the diagnosis of clinical cases of toxoplasmosis and have an almost 100% specificity and moderate

sensitivity, mainly due to the difficulty in DNA extraction from samples (Afonso, Thulliez & Gilot-Fromont 2006).

CHAPTER 2. MATERIALS AND METHODS

2.1. Description of study areas

2.1.1. Location

A cross-sectional study was conducted in the O.R Tambo District, in the Eastern Cape in South Africa, which covers a total area of 12 096 km^2 and is located 31^0 34' S and 28^0 46' E. The study area was divided along four state veterinary areas or municipalities namely ; Port St Johns/Nyadeni , Mhlontlo, King Sabata Dalindyebo and Ingquza Hill. The district is mainly rural with only 9.3% of the population living in the urban areas.

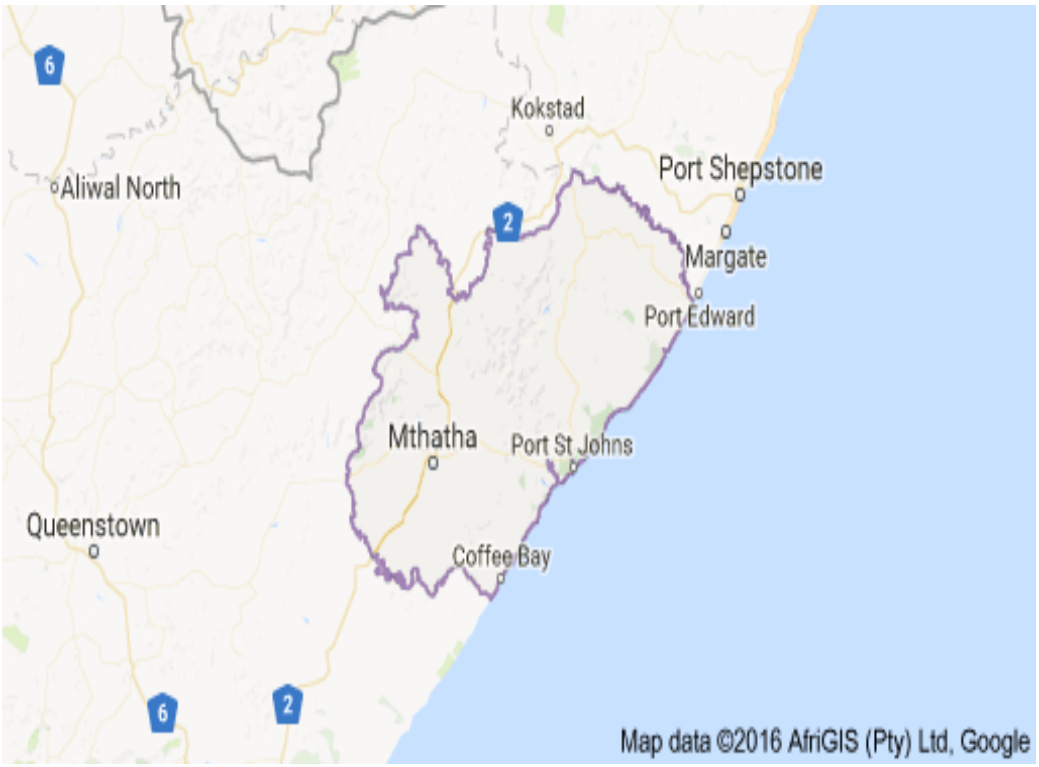

Figure 2.1. Location of O.R Tambo District

2.1.2. Climate

Some of these study areas within the district have different climatic and environmental patterns. The climate is highly varied and the district has two main types of agro-ecological zones which are subtropical and steppe (arid) as shown below:

Table 2.1: Temperatures and rainfall for the Eastern Cape Province (National Weather Service Climate Prediction Center 2016)

Agro-Ecological Zone	Mean rainfall (mm)		Mean temperature (^{0}C)	
	Summer	Winter	Summer	Winter
Steppe (Arid)	400 - 600	100 - 200	21	14
Sub-tropical	800	200	23	15

2.2. Animal management systems in study area

2.2.1. Livestock production systems in study area

The main type of livestock (cattle, sheep and goats) production system in the district is sedentary grazing. Mostly consists of over grazed rural areas where animals collectively graze on pasture owned by government and are enclosed in pens overnight. In the urban and peri-urban areas, semi-intensive farming is practiced and is characterized by overpopulation of animals living under unhygienic conditions. Sheep and goat farming is very popular in the region which has the highest population of sheep (6.9 million) and goats (2.2 million) in South Africa (Department of Agriculture, Forestry and Fisheries 2015).

2.2.2. Pig production systems in study area

The district is mainly characterized by backyard pig farming which is either semi-intensive or extensive free range. These free range pig production is characterised by pigs being raised in non-confinement systems and being free to scavenge around. These pigs are free to roam during the day and are placed in unhygienic pens at night for safe keeping. Backyard pigs have little access to drinking water and are sometimes

fed swill when it is available. A smaller percentage of the pigs in the district are reared commercially, in total confinement, under good hygienic conditions and have access to safe and adequate drinking water

2.2.3. Chicken production system in study area

Most households in the district own backyard chickens that are allowed to scavenge and roam freely around the homesteads during the day and are enclosed at night. Chickens under this traditional backyard farming system are exposed to various parasitic infections including, *Toxoplasma gondii* (Mtileni *et al.* 2009). A few farmers keep chickens under small scale, semi intensive systems, where the hygiene and management are much better.

2.2.4. Cat management in study area

Most domestic cats in the district live indoors and like strays are allowed to roam outdoors, where they hunt for food or live on scraps of garbage.

2.3. Study design and sample size.

The Eastern Cape Department of Rural Development and Agrarian Reform livestock census figures are given in Table 2.2. Sample size determination for detection of disease for each of the five species under study, was calculated using an expected disease prevalence threshold of 3% with a confidence of 95%. The formula for sample size determination is:

$$n = \log \alpha / \log (1 - p)$$

where n is sample size, α is 1 – confidence and p is the prevalence (Dohoo, Martin & Stryhn 2009). Thus at least 100 samples per species (cats, chickens, goats, pigs and sheep) were collected. To avoid any design effect resulting from intra-cluster correlation only one sample from each species was taken from each household. The inclusion criteria for the different animal species sampled were animals present at routine community visits by veterinary officials during primary animal health campaigns.

Table 2.2: Livestock Numbers in O.R Tambo District (Department of Agriculture, Forestry and Fisheries 2015).

Area	Municipality	Sheep	Goats	Pigs	Poultry	Cats
Qumbu	Ntabankulu	51 272	79 920	433	10 670	1 206
	Mhlontlo	306 361	110 513	1 223	62 818	366
Ingquza Hill	Mbizana	48 872	76 842	62	39 024	500
	Qaukeni	76 825	84 686	927	15 154	1 065
Port St Johns / Nyadeni	Port St Johns	16 711	28 683	7 692	7 692	1 084
	Nyandeni	178 784	81 452	33 764	33 764	2 110
Mthatha	King Sabata Dalindyebo	775 302	161 728	59 451	59 451	2 326
Total		**1 454 127**	**623 824**	**13 398**	**228 573**	**8 657**

2.3.1. Questionnaire survey

Data and sample collection occurred between June and September 2016. A questionnaire survey was conducted during sample collection by interviewing animal owners and/or herders so as to assess risk factors for toxoplasmosis. Risk factors assessed included location of household, climate, sex, age, flock size, presence of rodents, presence of cats (domestic and/or feral), grazing distance from household, type of animal management system, type of feeding regime, time spent in pasture, sources of drinking water and biosecurity. Determination of animal age was based on herders' information and confirmed by dentition, animals less than 1 year, were classified as juveniles while those older than one year were classified as adults based on the presence or absence of deciduous teeth (Shively 1985).

2.3.2. Blood collection and serum preparation

Venous blood was collected in plain serum tubes from each of the animals in the study. The jugular vein was used in sheep, pigs and goats, the cephalic vein in cats and the ulnar vein in chickens. Depending on the weight and age of the animal at least 3 ml of blood was collected from each animal and placed on ice, till it reached the laboratory. After overnight refrigeration, serum was separated by centrifugation at 2300 rpm for 20 minutes and stored at -20 °C in eppendorf tubes until analysis.

2.3.3. Latex Agglutination Test (Toxoreagent® RST701)

For this particular study, detection of *T. gondii* antibodies was done using a commercial latex agglutination test (LAT), (Toxoreagent® RST701). The assay was conducted according to the LAT, Toxoreagent®, Mast Group, United Kingdom manufacturer's instructions. All collected animal serum samples were tested for IgG antibodies against *T. gondii.* Each serum sample was diluted to obtain serial 2-fold dilutions from 1:16 to 1:1024. Agglutination at 1:64 or higher was regarded as positive according to the LAT kit instructions except in chickens where titres of 1:32 was regarded as positive (Zia-Ali *et al*. 2007, Hussien *et al.* 2016). The commercial LAT allows for qualitative and quantitative assessment of antibodies against *T. gondii* in collected sera. The test kit is supplied with a buffer, a freeze-dried positive control, latex suspension sensitised with a *T. gondii* antigen (Kobayashi *et al.* 1977). The test kit has a shelf life of almost a year when stored under the right conditions. Once reconstituted, the positive control is stable for two weeks only.

2.3.4. Latex Agglutination testing procedure

The test was performed in U-shaped microtitre plate wells. Each serum sample was first diluted 1:8, by adding 350 microliters of buffer supplied to 50 microliters of serum. Then, each sample was double diluted serially in a microtitre plate from 1:16 up to 1:1024. The double dilutions were carried out, using a pickup volume of 25 microliters and adding it to 25 microliters of buffer already in the microtitre plates. Finally, 25 microliters of a suspension containing, *Toxoplasma gondii* coated latex particles were added to each well. After mixing, the plates were covered and incubated at room temperature for at least 12 hours and negative test samples were indicated by formation of a compact

button while positive test samples showed agglutination. Positive and negative controls were run with each batch of test sera.

2.3.5. Determination of cut-off titres

The endpoint or titre was the highest dilution displaying a definite agglutination pattern. To determine whether the same titre (1:64) for determining positivity as indicated by the manufacturer's instruction for cats and pigs and by other studies (Ohshima, Tsubota & Hiraoka 1981, Kamani *et al.* 2010) was also acceptable for goats and sheep (Kyan *et al*. 2012, Rahman *et al.* 2014) and a titre of 1:32 or lower was acceptable for chickens (Zia-Ali *et al*. 2007, Hussien *et al.* 2016), an extensive literature review was conducted.

2.3.6. Data management and statistical analysis

The data generated was stored in Excel Spreadsheet and analysed using Studio R version 11.0 for Windows (R Core Team 2013). Maps were generated with R software using the package "RgoogleMaps". Prevalence estimates were adjusted for a test sensitivity of 94.2% and a specificity of 96.6% reported by Holliman (1990) in a study in humans, using the formula below:

$$TP = (AP + Sp - 1) / (Se + Sp - 1),$$

where TP = true prevalence, AP = apparent prevalence, Se = test sensitivity and Sp = test specificity (Rogan & Gladen 1978).

Confidence intervals (CI) were calculated using the following formula according to Dohoo *et al.* (2009)

$$CI = TP +/- Z_{\alpha/2}\sqrt{\frac{TP(1-TP)}{n}}$$

where TP is the true prevalence, n the sample size and $Z_{\alpha/2}$ is the value of Z from the normal law for an accepted risk α (in our case α=0.05 thus $Z_{\alpha/2}$ =1.96).

Depending on the nature of the variables, the Chi-Square test or the calculation of the odds ratio with 95% confidence intervals (CI 95%) confirmed by the Fisher's exact test was used to quantify the association between *T. gondii* seroprevalence and potential risk factors. Some of the important variables were categorized during data analysis, as follows; location (municipality), type of climate (subtropical, steppe), sex (male, female), age (juvenile, adult), animal management system (extensive, intensive), biosecurity (no fence, one fence, double fence), source of drinking water (dam, river, tap), presence of cats (domestic and/or feral), percentage of day spent on pasture, grazing distance from household (< 5 km, > 5 km), and also presence or absence of rodent control, cat-feed contact, rodent-feed contact and rodent-animal contact. The significance level was established at $p < 0.05$. The Moran Index calculation which measures the potential autocorrelation between points according to their value (either 0 or 1) was used to confirm spatial risk assumed by seropositivity associated to specific municipalities taking into account the spatial coordinates of the sampled households.

2.3.7. Ethical considerations

The animal ethics committee for animal experimentation at the University of Pretoria, South Africa, reviewed and approved the research proposal under ethical clearance number V067-16. Serum samples were collected by the researcher, veterinarian (Dr W.M. Tagwireyi) in accordance with the guidelines on research and testing of animals. The study was conducted with the assistance of the Eastern Cape Department of Rural Development and Agrarian Reform.

CHAPTER 3. RESULTS

3.1. Seroprevalence and geographical repartition

3.1.1. Seroprevalence in cats

Serum samples were collected from 109 cats and 35 (32.11%) tested positive for *T. gondii*. The number of cats per household ranged from 1 to 10 cats (mean 2, median 2). Amongst the 35 positive samples, 7 (20%) had a titre of 1:64, 10 (28.57%) had a titre of 1:128, 13 (37.14%) had a titre of 1:256 and 5 (14.29%) had a titre of 1:512. The overall cat seroprevalence in the district was 31.61% (95% CI: 21.97%, 41.27%).

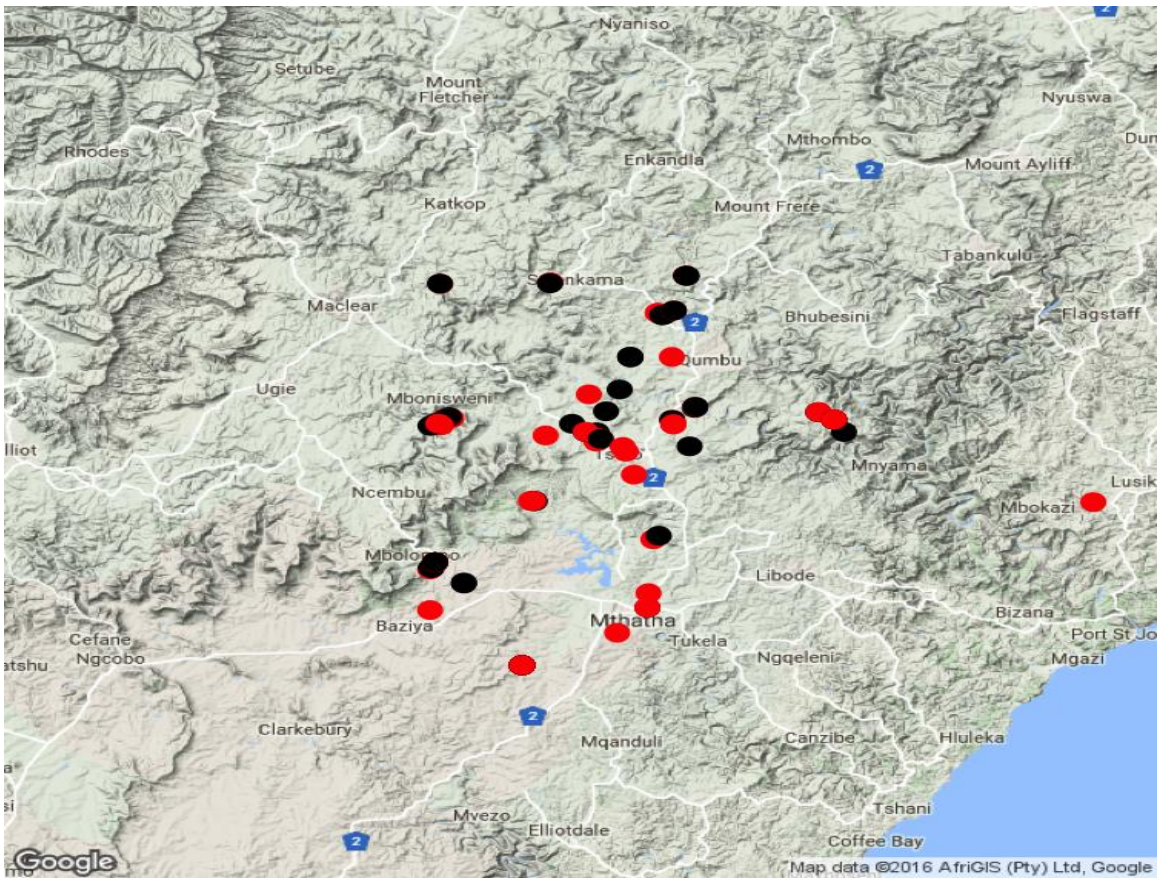

Figure 3.1: Geographic distribution of sampled house-holds with cats (red dots indicate positive test results and black dots indicate negative test results).

3.1.2. Seroprevalence in chickens

Forty six out of 137 chickens (33.58%) were seropositive for the parasite using a cut-off titre of 1:32. The number of chickens per household ranged from 2 to 45 chickens (mean17, median15). Of all the samples tested, 21 (15.33%) had a titre of 1:16, 25 (18.25%) had a titre of 1:32, 11 (8.03%) had a titre of 1:64, 7 (5.12%) had a titre of 1: 128, 2 (1.46%) had a titre of 1:256 and 1 (0.73%) had a titre of 1:512. The overall chicken seroprevalence in the district was 33.24% (95% CI: 24.53%, 41.95%).

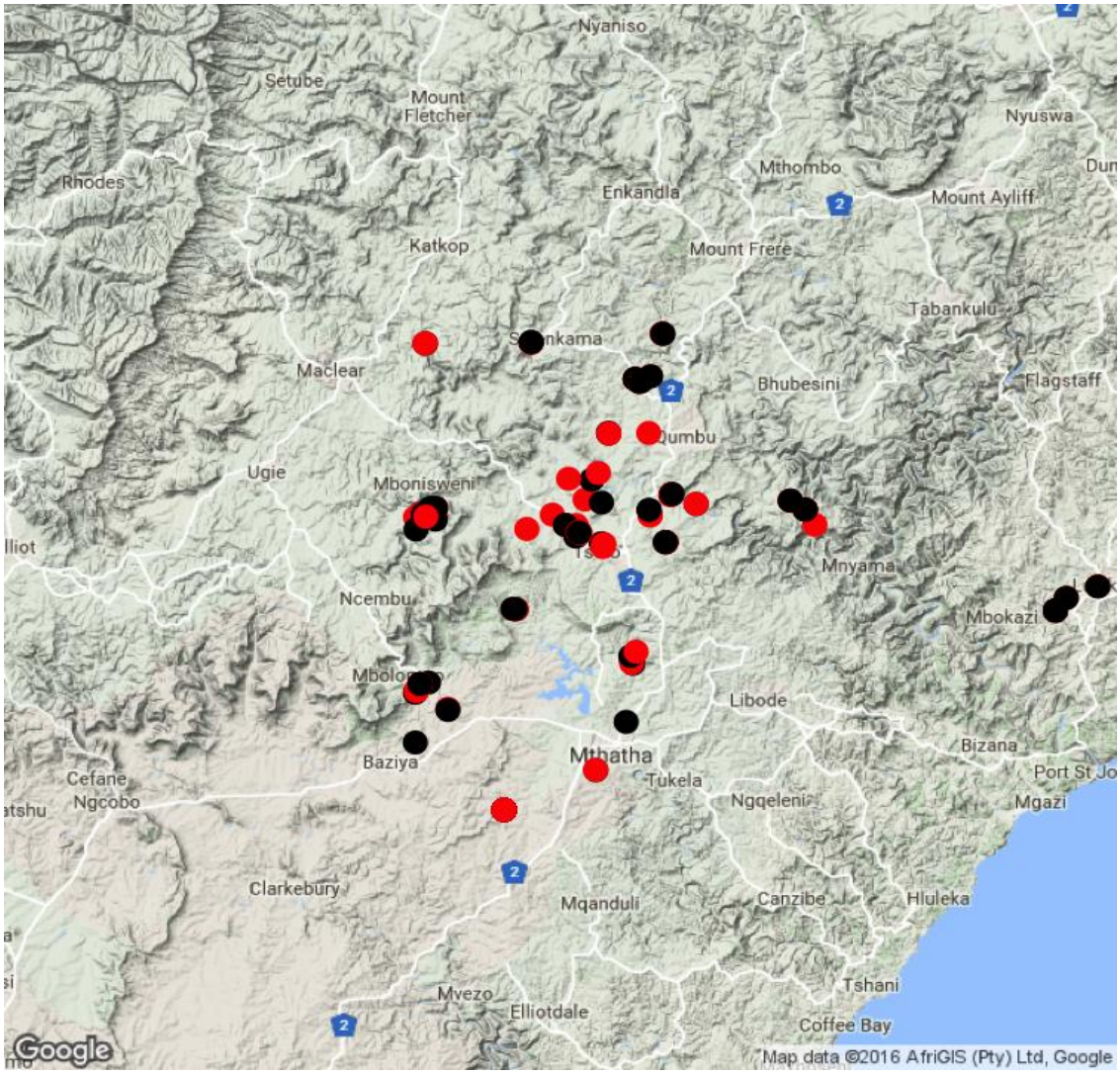

Figure 3.2: Geographic distribution of sampled house-holds with chickens (red dots indicate positive test results and black dots indicate negative test results).

3.1.3. Seroprevalence in goats

The study revealed that 69 out of 128 goats (53.91%) were seropositive for the parasite. The number of goats per household ranged from 2 to 105 goats (mean 21, median18). Thirteen (10.16%) had a titre of 1:16, 30 (23.44%) had a titre of 1:32, 33 (25.78%) had a titre of 1:64, 16 (12.5%) had a titre of 1: 128, 18 (14.06%) had a titre of 1:256 and 2 (1.56%) had a titre of 1:512. The overall goat seroprevalence in the district was 55.62% (95% CI: 46.11%, 65.14%).

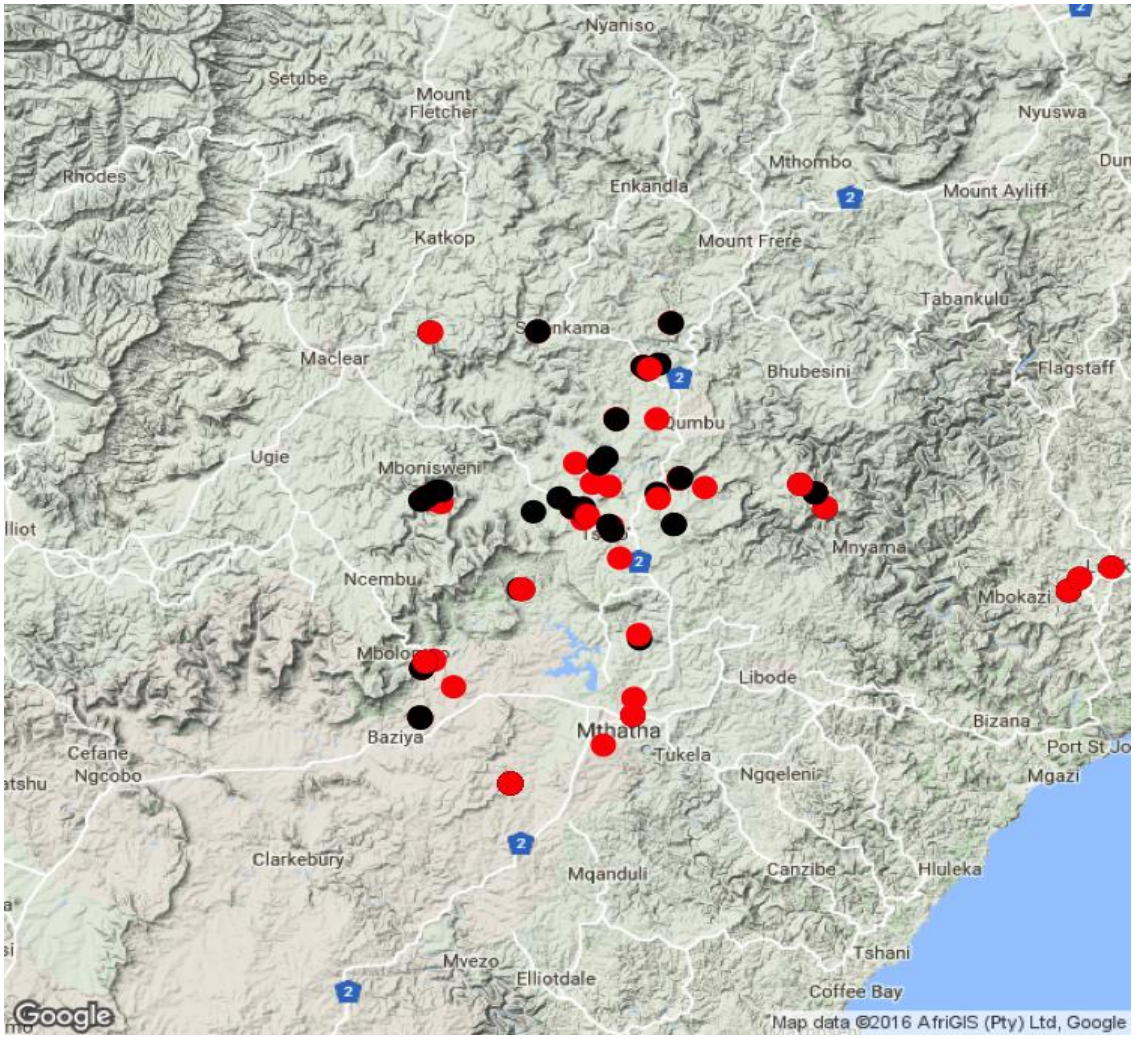

Figure 3.3: Geographic distribution of sampled house-holds with goats (red dots indicate positive test results and black dots indicate negative test results).

3.1.4. Seroprevalence in sheep

The study revealed that 78 out of 121 sheep (64.46%) tested positive for *T. gondii*. The number of sheep per household ranged from 2 to 880 sheep (mean 60, median 45). Nine (7.43%) sheep had a titre of 1:16, 19 (15.70%) had a titre of 1:32, 29 (23.97%) had a titre of 1:64, 19 (15.70%) had a titre of 1: 128, 23 (19.01%) had a titre of 1:256 and 7 (5.79%) had a titre of 1:512. The overall seroprevalence for sheep in the district was 67.25% (95% CI: 57.85%, 76.64%).

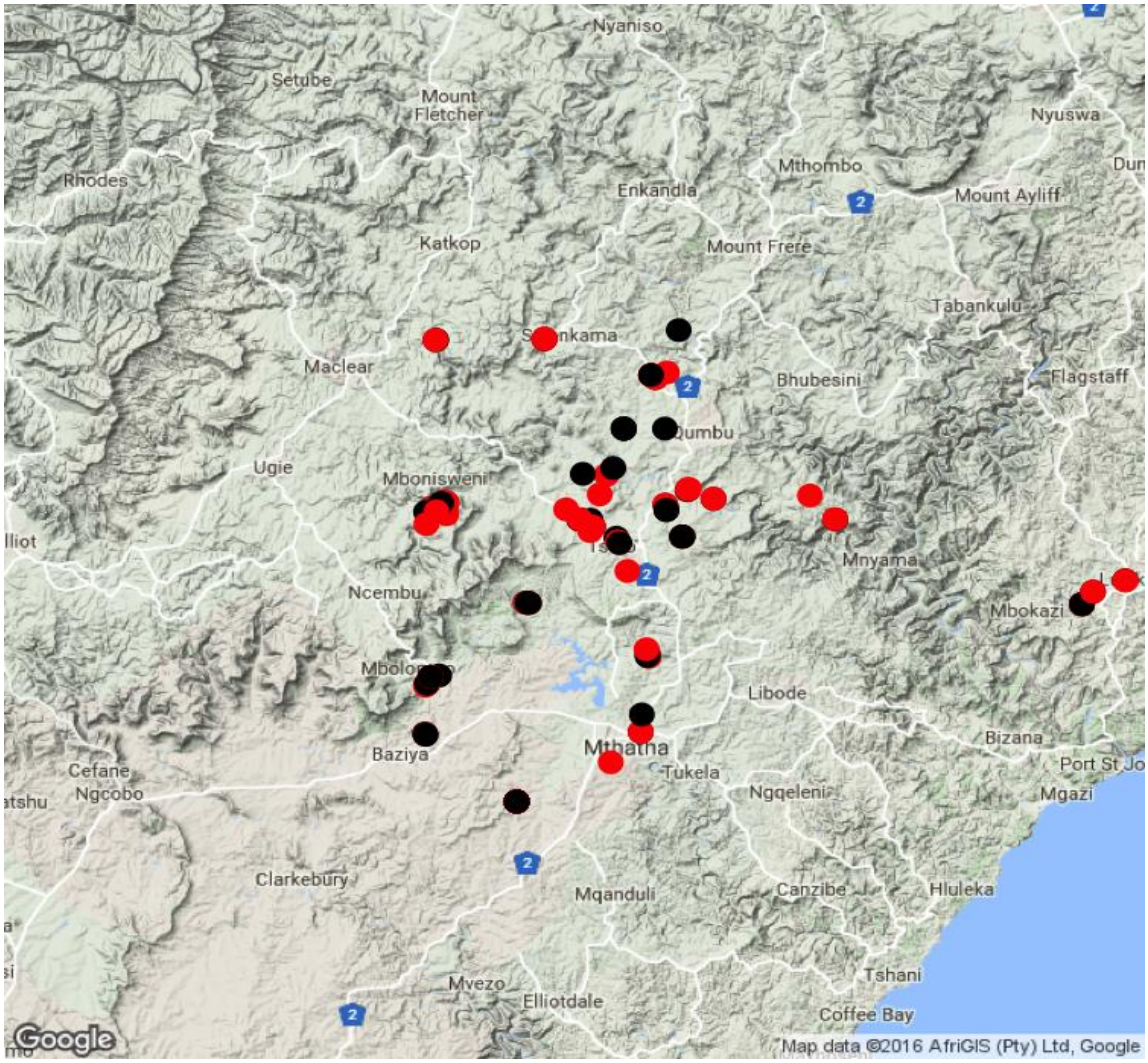

Figure 3.4: Geographic distribution of sampled house-holds with sheep (red dots indicate positive test results and black dots indicate negative test results).

3.1.5. Seroprevalence in pigs

Thirty six out of 106 pigs (33.96%) were seropositive for *T. gondii*. The number of pigs per household ranged from 1 to 40 pigs (mean 5, median 4). Amongst the 36 positive samples, 20 (55.56%) had a titre of 1:64, 9 (25%) had a titre of 1:128, 6 (16.66%) had a titre of 1:256, 1 (2.78%) had a titre of 1:512. The overall seroprevalence for pigs in the district was 33.65% (95% CI: 23.73%, 43.59%).

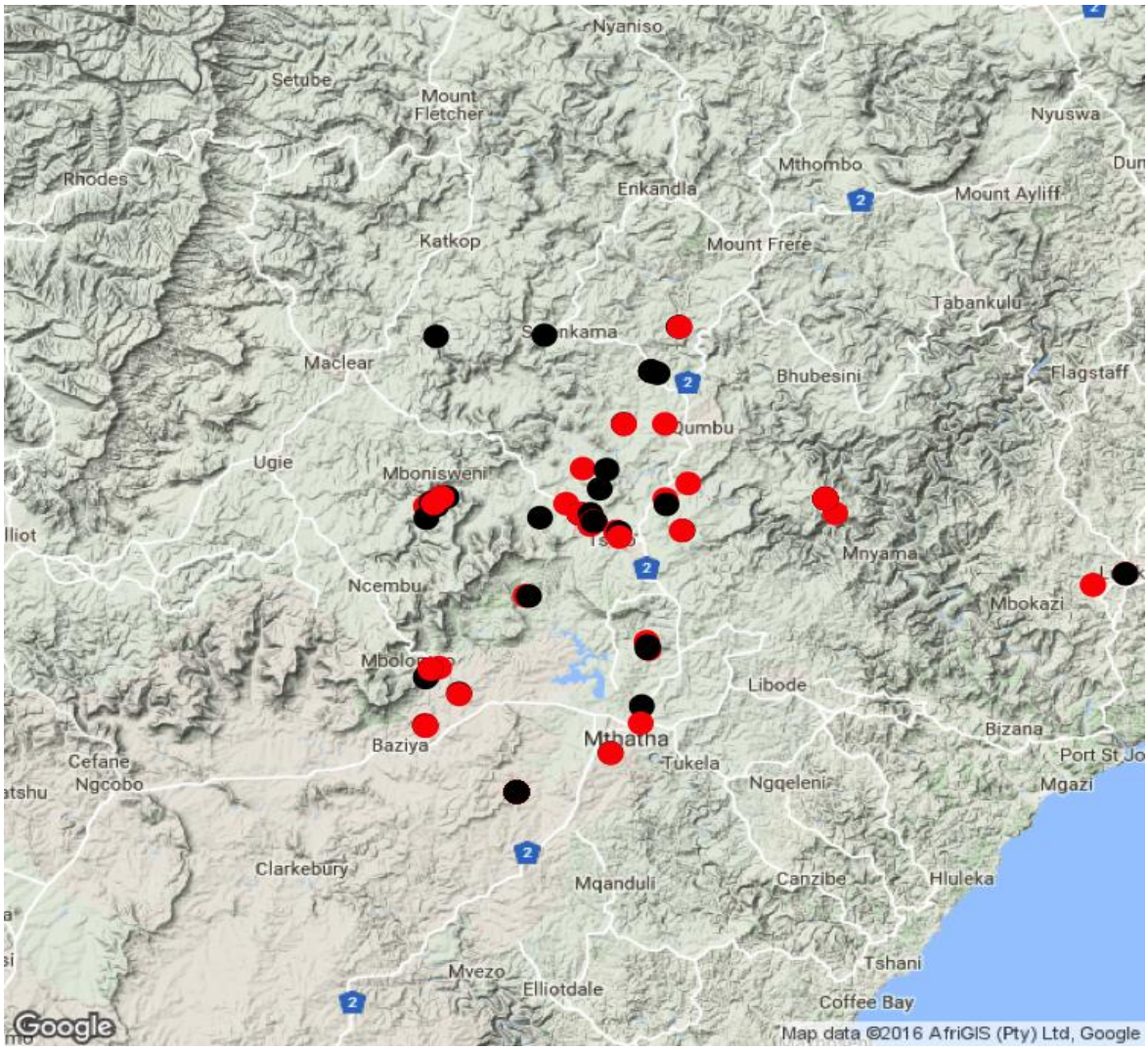

Figure 3.5: Geographic distribution of sampled house-holds with pigs pigs (red dots indicate positive test results and black dots indicate negative test results).

3.2. Risk factors for *Toxoplasma gondii* infection in different animal species

3.2.1. Risk factors in cats

In the bivariate analysis of potential risk factors for cats, age was statistically significant with an odds ratio of 3.43, $CI_{95\%}$[1.36 – 9.23]. Seropositivity for *T. gondii* increased with age from 41.89% for cats that were younger than 1 year old, to 71.43% for cats that were older than 1 year (Table 3.1).

Table 3.1: Seropositivity for *Toxoplasma gondii* in cats according to animal age.

	Number of cats		Percentage of Cats	
Age of Cat	**Positive**	**Negative**	**Positive**	**Negative**
< 1 year	31/74	48/74	41.89%	64.86%
> 1 year	25/35	10/35	71.43%	28.57%

3.2.2. Risk factors in chickens

Bivariate analysis of potential risk factors for chickens revealed that the type of chicken management system (extensive small scale and intensive small scale) were statistically significant. Chickens reared under intensive small scale conditions were 2.9 times $CI_{95\%}$[1.2 – 7.3] more likely to be seropositive for *T. gondii* infection while chickens under extensive small scale conditions were 2.9 times, OR= 0.34, $CI_{95\%}$[0.14 – 0.85], less likely to acquire infection.

3.2.3. Risk factors in goats

Toxoplasma gondii seroprevalence in goats was found to be statistically significant (p= 0.001374) to the geographical distribution (municipality).. The lowest *T. gondii* seroprevalence was seen in Mhlontlo municipality (43.75%), which increased to 63.16 % in King Sabata Dalindyebo municipality and was at its highest with100% in Port St Johns municipality.

3.2.4. Risk factors in sheep

Seroprevalence of *T. gondii* in sheep was found to be statistically significant to the geographical distribution (municipality), age, cat-feed contact and rodent control (Table 3.2). King Sabata Dalindyebo municipality had the highest seroprevalence of 81.58%, followed by Port St Johns municipality that had a seroprevalence of 70% and lastly Mhlontlo municipality had the lowest seroprevalence of 54.79%. Seropositivity for the parasite in sheep increased with age from 74.42%, for sheep younger than 1 year, to 91.03% for sheep that were older than 1 year. Households which reported cats having access to animal feed had a higher risk for toxoplasmosis compared with households that did not report cats having access to animal feed. *Toxoplasma gondii* infection was higher in households that had rodent control than those that did not have any rodent control.

Table 3.2. Risk factors for the presence of *Toxoplasma gondii* in sheep

Factors	Number of animals positive	Chi Squared test p value	Fisher's test p value	Odds ratio CI 95%
Age				
<1 year	32/43 (74.42%)	_	0.01797	3.447423
>1 year	71/78 (91.03%)			(1.10-11.50)
Municipality				
Mhlontlo municipality	40/73 (54.79%)	0.01857	-	-
King Sabata Dalindyebo municipality	31/38 (81.58%)			
Port St Johns municipality	7/10 (70%)			
Cat-feed contact				
Yes	47/78 (60.26%)	_	0.03669	2.302259
No	17/43 (39.50%)			(1.01-5.34)

3.2.5. Risk factors in pigs

Three risk factors for pigs were statistically significant as presented in the Table 3.3. Pigs from steppe (arid) climate had slightly higher seroprevalence of 52.8% as compared to those from subtropical climate (47.2%). Pigs in households that reported that cat faeces were covered up with soil were 5 times, OR=0.21 $CI_{95\%}$[0.06-0.72], less likely to test positive to *T. gondii* while those households that reported that cat faeces were left out in the open environment were 5.2 times $CI_{95\%}$[1.22-26.6] more likely to be positive for *T. gondii*.

Table 3.3. Risk factors for the presence of *Toxoplasma gondii* in pigs

Factors	Percentage of animals positive	Chi Squared test p value	Fisher's test p value	Odds ratio CI 95%
Climate				
Steppe (Arid)	52.8%	-	0.01797	3.37
Subtropical	47.2%	-		[1.35–8.89]
Cat faecal disposal				
Covered with soil	24.5%	0.008998	0.008537	0.2124281 [0.06-0.72]
Left in the environment	66.7%	-	0.01625	5.20189 [1.22-26.60]

3.2.6. Moran Index calculation

For goat and sheep the Moran Index was significant whereas for other animals it was not. Hence Chi-Square test statistic is confirmed taking into account the spatial coordinates of the sampled farms revealing a real spatial clustering of the positive cases in sheep and goats.

CHAPTER 4. DISCUSSION AND CONCLUSION

4.1 Discussion

The direct detection of the parasite, *Toxoplasma gondii* for the diagnosis of infection is rarely achievable and most clinical laboratories utilize serological tests (Tenter *et al*. 2000). Although the diagnostic method of choice is still the Sabin-Feldman dye test (Sabin & Feldman 1948), the LAT offers a good alternative in performing this serological survey. The latex agglutination test should, however, be considered as a screening test for *Toxoplasma gondii* because of its low specificity in animals. It should be used with caution in areas with low prevalence, as the number of seropositives is likely to be related to false positives. This means that in the case of low prevalence the positive predictive value is lower compared to the one in high prevalence areas (Xu *et al.* 2012).

Although a lot of animal studies have been conducted using the LAT kit, not much is known about its validity for the detection of *T. gondii* antibodies in animals except in cats and pigs. Even so, in these species and others, there is little or conflicting information on the sensitivity and specificity of this test kit. Higher specificities of 91.4% and 96.9% and lower sensitivities of 45.9% and 47.2% were reported by Dubey *et al.* (1995b) and Sroka *et al.* (2008) in pigs. However, Öncel *et al.* (2005) reported the inverse of this, a higher sensitivity of 78.6% and lower specificity of 61.9% in sheep. On the basis of lack of reliable information on test sensitivity and specificity in animals and the resultant urgent need to validate this test kit in most animals. The sensitivity and specificity reported by Holliman (1990) in a human study were used for the current study. These have been validated by numerous human studies, which gave similar results (Kobayashi *et al.* 1977, Balfour *et al.* 1982). Determination of the cut-off titre also presented a challenge as the 1:64 titre was only recommended for use in cats and pigs by the test kit manufacturer. The latex agglutination test does not require species-specific conjugates and can be used in any animal without alteration, since it is based on the agglutination of particles in the presence of IgG antibodies against *T. gondii* (Packham *et al.* 1998, Macrì *et al.* 2009). This strongly suggests that the criteria used for humans, cats and pigs can also be used for other domestic animals to determine seropositivity. Hence, a cut-off titre of 1:64 was used for all animals in the study, except for chickens. Chickens have *T. gondii* antibodies that are detectable for a short time and

produce much lower antibody titres as compared to mammals (Dubey 2010a). Therefore, a cut-off titre of 1:32, which is lower than that recommended for mammals (pigs and cats), was used in chickens.

The results of the study have shown variable seropositivity in different animal species, which is comparable to the findings of other studies in Africa. The seroprevalence reported in sheep (67.25%) in this study is not consistent with observations of previous investigators in South Africa. Abu Samraa *et al.* (2007) reported prevalence of 5.4 % using IFA test and 7.8% using ELISA in the Eastern Cape, while Hammond-Aryee (2015b) reported a prevalence of 8% in the Western Cape using ELISA. The results of the study are, however, similar to those obtained by Hove *et al*. (2005b) in Zimbabwe, where a seroprevalence of 67.7% and an eightfold difference in seroprevalence between sheep from rural areas (80%) and sheep from commercial farms (10%) was reported. This picture fits very well with that observed in O.R Tambo district, where almost all of the sheep sampled were from rural/communal grazing systems with overgrazed pastures. The high seroprevalence could also be attributed to the climatic conditions of the district, which are mild and humid, particularly in the coastal areas, which is conducive for sporulation of oocysts (Dubey *et al.* 1986). Differences between the seroprevalences found in the present study and those from the other two studies by Abu Samraa *et al*. (2007) and Hammond-Aryee, *et al*. 2015b conducted in sheep in South Africa could be due to the differences in diagnostic methods used, geo-climatic conditions and the nature of sampling, where mostly animals destined for slaughter at abattoirs were sampled. This would probably mean that animals used in the two studies were mostly from commercial or semi-commercial farms as opposed to those used in this study which were mainly from rural/communal areas. Rural farmers tend to practise informal slaughter of their animals without meat inspection at their homes.

Seropositivity for *T. gondii* in sheep increased with age from 74.42% in sheep younger than 1 year to 91.03% in sheep that were older than 1 year.Similar trends, have been reported where higher seroprevalances were seen in ewes or rams than in lambs (Dumètre *et al.* 2006, Gebremedhin *et al.* 2013). In this study, households that reported cats having access to animal feed had a higher risk for toxoplasmosis compared with households that did not report cats having access to animal feed. This finding is similar to findings from other studies in which the presence of cats on farms increased

seropositivity (Dubey 2010b, Andrade *et al.* 2013). *Toxoplasma gondii* seroprevalence was recorded in higher percentages in households that had rodent control than those that did not have it. This is unusual as rodent control is thought to decrease *T. gondii* seropositivity. However, in this study, the high density of rodents in the sampled households may have instigated the implementation of rodent control measures, which were mostly biological (cats), increasing the likelihood of contamination of the environment by infective oocysts, which would put the sheep at risk (Lehmann *et al.* 2006).

Goats in the study revealed a seroprevalence of 55.62%, which was higher than reports in neighboring Botswana, where a seroprevalences of 10% using IHAT was reported by Binta *et al* (1998) and one of 30% was reported by Sharma *et al* (2003), using the same method in goats with a history of abortion. The high seroprevalence reported in this study was similar and consistent with results from Zimbabwe, where a seroprevalence of 67.9% in small ruminants (goats and sheep) was reported (Hove *et al*. 2005b). The high seroprevalence in small ruminants could be attributed to the fact that animals sampled in the study were from overgrazed rural/communal areas to which cats have easy access. The highest *T. gondii* seroprevalence for goats was seen in Port St Johns municipality (100%) and Mhlontlo municipality had the lowest seroprevalence (43.75%), while King Sabata Dalindyebo municipality had a seroprevalence of 63.16%. Sheep showed a similar geographical distribution in seroprevalence as that reported in goats. Mhlontlo municipality also had the lowest seroprevalence (54.79%); Port St Johns municipality had one of 70%, while King Sabata Dalindyebo (KSD) municipality had the highest seroprevalence (81.58%). These geographical differences were confirmed by the Moran Index calculation for both goats and sheep. This trend could be due to the fact that both goats and sheep in all these municipalities are under similar production systems. Differences in the geo-climatic conditions of the three municipalities could have contributed to the differences in seroprevalences. Mhlontlo is an entirely inland municipality, while KSD is partially coastal and Port St Johns is mainly coastal. Coastal areas in the region generally have more rainfall and humidity, which is conducive to oocyst sporulation. Infection in small ruminants has been found to be more prevalent in cool and high rainfall areas as compared to hot and low rainfall areas (Van der Puije *et al.* 2000, Hove *et al*. 2005b).

Studies in feral cats in South Africa, using IFAT, reported a seroprevalence of 37.1% and another study in Egypt, using MAT, reported a seroprevalence of 97.4% (Al-Kappany *et al.* 2010, Hammond-Aryee *et al.* 2015a). This study reported a seroprevalence of 31.61%, which is comparable to the value reported by Hammond-Aryee (2015a) in the Western Cape (37.1%). The similarity in the two prevalences could be attributed to the fact that domestic cats in rural areas behave like feral cats as they are free to roam about, generally fending for themselves, hunting for food (rodents) and sometimes receiving leftovers from people. These cats may contaminate the environment with *T. gondii* oocysts and expose both humans and animals to infection (Dubey 2010b). Cats older than one year were 3.43 times more likely to test positive for *T. gondii* as compared to those younger than one year. This is consistent with reports by other researchers that found that the prevalence increases with age, which also supports the hypothesis of continuous exposure of cats to *T. gondii* oocysts in the environment with time (Ruiz & Frenkel 1980, Salant & Spira 2004, Afonso *et al.* 2006, Kamani *et al.* 2010, Opsteegh *et al.* 2012).

The findings of this study revealed a seroprevalence of 33.65% in pigs, which is in agreement with those of other researchers in Ghana, Zimbabwe and Ethiopia, who obtained a seroprevalence range of 32.1%- 39% (Arko-Mensah *et al.* 2000, Hove, Lind & Mukaratirwa 2005a, Gebremedhin *et al.* 2015a). This could be due to similarities in the management of backyard pigs, which have access to pasture and/or water contaminated with cat faeces. They are also putatively exposed to wildlife and rodents infected with *T. gondii*. Kijlstra *et al.* (2004) reported that access to pasture substantially increases the risk of *T. gondii* exposure to pigs. However, it is worth noting that much lower seroprevalences, 9.3% using MAT and 24.19% using ELISA, have been reported in Zimbabwe by Hove and Dubey (1999) and in Nigeria by Onyiche and Ademola (2015), respectively. The much lower prevalence could be due to the fact that animals sampled in these studies were mainly from abattoirs and slaughter houses, some of which only slaughter pigs from commercial or semi commercial operations, unlike rural backyard farmers, who normally practise informal home slaughter. Pigs from steppe (arid) climates had a slightly higher seroprevalence (52.8%) as compared to those from subtropical climates (47.2%). Backyard pigs have other possible sources of infection,

namely rodents and swill containing improperly cooked meat, and are not completely reliant on environmental contamination of pastures or water like ruminants.

The pigs in households that reported that cat faeces were covered up with soil were 5 times less likely to test positive to *T. gondii,* while those that reported that cat faeces were left out in the open environment were 5.2 times more likely to test positive for *T. gondii*. Environmental contamination by Toxoplasma oocysts is higher in cases where faeces are left in the environment as animals have access to them. Domestic cats bury their faeces, enhancing the survival of oocysts. However, this makes them inaccessible to intermediate hosts, reducing chances of them getting infected (Frenkel *et al.* 1970).

Free ranging chickens are considered important indicators of *Toxoplasma gondii* oocysts-contaminated soil, because of their habit of feeding off the ground (Ruiz & Frenkel 1980, Lehmann *et al.* 2006). Studies in free-range chickens in Africa have shown a wide range of seroprevalences from 30.5% to 100% (Gebremedhin *et al*. 2015b, Hussien *et al*. 2016). A seroprevalence of 33.24% was reported in chickens in this study. This is consistent with studies conducted in Ethiopia by Gebremedhin *et al.* (2015b) and in Nigeria by Ayinmode and Olaosebikan (2014), where similar prevalences of 30.5% and 40.4% were reported respectively. The majority of the chickens sampled in this study was free range during the day and enclosed at night in a perch housing system, where there was poor hygiene and sanitation. These chickens are likely to have become infected mostly during the day when they feed on ground contaminated with oocysts. Backyard chickens under intensive small scale management systems have limited access to pasture and are confined to small areas, usually around households. These are unhygienic and increase the probability of exposure to multiple sources of infective material (cat faeces, rodents, leftovers) as compared to extensively reared chickens, where the density of such infective material is lower. This suggests high contamination of the environment immediately around households with *T. gondii* oocysts.

4.2. Conclusion

All species included in the current study were exposed to *T. gondii*. Seroprevalences in the district varied from 31.61% to 67.25%. These variations in the *T. gondii* seroprevalences convincingly show that toxoplasmosis is widely prevalent in the O.R Tambo district. The study revealed that 78 out of 121 sheep (64.46%), 69 out of 128 goats (53.91%), 36 out of 106 pigs (33.96%), 35 out of 109 cats (32.11%) and 46 out of 137 chickens (33.58 %) were seropositive for the parasite. Additionally, the risk factors for infection for animals in the district were age, geographical location, climate, rodent control, cat-feed access and cat faecal disposal. Given the high seroprevalence of *T. gondii* observed in sheep and goats, which have different feeding patterns compared to the other species considered in the present study, it would be important for future research to examine the differences in the risk of infection between pastures and household surroundings. Knowledge of the prevalence and risk factors of toxoplasmosis in food-producing animals and cats could help in the implementation of measures that could further reduce the burden of the disease in the district. Information on the epidemiological status of *T. gondii* in the district should be disseminated to all stakeholders, particularly those responsible for public health. This would contribute to the implementation of routine testing, monitoring and prevention campaigns in people.

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APPENDIX 1

RISK FACTOR QUESTIONNAIRE

CONSENT :

I, _____ [insert owner name] hereby give permission for blood collection from my animal/s and thereby give my consent to take part in the research project entitled : **"A study of the seroprevalence and associated risk factors of *Toxoplasma gondii* in domestic animals in the Oliver Reginald Tambo District in the Eastern Cape"**
Signature of the owner: _____

I, Whatmore Munetsi Tagwireyi have sought permission from the owner for his/her participation in this study

Signature of the researcher_____

Date of sample collection : _____ Study reference :
_____

Location : O.R Tambo District **State Vet**
Area/Municipality_____

G.P.S coordinates :
E_____**S**_____

Address:
_____

Tel : _____ Email
Address_____

Agro- ecological zone : ☐ **Steppe (Arid)** ☐
Subtropical

	Animals present on farm/household	**Animal bled**	**Sex of animal bled (M / F)**	**Age of animal bled (< 1 yr) (>1 yr)**	**Herd size**
Cats					
Chickens					
Goats					
Pigs					
Sheep					

1. Type feeding regime and animal production system used by farmer.

Key for animalproduction system :

1=Extensive and small scale **2**=Extensive and large scale **3**=Intensive and small scale

4=Intensive and large scale **5**=Free-roaming **6**=Indoors

Species	**Production system used**	**Feeding regime**			
	(1 /2 /3 /4 /5 /6)	**Pasture access only**	**Pasture access with supplementary feeding**	**Additional / Pen feeding only**	**Feeding of domestic waste or swill**
Cats					
Chickens					
Goats					
Pigs					
Sheep					

2.Where are the animals fed or grazed?

☐ Around homestead (< 5 kms away) ☐ Far away from homestead (> 5 kms away)

3. Do you see cats/rodents on your property?

☐ No ☐ Yes, cats only

☐ Yes, rodents only ☐ Yes, both cats and rodents

4. Do rodents have access to feed storage areas/feeding pens/troughs?

☐ Yes ☐ No

5. Do cats have access to feed storage areas/feeding pens/troughs?

☐ Yes ☐ No

6. Any rodent control done?

☐ Yes ☐ No

7. Which products or technics are used for rodent control?

☐ Rodenticide ☐ Rodent trap ☐ Biological control
☐ Others (Specify)_____

8. How often is the control carried out and does it result in a significant reduction in the number of rodents?

☐ Daily and marked reduction ☐ Daily and minimal reduction
☐ Weekly and marked reduction ☐ Weekly and minimal reduction
☐ Monthly and marked reduction ☐ Monthly and minimal reduction

9. Any presence of feral cats?

☐ Yes ☐ No

10. Do you own a cat/s?

☐ Yes ☐ No

11. What do you feed your cat/s?

☐ Leftovers ☐ Raw offals ☐ Commercial pet food ☐ Not fed

12. How are cat faeces disposed of?

☐ Left exposed in the environment ☐ Covered up with soil
☐ In a bin ☐ In a pit

13. Who is responsible for cat faeces disposal?

☐ No one ☐ Males ☐ Females
☐ Adults ☐ Children

14. Do your animals have any contact with wildlife? ☐ Yes ☐ No

If yes (Specify) _____

15. What percent of time do animals spend on pasture and in their housing during the different seasons of the year?

Season	**Pasture %**	**Stable// Kraal / Housing**
Summer		
Winter		

16. How often is cleaning and disinfection of animal housing done?
☐ Once a day ☐ Once a week ☐ Once a month
☐ Once a year ☐ Never.

17. Where do animals most commonly drink from?
☐ River ☐ Dam water ☐ Borehole water ☐ Municipal water

18. Has the animal bled ever aborted?
☐ Yes ☐ No ☐ Not sure

19. Level of biosecurity of animal husbandry practices on farm.
Refer to Biosecurity Appendix 3
☐ High biosecurity (double fence)
☐ Moderate biosecurity (one fence)
☐ Low biosecurity (no fence)

20. What other species of animals do you have other than the one bled?
☐ Cats ☐ Sheep ☐ Goats ☐ Cattle ☐ Pigs
☐ Chickens ☐ Others (Specify) _____

21. Do you have easy access to veterinary services?
☐ Yes ☐ No ☐ Sometimes

22 .What is the destination of animals from this farm?

Species	**Destination of Animal**			
	Live Market	Direct Slaughter	Sell to other farmers	Other
Cats				
Chickens				
Goats				
Pigs				
Sheep				

23. From which income group is the participant?
☐ > R15 000 / month ☐ R15 000 – R5 000 / month ☐ < R5 000 / month

24. Level of education of participant?
☐ None ☐ Primary ☐ Secondary ☐ Tertiary

25. Any knowledge of zoonotic diseases with specific reference to Toxoplasmosis by farmer surveyed?

☐ No knowledge of zoonotic diseases at all.
☐ Knowledge of zoonotic diseases but no knowledge about Toxoplasmosis.
☐ Knowledge of zoonotic diseases with knowledge about Toxoplasmosis.

26 . Source of Toxoplasmosis knowledge.

☐ Newspapers ☐ Internet/ Social Media ☐ Word of mouth
☐ Health/Veterinary Information Days ☐ Other (Specify)_____

27. Does farmer engage in traditional rituals which entail domestic slaughter and consumption of meat without meat inspection?

☐ Yes ☐ No

28. Which ethnic group does farmer belong to?

☐ Xhosa ☐ Zulu ☐ Pedi ☐ Tswana ☐ Venda

☐ Afrikaner ☐ Others (Specify) _____

29. Does the household have access to game meat?

☐ Yes ☐ No ☐ Sometimes

30. Do you eat raw or half done (rare) meat?

☐ Yes ☐ No

31. Has anyone in the household ever had a miscarriage?

☐ Never ☐ Yes

32. Has there been any health education especially to women of child bearing age or immunocompromised individuals on preventing *T. gondii* transmission.

☐ Non ☐ Yes

33. Do you drink unpasteurized goat/sheep milk?

☐ No ☐ Yes

34. Do you eat raw oyster, clams or mussel?

☐ No ☐ Yes ☐ Sometimes

35. Do you eat unwashed vegetable or fruits?

☐ No ☐ Yes ☐ Sometimes

…… …..**END**……

Thank you for your cooperation and time spent. Enjoy your day!

RISK FACTOR QUESTIONNAIRE

I give permission for Dr Tagwireyi to contact me for more information about the general management of my animals in order to identify risk factors that may play a role in why the disease occurs in most animals in the Eastern Cape. Please don't hesitate to contact us if you have any queries :

□ (Please tick this box if you agree)

APPENDIX 2

ANIMAL ETHIC APPROVAL CERTIFICATE

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**A study of the Seroprevalence and associated risk factors of Toxoplasma Gondi in domestic animals in the Oliver Reginald District EC, SA.**
PROJECT NUMBER	**V067-16**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Dr. W Tagwireyi**

STUDENT NUMBER (where applicable)	**UP_14434289**
DISSERTATION/THESIS SUBMITTED FOR	**MSc**

ANIMAL SPECIES	**Chickens, pigs, goats**	**Sheep, cats**
NUMBER OF ANIMALS	**100 of each species**	**100 of each species**
Approval period to use animals for research/testing purposes		**June 2016 –June 2017**
SUPERVISOR	**Prof. L Neves**	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date 25 July 2016
CHAIRMAN: UP Animal Ethics Committee	Signature

S4285-15