SERO-PREVALANCE AND ZOONOTIC IMPLICATION OF TOXOPLASMOSIS IN SHEEP IN SOUTH AFRICA

by

NADA ABU SAMRA

Submitted in partial fulfilment of the requirements for the degree of *Magister Scientiae* (Veterinary Science) in the Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria

Date submitted: December 2006

ACKNOWLEDGEMENTS

This work involved participation of many colleagues, without whose assistance this project would not have been possible. My sincere appreciation goes to:

- My supervisor Prof C M E McCrindle for her valuable guidance, advice, encouragement and the necessary pressure throughout this study. Without her support this study would not have been possible.

- My co-supervisor Prof B Penzhorn for his interest and support to my study.

- Dr Ferran Jori for statistical analysis and advice.

- My collaborator Dr Anton Stoltz and his assistants Carmen Kroon and Maris who made it possible to have access to human data and who assisted me with the ethics committee.

- The following laboratories who supplied me with valuable data on humans; Tygerberg Hospital and National, Cape Town; Pretoria Academic Hospital; National Health, Pretoria; Public Health, Durban.

- Jeannette Wentzel for kindly sending me samples from the Free State.

- All state veterinarians and animal health technicians for their hospitality and assistance.

- Farmers who allowed me to use their sheep for this study.

- The National Wool Association for assisting me with important information.

- To all my friends I met in South Africa.

DEDICATION

To my parents for their encouragement and keen support

DECLARATION

I, *Nada Abu Samra*, hereby declare that the work on which this thesis is based is original and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree at this or any other University.

SIGNATURE DATE

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

SUMMARY

Title:
SERO-PREVALENCE AND ZOONOTIC IMPLICATION OF TOXOPLASMOSIS IN SHEEP IN SOUTH AFRICA

Researcher Name : Dr N Abu Samra
Study Supervisor : Prof. C M E McCrindle
Co-Supervisor : Porf B L Penzhorn
Department : Paraclinical Sciences
Degree : *Magister Scientiae* (Veterinary Science)
Institution : University of Pretoria

Key words: Toxoplasmosis in sheep in South Africa, source of infection to humans, medical opinion on the importance of toxoplasmosis

Abstract:

Toxoplasmosis is a zoonotic disease with severe manifestations in HIV-positive human patients. In 1978 the overall sero-prevalence of toxoplasmosis in human patients in South Africa was found to be 20%. Toxoplasmosis in immunocompromised patients is known to be a cause of sometimes fatal complications, such as encephalomyelitis and ocular lesions. According to the literature, mutton infected with the cysts of *Toxoplasma gondii* is an important route of transmission to humans who ingest under-cooked meat, or eat with unwashed hands after working with meat. There is no data on the sero-prevalence in sheep in South Africa, although this is available for most other countries, including Zimbabwe.

The aim of this study was to estimate the sero-prevalence of *T.gondii* in sheep in South Africa and to discuss the zoonotic aspects related to the prevalence of toxoplasmosis in humans.

Three-stage cluster sampling was done where five different provinces randomly chosen from all the provinces in South Africa were the primary units: Gauteng, KwaZulu-Natal, Free State, Eastern Cape and Western Cape. Two sheep abattoirs and one rural location per province, selected randomly from a list supplied by the provincial Departments of Agriculture, were the secondary units. A total of 677 serum samples from these sheep were tested for IgG using the Indirect Fluorescent Antibody (IFA) test (Diagnostic & Technical Services CC, Randburg, South Africa) and the commercial Enzyme-linked Immunosorbent-Assay (ELISA) kit.

Informal interviews were conducted with doctors (n=5), doctors regarded as experts (n=17) were selected for an expert opinion survey and National Laboratories (n=3) supplied data on human serum tested for toxoplasmosis in different provinces.

The sero-prevalence in sheep, per province, was found to be: Gauteng 6%, Eastern Cape 7.8%, Western Cape 6%, KwaZulu-Natal 6.3% and Free State 2.7% when tested with the IFA test. The results obtained with the ELISA test were: Gauteng 6%, Eastern Cape 5.4%, Western Cape 4%, KwaZulu-Natal 3.6% and Free State 2.7%. Overall prevalences of 5.6% (IFA) and 4.3% (ELISA) were obtained. From the results it appears that toxoplasmosis in sheep has a lower sero-prevalence in South Africa than in other countries. Zimbabwe has an average sero-prevalence in sheep of 67.9%, there is a 80% sero-prevalence in sheep in France and 20-30% in different states in the USA. There was no significant difference between the levels in rural and commercial sheep at the 95% confidence level in South Africa, although there was a significantly higher prevalence in intensively farmed sheep in contrast to those farmed extensively.

The informal interviews with the medical doctors indicated that they do not consider toxoplasmosis as an important disease. In contrast to these findings, the experts regard toxoplasmosis as a significant disease and the data obtained from the National Laboratories substantiated this opinion.

The seroprevalence in humans was found to be between 14 and 32 % in the three provinces from which data were obtained.

It can be concluded that the lower sero-prevalence of toxoplasmosis in sheep in South Africa, as compared with international levels, was probably due to more extensive methods of sheep farming and the relatively low rainfall in southern Africa. It must be noted, however, that comparison of sero-prevalence in different countries is made difficult by the many different tests and end-titres used in both humans and animals. Standardisation is recommended.

The presence of toxoplasmosis in sheep in South Africa should be considered as significant because in this country we have a high consumption of mutton. Medical practitioners underestimate the importance of toxoplasmosis in humans. It was recommended that a pamphlet for education of veterinarians, doctors, health workers and patients be produced to increase the knowledge and understanding of this disease and its prevention in South Africa.

CHAPTER 1

INTRODUCTION

1.1. Background and Justification

1.1.1. Background

Toxoplasma gondii is an intracellular protozoan organism with a large number of intermediate hosts, including all warm-blooded animals and humans. Felids, particularly the domestic cat, are its definitive hosts and the only animal species in which oocysts develop (Dubey, 1986; 2004). Because of its broad host range, its high infection rates and its benign co-existence with the host, *T. gondii* is regarded as one of the most successful parasites on earth. *Toxoplasma gondii* is a global parasite with no known geographical boundaries (Carruthers, 2002). Serological surveys done in various parts of the world show that in some countries more than a third of the human population have antibodies against *T. gondii*. This high prevalence of infection in man proves the importance of toxoplasmosis as a zoonotic disease, particularly in pregnant women and immuno-compromised patients (Bhigjee *et al.,* 1999; Tenter *et al.,* 2000). In most adults it does not cause serious illness, but it can cause blindness and mental retardation in congenitally infected children and in immunocompromised individuals. *Toxoplama gondii* is a highly adapted parasite that establishes a life-long chronic infection and only rarely causes acute disease in healthy individuals. In South Africa, toxoplasmosis has been described in human patients but there are few references to the seroprevalence of this disease in livestock. South Africa, the study area for this investigation, has a high level of immunocompromised people in its population, due to disease caused by the human immuno-deficiency virus (HIV).

1.1.2 **Justification**

Consumption of mutton is regarded as an important source of infection for toxoplasmosis in humans (Skjerve *et al.*, 1998). In South Africa, mutton also comprises an important part of the diet (NDA, 2003). Although there is some literature on toxoplasmosis in dogs, lions, cheetah, chinchillas and ferrets in South Africa (Bigalke *et al.,* 1966; Du Plessis *et al.,* 1967; Penzhorn *et al.,* 2002; Van Heerden & van Rensburg, 1979; Van Rensburg & Silkstone, 1984), there are no data on the sero-prevalence in sheep, although this is available for most other countries (Dubey *et al.,* 1986; Skjerve *et al.,* 1998).

In South Africa, three factors contribute to the risk of toxoplasmosis for immune-compromised individuals and pregnant women. These are:

- Informal slaughter and home consumption of sheep is common, so many people are in contact with meat – not just abattoir workers and butchers.
- Due to lack of water, hand washing is not always rigorously practised after preparation of meat or working with soil.
- People often eat with their hands, not utensils.

The prevalence of toxoplasmosis in sheep should be known, in order to properly assess the risk of toxoplasmosis in humans and produce educational and extension material for risk communication.

1.2. Research Problem

There are no data on the prevalence of toxoplasmosis in sheep in South Africa although toxoplasmosis is linked to the consumption of mutton in humans.

The actual distribution of toxoplasmosis in sheep in South Africa is not known but it is important to study the situation, because of the high consumption of mutton in this

country. Mutton infected with the cysts of *T. gondii* probably presents a major source of transmission to humans (Dubey, 1996; Turner, 1976). Therefore, the prevalence of *T. gondii* in humans in South Africa is most likely to be related to the prevalence of sero-positive cases in sheep.

1.3. Hypothesis

Data will be obtained on the seroprevalence of toxoplasmosis in sheep in South Africa that can be used to estimate zoonotic significance.

1.4. Objectives

The objectives are to:

- Study the literature on the epidemiology of toxoplasmosis and its symptoms in animals and humans worldwide and in South Africa.
- Design a sampling frame for collection of samples of sera from sheep at abattoirs and rural areas in South Africa.
- Obtain data on mutton consumption in different geographical areas in South Africa.
- Collect a representative sample (n=600) of sera from five different provinces in South Africa and test these samples for antibodies against *T. gondii* using the commercial ELISA kits and the Immuno-Fluorecsence Agglutination (IFA) test.
- Record opinions through informal interviews and questionnaires submitted to medical practitioners working with HIV/AIDS-positive patients in different geographical areas of South Africa to gain their opinions on the prevalence and significance of *T. gondii* infections in AIDS patients in South Africa.
- Develop extension materials based on risk mitigation and risk communication of toxoplasmosis as a possible zoonosis.

1.5. Work Plan

The work plan for the study is as follows;

- Identify and list the sheep abattoirs in each of the five provinces.
- Randomly selection of two abattoirs and one rural area per region.
- Identify key role-players in the state veterinary services and abattoirs to get permission to bleed sheep at abattoirs and in rural areas.
- Travel to selected abattoirs and use systematic random sampling to select a minimum of 25 sheep per abattoir and 50 sheep per rural area.
- Contact medical practitioners to evaluate the importance of toxoplasmosis in humans.
- Estimate the sero-prevalence of toxoplasmosis in sheep.
- Design educational materials for use in human patients, in consultation with medical practitioners.

CHAPTER 2

LITERATURE REVIEW

2.1. Introduction

Toxoplasmosis, caused by *Toxoplasma gondii,* has been known as a zoonotic disease for more than 100 years. It has a wide host range and causes cysts in the tissues of the intermediate host. The only definitive host, in which the sexual reproduction takes place, is the felid. This will be discussed further under the following subheadings: Historical aspects; the parasite; its life cycle and transmission; prevalence of toxoplasmosis in animals in South Africa and worldwide.

2.2. Historical aspects

In 1908 Nicolle and Manceaux described *T. gondii* merozoites for the first time in the spleen, liver and blood of the rodent *Ctenodactylus gundi.* It was later discovered, however, that *C. gundi* had acquired the infection in captivity. At about the same time, Splendore independently described *T. gondii* in a laboratory rabbit in Sao Paulo, Brazil, and Darling described it in a human patient in Panama (cited by Dubey & Beattie, 1988). In 1923 Dr Jeanku had reported a congenital infection. He discovered parasitic cysts in the retina of an eleven-month-old child (cited by Turner, 1976), but the role of the parasite as a human pathogen was not widely known until Wolf & Cowen (1937) reported congenital *T. gondii* infection. Weinman and Chandler (1956) suggested that trasmission might occur through the ingestion of undercooked meat. Jacobs *et al.* (1960, cited by Dubey & Beattie, 1988) provided evidence to support this idea by demonstrating the resistance of tissue cysts of *T. gondii* to proteolytic enzymes. In 1970, more than 60 years after the first description of the asexual stage of *T. gondii* in intermediate hosts, the sexual stage was discovered in the small intestine of cats and the life cycle of *T. gondii* was fully understood (Tenter *et al.,* 2000). *Toxoplasma gondii* oocysts, the product of schizogony and

gametogony, were found in cat faeces and characterized morphologically and biologically by Dubey & Frenkel in1976.

2.3. The parasite, its life cycle and transmission

Understanding of the intracellular nature of this parasite and its life cycle is important to the epidemiology and risk assessment of its zoonotic potential.

Toxoplasma gondii is part of the Phylum Apicomplexa, class Sporozoasida and subclass Coccidiasina. The life cycle of *T. gondii* can be divided into an oocyst-oral cycle in the intermediate host and a tissue cyst-oral cycle in felines (Tenter *et al.,* 2000). *Toxoplasma gondii* has three infectious stages, namely tachyzoites in the proliferative forms, bradyzoites in the tissue-cyst stage and sporozoites in sporulated oocysts. Bradyzoites released in the intestine from tissue cysts, or sporozoites from oocysts, transform to tachyzoites, which multiply inside gut epithelial cells. After being released from the epithelial cells, they invade the body via blood and lymph, possibly in circulating cells such as monocytes and macrophages. The acute phase of infection occurs in the first 8 - 10 days when tachyzoites replicate asexually in a variety of tissues. In the host cell they transform into bradyzoites and become surrounded by cyst walls (Dubey & Beattie, 1988; Tenter *et al.,* 2000).

In the tachyzoite stage, *T. gondii* is particularly proficient at crossing tissue boundaries such as the blood-brain barrier and the placenta, to be transmitted to the foetus (Carruthers, 2002). The tissue cysts have a preference for the central nervous system (CNS), skeletal muscles, liver and myocardium but are also found in other organs. The appearance of bradyzoites marks the beginning of the chronic phase of infection, when parasite replication slows down. *Toxoplasma gondii* cysts are usually hemispherical and contain hundreds of bradyzoites. There is little or no evidence of inflammation or immune cell infiltrates surrounding the cysts (Carruthers, 2002), and they may persist indefinitely (Dubey & Towle, 1986). In this way, the parasite remains a life-long infection and individuals remain seropositive indefinitely. This

immunity can be defined as latent infection and may last for life, even in the absence of reinfection. The presence of latent infection is known as premunity and was thought to be a prerequisite for protective immunity but it is now known that persistence of infection is not necessary to ensure immunity (Carruthers, 2002).

Cats ingest the tissue cysts that are present in birds, rodents or other animals, after which the cyst wall is digested, the bradyzoites are released and they penetrate the epithelial cells of the small intestine. After a series of genetically determined asexual generations the sexual cycle begins with the formation of the gametocytes, and the micro- and macrogametes. Once a macrogemete is fertilized the oocyst develops (Dubey, 1986).

Enterocytes rupture, releasing the oocysts which are excreted in the faeces. Sporulation takes place only outside the host. Although *T. gondii* is found in many animals, felids are the only animal species in which oocysts develop (Dubey *et al.,* 1986). Cats (both wild and domestic) become infected by ingesting any of the three infectious stages of *T. gondii*. Cats shed oocysts after the ingestion of any of three infectious stages (tachyzoites, bradyzoites, or sporozoites) but the main form of infection is through ingestion of tissue cysts (sporozoites). Dubey & Frenkel (1976) observed the prepatent periods before oocyst shedding, after the ingestion of chronically infected mice, to be 3-5 days. After the ingestion of acutely infected mice, it was 5-10 days and after the ingestion of oocysts, 20 days or longer. This suggests that different developmental cycles might follow the ingestion of each of the three infectious stages.

As a rule, the duration of excretion is from 1 - 3 weeks and is rarely reported. The proportion of infected cats excreting oocysts at any one time is not high and usually not more than 2% in most countries. A cat may shed millions of oocysts, which are very hardy, capable of surviving in the soil for a year or more, increasing the risk of transmission (Dubey & Beattie, 1988).

2.3.1. Epidemiology and transmission of toxoplasmosis

Although omnivores and carnivores can be infected by various means, the ingestion of oocysts is the only way herbivores can be infected. The prevalence of toxoplasmosis in sheep is higher in areas where cats (and therefore oocysts) are present than where they are absent (Dubey, 1980; Dubey & Beattie, 1988). The frequency with which humans acquire postnatal toxoplasmosis is due mainly to their habit of eating raw or uncooked meat such as mutton and pork, (Dubey & Towle, 1986). *Toxoplasma gondii* is highly prevalent in sheep, goats and pigs used for human consumption (Dubey, 1990; Dubey & Beattie, 1988). The United States Department of Agriculture (USDA) estimates that one-half of *T. gondii* infections in the United States are caused by ingestion of raw or undercooked infected meat. A community-based study in Maryland, comparing persons who did not eat meat with those who did, supports the USDA estimate (Jones *et al*., 2003). A large European case-control study showed that undercooked meat accounted for the largest risk of infection with *T. gondii*. According to this study, between 30% and 63% of infections could be attributed to consumption of undercooked or cured meat products and 6% to 17% to soil contact (Cook et al., 2000). In a study of toxoplasmosis in the United States and elsewhere, (Dubey, 1986; Dubey *et al.,* 2003; 2004), poultry was found to be infected but did not play an important role in transmission, as the meat is usually frozen or well cooked. *Toxoplasma gondii* cysts have also been found in horses, but horse meat is rarely eaten. Venison may also be a source of *T. gondii*, as cysts have been found in the muscles of wild moose, pronghorn, elk, deer and probably in other cervids (Dubey, 1986). Pork remains the main source of *T. gondii* infection in the United States. In another study, Dubey (1980) isolated tachyzoites from milk of infected goats but according to him the risk of human transmission is low because tachyzoites would be easily destroyed by gastric juices. In contrast to his statement, Figueiredo *et al.* (2001) considered that there was a significant correlation between positive serology for *T. gondii* in humans and drinking of goats' milk. In fact, the consumption of goat's milk is considered a public health issue, since more goats' milk is drunk by children with cows' milk allergies (Figueiredo *et al.,* 2001) and goats

are regarded as the most important source of milk for Islamic people (Jittapalapong *et al.,* 2005).

Compared to the consumption of meat, contact with cats or kittens was considered a low risk factor because cats excrete oocysts for only two weeks of their life, when they first acquire the infection (Dubey, 1986). Oocysts become infective 1-5 days after excretion and can survive for more than a year. Contact with soil and water, rather than direct contact with cats, is a risk factor for infection (Cook *et al.,* 2000). Outbreaks of acute toxoplasmosis have been associated with contamination of the environment with oocysts, as they survive short periods of cold and dehydration, but are killed easily within 1-2 minutes by heating to 55-60° C. Wind, rain, surface water and even coprophagous invertebrates contribute to the distribution of this parasite in the environment (Tenter, 2000). A large outbreak of toxoplasmosis in humans was reported in Canada in 1994 after widespread contamination of drinking water (Dubey, 2004). In Atlanta, an outbreak was associated with aerosolized oocysts in a riding stable where infected cats were present (Dubey, 2004). Cysts were aspirated with the dust, leading to disease in humans.

2.4. Prevalence of toxoplasmosis in different species

A serological survey of antibodies to *T. gondii* in Iran showed a prevalence of 9% for cattle, 30% for sheep and 15% for goats (Ghazaei, 2005). According to Blewett (1983), the median values of seroprevalence are 12.5% for cattle, 30% for sheep, 6.5% for horses, 23.5% for pigs, and 40% for cats. These results show that the prevalence of *T. gondii* antibodies in sheep is generally higher when compared to other domesticated animals.

Viable *T. gondii* is rarely found in beef (Dubey, 1996). Although cattle may be sero-positive, it is rare to find tissue cysts in beef and *T. gondii* has rarely been isolated from bovine tissues (Dubey, 1986). Adult cows that were experimentally fed *T. gondii* oocysts became infected, as was evident by detection of antibodies, but the number

of *T. gondii* tissue cysts was below the detection threshold of a bioassay using mice (Dubey, 1996). The parasite could also not be detected in a study to investigate whether milk from cows infected with *T. gondii* is of epidemiological importance (Dubey, 1986).

A serological survey in the USA and other countries indicated a high prevalence of *T. gondii* in pigs, as 23% of 11 229 pigs slaughtered in the USA from 1983 to 1984 were found to be seropositive (Dubey, 1996). The seroprevalence in domestic swine on large commercial farms in developed countries has declined over the years, probably due to improvement in managemental practices. Domestic pigs in Zimbabwe were tested for antibodies to *T. gondii* and the results showed that the infection is widespread. The seroprevalence was directly related to the hygienic conditions under which these animals were kept. The seroprevalence was lowest in fattening pigs from large commercial farms (19.75%, *n* = 238) and highest in backyard scavenging pigs (35.71%, *n* = 70) (Hove *et al.,* 2005).

Little is known of the prevalence of *T. gondii* in chickens. Recent studies have been done on 96 free-range chickens from a commercial farm in Israel. *Toxoplasma gondii* was isolated from 42.2% of the tested chickens by a bioassay using mice (Dubey *et al.,* 2004). In Egypt, the prevalence of *T. gondii* antibodies in house-bred chickens was 30% and in farm-bred chickens 11.1% (overall prevalence of 18.7%) (Deyab & Hassanein, 2005). Dubey *et al.* (2003*,* 2005) considered the seroprevalence of toxoplasmosis in free-ranging chickens to be a good indicator of the general prevalence of *T. gondii* oocysts in the soil because chickens feed from the ground. The seroprevalence was 16.9% and 44.4%, respectively, in 118 chickens from Ohio, USA and 77 free-range chickens from Colombia, South America.

Cats are pivotal to the transmission of toxoplasmosis. Epidemiological data indicated that most cats became infected with *T. gondii* in nature soon after they were weaned, either by eating raw pet food or by sharing infected food brought by the mother.

Toxoplasma gondii infection is therefore higher in feral cats than in domestic cats (Dubey, 1986). Besides the domestic cat, other members of the Felidae family such as mountain lion (*Felis concolor*), ocelot (*Felis paradalis*), margay *(Felis weidii*), jaguarundi (*Felis yagouaroundi*), bobcat (*Lynx rufus*) and Bengal tiger (*Felis bengalensis*) can also shed *T. gondii* oocysts. In free-ranging large felids, such as lions and leopards, the seroprevalence of *T. gondii* was found to be high. Serum antibodies were detected in lions from Botswana, Zimbawe and the Kruger National Park (Penzhorn *et al.,* 2002).

Van Rensburg & Silkstone (1984), described the concomitant presence of feline infectious peritonitis and toxoplasmosis in captive cheetah at the Johannesburg Zoological Gardens. In chinchillas, the first major outbreak of toxoplasmosis in South Africa occurred in 1966 in the Cape Province (Du Plessis *et al.,* 1967) and in the same year *T. gondii* was isolated from two ferrets that presented with cysts in the brain (Bigalke *et al.,* 1966).

A large percentage of dogs show acquired or congenital forms of toxoplasmosis. Although most dogs develop asymptomatic infections, immaturity and concurrent distemper infection leads to clinical symptoms such as disturbance of the central nervous system (Van Heerden & van Rensburg, 1979). In the Pretoria area seven cases of toxoplasmosis in dogs have been reported but the occurrence in other areas of South Africa is uncertain (Smit, 1961). Nesbit *et al.* (1981) reported two cases of toxoplasmosis in pups which manifested spastic paresis of the pelvic limbs. This may have been a misdiagnosis, and confusion with another morphologically similar protozoan parasite, *Neospora canis*. The differences between the two organisms were well described by Dubey *et al.,* in 2003.

Recently, *T. gondii* was recognized as the cause of encephalitis in marine mammals. Antibodies were found in 19 of 45 (42%) sea lions studied (*Zalophus californianus*), in 51 of 311 (16%) Pacific harbor seals (*Phoca vitulina*), in 5 of 32 (16%) ringed seals (*Phoca hispida*) and in 4 of 8 (50%) of bearded seals (*Erignathus barbatus*).

oxoplasma gondii has also been identified in Atlantic bottlenose dolphins (*Tursiops truncatus*) and in 89 of 115 (77%) dead, and 18 of 30 (60%) apparently healthy sea otters (*Enhydra lutris*) (Dubey *et al.,* 2003). Further studies have been done on toxoplasmosis of southern sea otters along the California coast. Miller *et al.* (2002) investigated the extent of exposure to *T. gondii* in southern sea otters and compiled environmental, demographic and serological data from 223 live and dead sea otters examined between 1997 and 2001. The seroprevalence was 42% for live otters and 62% for dead otters and *T. gondii* encephalitis appeared to be an important cause of sea otter mortality. The marine source of *T. gondii* exposure for sea otters is not known. One possible route is through direct ingestion of infective oocysts present in contaminated water (Frenkel & Dubey, 1972).

The lesions caused by toxoplasmosis have been described by Canfield *et al.* (1990) in Australian marsupials. Clinical signs, necropsy findings and histopathological changes of 43 macropods, two common wombats, two koalas, six possums, 15 dasyurids, two numbats, eight bandicoots and one bilby were described. The first studies of *T. gondii* in wombats (*Vombatus ursinus*) were done by collecting serum from 23 wild common wombats in the Southern Tablelands of New South Wales, Australia, between August 2001 and March 2002. Six animals (26.1%) were shown to have antibodies to *T. gondii* (Hartley & English, 2005). An epidemic of toxoplasmosis among captive black-faced kangaroos (*Macropus fuliginosus melanops*) has been described and eight out of 25 adult kangaroos were shown to have antibodies to *T. gondii* (Dubey *et al*., 1988).

2.5. Toxoplasmosis in sheep

2.5.1. Worldwide prevalence

Sheep are reliable indicators of the prevailing rates of *T. gondii* infection as they are fully susceptible to infection and once infected, antibodies are detectable for a long time (Blewett, 1983). As a result, the prevalence of antibody in ewes is more than

twice that in lambs. The prevalence increases with age, reaching 80% in six-year old ewes in some flocks and in general most sheep acquire infection before the age of four. In the 0-5, 6-24 and >24 months age groups, seroprevalences of 7.6%, 30.1% and 46% were found, respectively (Van der Puije *et al.*, 2000).

Sheep have a higher overall seroprevalence for *T. gondii* than goats. Recently, a serological survey in sheep and goats in Ethiopia detected a seroprevalence of 52,6% in sheep and 24% in goats (Negash *et al*., 2004). In Iran, antibodies to *T. gondii* were found in 24,5% of sheep and 19,3% of goats (Hashemi-Fesharki, 1996). In Ghana, the seroprevalence was 33.2% for sheep and 26.8% for goats (Van der Puije, *et al.,* 2000); in Tanzania, the prevalence in sheep was 31,9% and for Sudan 63% (Negash *et al.,* 2004). In Uganda, the combined seroprevalence of *T. gondii* in domesticated goats was 31% but there was a significantly higher prevalence of antibodies in goats sampled from an urban environment compared with those reared in rural locations within the same geographical region (Bisson *et al*., 2000). Similar observations have been made in a study done in Zimbabwe on the prevalence of *T. gondii* infection in goats and sheep (Hove *et al.,* 2005). In this study, a high prevalence of 67,2% in adult sheep and goats was determined, but the sero-prevalence in sheep from a large commercial farm (10%) was significantly lower than that of sheep reared under the communal grazing system (80%). This variation is probably due to the fact that in rural areas animals graze around the households where the highest concentration of domestic cat faeces is most likely to be (Hove *et al*., 2005). Both cat and rodent populations are increasing in rural areas, which results in an increased availability of both definitive and intermediate hosts for the parasite (Bisson *et al*., 2000).

Other factors that influence the seroprevalence of *T. gondii* are the climatic conditions and geographic locations. Climatic conditions, especially rainfall, have caused differences in the prevalence of *T. gondii* infection, since oocysts are known to survive longer in cool, moist conditions. In Ghana, studies have been done to obtain data on the prevalence of toxoplasmosis in three main ecological zones, the

Coastal Savannah, the Forest zone and the Guinea Savannah zone (Van der Puije *et al.,* 2000). The overall prevalence in sheep was 33,2%. Sheep from the Coastal Savannah had the highest prevalence and those from the Guinea Savannah the lowest, but no differences were found in the prevalence between sheep from the Coastal Savannah and the Forest zones (Van der Puije *et al*., 2000). Hove *et al.* (2005) suggested that the relatively high prevalence in sheep and goats in Zimbabwe compared to the lower national prevalence in Botswana (10%) was due to climatic conditions. Botswana has a semi-desert climate with extensive grazing systems and in this environment oocysts can only survive for a limited period.

It has been suggested that female animals are more susceptible than males to infections of protozoan parasites (Alexander & Stimson, 1988). The sero-prevalence of *T. gondii* in Ghanaian female sheep (= ewes) and goats (= nannies) was significantly higher than those of males. Male sheep (= rams/wethers in sheep, billy goat) had an overall sero-prevalence of 23,1% compared to 40,9% for female sheep (Van der Puije *et al*., 2000). Further studies done in Satun Province, Thailand, also showed that female meat goats were more likely to be sero-positive than males (Jittapalapong *et al.,* 2005).

2.5.2. Symptoms of *T. gondii* in small ruminants

There is a high incidence worldwide of abortion and neonatal mortality in sheep and goats infected with toxoplasmosis (Dubey *et al.,* 1986) and it is considered to be second only to *Chlamydophila* as the most common cause of abortion in most countries (Negash *et al.,* 2004).

The prevalence of flocks showing clinical toxoplasmosis was 18% and 20% in England and 16% in Scotland, while it has been reported that 33% of sheep in the UK have experienced infection with *T. gondii* (Blewett & Watson, 1983). There is an enormous discrepancy between the prevalence of antibodies to *T. gondii* and prevalence of diagnosed clinical toxoplasmosis (Blewett & Watson, 1983). Clinical

ovine toxoplasmosis (evident as abortions) results from primary infection with *T. gondii* during pregnancy. The period of risk is therefore short and well defined, possibly no more than two or three months in mid to late pregnancy (Blewett & Watson, 1983). The ewes themselves are generally clinically normal at the time of abortion. Lambs may be mummified, macerated, aborted, stillborn or may be born weak or die within a week of birth (Dubey & Towle, 1986). Unlike in sheep, *T. gondii* can also cause encephalitis, nephritis, hepatitis, enteritis and cystitis in adult goats. Among domestic food animals, *T. gondii* is most pathogenic for goats (Dubey, 1990).

In 1980, Dubey demonstrated that *T. gondii* cysts frequently develop in the liver and kidneys of goats and he also showed that cysts persist longer in skeletal muscles than in the brain.

2.6. The sheep farming industry in South Africa

2.6.1. Sheep farming in South Africa

In South Africa there is a dual agricultural economy, with both commercial and subsistence-based farming. Approximately 68.6% of the land mass can sustain livestock, particularly cattle, sheep and goats. Sheep and goat farming occupies approximately 590 000 km^2 of land in South Africa, which represents 53% of all agricultural land in the country. It includes the vast Karoo areas of the Northern and Western Cape Provinces and the mixed veld types of the Eastern Cape Province and southern Free State. Commercial sheep farms are also found in other areas such as the Kalahari, the winter rainfall area, and the grassland of Mpumalanga, eastern Free State and KwaZulu-Natal. Although sheep farms are found in all provinces, they are concentrated in the more arid parts of the country. The largest number of sheep is found in Eastern Cape (30,1%), Northern Cape (25,3%), Free State (20,4%) and Western Cape provinces (10,8%). At the end of August 2005, the total number of sheep in South Africa was estimated at 25,3 million. Sheep farming

is mainly extensive, both commercially and in the case of small-scale and communal farmers (NDA, 2003).

2.6.2. Climate of South Africa

South Africa is characterized by a semi-arid to arid climate. The average mean of annual rainfall in South Africa is only around 500 mm compared to a world mean of 860 mm. There is a monthly variation of rainfall; summer rainfalls occurs between October and March in most parts of the country excluding the south-western Cape coast where winter rainfall occurs. Devastating droughts regularly occur in South Africa and the worst-hit drought areas are the semi-arid and arid plains of the central and western interior, while areas that are less susceptible to droughts are the highveld and the coastal plain. Temperatures in South Africa depend to a great extent on the altitude and configuration of the land. From sea level to the plateau the altitude varies about 1250 m and the temperature range is considerable (Tyson, 1986).

2.6.3. Transport and slaughter of sheep for mutton in South Africa

In South Africa livestock are mainly transported to the abattoirs by road. Over the past five years, animals have mainly been transported over short distances (less than 200 km), due to the high price of petrol and to the closure of several large abattoirs. Consequently, there has been an increase of local abattoirs, which have the facilities to slaughter only a small number of animals per day. Prior to the deregulation process, the abattoir industry comprised mainly of large abattoirs with high throughput. The deregulation process accomplished an increase in the number of abattoirs to over 470. Vast numbers of smaller E-grade abattoirs with a low throughput are included in this number. Not only A grade abattoirs, but also B and C grade abattoirs now contribute significantly to slaughtering in South Africa (McCrindle et al., 2006).

The red meat industry is one of the most important industries in the agricultural sector and contributes approximately 13% to the gross value of agricultural production in South Africa. The Red Meat Abattoir Association (RMAA) is an independent membership-based organization, formed in February 1991. The abattoir industry ensures a safe product to the consumers and the Meat Safety Act (act no. 40 of 2000) describes measures to promote safety of meat and animal products and to establish and maintain essential national standards in respect of abattoirs.

2.7. Human cases of toxoplasmosis

2.7.1. Symptoms

Human toxoplasmosis can result from a congenital or an acquired infection. The congenital infection is mainly characterized by neurological abnormalities, such as hydrocephaly, microcephaly, convulsion, chorioretinitis, cerebral calcification, blindness, mental retardation, epilepsy and deafness. Toxoplasmosis is important for sero-negative women who become primarily infected during pregnancy, because of the risk to the foetus. Toxoplasmosis may be clinically apparent in the neonate either in the first months of life or later during infancy, childhood, or adolescence. People may remain subclinically infected for life (Jones *et al.,* 2003). Approximately 30% of women of childbearing age in the United States have antibodies to *T. gondii* and are therefore immune to congenital toxoplasmosis, which leaves 70% of the female population at risk of acquiring *T. gondii* infection during pregnancy (Dubey, 1986). An estimated 400 to 4 000 cases of congenital toxoplasmosis occur each year in the United States (Jones *et al.,* 2003).

The acquired form is frequently characterized by lymphadenitis (Jacobs, 1981) but myocarditis, encephalomyelitis, ocular lesions and/or acute fulminating pneumonia may also occur (Bhigjee *et al.,* 1999). The incidence of toxoplasmosis has increased dramatically since the high frequency of patients with acquired immunodeficiency syndrome (AIDS) has become a serious problem. Infection with HIV (Human

Immunodeficiency Virus, the cause of AIDS) has increased considerably in the last ten years. It is estimated that more than 33 million adults and 1.3 million children are infected worldwide (Del Valle & Pina-Oviedo, 2006). South Africa has one of the fastest growing HIV epidemics in the world with an estimated 5.3 million adults and children currently living with HIV in South Africa (Motloung *et al.,* 2004).

Neuropathological conditions are present in approximately 70 to 90% of AIDS patients and can be the result of opportunistic infections such as toxoplasmosis, cryptococcal meningitis, primary central nervous system (CNS) lymphoma, tuberculosis or progressive multifocal leukoencephalopathy (PML) (Del Valle & Pina-Oviedo, 2006). There are at least two ways to classify the neurological complications of HIV infection, i.e. i) according to their pathogenesis and ii) according to their neuroanatomical localization as follows:

- *Toxoplasma gondii* tends to infect the grey matter of the brain by haematogenous spread, leading to abscesses in the diencephalon and cortex.
- *Cryptococcus neoformans* causes meningitis because the subarachnoid space provides a favorable milieu for growth of this fungus.
- Polyomavirus (JC virus) (the cause of PML) infects oligodendrocytes and so afflicts the white matter with demyelinating lesions.

Cerebral toxoplasmosis, primary CNS lymphoma and PML cause predominantly focal cerebral diseases, which often present with focal hemispheric dysfunction such as hemiparesis, aphasia and apraxia. Clinical signs of cerebral toxoplasmosis develop rapidly, while with primary CNS lymphoma and PML the signs develop over several weeks. On neuro-imaging, the focal lesion of toxoplasmosis and primary lymphoma show a mass effect and surrounding oedema, which is usually more distinct and ring-like in toxoplasmosis and more diffuse in lymphoma (Price, 1996). In an analysis of data from a large HIV-infected study group, toxoplasmosis was found to be the most frequent severe neurologic infection among persons with AIDS in the United States (Jones *et al.,* 2003).

2.7.2. Worldwide studies on human cases of toxoplasmosis

An epidemiological study on the seroprevalence of acquired toxoplasmosis in HIV-infected patients in Nigeria showed that there was a higher seroprevalence of *T. gondii* antibodies among individuals aged 31 - 40 years (36.5%). Patients with concomitant toxoplasmosis and HIV infection manifested fever (63.5%), headache (44.7%), rashes (41.2%) and anorexia (34.1%) (Uneke *et al.,* 2005). In the Neurological Department of a hospital in Mexico City, neurological manifestations in 149 patients with AIDS were recorded between 1990 and 1998. The most common infections were brain toxoplasmosis (32.2%), meningeal cryptococcosis (21.5%) and tuberculosis (8.7%). Further studies done by the Yucatan University in Mexico showed that neurological manifestations in AIDS patients in the state of Yucatan are due to toxoplasmosis in 47% of the cases (Castro-Sansores *et al.,* 2004). Luft & Remington (1992) also consider toxoplasmatic encephalitis as one of the most common and most treatable causes of AIDS-associated Central Nervous System (CNS) pathologies. Among patients with AIDS, more than 95% of toxoplasmatic encephalitis is due to the reactivation of a chronic/latent infection caused by the loss of cellular immune surveillance. Toxoplasmatic encephalitis develops in most cases when the CD4 cell count falls below 100/mm^3. A study done in Thailand at the Mahidol University of Bangkok showed that among HIV positive and *T. gondii* antibody positive groups, 43.2% had symptoms and signs of acute toxoplasmosis involving eye and/or the central nervous system (Sukthana *et al.,* 2000). Furthermore, toxoplasmosis remains a major cause of mortality in AIDS patients in underdeveloped countries with limited financial resources and infrastructure for purchasing and distributing anti-HIV drugs (Carruthers, 2002). According to Walls (1988), 70% - 80% of patients with AIDS showed toxoplasmatic encephalitis.

As elsewhere in the world toxoplasmosis was found to be the most frequent cause of an Intracranial Mass Lesion (IML) reported by Bhigjee *et al.* (2005) from the Wentworth Hospital in Durban (South Africa). In a previous study over a period of 17 months, Bhigjee *et al*. (1999) tested HIV-seropositive patients to determine the type

and frequency of IML. The most frequent cause of the IML was toxoplasmosis followed by encephalitis of obscure origin, brain abscesses and microbacterial infections. The clinical presentation of toxoplasmosis in HIV-seropositive patients is non-specific. The diagnosis of toxoplasmosis, using techniques such as computed tomography (CT) and magnetic resonance imaging (MRI), is also non-specific. Even though MRI is more sensitive than CT in picking up multiplicity of lesions, MRI is not widely available in South African hospitals. Since specific therapy is available, patients suspected of acute cerebral toxoplasmosis who reacted positively to treatment were considered as *T. gondii* positive. Bhigjee *et. al.* (1999) did not test the sera of their HIV-positive patients for *T. gondii* antibody. A negative result does not exclude the diagnosis of toxoplasmosis because the detection of antibodies becomes more difficult in HIV-infected people. In addition, of the patients who are seropositive, only 25-50% will go on to develop toxoplasmatic encephalitis.

Consequences of postnatal infection of immunocompetent people usually receive little consideration, because most of these cases are thought to be subclinical (Hill & Dubey, 2002; MacAllister, 2005). Recent studies showed that nervous involvement of toxoplasmosis in the healthy population may have greater consequences than previously realized. Animals infected with *T. gondii* can show altered behaviour and neurotransmitter function. The organisms have been shown to weaken learning and memory in mice and to produce behavioural changes in both mice and rats. These changes increase the chances of predation and provide an example of evolution-driven manipulation of host behaviour by the parasite. In humans, acute infection with *T. gondii* can produce psychotic symptoms similar to those displayed by persons with schizophrenia (Torrey & Yolken, 2003; Yolken *et al.,* 2001). Studies done in three different countries found that schizophrenic patients had higher antibody levels to *T. gondii* compared to control subjects. The proportion of schizophrenia that may be attributable to infection with *T. gondii* has not been determined, but recent studies showed that 42% of schizophrenic patients were *T. gondii* positive compared with 11% of the matched control subjects (MacAllister, 2005). Toxoplasmosis may also cause more subtle neurological results than overt

psychosis, by affecting personality traits and intelligence. In a recent study of military applicants, 628 men that were seronegative for *T. gondii* had higher scores on intelligence test and had achieved a higher level of education compared to 229 seropositive men. Additional studies in which personality questionnaires were administered to healthy adults have indicated that serum antibodies to *T. gondii* are associated with alterations in behaviour and psychomotor skills. In schoolchildren, tiredness and inattentiveness have been linked to seropositivity to *T. gondii*. France, which has a high *T. gondii*-infected population, was reported to have approximately 50% higher rates of schizophrenia than those in England (Torrey & Yolken, 2003).

Ocular toxoplasmosis used to be attributed to congenital infection, but Gilbert and Stanford (2000) recently compared prenatal and postnatal toxoplasmosis. They concluded that at least two thirds of ocular toxoplasmosis is caused by postnatal infection, which has major public health implications. An outbreak of acquired *T. gondii* infection occurred in the greater area of British Columbia in 1994-1995. In total, 100 patients with acquired and 12 patients with congenital toxoplasmosis were identified. Lesions were consistent with acute toxoplasmosis retinitis, and serologic testing confirmed the acute acquired nature of the infection (Burnett *et al.,* 1998).

2.7.3. Epidemiology of toxoplasmosis in people

Toxoplasma gondii infection is widespread in humans and its prevalence varies widely between different ecological regions. In the United States of America, a seropositivity of 20-30% has been reported in the human population along the sea coast and 1% in the population in the Rocky Mountains and the South Western desert areas. These results show a direct correlation between climate and prevalence, where cold and dry conditions are less suitable for transmission. Seroprevalence is also low in Scandinavian countries (11-28%), where the climate is cold (Walls, 1988). A great variation in prevalence has also been reported in Africa, where the ethnic and geographic populations are very different from each other. In some areas of Egypt and South Africa, the prevalence is less than 10%, while in

Nigeria, Kenya and Tanzania; it is greater than 60% (Jacobs & Mason, 1978). This is probably related to conditions favouring sporulation and survival of oocysts (Dubey & Beattie, 1988).

The seroprevalence of infection varies between ethnic groups, but this is due to sanitary and cooking habits rather than to genetic differences. In Paris, for example, where it is customary to eat raw or undercooked meat, over 80% of the adult population had antibodies to *T. gondii* (Dubey & Beattie, 1988).

Populations that were matched with respect to age, cultural habits or environmental factors, showed similar levels of *T. gondii* prevalence. For example, in the 1990s, central European countries such as Austria, Belgium, Germany and Switzerland had an estimated sero-prevalence of 37-58 % in women of childbearing age. In similar populations in Croatia, Poland, Slovenia, Australia, and Northern Africa, comparable sero-prevalence has been observed. Higher sero-prevalence has been reported in several Latin-American countries, including Argentina, Jamaica, Brazil, Venezuela and Cuba (51-72%), and in West African countries on the Gulf of Guinea, i.e. Benin, Cameroon, Congo, Gabon, and Togo (54-77%). The seroprevalence is lower in Southeast Asia, China, and Korea (4-39%) (Tenter *et al.*, 2000).

In meat-producing animals, tissue cysts of *T. gondii* are most frequently observed in tissues of infected pigs, sheep, and goats. Tissue cysts are relatively resistant to changes of temperature and remain infectious in carcasses refrigerated at 1-4°C or in minced meat for up to 3 weeks. Tissue cysts also survive freezing at temperatures between –1 and -8 °C for longer than a week. In contrast, tissue cysts in meat are killed by heating to 67°C and survival of tissue cysts at lower temperatures depends on the duration of cooking (Hill & Dubey, 2002). Therefore, the eating habits of a population have an influence on the distribution of *T. gondii* and the presence of mutton or pork in the diet increases the risk factor. Indians in South Africa eat a lot of mutton, which might explain the higher incidence compared to Whites (Jacobs & Mason, 1978). This is in contrast to the theory that toxoplasmosis is more related to

keeping of “pet cats”, as this is more culturally associated with the “White” group in South Africa (McCrindle, 2005).

2.7.4. Prevalence in South Africa

Climatic conditions vary considerably in South Africa, from the dry climate in the north-western part to the winter-rainfall, temperate climate of Western Cape, and the humid, tropical climate of KwaZulu-Natal. The prevalence of *T. gondii* antibodies in people in South Africa was studied by Jacobs & Mason (1978). The results of this study indicate that the overall sero-prevalence was 20%. The article divided the population on the basis of race and found that the highest prevalence occurred in Blacks (34%) and Indians (33%) from Natal, while the lowest was in the San people (9%) and the Whites (12%) from the western part of South Africa. In the Black population the highest incidence was found in the age group of 31-40 years, while the incidence in the Whites was in the 21-30 year age group in a study done in Bloemfontein, Free State province (Brink *et al.,* 1975).

2.8. HIV/AIDS prevalence in South Africa

Approximately 36 million individuals worldwide were living with HIV/AIDS in 2001 and 25 million of these were in Sub-Saharan Africa, the most affected region worldwide (Barnett & Whiteside, 2002). In other words, almost two-thirds of all people living with HIV are situated in Sub-Saharan Africa (CSA, 2005). According to the UNAIDS December 2004 epidemic update, an estimated 4.9 million people became infected with HIV in 2004 and UNAIDS also found that the most infection occurred in 2003 compared to any single year since the start of the epidemic (Barnett & Whiteside, 2002).

The South African epidemic has been the last to develop and is currently one of the most severe in the world. The Nelson Mandela/HSRC Study of HIV/AIDS estimated

in 2003 that 11,4% of all South Africans are infected with HIV and that an estimated 1700 new infections occur in South Africa every day (CSA, 2005).

South Africa's peculiar history has made it a fertile ground for the spread of HIV (Barnett & Whiteside, 2003). The disruption of family and communal life resulting from apartheid and migrant labour, is one of the factors contributing to the epidemic. South Africa's black population was forced into crowded, impoverished homelands, which led to the breakdown of traditional culture structures and livelihoods, as for example the migration of adults to urban areas in order to work in white-owned factories and mines where they lived in single-sex hostels. These labourers came into an area of high sero-prevalence and infected men returned to their home communities where they spread the disease. Other factors involved in the spread of HIV are: a good transport infrastructure, the low status of women in society and relationships, social norms which accept high numbers of sexual partners; and extensive resistance to condom use (CSA, 2005). The current HIV epidemic situation in South Africa indicates that :

- approximately 15% of all South African adults between 20 and 64 are HIV positive;
- 10% of all children in South Africa are orphans (an estimated number of over 1.5 million) and 43% of all orphans are orphaned by AIDS (CSA, 2005);
- a prevalence of 9.9-11.6% in the whole population (an estimated 10.8%) (CSA, 2005);
- a prevalence of 14.9-17.7% amongst all people aged 15-19 years old (an estimated 16.2%) (CSA, 2005).

2.9. Sampling frame and sampling methods

2.9.1. Sampling frame

The sampling frame is defined as the list of all sampling units in the target population. The target population is the total population about which information is required (population at risk), while the study population is the population from which a sample is drawn. The study population should be representative of the target population and consists of elementary units, which cannot be divided further. A collection of elementary units, grouped according to a common characteristic, is a stratum (Thrusfield, 2005). Veterinary sampling frames include lists of abattoirs, farms and veterinary practices (Thrusfield, 2005). This complete list of all sampling units is required in order to draw a simple random sample.

2.9.2. Sampling methods and sampling procedure

Surveys and analytic studies need a good sample design and planning the sample strategy is of major importance. The reason for taking a planned sample of a population is to describe the characteristics of a population (frequency of disease or production levels) and to estimate specific associations (test hypothesis) in the population (Gummow, 2006).

There are two main types of sampling: non-probability sampling and probability-sampling.

According to Thrusfield (2005) non-probability sampling, in which the choice of the samples is left to the investigator, includes judgment sampling, convenience sampling, and purposive sampling. In judgment sampling representive units of the population are selected by the investigator, in convenience sampling the sample is selected because it is easy to obtain and in purposive sampling the selection of units is based on known exposures (Gummow, 2006).

Probability sampling is the sampling method in which each sampling unit in a group has an equal probability to be selected; hence an individual can only be chosen once without replacement. Probability sampling includes simple random sampling, systematic random sampling, stratified random sampling, cluster sampling and multistage sampling (Thrusfield, 2005; Gummow, 2006).

In simple random sampling, the samples are selected randomly from the study population by using random numbers, while in systematic random sampling the samples are selected at equal intervals and only the first animal is selected randomly. In stratified sampling the study population is divided into groups (strata) and samples are selected randomly from each group. These sampling methods improve accuracy because they ensure that each group in the population is represented. If the strata are geographical locations (different countries, regions, districts and villages), different sampling periods or other categories, the strata is termed a cluster. When all the animals in each cluster are sampled, the sampling method is defined as one-stage cluster sampling. If the cluster is divided into sub-units and the clusters are the primary units, while the selected members of the sub-samples are the secondary units, we talk about two-stage cluster sampling. If further stages of sampling are undertaken and in this way a higher level of sub-sampling is created, the sampling method is defined as multistage cluster sampling.

Cluster sampling is a technique that is often used in the study population where the members are not all known (Thrusfield, 2005).

2.10. Risk communication and Veterinary extension

The aim of risk communication is to identify and impose priorities and take appropriate actions to minimize risks.
Three stages have to be considered in assessing and managing risk:

- Risk identification
- Risk analysis

- Risk management

Once the risk has been identified and analyzed it has to be communicated to the target audience that can be, for example, a community (Thrusfield, 2005). In the process of risk communication the sender (for example a veterinarian) needs to communicate the message, by using various communication channels, to the receiver. The feedback from the received message can be considered as the effect of the risk communication. This communication between a veterinarian and the target audience can be represented in terms of SMCRE (Sender-Message-Communication Channel-Receiver-Effect). The sender has to build up a good relationship with the community and the message must be relevant to the situation, resources and assets of the target audience. The communication channel should be chosen according to the characteristics of the target audience and should stimulate as many senses as possible in order to get their full attention (sight, hearing, smell, touch, taste). The receiver or target audience can be a single farmer, a group of farmers or an entire community, which should have the same educational and economic level. The likely effect of the message should be considered by the sender in order to evaluate the success of the communication (McCrindle, 2006).

2.11. Diagnostic tests

Serological testing is a useful method to determinate the epidemiological distribution of toxoplasmosis in South Africa.

The first serological test to be widely used for the detection of antibodies of *T. gondii* was the Sabine-Feldman test, also called the *Methylene blue dye test* (DT). The DT was developed in 1948 by Sabin and Feldman and has been recognized since then as the gold standard by which all other tests are judged. This test is thought to be highly sensitive and specific, but has the major disadvantage of requiring the use of live organisms and has therefore been replaced by other tests (cited by Wilson *et al.,* 1990).

The most common serological tests used to identify humeral antibodies are the enzyme-linked immunosorbent assay (ELISA), the indirect haemoagglutination test (IHA), the immunosorbent agglutination assay test (IAAT), the modified agglutination test (MAT), the latex agglutination test (LAT), the direct agglutination test (DA) and the indirect fluorescent antibody test (IFAT) (Gamble *et al.,* 2005; Wilson *et al.,* 1990).

Detection of an increase in antibody titres or the presence of IgM or IgG antibodies, is the main tool for diagnosis. The detection of IgM is important because it appears sooner after the infection than IgG and disappears earlier than IgG. The IgG antibody stays high for as long as the *T. gondii* antigen is present but does not prove recent or acute infection. The IFAT, IAAT and ELISA have been modified in order to detect IgM antibodies. The commercial ELISA and the MAT have been compared and it was found that the MAT is not suitable for use in the abattoir or in the field because of the length of time required for the results to become available.

2.11.1. Enzyme-linked immunosorbent assay

The ELISA test allows a wide use of serological testing and is a good tool for epidemiological studies. This method has been used, for example, in a study done on the presence of antibodies in lambs in Norway, with titres of 1:512 and 1:16 (Skjerve *et al*., 1998). Another study was done in the United States for comparison of a commercial ELISA with the MAT for the detection of *T. gondii* infection in domestic pigs. The MAT was performed at serum dilutions of 1:10, 1:25, 1:100 and 1:500. A titre of 1:25 was considered positive and a titre of 1:10 was considered suspect, while the sera tested using the ELISA commercial test were diluted 1:100. Based on the results of this study, the ELISA test is as good as, or better than, the MAT for detecting antibodies to *Toxoplasma* in pigs. ELISA is considered to be a more useful test for routine screening on farms or at abattoirs, compared to the MAT, which is difficult to interpret (Gamble *et al.,* 2005).

ELISA tests have become the most widely used tests in the United States for initial screening of toxoplasmosis in humans (Wilson *et al.,* 1990).

An evaluation of ELISA methods for the diagnosis of acute toxoplasmosis was done by Johnson *et al.,* (1992) at the Flinders University in Australia. In this study a laboratory-produced ELISA, containing two recombined *T. gondii* polypeptides as antigen, was compared to three commercially available ELISAs, which employed antigen derived from whole tachyzoites. The ELISA based on the recombinant *T. gondii* polypeptides appeared to be the most specific ELISA in this comparison.

2.11.2. Indirect Fluorescent Antibody Test

The *Toxoplasma* IFAT is considered to be highly specific and has been largely used for serodiagnosis in humans. In the Bloemfontein area in 1975, IFAT was used for detecting antibodies in sera of humans. Most positive sera had antibody titres between 1:30 and 1:120 (Brink *et al.,* 1975).

The IFA test results compare well with those of the Methylene blue dye test (DT), The IFA test for toxoplasmosis uses whole organisms as antigen (tachyzoites are fixed in formalin and air dried on slides) and therefore became widely available in most laboratories because it removed the necessity of maintaining live parasites (Wilson *et al.,* 1990).

The IFA test has also been used for detecting antibodies to *T. gondii* in swine, sheep, cattle and other domestic animals. The seroprevalence of *T. gondii* in domestic pigs was studied in Zimbabwe using the IFAT. Serum samples were screened at dilutions of 1:100, 1:200 and 1:400, where fluorescence at a serum dilution of 1:100 was considered positive (Hove *et al.,* 2005). Similar studies have been done in Brazil where the IFAT has been used for detecting antibodies to *T. gondii* in goats and the optimum titre was considered to be at a serum dilution of

1:50 (Figueiredo *et al.,* 2001). *Toxoplasma* IFAT titres in sheep serum equal to or exceeding 1:20 were regarded as positive (Foodsafety website**,** 2006).

2.11.3. ELISA test in comparison with IFA test

Further research on the seroprevalence of *T. gondii* was done in Spain using IFAT and ELISA. The results of both serological techniques were shown to be identical (Pereira-Bueno *et al*., 2004). The IFA and the ELISA test have the disadvantage that they require individual fluorescin- or enzyme-labeled antisera for each animal species tested. The ELISA has advantages over the IFA test because of its higher automation (Wilson *et al.,*1990). According to studies done by Bartoszcze *et al*. (1991) on the detection of antibodies in pigs, ELISA has been proven to be the most sensitive test with the best results obtained using antigen diluted 1:10 and the conjugate diluted 1:100. Although the results of the ELISA compare favourably with results of the IFA test in regard to sensitivity, some false positives have been reported, due to heat inactivation of serum that can cause false positive ELISA reactions (Wilson *et al.,* 1990).

In serological surveys on toxoplasmosis in sheep in Australia, significant correlations between the positive rates obtained by IFAT and ELISA were found and high antibodies titres found in IFAT and ELISA, compared to the IHA, justify the great sensitivity of these former tests (Figueiredo *et al.,* 2001). In further studies, like the detection of anti-*T. gondii* antibodies in small ruminants in Ghana, a high sensitivity and specificity of the ELISA test and the IFAT were demonstrated, where the IFAT was used as a reference test (Van der Puije *et al.,* 2000).

Currently, many ELISA kits are available and have been approved for use in humans. In the United States, *Toxoplasma* kits are not standardized as they are partly in Europe. Only one company in the United States uses a positive control that has been standardized against the WHO *Toxoplasma* International Standard serum. Consequently, there is no uniform expression of results among the ELISA kits and therefore it is impossible to compare results from two different kits besides the strong

positive and strong negative results. Seven IgG/IgM kits have been evaluated in the United States by testing 100 blood samples once with each test and all results were compared with IFA results. The results with a high discrepancy, compared with those of the IFA test, were retested. The positive results of the kits agreed 100% with the IFA-positive sera, but two ELISA kits incorrectly detected as positive several of the IFA-negative sera (Wilson, *et al.,* 1990).

CHAPTER 3

MATERIALS AND METHODS

3.1. Model system

A prospective survey of the sero-prevalence of toxoplasmosis in sheep in South Africa was done. In addition a retrospective survey of sero-prevalence of *T. gondii* antibodies in human patients was done as well as informal interviews and expert opinion questionnaires in South Africa. Two types of cross-sectional studies were done: on the communal sheep production systems and on the informal sheep production system. A comparison of diagnostic methods was done by using two different serological tests, the ELISA and the IFA test, on the same serum samples.

3.2. Experimental design and procedure

Randomised selection of five of the nine provinces was used, followed by random selection of abattoirs and rural areas where there was small-scale and subsistence sheep farming (representing the informal sector). Purposive selection of medical doctors who were recommended by their peers was used to select experts for the expert opinion survey. The serums of the sheep sampled were tested using ELISA and IFA tests. The data obtained were compared.

3.3. Sampling methods

3.3.1. Sampling procedure for livestock

A three-stage sampling method as described by Thrusfield (2005), was used to select 500 sheep (Fig.3.1).

Provinces, abattoirs, State Veterinary (SV) districts and sheep were randomly selected. Blood samples were obtained from sheep between August 2005 and May 2006 from five provinces: KwaZulu-Natal, Gauteng, Eastern Cape, Western Cape and Free State (Stage 1).

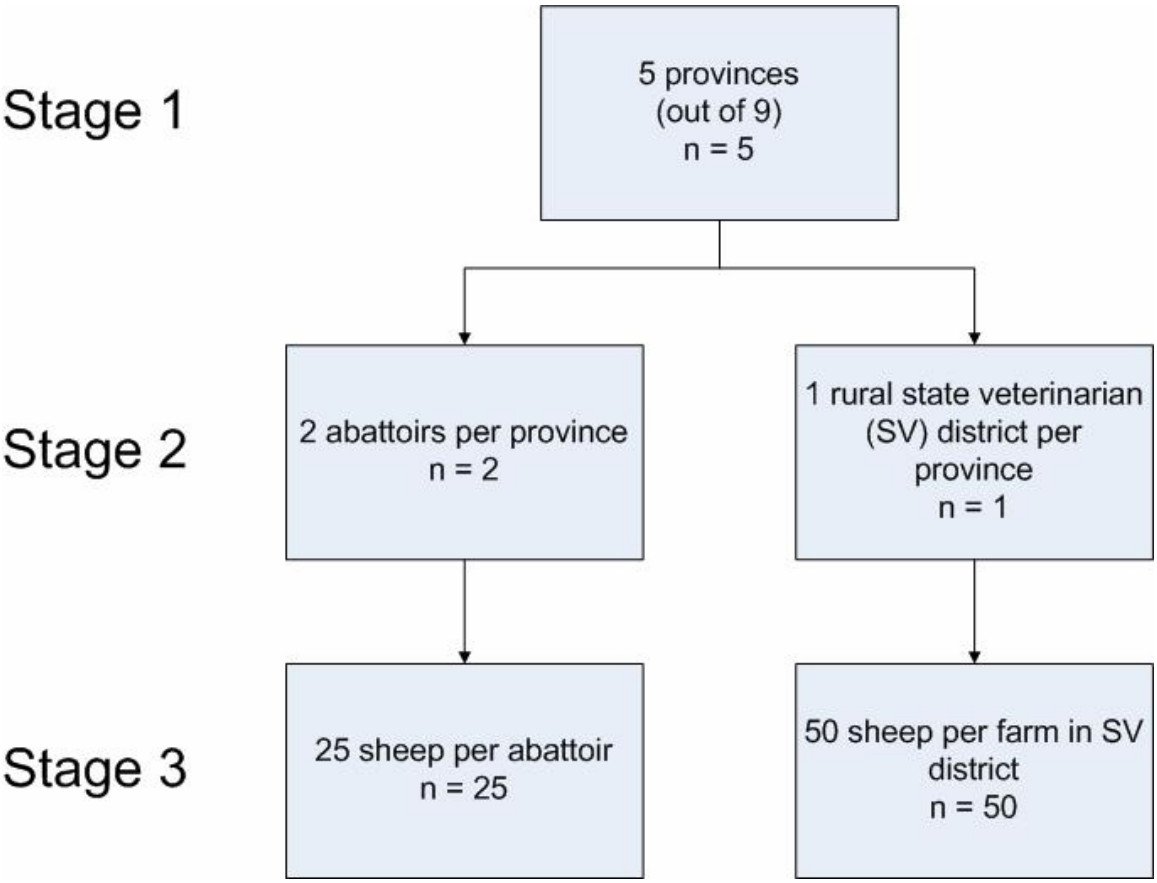


Fig 3.1 Diagram of multi-stage sampling procedure

As can be seen in Fig 3.1, Stage 2 was divided into abattoirs (n=10) and rural areas (n=5). The rural areas were selected as representative of informal slaughter and the state veterinarian in each area assisted in identifying at least five different farms/communal areas where sheep were not marketed through formal channels.

At the abattoirs, systematic random sampling was done. Taking into account that more then one supplier was usually involved, the sampling was also stratified by supplier/owner, so that all the sheep did not come from a single owner. No attempt was made to estimate the age of the sheep.

Plate 3.1 shows a farmer restraining a randomly sampled sheep so that blood could be taken from the jugular vein.

Plate 3.1 Sampling of sheep in Ngingxolo village, East London District

3.3.2 Sampling procedure for medical practitioners

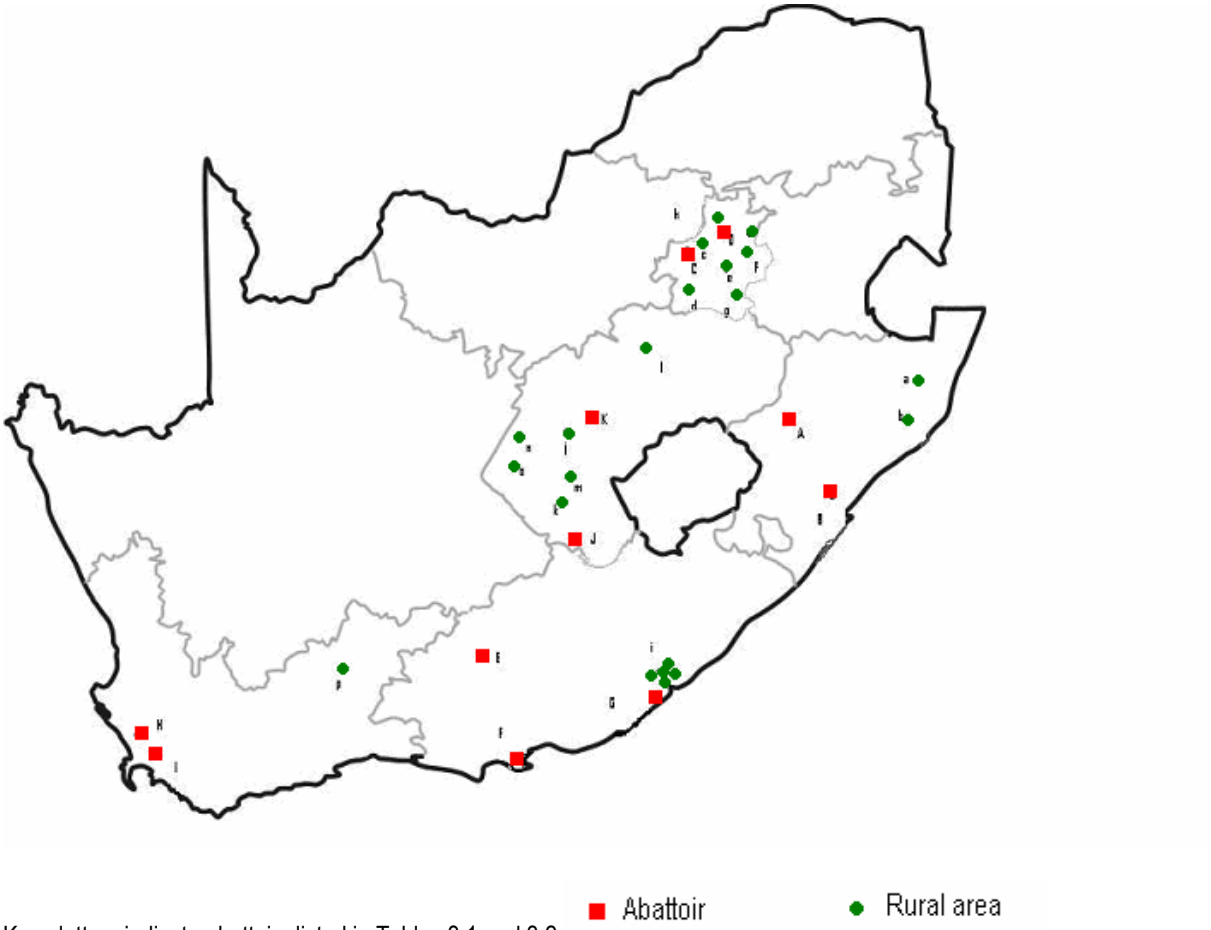
So as to understand the magnitude of the problem of toxoplasmosis in humans and seek advice on selection of expert opinion, informal personal interviews were conducted with doctors (n=6) at hospitals in Gauteng Province.

Doctors (n=10) regarded as experts by their peers were purposely selected for an expert opinion survey (Thrusfield, 2005) on the significance of toxoplasmosis in human patients.

The National Laboratories of South Africa (n=5) were contacted in each province in order to obtain data on the prevalence of *T. gondii* antibodies in humans.

3.4. Study area

Abattoirs and rural areas were sampled as described in Section 3 and Plate 3.2 shows the areas and locations where samples were taken. The number of samples taken from each abattoir and rural district is given in Table 3.1.



Key: letters indicate abattoirs listed in Tables 3.1 and 3.2

Plate 3.2 : Map of South Africa showing the areas where sheep sampling took place

Table 3.1: Localities of the abattoirs where samples were collected (see Fig 3.1)

Province	Name of Abattoir*	Locality	No of samples
KwaZulu-Natal	A)Ladysmith Abattoir B)Cato Ridge Abattoir	Ladysmith Cato Ridge	24 25
Gauteng	C)Krugersdrop Abattoir D)Bukeret Abattoir	Krugersdrop Pretoria	26 25
Eastern Cape	E)Graaff-Reinet Abattoir F)Port Elizabeth Livestock Abattoir G)East London Abattoir	Graaff Reinet Port Elizabeth East London	25 30 23
Western Cape	H)Paarl Abattoir I)Elsenburg Agriculture Department	Paarl Stellenbosch	25 25
Free State	J)Bethulie Abattoir K)Brandfort Abattoir	Bethulie Brandfort	25 25
Total			**278**

Key: * the letters next to the name indicates the map reference in Fig 3.1

Table 3.2: Localities in the rural areas where samples were collected (see Fig 3.1)

Province	Locality of Farm*	No of samples
KwaZulu-Natal	a)Nongoma area: • Sindane • Nongoma • Ebukhalemi • Mbanjeni b)Ulundi Dlebe Area: • Babanango • Kwa-Nodwengu	5 5 5 5 15 20
Gauteng	c)Krugersdorp d)Randfontein e)Elandsfontein f)Bronkhorstspruit g)Heidelberg h)Onderstepoort	10 10 15 10 10 10
Eastern Cape	i)East London District • Cove Ridge • Kwelera • Mooiplaas • Komgha • Ngxingxolo	 10 10 10 15 5
Free State	j)Bloemfontein k)Springfontein l)Kroonstad m)Edenburg n)Jacobsdal o)Kofflefontein	20 10 15 15 20 15
Western Cape	p)Beaufort West area	50
Total		**322**

Key: * the letters next to the name indicates the map reference in Fig 3.1

3.5. Location and climatic description of study areas

Different natural regions (vegetation zones) and management conditions of sheep farming are present in the five provinces.

3.5.1. Different vegetative zones of study areas

The vegetation zones of South Africa are shown in Plate 3.3.

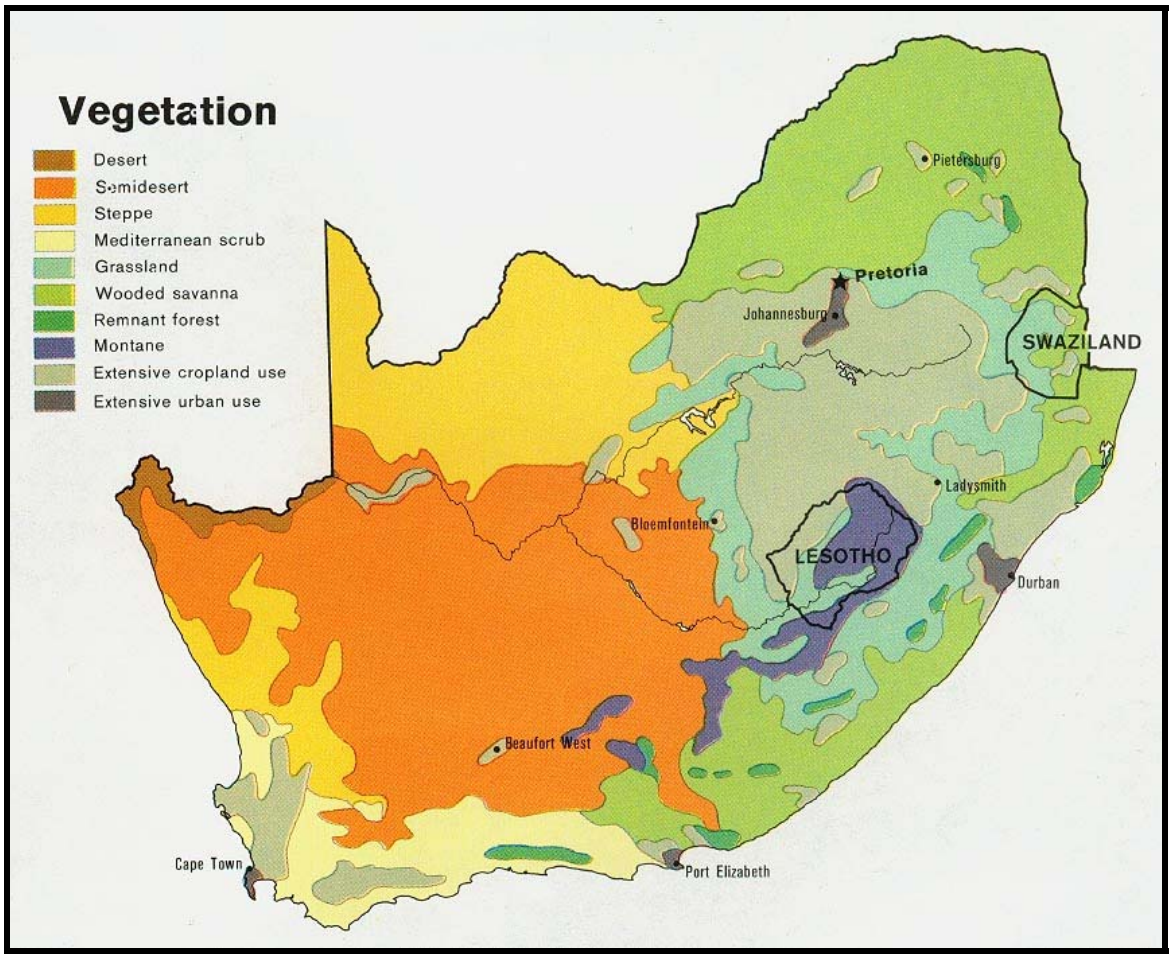


Plate 3.3: Vegetation Map of South Africa

KwaZulu-Natal was sampled in two different zones of vegetative types: the Karoo and Karroid Bushveld types and the coastal tropical forest. The sub-zones are described in Table 3.3.

Table 3.3: Sub-types of vegetation in KwaZulu-Natal where sampling took place

PLACE	TYPE OF VEGETATION
Ladysmith	Valley Bushveld
Cato Ridge	Ngongoni Veld of Natal Mist-belt
Nongoma	Zululand Thornveld
Ulundi	Zululand Thornveld

Eastern Cape was sampled in the Central lower Karoo and the coastal tropical types (Table 3.3).

Table 3.4: Sub-types of vegetation in the Eastern Cape where sampling took place

PLACE	TYPE OF VEGETATION
Graaff-Reinet	Central Lower Karoo
Port Elizabeth	Coastal forest and Thornland
East London	Eastern Province Thornveld
Cove Ridge	Eastern Province Thornveld
Kwelegha	Eastern Province Thornveld
Mooiplaats	Eastern Province Thornveld
Komgha	Eastern Province Thornveld
Ngxinxolo	Eastern Province Thornveld

The Western Cape was sampled in the temperate and transitional forest and subtypes and the Karoo and Karroid Bushveld types (Table 3.5).

Table 3.5: Sub-types of vegetation in the Western Cape where sampling took place

PLACE	TYPE OF VEGETATION
Paarl	Coastal Renosterbosveld
Elsenburg/Stellenbosh	Coastal Renosterbosveld
Beaufort West	Karroid Broken Veld

Gauteng was sampled in the False Grassveld vegetation. The subtypes of vegetation are shown in Table 3.6.

Table 3.6: Sub-types of the vegetation in Gauteng where sampling took place

PLACE	TYPE OF VEGETATION
Krugersdorp	Bankenveld
Randfontein	Turf Thornveld
Bronkhorstspruit	Turf Thornveld
Heidelberg	Turf Highveld
Onderstepoort	Turf Thornveld

The Free State was sampled in the Pure Grassveld types and the False Karoo types (See Table 3.7).

Table 3.7: Sub-types of the vegetation in the Free States where sampling took place

PLACE	TYPE OF VEGETATION
Bethulie	False Upper Karoo
Brandfort	Dry Cymbopogon-Themeda Veld
Bloemfontein	Dry Cymbopogon-Themeda Veld
Springfontein	False Upper Karoo
Kroonstad	Dry Cymbopogon-Themeda Veld
Edenburg	False Upper Karoo
Jacobsdal	False Upper Karoo
Koffiefontein	False Upper Karoo

3.5.2. Management systems for sheep farming and climatic conditions in the study areas

In Gauteng (Krugersdorp and Pretoria) sheep farming is mostly semi-intensive and animals are kept in pens overnight. Most farmers provide their sheep with concentrate and hay during the night. About 80% of sheep in the Pretoria/Krugersdorp area are used for mutton consumption and only very small percentages are kept for wool production (Klopper, 2006). This area lies at an altitude range of 1250 to 1500 m, the average annual rainfall is 784 mm and the average min temperature is 10.4 C and the average maximum temperature is 24.3 C (BBC, 2006).

In the rural areas of Gauteng a communal free-range livestock grazing system is common, where animals are kept in pens overnight and share communal grazing fields owned by the state during the day (BBC, 2006).

In KwaZulu-Natal, the Cato Ridge area is classified as the Moist Midlands Mistbelt. Lying at an altitude range of 900 to 1 400m above sea level, it is generally hilly, rolling country with a high percentage of arable land, approximately 47% being suitable for cropping. The annual rainfall ranges from 800 to 1280mm. The average

annual minimum temperature is 16.6°C and the average annual maximum temperature is 24.6°C (BBC, 2006). Generally, intensive sheep farming is practised in this area. The suitability for sheep farming is high. Limitations to maximal productivity are high input costs, high rainfall (wool), disease, foot rot, stock theft and predators (de Villiers, 2006).

The Ladysmith farming area is classified as Moist Tall Grassveld and Dry Tall Grassveld. This farming area is highly suitable for sheep farming, mainly for wool production. Limitations to maximal productivity are short-term droughts resulting in inadequate fodder flow, stock theft and predators. Different strategies are followed to overcome the short-term drought periods (winter months) in the area. Semi-extensive farming is practised in this area (de Villiers, 2006).

Moist Tall Grassveld – The mean annual rainfall range of this bioresource group is 712 to 805 mm and the mean annual temperature is 17°C. The veld (natural grazing) is in a transitional stage between the sour and mixed veld and has a summer grazing period, before winter licks are required, of approximately 275 days. Sheep need to adapt to a grazing system to avoid serious deterioration in the condition of the veld (de Villiers, 2006).

Dry Tall Grassveld – The mean annual rainfall range is 666 to 745mm and the mean annual temperature is 17.3°C. Annual droughts are between 3 and 4 months – winter period. This area is regarded as an extensive farming area due to the low and erratic rainfall. Stock farming is the most important line of farming (de Villiers, 2006).

The Ulundi area is classified as Lowveld and lies below 450m, the mean annual rainfall range is 587 to 750 mm and the mean annual temperature is 21.9°C. Summers are hot and winters mild to warm. The area is best suited to extensive farming because of the low and erratic rainfall. The sweetveld is able to sustain beef animals throughout the year without the need for supplementation. The suitability for sheep farming is low. This area is not a traditional sheep area and sheep are farmed

under an extensive system. Mutton is the major product of sheep. Limitations to maximal productivity are the high temperature, disease, inconsistent fodder flow, predators and stock theft (de Villiers, 2006).

In the Western Cape, sheep farming is semi-extensive; sheep are sent out on pasture and are not kept in housing. An average of 2.5 sheep per hectare is kept where the tendency of farming is half wheat and half pasture farming. The total annual rainfall is 509 mm at the coast and of 236 mm inland. The altitude of this part of the Western Cape is from 10 to 160 m above the sea level (Klingbill, 2006).

In the Eastern Cape, intensive to semi-intensive farming is mainly practised along the sea border, while towards the inland of the Eastern Cape the farming system becomes extensive. The Eastern Cape can be divided into two climate zones, the moist coastal area and the dry inland. The total annual rainfall is 800-900 mm along the sea border and 300 mm inland. The average annual minimum temperature is 12°C and the average annual maximum temperature is 22.2°C (Debishani, 2006).

3.5.3. Informal Interviews with state veterinarians and AHT

During the visits to the rural areas the state veterinarians and animal health technicians (AHT) were informally interviewed to give an opinion on the number of feral cats present in their district and the habit of keeping cats as pets by the people living in their province.

3.6. Evaluation of medical opinions and human data on toxoplasmosis

At the outset of the study, an overview of doctors' opinions was gathered by informal interviews with doctors (n=5). Four of the doctors were in Gauteng close to the University and one in a major hospital in the Eastern Cape. In addition, a structured questionnaire was developed for medical doctors (n=17), that were specialized in Infectious Diseases, to evaluate expert opinion on toxoplasmosis (Thrusfield, 2005).

As a consequence of a contact with the Pretoria Academic Hospital (Gauteng Province), collaboration was established with a doctor from the Department of Infectious Diseases. Together with this collaborator, data on patients tested for toxoplasmosis from different laboratories in South Africa was requested. Only three laboratories were willing to collaborate (Tygerberg Hospital, Cape Town; Public Health Laboratory, Durban; Pretoria Academic, Pretoria). The permission of the Research Ethics Committee, University of Pretoria, Faculty of Veterinary Science, was given to allow these data to be published.

3.7. Collection and processing of serum samples from sheep

Sheep were bled from the jugular vein by venipuncture into vacutainer tubes which contained no anti-coagulant or preservatives, Vacutainer ® (BP Vacutainer, Preanalytical Solutions, CAT, Belliver Industrial Estate, Plymouth, United Kingdom). Approximately 4 ml of blood was collected per live sheep at abattoirs (before the slaughtering process) and from rural farms. The blood samples were transported on ice to the laboratory and after clotting, the samples were centrifuged at 3000 g for 10 min. The serum was collected into 1.5 ml eppis (®Eppendorf-Netheler-Heinz GmbH, Hamburg, Germany), stored at -20ºC and analyzed in the Serology Laboratory of the Department of Veterinary Tropical Diseases, University of Pretoria, Onderstepoort.

3.8. Laboratory testing

Two serological tests were used for the detection of antibodies of *T. gondii*: the ELISA test and the IFA test. Details of the tests are shown in 3.8.1 and 3.8.2.

3.8.1. ELISA test

The first diagnostic test used was the ELISA, using a commercial enzyme immuno-assay (EIA) test kit (CHEKIT-Toxotest, distributed by IDEXX Laboratories and manufactured by Dr Bommeli AG, Switzerland).

The method used for the ELISA was:

- 100 µℓ of 1:400 diluted samples and controls (diluted by using the CHEKIT-Washing & Dilution-Solution) were distributed into the appropriate wells of the microtitre plate.
- The plate was shaken gently and then incubated for 60 min at 37°C.
- Each well was emptied and washed three times with at least 300 µℓ CHEKIT-Washing & Dilution-Solution.
- 100 µℓ of the CHEKIT-TOXOTEST-Anti-Ruminant-Ig-PO-Conjugate was added to each well and the plate had to be incubated for 60 min at 37°C in a humid chamber.
- The washing process was repeated
- 100 µℓ of the CHEKIT-TMB-Substrate was added to each well and incubated for 15 min until the colour reaction was stopped by adding 100 µℓ of CHEKIT-Stopping-TMB-Solution.
- Within two hours after adding the stopping solution the plates were read by using a photometer.

The optical density (OD) of the positive control was not supposed to exceed 2.0 and the negative control was supposed to be 0.5. The samples were analyzed in relation to the positive and negative controls; the optical density (OD) of the positive control as well as the optical density (OD) of the samples was corrected by subtracting the optical density (OD) of the negative control. The formula used according to the manufacturer instructions in the kit, was the following:

$$\text{Value \%} \quad \frac{\text{(OD of samples – OD of negative control)}}{\text{(OD positive control – OD negative control)}} \quad \text{X } 100$$

The raw data (OD) obtained by this test were typed into the software programme Microsoft Excel and the Value (%) formula was used to calculate the values as a percentage.

The values resulting from the calculation were interpreted as negative if the value was < 20%, as ambiguous if the value was between 20% and 30%, as weak positive if the value was between 30 and 100% and as positive if the value exceeded 100%.

3.8.2. IFA test

The *Toxoplasma* IFA Test is available in the form of slides (Diagnostic & Technical Services C.C., Randburg, South Africa), which contain the whole tachyzoites fixed in formalin, distributed in twelve wells on each slide. The conjugate to be used in this test is provided from KPL (Kirkegaard & Perry Laboratories, Maryland, USA) and is a rabbit anti-sheep Immunoglobin G (IgG) with affinity for purified antibody and fluorescent labelled.

The samples screened at dilutions of 1:64, were considered positive.

- 5µℓ of each serum sample was diluted with 315 µℓ of Phosphate Buffered Sodium Solution (PBS) and 10 µℓ of this dilution was distributed in each well.
- The slides were incubated for 30 min at 37°C.
- The slides were washed in PBS.
- IgG anti-sheep conjugate was added in form of a dilution with PBS Evans Blue of 1:200.
- The slides were incubated for 30 min at 37°C.
- The washing process was repeated.

The slides were examined under an immunofluorescence microscope. If the *Toxoplasma* organisms reflected a bright immunofluorescent light, they were considered positive, otherwise the organisms were of a red to red-green colour and as such they were considered negative.

3.9. Data Analysis

Data were entered into the Microsoft Excel programme and then transferred to Epi Info statistical program (Epi Info Version 3.3.2. CDC Atlanta, 2005). Quantitive data were analysed for significant co-relations.

CHAPTER 4

RESULTS

4.1. Sampling results

Sampling results are important in relation to the abattoirs and rural areas that were randomly selected from the five provinces chosen and the traceability of the sheep. These are discussed in detail in the sub-sections below.

4.1.1. Abattoirs and traceability of animals

List of abattoirs were obtained from the Red Meat Association for each of the five provinces and were randomly selected from the list for this study.

Two abattoirs per province were randomly sampled from these lists and the sheep that were sampled, as described in Chapter 3, were traced using records supplied by the abattoir managers. Traceability comprised of both backward tracing to the location of the farm of origin as well as forward tracing to the location of the butchers that bought the carcasses for retail distribution and sale to consumers.

The origin of the sheep and their destination after slaughter are shown in Table 4.1. It is to be noted that sometimes the selected abattoir obtained the sheep from a different province. The destination of the mutton was also found to involve more than one province in many of the abattoirs.

The findings reflected in Table 4.1 are of considerable interest beyond what was required for this study, as very little published data are available on the traceability and distribution of live sheep and mutton in South Africa.

Table 4.1 Origin of sheep and destination of meat

LOCATION OF ABATTOIR	ORIGIN OF SHEEP	DESTINATION OF MUTTON	SLAUGHTER PROCESS
Ladysmith(KZN)	Ladysmith district (KZN)	Ladysmith Butchery (KZN)	H
Cato Ridge (KZN)	Summerville(EC) Zastron (FS)	Durban Butchery (KZN)	N
Krugersdorp(G)	Gauteng	Central Meat Market, Krugersdorp (G) Seemans,Randburg (G) Broederstroom Butchery(G)	N
Bukeret (Pretoria) (G)	Mpumalanga (MP)	Johannesburg Butcheries(G)	N
Graaff-Reinet (EC)	Graaff-Reinet (EC)	Graaff-Reinet Butcheries (EC)	N
Port Elizabeth(EC)	Grahamstown (EC) Steytlerville (EC)	Port Elizabeth Butcheries (EC)	N
East London (EC)	Port Elizabeth (EC)	East London Butcheries (EC)	N
Paarl (WC)	Piketberg (WC) Malmesbury (WC) Moorreesburg (WC)	Cape Town Butcheries (WC)	N
Bethulie (FS)	Colesberg (NC) Bethulie (FS)	Bethulie Butchery (FS) Durban Butcheries (KZN)	N
Brandfort	Brandfort (FS)	Brandfort Butchery (FS)	N

Key: H = Halaal slaughter for Islamic consumption; N= Non-Halaal slaughter

4.1.2. Rural areas and identification of farms

One state veterinary district was selected randomly per province from a list obtained from the National Department of Agriculture. The veterinary services knew which farms or areas in the province had sheep used for informal slaughter or home consumption and therefore this advice was useful in randomly selecting the district. Each state veterinarian in the districts randomly selected was contacted and asked to select an area or farms in his or her district that were characterized by informal small-scale sheep farming and informal slaughter, as described in Chapter 3.

Documenting the name and phone number of the farmer or owner of the sheep identified each farm used. Each farmer was allocated a letter code.

During visits to these areas, it was noted that all the farmers seemed to have a close relationship to their animals and showed interest in the study, asking the state veterinarian (who was able to communicate in their language) for information about toxoplasmosis and its consequences.

The farmers, their contact numbers and the approximate size of the flocks are shown below for each province selected (Tables 4.2 to 4.7).

Table 4.2: Rural farmers visited in the Eastern Cape (East London District)

Location of farm	Name of owner	Average size of herd
Cove Ridge	A	>30
Kwelera	B	10-20
Mooiplaas	C	>30
Komga	D	>30
Ngxingxolo	E	<10

In part, subsistence farming was practised, with an average number of seven sheep, in the villages that were visited in the Eastern Cape and KwaZulu-Natal (Plate 4.1).

Plate 4.1 Small-scale farming in rural area of KwaZulu-Natal

These farmers slaughter their animals informally only for family celebrations such as marriage and religious festivities.

Table 4.3 Rural farmers visited in KwaZulu-Natal (Nongoma/Ulundi)

LOCATION OF FARM	NAME OF OWNER	AVERAGE SIZE OF HERD
Nongoma	F	10-20
Nongoma	G	20-30
Ebukhalemi	H	10-20
Mbanjeni	I	<10
Ulundi / Dlebe Area	J	10-20
Ulundi / Dlebe Area	K	<10
Ulundi / Dlebe Area	L	10-20
Ulundi / Dlebe Area	M	10-20
Babanango / Nodwengu Area	N	>30

Some farmers had a larger herd of sheep, an average of twenty to thirty sheep, which were used for selling on auctions or to private people.

Table 4.4 Rural farmers visited in Gauteng

LOCATION OF FARM	NAME OF OWNER
Krugersdorp	O
Randfontein	P
Kempton Park (Elandfontein)	Q
Heidelberg	R
Bronkhorstspruit	S

In the Western Cape the state veterinarian, without details, sent the samples. No names of the farm owners were given.

Table 4.5 Rural farmers visited in the Free State

LOCATION OF FARM	NAME OF OWNER
Bloemfontein	T
Springfontein	U
Kroonstaad	V
Edenburg	W
Jacobsdal	X
Koffiefontein	Y

4.2. Mutton consumption in different provinces in South Africa

Information on proportional mutton consumption per province in South Africa was obtained from the Shoprite/Checkers Supermarket Chain (personal communication; Kritzinger, 2006). The Checkers is one of the leading supermarket Chain in South Africa. The data are shown in Table 4.6.

The Western Cape (29.9%) had the highest percentage consumption of all the provinces investigated, followed by Kwa-Zulu Natal with 24.1% There were no data for North West Province.

Table 4.6 Proportional consumption of mutton per province, 2006 (Shoprite Checkers, 2006)

PROVINCE	TOTAL MUTTON AS %
Western Cape	29.9%
KwaZulu-Natal	24.1%
Gauteng	21.1%
Eastern Cape	12.6%
Limpopo, Mpumalanga	6.4%
Northern Cape, Free State	6.0%

4.3. Results of serology testing

The Enzyme-linked Immunosorbent-Assay (ELISA) and the Indirect Fluorescent Antibody Test (IFA) were used for estimating the prevalence of *T. gondii* in sheep. The test results are shown in the sub-sections below. The accuracy (sensitivity and specificity) of the IFAT for demonstration of anti-*T. gondii* antibodies in sheep sera used in this study was determined using the ELISA kit as a reference assay. The ELISA kit was found to be highly sentive (93.4%) and specific (100%) and its cut-off was based on the OD of 0.45. A titre > 1:64 were used as a standard cut-off point for the IFA test.

4.3.1. ELISA test

The results of the ELISA test have been summarized in Table 4.7 and Table 4.8. Out of the 600 serum samples tested a total of 26 were positive. The prevalence per province is shown in Table 4.7.

Table 4.7 Serological results from the ELISA test

PROVINCE	PREVALENCE PER PROVINCE IN %
KwaZulu-Natal	3.6
Eastern Cape	5.4
Western Cape	4.0
Gauteng	6.0
Free State	2.7

The findings show some variation between provinces, with a range of 2.7% to 6.0%. The mean sero- prevalence for all the samples (n=600) was 4.3%

Table 4.8 Serological results from the ELISA test considering rural and urban as variables

BIOME/ PROVINCE	NUMBER OF TESTS	FREQENCY		PERCENTAGE	
		RURAL	URBAN	RURAL	URBAN
KwaZulu-Natal	110	2	2	3.2	4
Eastern Cape	129	1	6	1.9	7.6
Western Cape	100	0	4	0	8
Gauteng	116	3	4	4.6	7.8
Free State	145	4	0	4.2	0
TOTAL (n=)	**600**	**10**	**16**	**4.3**	

4.3.2. IFA test

A total of 34 positive samples were detected using the IFA test, using the same 600 serum samples. The results of the IFA tests have been summarized in Table 4.9 and 5.0.

Table 4.9 Serological results from the IFA test

PROVINCE	PREVALENCE PER PROVINCE IN %
KwaZulu-Natal	6.3
Eastern Cape	7.8
Western Cape	6.0
Gauteng	6.0
Free State	2.7

The prevalence per province also varied, with a range of 2.7% to 7.8%. The mean sero-prevalence for South Africa, using the IFA test on the same serum samples (n=600) was 5.6%.

Table 4.10: Serological results from IFA test considering rural and urban as variables

PROVINCE	NUMBER OF TESTS	FREQENCY		PERCENTAGE	
		RURAL	URBAN	RURAL	URBAN
KwaZulu-Natal	110	5	2	8.2	4
Eastern Cape	129	1	9	1.9	11.5
Western Cape	100	1	5	2	10
Gauteng	116	2	5	3	9.8
Free State	145	3	1	3.2	2
TOTAL (n=)	**600**	**12**	**22**		

4.3.3. Comparison of IFA and ELISA test

The results of both tests were compared, as shown in Table 4.11 below.

Table 4.11: Comparison of IFA and ELISA test results

SAMPLES	NO.
Positive by IFA/negative by ELISA	10
Negative by IFA/positive by ELISA	3
Positive by IFA/positive by ELISA	23
Negative by IFA/negative by ELISA	557

The agreement between the two tests was calculated by using the kappa value, as shown below in Table 4.12.

Table 4.12: Calculation of Kappa value

	ELISA +	ELISA -	Total
IFA+	23 (a)	10 (b)	33
IFA-	3 (c)	557 (d)	560
Total	26	567	593

Where:

OP (observed proportional agreement) = a+d/N =580/593 = 0.978 = 97.8%

EP (expected proportional agreement)

= (a+b/N x a+c/N) + (c+d/N x b+d/N)

= 0.904

= 90%

Kappa = (OP-EP)/(1-EP) = 0.77 = 77%

The agreement between the IFA test and the ELISA test is thus 77 %.

4.3.4. Positive samples and their origin

The number of positive cases as per abattoir or rural area where the serum samples were taken is shown in Table 4.13.

Table 4.13: Positive samples from the different sampling locations in each province

	KWAZULU-NATAL	EASTERN CAPE	WESTERN CAPE	GAUTENG	FREE STATE
ABATTOIR	Ladysmith Abattoir [2] Cato Ridge Abattoir [2]	Graaff-Reinet Abattoir [1] Port Elizabeth Livestock Abattoir [6] East London Abattoir [2]	Paarl Abattoir [3] Elsenburg University [2]	Krugersdorp Abattoir [4]	Brandfort Abattoir [1]
RURAL		Ngxingxolo [1]	Beaufort West [1]	Onderstepoort [1] Elandsfontein [1] Randfontein [2]	Zastron [1] Koffiefontein [1]

Key: The numbers of positive samples are shown in brackets next to each abattoir or rural area in the relevant province

However, as noted previously, the actual origin of the sheep that were bled was different from the place where they were slaughtered. The two sheep testing positive at Cato Ridge were originally from Summerville (EC) or Zastron (FS); the six testing positive at Port Elizabeth were originally from Grahamstown (EC) or Steytlerville (EC) and the two testing positive at East London were originally from Port Elizabeth. At Paarl abattoir the three sheep that were tested positive originally came from different farms in the inland of the Western Cape (Piketberg, Malmesbury, Moorreesburg).

4.3.5. Seroprevalence of anti *T. gondii* antibodies in sheep from different climatic zones

Sheep from the coastal area (the Eastern Cape, KwaZulu-Natal, Western Cape) and Gauteng had the highest sero-prevalence while those from the Karoo-Inland (Free State and interior of Western Cape) had the lowest sero-prevalence. The sero-prevalence of antibodies against *T gondii* found in sheep sampled in different climatic areas is shown in Table 4.14.

Table 4.14: Seroprevalence of anti-*T. gondii* antibodies in sheep sampled from the different climatic areas of South Africa

PROVINCE	ANNUAL RAINFALL (MM)	MIN AVERAGE TEMPERATURE ° C	MAX AVERAGE TEMPERATURE ° C	% OF INFECTION
Gauteng	784	10.4	24.3	6
KwaZulu-Natal	750/1050	14/17	25	6.3
Western Cape	200/750	8/14	23/25	6
Eastern Cape	750/1050	14/17	20/23	7.75
Free State	500	8	20/23	2.7

A significant correlation ($p>0.05$) was detected between the sero-prevalence of IgG in sheep and the minimum average temperature (Table 4.15).

Table 4.15: Analysis of minimum mean daily temperature

MINIMUM MEAN DAILY TEMPERATURE ° C	ELISA		IFA	
	POS*	NEG*	POS*	NEG*
8-10	5	278	9	274
10-14	10	157	12	167
14-17	7	143	12	150
Total	22	578	33	567

Key: Pos = positive tests; Neg = negative tests

No significant correlation was found between the sero-prevalence and the total annual rainfall ($p > 0.05$) in the different provinces.

4.3.6. Sero-prevalence in sheep under different systems of management

The sero-prevalence of antibodies against *T. gondii* in sheep sampled from farms/areas with different management systems is shown in Table 4.16.

Table 4.16: Sero-prevalence of anti-*T. gondii* antibodies in sheep under different systems of management

Province	Management system	Number of sampled
KwaZulu-Natal Abattoir Rural	• Semi-intensive • extensive	110
EasternCape: Abattoir Rural	• semi-intensive • Extensive	129
Western Cape: Abattior Rural	• Semi-intensive • Extensive	100
Gauteng Abattior and Rural	• Semi-intensive	116
Free State Abattoir and Rural	• extensive	145

Table 4.17 contrasts the level of positive cases according to management systems. Sheep managed extensively had a sero-prevalence of 1.8%, which was significantly

lower ($p<0.05$) than the sero-prevalence in sheep under semi-intensive management (5.3%).

Table 4.17: Comparison between different farm management systems

FARM MANAGEMENT SYSTEM	IFA		ELISA	
	POS*	POS*	NEG*	NEG*
Extensive	10	5	273	268
Semi-Intensive	23	17	305	299
Total	33	22	578	567

Key: Pos = positive tests; Neg = negative tests, *Difference because 10 sera were insufficient volume for two tests to be done

4.3.7. Sero-prevalence of toxoplasmosis in sheep originating from rural and commercial farms

The highest overall sero-prevalence among sheep tested was found in commercial sheep, mainly sampled at abattoirs, while the lowest occurred in sheep from rural farms (Table 4.18). A very significant difference ($p< 0.01$) between these two groups was found, with an Odds Ratio (OD) of 0.4. This is reflected in Table 4.18.

4.18: Association between different farm types

TYPE OF FARM	IFA		ELISA	
	POS*	NEG*	POS*	NEG*
Commercial	22	257	15	264
Rural	11	309	7	313
Total	33	566*	22	577*

Key: Pos = positive tests; Neg = negative tests, *Difference because 10 sera were insufficient volume for two tests to be done

4.4. Results of the evaluation of medical opinion

Both formal expert questionnaires and informal interviews were done with medical practitioners in different parts of South Africa.

4.4.1. Informal interviews

In the Gauteng province five medical practitioners were informally interviewed about toxoplasmosis. The results of the interviews are summarised in Table 4.19. In order to keep their names confidential, the doctors have each been allocated a code letter.

Table 4.19: Informal interviews with medical practitioners, 2006

CODE	HOSPITAL	PRACTICE OR SPECIALISATION	NO.OF CASES SEEN	DIAGNOSIS METHOD
Dr A	Medical Centre (Hatfield/Pretoria)	General practitioner	3	Clinical exam
Dr B	Muelmed Hospital (Hatfield/Pretoria)	Immunologist/ General practitioner	4	Clinical exam
Dr C	Kalafong Hospital (Pretoria)	Internal medicine	Not common	Radiology, Brain scan
Dr D	Kalafong Hospital (Pretoria)	Immunologist	3	Clinical exam
Dr E	Port Elizabeth Wellness Clinic (Port Elizabeth)	Immunologist/ General Practiotioner	Not common	Clinical exam

Key: CT* = Computed Tomography

All the doctors interviewed considered toxoplasmosis as a minor problem for HIV-positive patients because they routinely administer prophylactic treatment with three months of sulphonamides, to their HIV-positive patients. This is usually considered to be an effective prophylactic against toxoplasmosis. They do not routinely use

computed tomography (CT) to scan patients for neural toxoplasmosis as it is expensive and few of the hospitals have the equipment. Suspected cases of granulomatous intra-cranial lesions are referred to specialist neurologists.

4.4.2. Expert opinion questionnaires

The data obtained from the expert opinion survey is given in Tables 4.20 to 4.25. The questionnaire is attached in Appendix 1. Most of the experts who filled in the questionnaire are based in Pretoria and specialized in infectious disease. Therefore these doctors are in contact with HIV patients in various stages on a daily basis. Only one questionnaire was completed by a neurologist who is working in Durban, KwaZulu-Natal. A total of 17 medical doctors completed the questionnaire.

The question, how significant toxoplasmosis is as a pathogen related to human HIV infection, is answered by the experts as shown in Table 4.20.

Table 4.20: The significance of toxoplasmosis in HIV positive patients

SIGNIFICANCE OF TOXOPLASMOSIS	EXPERT OPINIONS	% OF EXPERT OPINION	RANKING
Very significant	7	41	1
Significant	6	35	2
Moderately significant	4	23	3
Not very significant	0	0	-
No significance	0	0	-
Total	17	100	

If the answers in Table 4.20 are ranked, it can be seen that the majority of experts regard toxoplasmosis as very significant, with none of them regarding it as “not very significant” or “of no significance”. This differs from the opinions in the informal interviews done with medical practitioners (Table 4.19).

The opinions of the medical doctors on the main syndromes of toxoplasmosis in HIV-positive patients are listed in Table 4.21. They have also been ranked and it can be seen that the syndrome considered most important is cerebral toxoplasmosis (n=17) followed by ocular (n=6) and disseminated toxoplasmosis (n=5).

Table 4.21: The main syndromes associated with patients suffering from toxoplasmosis as a complication of HIV positive patients

MAIN SYNDROMES OF TOXOPLASMOSIS	EXPERT OPINIONS	RANKING
Cerebral toxoplasmosis	17	1
Disseminated toxoplasmosis	5	3
Seizures	2	6
Pneumonia	4	4
Ocular toxoplasmosis	6	2
Hepato-splenomegaly	3	5

In Table 4.22, it can be seen that the expert opinion was divided on the percentage of HIV-AIDS patients who showed toxoplasmosis. Between 5-10% had a ranking of 1 and between 30-40% had a ranking of 2. The reason is unclear and the finding is very interesting and probably reflects the epidemiology of the disease in some way.

Table 4.22: The percentage of HIV/AIDS patients that suffer from toxoplasmosis as a complication

PERCENTAGE OF HIV/AIDS PATIENTS SUFFERING FROM TOXOPLASMOSIS	EXPERT OPINIONS	PERCENTAGE	RANKING
0-5%	1	6	5
5-10%	6	38	1
10-20%	3	19	3
20-30%	2	12	4
30-40%	4	25	2
No opinion	1	-	-
Total	17	100	

Opinions on toxoplasmosis-related mortalities are shown in Table 4.23, below.

Most respondents agreed that toxoplasmosis was a cause of death in 0-5% of patients. In this case three of the experts felt unable to give an opinion. There are very little data on causes of mortality in human patients because cultural and religious norms in South Africa in regard to desecration of dead bodies, means that very few autopsies are done.

Table 4.23: The percentage of HIV/AIDS-related mortalities linked to toxoplasmosis as a complication

HIV/AIDS-RELATED MORTALITY LINKED TO TOXOPLASMOSIS	EXPERT OPINIONS	PERCENTAGE	RANKING
0-5 %	8	47	1
5-10 %	4	23	2
10-20%	2	12	3
No opinion	3	18	-
Total	17	100	

The different treatments for toxoplasmosis chosen by the medical doctors are listed in Table 4.24 and the success of such treatments in HIV positive infected patients are shown in Table 4.25.

Table 4.24: The choice of treatment for toxoplasmosis as a complication of HIV/AIDS

TREATMENT OF TOXOPLASMOSIS IN HIV/AIDS PATIENTS	EXPERT OPINIONS	RANKING
Suphadiazine+pyrimethamine	5	2
Clindamycin	3	3
Cotrimoxazole	2	4
Bactrim	8	1
Trimethoprim	1	5
Sulphadiazine	1	5

In this case, the experts were allowed to choose more than one option and these were added together. Bactrim was ranked first as the treatment of choice.

Table 4.25: The success of treatment of toxoplasmosis as a complication of HIV/AIDS

SUCCESS OF TREATMENT	EXPERT OPINION	% OF EXPERT OPINIONS	RANKING
Very successful	1	6	4
Successful	6	35	2
Moderately successful	7	41	1
Not very successful	3	17	3
Never successful	0	0	-
Total	17	100	

The findings shown in Table 4.25 are surprising in the light of the results of the informal interviews which indicated that toxoplasmosis is not a problem because it can be treated. However, this agrees with the published fact that cerebral

toxoplasmosis is often fatal. The use of suphonamides against this parasite is supported by the pharmacodynamics of this group of drugs, which bind to plasma proteins. In ocular fluids they reach 50-90% of their blood levels and in the cerebro-spinal fluid this can be as high as 80% (Merck, 2005).

4.5. Elaboration of data on human toxoplasmosis

Laboratories from three different provinces were willing to collaborate and provided data on humans tested for toxoplasmosis in the last years. These data include information on gender (females and males), but for ethical reasons no detailed information is available on the patients. As a result the overall prevalence of toxoplasmosis in Gauteng, KwaZulu-Natal and Western Cape was calculated to be 28%. Further details per province are shown in the sub-sections below.

4.5.1. Data from Tygerberg Hospital, Cape Town, Western Cape

A total of 2928 patients were tested for IgG and IgM antibodies from January 2003 to August 2006. Table 4.26 shows the results when tested for IgG and Table 4.27 shows the results when tested for IgM.

Table 4.26: Patients tested for toxoplasmosis IgG and IgM antibodies in Western Cape

PATIENTS TESTED FOR IgG			PATIENTS TESTED FOR IgM		
RESULT	FREQUENCY	PERCENTAGE	RESULT	FREQUENCY	PERCENTAGE
Negative	1991	68	Negative	2661	91
Positive	934	32	Positive	265	9
Total	2928	100	Total	2928	100

The difference between the two levels is explained by the fact that IgG antibodies are detectable in the chronic stage of infection and IgM antibodies are present in the acute stage.

4.5.2. Data from the Public Health Laboratory, Durban, KwaZulu-Natal

The data provided by the Public Health Laboratory of Durban are shown in Table 4.27.

A total of 3554 patients were tested over a period of two years; from January 2005 to September 2006.These patients were only tested for IgG.

Table 4.27: Patients tested for IgG antibodies against toxoplasmosis in KwaZulu-Natal

RESULT	FREQUENCY	PERCENTAGE
Negative	2541	72
Positive	1010	28
Total	3554	100

4.5.3. Data from the Pretoria Academic Hospital, Pretoria, Gauteng

A total of 770 patients were tested from January 2005 to December 2005. Table 4.29 shows the number and percentage of patients testing positive and negative for IgG antibodies against toxoplasmosis

Table 4.28: Patients tested for IgG and IgM antibodies against toxoplasmosis in Gauteng

PATIENTS TESTED FOR IGG			PATIENTS TESTED FOR IGM		
RESULT	FREQUENCY	PERCENTAGE	RESULT	FREQUENCY	PERCENTAGE
Negative	638	82	Negative	697	91
Positive	112	14	Positive	17	2
Total	770	96	Total	770	93

It appears from the above tables that the seroprevalence in humans varies from 14-32% when IgG antibodies are tested to between 2 and 9% when IgM antibodies are tested.

CHAPTER 5

DISCUSSION

5.1. Introduction

In the literature review in Chapter 2, several authors maintained that toxoplasmosis was an important problem in HIV-positive patients and that the sero-prevalence in sheep was high. There is a 80% sero-prevalence in humans in France (Dubey & Beattie, 1988) and 20 to 30% in different states in the USA (Walls, 1988). The central European countries (Belgium, Germany, Austria, Switzerland) report a sero-prevalence of between 37 and 58% in humans, Latin America (Argentina, Jamaica, Brazil, Venezuela) between 51 and 72%, West Africa (Benin, Cameroon, Congo, Cuba, Togo) between 54 and 77% and South East Asia (China and Korea) between 4 and 39% (Tenter *et al.,* 2000). Toxoplasmosis is an important zoonotic disease and the consumption of mutton is considered an important source of infection to humans (Skjerve *et al.,* 1998). A similar high prevalence was reported in sheep in the following countries: Ethiopia (52.6%) (Negash *et al*., 2004), Iran (24.5%) (Hashemi-Fesharki, 1996), Ghana (33.2%) (Van der Puije *et al*., 2000), Tanzania (31.9%) (Singh & Moslla, 1984), Sudan (63%) (Zain Eldin *et al.,* 1985; Negash *et al.,* 2004) Zimbabwe (67.2%) (Hove *et al.,* 2005) and Botswana (10%) (Binta *et al.,* 1998).

5.2. Traceability of slaughter sheep and mutton consumption

In Table 4.1, Chapter 4, the traceability of sheep slaughtered at the abattoirs and the destination of mutton is shown. It can be noticed from these data that sheep slaughtered at Cato Ridge (KwaZulu- Natal) originated from two different provinces: the Eastern Cape (Summerville) and the Free State (Zastron).

The sheep slaughtered in Cato Ridge were sold as mutton to different butcheries in Durban (KwaZulu-Natal). In the Free State it was observed that the Bethulie Abattoir slaughtered sheep that mainly originated from the Northern Cape (Colesberg) and only a small proportion originated in Bethulie. The Bethulie Abattoir transports most of the produced mutton to Durban butcheries. The Bukeret Abattoir in Pretoria (Gauteng) received animals from Mpumalanga and sold the meat to different butcheries in Johannesburg (Gauteng).

As can be seen from the trace-back of the abattoirs in KwaZulu-Natal and Free State there is considerable movement of sheep for slaughter in South Africa.

In Port Elizabeth (Eastern Cape), the animals slaughtered came originally from the centre of the same province, and sheep slaughtered in East London (Eastern Cape) came originally from Port Elizabeth. In the Western Cape, the Paarl Abattoir received sheep from different parts of the province and their meat was sold in Cape Town butcheries. From this it can be seen that there is also long-distance movement of animals within provinces. The movement of sheep to the abattoirs and the destination of meat to the different butcheries is illustrated in Figure 5.1.

As reported in Chapter 2, the deregulation process caused the closure of several large abattoirs. Therefore local abattoirs of small size increased and several large abattoirs with high throughput closed down. The intention was to reduce the long-distance transport of livestock.

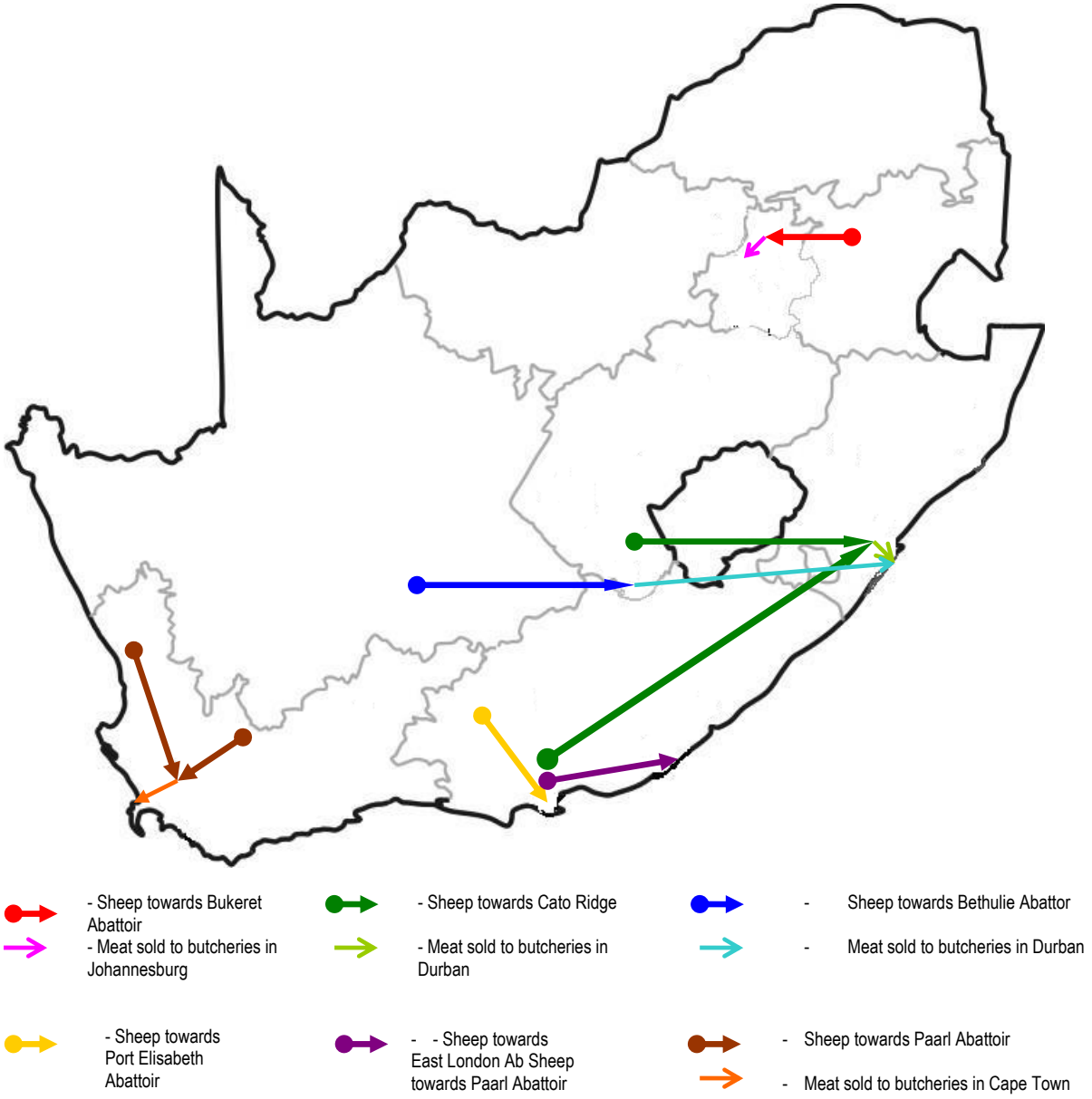


Plate 5.1: Trace-back of sheep slaughtered and the destination of meat

As is evident from the trace-back shown in Figure 5.1, the deregulation process has not been applied to the Cato Ridge and the Bukeret Abattoir, but it has been applied for several local abattoirs that were sampled, such as Ladysmith, (KwaZulu-Natal), Graaff-Reinet (Eastern Cape) and Brandfort (Free State). The consumption of mutton in South Africa was summarized in Table 4.6, Chapter 4, and shows the highest percentage in the Western Cape, followed by KwaZulu-Natal and Gauteng.

The highest number of sheep in South Africa is present in the Eastern Cape (30.1%), Northern Cape (25.3%) and the Free State (20.4%).

Comparing the information of the consumption of mutton per province and the number of sheep per province, there is no agreement; the Western Cape with the highest consumption of mutton has the lowest number of sheep, the Eastern Cape which has the highest number of sheep has a consumption of only 12.6% out of the total for South Africa. Considering this information, the trace-back of sheep at the abattoirs can be explained. KwaZulu-Natal receives sheep for slaughter from the Eastern Cape and the Free State, which are the two provinces with the highest number of sheep; the Bethulie Abattoir sends the meat to KwaZulu-Natal. KwaZulu-Natal has a high consumption of mutton, but does not have a high number of sheep. Therefore, sheep are transported to this province from the Free State and the Eastern Cape and meat is transported to KwaZulu-Natal from the Free State.

5.3. Sero-prevalence at different titres

Titres between 1:32 and 1:64 were used by different authors for detecting the prevalence of toxoplasmosis in sheep or goats. This is discussed in more detail below. Titres used in these different studies are listed in Table 5.1.

O'Donoghue *et al.,* (1987) detected a sero-prevalence of 7.4% and 9.2% in sheep in South Australia with titres > 64 (serum dilution > 1:64) using the IHA and ELISA test. In this study, statistical analyses indicated a significant positive correlation between the two tests applied and this provided a strong support that titres >= 64 can be regarded as significant.

The results obtained by the study of Trees *et al*. (1989), also indicated that with the use of a serum dilution of 1:64, the latex agglutination test (LAT) is a sensitive and reliable test for *T. gondii* infection.

Table 5.1: Titres used in different studies

COUNTRY	ANIMAL SPECIES	TEST USED	SERUM DILUTION	SERO-PREVALENCE
Zimbabwe (Hove *et al.,* 2005)	Sheep and goat	IFA test	1:50	67.2%
Brazil (Figueiredo *et al.,* 2001)	Goat	IHA,IFA test,ELISA	1:32 and 1:64	18.4%
Turkey (Sevgili *et al.,* 2005)	Sheep	SF dye test	1:64	34%
USA (Malik *et al.,* 1990)	Sheep	IFA test	1:32	21.3%
Zimbawe (Pandey & Van Knapen, 1992)	Sheep,goat	LAT (latex agglutination test)	1:64	10%
Ghana (Van der Puije *et al.,* 2000)	Sheep and goat	ELISA	1:64	33.2%
Australia (O'Donoghue *et al.,* 1987)	Sheep	ELISA, IHA	1:64	7.4%, 9.2%
United Kingdom (Trees et al., 1989)	Sheep	LAT	1:64	Experimental study
United States (Riemann *et al.,* 1977)	Sheep	IHA	1:64	24%

According to Plant *et al*., (1982), flocks that contain rams with a titre >= 64 were considered to be infected flocks. In the current study a titre of > 64 was used as a standard dilution for the IFA test, as this was the titre at which the majority of other studies were done. The titre of >50, as used in Zimbabwe, gave a higher sero-prevalence and it is suggested that this may have been due to false positives at the lower dilution.

5.3.1. Comparison of the commercial ELISA with the IFA test

Previous studies have shown a significant correlation between the IFA and the ELISA test. In South Australia a significant correlation between the IFA (7.4%) and ELISA (9.2%) test was found by O'Donoghue *et al.,* (1987) during their serological survey for toxoplasmosis in sheep. In 2001, Figueiredo *et al.,* found a high correlation between IFA test (19.5%) and ELISA (19.5%) in their study on the

seroprevalence of toxoplasmosis in sheep in Uberlandia (Brazil). A positive correlation between the two tests was also found in a study done in France by Berthet and Bourdin (1982). Pereira-Bueno *et al*., (2004) used several diagnostic techniques for detecting *T. gondii* antibody in aborted foetuses and a sero-prevalence of 28.3% was found by using IFA test and ELISA. They compared the serological results and obtained a perfect agreement between the two tests. In the northwestern United States the sero-prevalence using IFA test (58.5%) was similar to that using ELISA (59%) (Malik *et al.,*1990).

The results of the current study revealed a fair to good overall correlation between the IFA test (5.6%) and ELISA (4.3%) in sheep (kappa= 77%).

5.3.2. Prevalence of toxoplasmosis in sheep compared to other animals in South Africa

An overall sero-prevalence of 4.3% (ELISA test) and 5.6% (IFA test) for toxoplasmosis was found for sheep in South Africa. These is the first data available on toxoplasmosis in sheep for this country, although the sero-prevalence in free-ranging large felids, such as lions and leopards, has previously been published by Penzhorn *et al.* (2002). The sero-prevalence in these felids was found to be high: 86% of 7 leopard and 100% of 12 lion sera from the Kruger National Park were positive. From Hluhluwe-Umfolozi Park 100% of 30 lions tested sero-positive.

In contrast to the human and felid sero-prevalence in South Africa, as described above, our study revealed an unexpectedly low prevalence in sheep. Mutton is an important way of transmission to humans and we can therefore expect that the prevalence in humans is related to the actual data found in sheep.

5.3.3. Prevalence of toxoplasmosis in sheep sampled from different climatic zones in South Africa

The sero-prevalence in the five sampling areas (ranging from a dry climate in the north-west to a temperate climate in the Western Cape and a humid tropical climate of KwaZulu-Natal) was found to be the highest in the Eastern Cape, followed by KwaZulu-Natal, Western Cape and Gauteng. The lowest prevalence of infected sheep was found in the Free State.

Previous epidemiological studies have found infections in sheep to be more prevalent in cool, moist areas than in hot, dry areas (O'Donoghue *et al.,* 1987). Hove *et al.* (2005) found similar results in the study done in Zimbabwe: a significantly higher seroprevalence in the Mt. Darwin and Bikita districts of Zimbabwe, which receives a higher rainfall, as compared to the Mudzi and Gwanda districts.

Similar studies have also been done by Van der Puije *et al.* (2000) who justified the higher prevalence found in the wet coastal savannah and the humid forest zones of Ghana as a factor responsible for the higher prevalence in these areas, especially if compared to the drier Guinea savannah of Ghana. It has been suggested that a wet moist climate favours the time of survival of oocysts (leading to a prolonged period of exposure to sheep) because the oocysts are known to survive only a short period of dehydration and cold (Munday 1975; Riemann *et al.,* 1977; Uggla & Hjort 1981). The analysis of the minimum average temperatures in our five different study areas revealed a significant correlation between the prevalence of toxoplasmosis and this climatic factor ($p < 0.05$). The Free State with the lowest prevalence has a minimum temperature of 8.6 ºC while Eastern Cape with the highest prevalence has a minimum average temperature of 13 ºC. The lower prevalence in the Free State (2.7%) compared to the prevalence of toxoplasmosis found in the Eastern Cape, Western Cape and KwaZulu-Natal (7.75%, 6% and 6.3%) might also be due to the difference in rainfall along the coast. Statistical analyses did not reveal any significant correlation between the sero-prevalence of toxoplasmosis in sheep and

the total annual rainfall in each province. This might be due to the considerable movement of sheep demonstrated by trace-back. It is also possible that sheep listed as coming from a particular province may actually have originated elsewhere and been merely fattened for slaughter in that province.

5.3.4. Sero-prevalence in other countries under different climatic conditions

As the climatic factors are correlated to the prevalence of toxoplasmosis (explained in Section 5.3.3), the relatively low rainfall in southern Africa might justify the much lower overall prevalence of *T. gondii* in sheep in South Africa. South Africa has an average annual rainfall of 464mm, compared with a world average of 860mm (Toyson, 1986) and in addition South Africa is regularly hit by droughts with the last one occurring as recently as 2004.

The sero-prevalence of *T. gondii* in African countries is shown in Table 5.3. In neighbouring countries such as Zimbabwe, an overall sero-prevalence of 67.7% was found in adult sheep and goats (Hove *et al.,* 2005) while in 1992 Pandey and van Knapen detected a low prevalence of *T. gondii* infection of 9.2% in adult sheep and 2.9% in goats from different parts of Zimbabwe (Pandey & van Knapen, 1992).

Table 5.2: Sero-prevalence of *T. gondii* in sheep in different African countries and climatic conditions

COUNTRY	SERO-PREVALENCE %	CLIMATE
Ethiopia	52.3	Tropical monsoon
Ghana	33.2	Tropical, warm, hot and humid in southwest, hot and dry in the north
Sudan	63	Tropical in south, arid desert in north, rainy season in April to November
Tanzania	31.9	Varies from tropical along the coast to temperate in highlands
Botswana	10	Semi-arid, warm winters and hot summers
Zimbabwe	67.2	Tropical, hot, wet summer with mild dry winters, harsher in interior
South Africa	5.6	Semi-arid, subtropical along east coast, sunny days, cool nights

In Botswana a sero-prevalence of 10% was detected in goats (Binta *et al.,* 1998). Further sero-prevalence rates ranging from 22.9% to 63 % have been reported in different African countries; in Ethiopia a prevalence of 22.9% was detected in sheep in 1989 (Bekele & Kasali, 1989) and in a later study a sero- prevalence of 52.6% was reported (Negash *et al.,* 2004); in Ghana the overall sero-prevalence in sheep was shown to be 33.2% (Van der Puije *et al.,* 2000), in Tanzania 31.9% and in Sudan 63% (Negash *et al.,* 2004).

Compared to the data obtained from Zimbabwe in 2005 and other African countries, South Africa has a low prevalence of toxoplasmosis. The highest prevalence levels (> 50%) were detected in Zimbabwe, Sudan and Ethiopia, which can possibly be associated with the tropical climate in these countries. Botswana and South Africa report a low prevalence (< 10%) of toxoplasmosis in sheep, probably due to their mainly semi-arid climate.

The sero-prevalence of *T. gondii* in sheep in South Australia has been studied by O' Donoghue *et al.,* (1987). South Australia, which is at a similar latitude as South Africa, also has similar climatic conditions (arid to semi-arid climate with a winter rainfall temperate climate). The sero-prevalence of infected sheep in that country was found to be 7.4% (IHAT) and 9.2% (ELISA). These results do not differ very much from the sero-prevalence of 4.3% (IFAT) and 5.6% (ELISA) found in South Africa. South Africa's temperatures tend to be lower than in Australia, due to the greater elevation above sea level of the subcontinent (Toyson, 1986). This factor might also justify the slight difference in sero-prevalence.

In the northeastern United States the prevalence of toxoplasmosis in sheep was found to be 59.5 % (ELISA) and 59% (IFAT). The climate in this part of the United States is characterized by hot, wet summers and cold humid winters and these climatic conditions might justify the much higher prevalence found (Malik *et al.,* 1990).

In the Madrid region (Spain) the sero-prevalence of toxoplasmosis in sheep and goats was found to be 11.8%. This relatively low prevalence might be associated with the continental climate in the Madrid region (hot and dry summer and cold winter) (Mainar *et al.,* 1996).

5.3.5 Prevalence of toxoplasmosis under different systems of management

Another factor that increases the risk of exposure of sheep to *T. gondii* is the management system. Previous epidemiological studies have found that infections are more prevalent in sheep kept under intensive or semi-intensive systems of management (Waldeland, 1976; Plant *et al.,* 1974 and 1982). Van der Puije *et al.* (2000) associated the higher prevalence of *T. gondii* in sheep to the intensive and semi-intensive farming system in Ghana. An association between management and

prevalence was also observed in Norway (Waldeland, 1976) and in the United States of America (Riemann *et al.,* 1977).

Plant *et al.* (1982) suggested that the prevalence of infection was even more influenced by management factors than by environmental factors. In their study, a higher prevalence was recorded in sheep kept under more intensive systems of management and they did not find a positive association between the prevalence of toxoplasmosis in different geographic areas. In Tasmania, Munday (1975) concluded from his study that the prevalence of *T. gondii* was related to the management system. A similar trend was found in the current study and Table 4.16 summarizes the different management systems per province and compares this with sero-prevalence. The prevalence of infections was high in the coastal areas and in the urban areas (Gauteng) where sheep management practices were more intensive compared to the Karoo area and the Free State, which are characterized by its vast grazing areas. In an intensive to semi-intensive farming system sheep are housed together entirely or partly during the day. Domestic cats are more probably living near the food stores on the farm where they are used for controlling rodents. For this reason sheep under semi-intensive or intensive farming conditions might be more exposed to *Toxoplasma* oocysts shed by cats. An experimental study was done by Plant *et al.* (1974), where an outbreak of ovine congenital toxoplasmosis was simulated by feeding sheep with grain contaminated with cat feces. This study confirmed that, in an intensive farming system, where cats are commonly kept on farms, feed could easily be contaminated with oocysts and be responsible for a rapid spread of infection in a flock.

5.3.5. Prevalence in urban areas compared to rural areas

The study done on the sero-prevalence of *T. gondii* infection in sheep and goats in Zimbabwe showed that sheep and goats originating from commercial farms had an eight times lower prevalence (10%) compared to sheep and goats originating from rural areas (80%) (Hove *et.al*., 2005). It was expected that a similar situation would

be seen in the study done in South Africa and therefore the difference in prevalence between rural and commercial farms was examined.

Statistical analyses revealed a significant difference between the sero-prevalence on commercial and rural farms ($p<0.01$). However, there is no constant trend of higher levels in rural areas as reported in Zimbabwe.

As shown in Table 4.8 in Chapter 4, this was not found in all provinces, only KwaZulu-Natal showed a higher level in rural communities. In KwaZulu-Natal 8.2% of the rural sheep population was sero-positive while 4% of sheep sampled from commercial farms (abattoirs) were sero-positive. This province would fit into the picture given by the study done in Zimbabwe by Hove *et al.* (2005), while the other provinces show the opposite picture from Zimbabwe: Eastern Cape (1.9% rural and 11.5% urban), Western Cape (2% rural and 10% urban) and Gauteng (3% rural and 9.8% urban).

This disagreement of our study with the study done in Zimbabwe in 2005 might be due to the different ethnic groups living in South Africa and their different relationship to cats. The lower sero-prevalence of toxoplasmosis in rural areas in the Cape area and Gauteng, compared to urban areas, may be related to the fact that rural communities seldom keep cats because of their traditional beliefs while in the urban areas cats are regarded as pets and are also used for rodent control on farms. KwaZulu-Natal is the province with the highest Muslim and Hindu community in South Africa. Muslims and Hindus living in rural areas prefer to keep cats rather than dogs, according to informal interviews with such owners. A previous study done on the sero-prevalence of toxoplasmosis in humans in South Africa revealed a higher prevalence (33%) in the Indian population, mainly living in KwaZulu-Natal (Jacobs & Mason, 1978), compared to other ethic groups. Although accurate statistics are not available on the distribution of domestic cats or feral cats in South Africa, during visits to rural communities very few feral cats were seen in the houses and

surroundings and informal interviews with veterinary staff indicated a cultural prejudice against keeping cats.

5.4. Medical opinions

In contrast to findings in other countries, the South African study showed a much lower prevalence in sheep. The sero-prevalence of *T. gondii* antibodies in humans in South Africa, which was published by Jacobs and Mason nearly 40 years ago, at a time when HIV-AIDS was not a problem, was found to be 20% (Jacobs & Mason, 1978). Medical practitioners who were informally interviewed did not regard toxoplasmosis as an important zoonosis of HIV-positive patients in South Africa. In contrast to these findings, medical doctors considered as experts by their colleagues regarded toxoplasmosis as a significant pathogen related to HIV-positive patients. Their estimation of the problem was validated by the data obtained from three laboratories in South Africa that routinely tested humans for toxoplasmosis. A sero-prevalence in humans, ranging from 2% (IgM in Gauteng) to a high of 32% (IgG in the Western Cape) was found by the analysis of these data.

5.4.1. Evaluation of informal interviews and expert opinion questionnaires

The medical doctors who were informally interviewed were mainly general practitioners or immunologists and their colleagues did not nominate them as experts. They do not consider toxoplasmosis as a major problem in HIV-positive patients and regard the treatment as successful. HIV-positive patients are treated prophylactically with sulphonamides and no further tests on toxoplasmosis are performed. They estimated that they saw three or four cases of toxoplasmosis related to HIV-infected patients per year.

The outcome of the informal interviews with the general practitioners and immunologist differed from the results obtained from the expert opinion questionnaires. The doctors selected as experts are mainly specialists on infectious

disease and are therefore exposed to many HIV-infected patients. Most of them (41%) considered toxoplasmosis as a very significant pathogen, as a significant pathogen (35%) and as a moderately significant pathogen (23%). Their opinion on the percentage of HIV-infected patients who suffer from toxoplasmosis was much higher compared to the number of cases estimated by the informal interviews with doctors. The questionnaires showed that 38% of the experts estimate 5 to 10% of HIV-positive patients suffer from toxoplasmosis while 25% of the experts estimated toxoplasmosis to be as high as 30 to 40%. This disagreement might be due to the fact that they are exposed to patients from different areas. The disease might be spread differently in diverse areas where the eating habits and hygienic conditions vary. The treatment of toxoplasmosis was considered by 41% of the experts as moderately successful and by 35% as successful. Only 6% considered the treatment as very successful and 17% as not successful. This outcome shows that the treatment can be considered on average as moderately successful with a possibility of being not very successful. In the informal interviews toxoplasmosis was considered to be a treatable disease and therefore not viewed as a problem.

The opinion on toxoplasmosis obtained from the two different groups (the expert and the informal interviews) showed a very different outcome. This might be due to the fact that the general practitioners and immunologists do not give importance to a disease that in their opinion can be easily treated.

5.4.2. Evaluation of human data

The data obtained from laboratories in three provinces in South Africa (Western Cape, KwaZulu-Natal and Gauteng) may be biased because the patients tested for toxoplasmosis were not selected randomly. Therefore these data only help to estimate the prevalence of toxoplasmosis in humans in South Africa.

The estimated seroprevalence varied from 32% in the Western Cape, to 28% in KwaZulu-Natal and 14% in Gauteng.

In South Africa we have diverse ethical groups and religions, which follow different eating habits, have different relationships to pets and different housing habits. Therefore each group is exposed to different risk factors of becoming infected with toxoplasmosis.

KwaZulu-Natal and the Western Cape have a large concentration of Muslims and Hindus who are known to prefer mutton. As the information obtained from Shoprite-Checkers confirms, the Western Cape is the region with the highest consumption of mutton, followed by KwaZulu-Natal (Chapter 4, Table 4.6). The higher sero-prevalence of toxoplasmosis in humans in the Western Cape and KwaZulu-Natal might be due to the higher consumption of mutton compared to other provinces and it has been confirmed from the literature that the consumption of undercooked meat is the main source of infection for humans (Cook *et al*., 2000).

In 1978, Jacobs and Mason studied the seroprevalence of toxoplasmosis in humans in South Africa and compared the differences between different ethnic groups. Their study indicated sero-prevalence rates that varied from 9% in the San population to 34% in the black population of KwaZulu-Natal. The Indian population of KwaZulu-Natal showed a sero-prevalence similar to that of the black population (33%). They justified the discrepancy in sero-prevalence between the different ethnic groups by suggesting that there was a higher risk of becoming infected in rural than in urban areas. According to Jacob and Manson, the Black population around Durban and Port Elizabeth was mainly living in rural areas and therefore had a relative high prevalence of toxoplasmosis.

The findings for human sero-prevalence in different provinces described in this study are similar to those found by Jacobs and Mason (1978). However, the results obtained lead us to suggest that the differences in human seroprevalence may be

linked to different eating habits, particularly mutton consumption, of populations in different provinces.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

As an outcome of this study, the sero-prevalence of toxoplasmosis in sheep in South Africa was found to be 5.6% using the IFA test and 4.3% using the ELISA test. Although the sero-prevalence of sheep in this country is lower than expected, these data prove that *T. gondii* is present in sheep in South Africa. The lower level of toxoplasmosis in sheep may be due to the arid conditions of this country as well as to the extensive sheep farming systems mainly practised by South African farmers. The fact that we have a high turnover of sheep, with most slaughter stock being less than a year old, also explains the low sero-prevalence of toxoplasmosis. Sheep get slaughtered at a young age and are therefore exposed to the risk of becoming infected for a shorter period of time.

South Africans consume a lot of mutton, especially members of the Muslim and Hindu communities. Informal slaughtering is frequently used in rural areas. About 30% of sheep are slaughtered informally among indigenous rural communities who are mainly subsistence farmers (McCrindle, 2005). In these communities many family members participate in the slaughter process, the cleaning of the carcass and the preparation of the meat. Each person involved in the whole working process has a higher risk of becoming infected with toxoplasmosis. In the rural areas, the lack of running water and the habit of eating with their fingers also contributes to people becoming infected with toxoplasmosis.

Mutton can be considered as the main source of *T. gondii* infection to humans in South Africa, while contact with cats is a lower risk factor. This statement is based on the fact that in South Africa the number of feral cats is relatively low. Informal

interviews with state veterinarians and animal health technicians, as well as personal observations indicated that feral cats are less common in South Africa than in Europe. Cats kept as pets mainly live in good hygienic conditions, are fed canned cat food and are therefore not considered as a high risk factor.

6.2. Recommendations

Informal interviews with medical doctors (mainly general practitioners) indicated that their knowledge about toxoplasmosis is low and that they underestimate the importance of this parasite. A disagreement between the informal interviews with medical practitioners and structured interviews with the medical doctors selected as experts underlines this fact. The experts have a good general knowledge on toxoplasmosis and considered this disease as significant, especially when related to HIV-positive patients. In addition, the data obtained from human serum from the laboratories indicate that the sero-prevalence in humans in South Africa is higher than estimated by the informal interviews with general practitioners.

Collaboration between medical doctors and veterinarians should contribute to a better education in both professions. The present research was undertaken because of such collaboration. Apart from a few medical doctors, collaboration turned out to be very difficult.

These conclusions have resulted in the production of a pamphlet which is included as an annexure. This pamphlet could be considered as the first step to contribute towards general education of the public about toxoplasmosis and would hopefully motivate medical doctors and veterinarians to work together in future on many other potentially zoonotic diseases.

CHAPTER 7

REFERENCES

Alexander J & Stimson W H 1988 Sex hormones and the course of parasitic infection. *Parasitology Today* 4 (7): 189-193

Barnett T & Whiteside A 2002 *AIDS in the twenty-first century* 1st Edition.

Bartoszcze M, Krupa K & Roszkowski J 1991 ELISA for assessing *T.gondii* antibodies in pigs. *Journal of Veterinary Medicine*, series B, 38 (4): 263-264

Bekele T & Kasali OB 1989 Toxoplasmosis in sheep, goats and cattle in central Ethiopia. *Veterinary Research Communications* 13 (5): 371-375

Berthet B & Bourdin P 1982 Application of the ELISA technique to the serological diagnosis of ovine toxoplasmosis: its value for small Sahelian ruminants. *Revue d'Elevage et de Medecine Veterinaire des Pays Tropicaux Pays Trop*, 35: 27

Bhigjee A I, Naidoo K, Patel V B & Govender D 1999 Intracranial mass lesions in HIV-positive patients the KwaZulu-Natal experience. *South African Medical Journal* 89: 1284-1288

Bhigjee A I 2005 Neurological manifestations of HIV infection in KwaZulu-Natal, South Africa. *Journal of Neurovirology* 11 (1): 17-21

Bigalke R D, Tustin R C, Du Plessis J L, Basson P A & Mc Cully R M 1966 The isolation of *Toxoplasma gondii* from ferrets in South Africa. *Journal of the South African Veterinary Medical Association* 37: 243-247

Binta M G, Mushi E Z, Raborokgwe M & Ndebele T R 1998 The prevalence of antibodies to *Toxoplasma gondii* in goats with history of abortion. *Zimbabwe Veterinary Journal* 29: 47-50

Bisson A, Maley S, Rubaire-Akiiki C M & Wastling J M 2000 The seroprevalence of antibodies to *Toxoplasma gondii* in domestic goats in Uganda. *Acta Tropica* 76 (1): 33-38

Blewett D A & Watson F 1983 The epidemiology of ovine toxoplasmosis II. Possible sources of infection in outbreaks of clinical disease. *British Veterinary Journa*l 139: 546-555

Brink J D, De Wet J S & Van Rensburg A J 1975 A serological survey of toxoplasmosis in the Bloefontein area. *South African Medical Journal* 49: 1441-1443
Burnett A J, Shortt S G, Isaac-Renton J, King A, Werker D & Bowie W R 1998 Multiple cases of acquired toxoplasmosis retinitis presenting in an outbreak. *Ophthalmology* 105 (6): 1032-1037

Canfield P J, Hartley W J & Dubey J P 1990 Lesions of toxoplasmosis in Australian marsupials. *Journal of Comparative Pathology* 103 (2): 159-167

Carruthers V B 2002 Host cell invasion by opportunistic pathogen *Toxoplasma gondii*. *Acta Tropica* 81 (2): 111-122

Castro-Sansores C J, Go'ngora-Biachi R A & Gonza'lez-Martinez P 2004 HIV/Encephalopathy as initial manifestation of acquired immunodeficiency syndrome in Yucatan State, Mexico. *Archives of Medical Research* 35 (1): 49-35

Centre for the Study of AIDS (CSA) 2005 Education course and community work, University of Pretoria

Cook A J C, Gilbert R E, Buffolano W, Zufferey J, Petersen E, Jenum P A, Foulon W, Semprini A E & Dunn D T 2000 Sources of *Toxoplasma* in pregnant women: European multicentre case control study. *British Medical Journal* 321: 142-147

Debishani L 2006 National Wool Growers Association, Personal Communication
De Villers H 2006 Farming System Research Section, South Region, Hilton, Western Cape: Personal communication

Del Valle L & Pina-Oviedo S 2006 HIV disorders of brain: pathology and pathogenesis. *Frontiers in Bioscience* 11: 718-732

Deyab A K & Hassanein R 2005 Zoonotic toxoplasmosis in chickens. *Journal of the Egyptian Society of Parasitology* 35(1): 341-350

Dubey J P 1980 Persistence of encysted *Toxoplasma gondii* in caprine livers and public health significance of toxoplasmosis in goats. *Journal of the American Veterinary Medical Association* 177 (12): 1203-1207

Dubey J P 1986 A review of toxoplasmosis in cattle. *Veterinary Parasitology* 22: 177-202

Dubey J P 1990 Status of toxoplasmosis in sheep and goats in the United States. *Journal of the American Veterinary Medical Association* 196 (2): 259-262

Dubey J P 1996 Strategies to reduce transmission of *Toxoplasma gondii* to animals and humans. *Veterinary Parasitology* 64: 65-70

Dubey J P 2004 Toxoplasmosis - a waterborne zoonosis. *Veterinary Parasitology* 126 (1-2): 57-72

Dubey J P and Beattie C P 1988 *Toxoplasmosis of animals and man* Boca Raton, Edition CRC Press, Florida, USA

Dubey J P & Frenkel J K 1976 Feline toxoplasmosis from acutely infected mice and the development of *Toxoplasma* cysts. *Journal of Protozoology* 23 (4): 537-546

Dubey J P, Gomez-Marin J E, Bedoya A, Lora F, Vianna M C, Hill D, Kwok O C, Shen S K, Marcet P L & Lehmann T 2005 Genetic and biologic characteristics of *Toxoplasma gondii* isolates in free-range chickens from Colombia, South America. *Veterinary Parasitology* 134 (1-2): 67-72

Dubey J P, Graham D H, Dahl E, Sreekumar C, Lehmann T, Davis M F & Morishita T Y 2003 *Toxoplasma gondii* isolates from free-ranging chickens in the United States. *Journal of Parasitology* 89 (5): 1060-1062

Dubey J P, Miller S, Powell E C & Anderson W R 1986 Epizootiologic investigations on a sheep farm with *Toxoplasma gondii*-induced abortions. *Journal of the American Veterinary Medical Association* 188: 155-158

Dubey J P, Ott-Joslin J, Torgerson R W, Topper M J & Sundberg J P 1988 Toxoplasmosis in black-faced kangaroos (*Macropus fuliginosus melanops*). *Veterinary Parasitology* 30 (2): 97-105

Dubey J P, Salant H, Sreekumar C, Dahl E, Vianna M C, Shen S K, Kwok O C, Spira D, Hamburger J & Lehmann T 2004 High prevalence of *Toxoplasma gondii* in a commercial flock of chickens in Israel, and public health implications of free-range farming. *Veterinary Parasitology* 121 (3-4): 317-322

Dubey J P & Towle A 1986 *Toxoplasmosis in sheep*, Cambrian News Ltd, Edition C.A.B. International, Slough, UK

Dubey J P, Zarnke R, Thomas N J, Wong S K, Van Bonn W, Briggs M, Davis J W, Ewing R, Mense M, Kwok O C H, Romand S & Thulliez P 2003 *Toxoplasma gondii, Neospora caninum, Sarcocystis neurona*, and *Sarcocystis canis*-like infection in marine mammals. *Veterinary Parasitology* 116 (4): 275-296

Du Plessis J L, Bigalke R D & Gurnell T O 1967 An outbreak of toxoplasmosis in chinchillas in South Africa. *Journal of the South African Veterinary Medical Association* 38 (1): 79-83

Figueiredo J F, Silva D A O, Cabral D D & Mineo J R 2001 Seroprevalence of *Toxoplasma gondii* infection in goats by the Indirect Haemagglutination, Immunofluorescence and Immunoenzymatic Test in the region of Uberlandia, Brazil. *Memorias do Instituto Oswaldo Cruz, Rio de Janiero* 95 (5): 687-692

Foodsafety website 2000, http://www.foodsafety

Frenkel J K & Dubey J P 1972 Toxoplasmosis and its prevention in cats and man. *Journal of Infectious Disease* 126: 664-673

Gamble H R, Dubey J P & Lambillotte D N 2005 Comparison of a commercial ELISA with the modified agglutination test for detection of *Toxoplasma* infection in domestic pigs. *Veterinary Parasitology* 128 (3-4): 177-181

Ghazaei C 2005 Serological survey of antibodies to *Toxoplasma*. *African Journal of Livestock Extension* 4 (3): 306-308

Gilbert R E & Stanford M R 2000 Is ocular toxoplasmosis caused by prenatal or postnatal infection? *British Journal of Ophthalmology* 84: 224-226

Gummow B 2006 Veterinary Epidemiology, Course EPI 751, University of Pretoria, South Africa

Hartley M & English A 2005 A seroprevalence survey of *Toxoplasma gondii* in common wombats (*Vombatus ursinus*). *European Journal of Wildlife Research* 51 (1): 65-67

Hashemi-Fesharki R 1996 Seroprevalence of *Toxoplasma gondii* in cattle, sheep and goats in Iran. *Veterinary Parasitology* 61: 1-3

Hill D & Dubey J P 2002 *Toxoplasma gondii*: transmission, diagnosis and prevention. *Clinical Microbiology & Infection* 8 (10): 634-642

Hove T, Lind P & Mukaratirwa S 2005 a. Seroprevalence of *Toxoplasma gondii* infection in domestic pigs reared under different management systems in Zimbabwe. *Onderstepoort Journal of Veterinary Research* 72: 231-237

Jacobs M R & Mason P R 1978 Prevalence of *Toxoplasma* antibodies in Southern Africa. *South African Medical Journal* 53: 619-621

Jittapalapong S, Sangvaranond A, Pinyopanuwat N, Chimnoi W, Koizumi S & Maruyama S 2005 Seroprevalence of *Toxoplasma gondii* infection in domestic goats in Satun Province, Thailand. *Veterinary Parasitology* 127 (1): 23-28

Johnson A M, Roberts H & Tenter A M 1992 Evaluation of a recombinant antigen ELISA for the diagnosis of acute toxoplasmosis and comparison with traditional antigen ELISAs. *Journal of Medical Microbiology* 37 (6): 404-409

Jones J L, Kurszon-Moran D & Wilson M 2003 *Toxoplasma gondii* infection in the United States, 1999-2000. *Emerging Infectious Disease* 9 (11) URL hhtt://www.cdc.gov/ncidod/EID/vol9no11/03/0098.htm

Klingbill G 2006 National Wool Growers Association, Personal Communication

Kritzinger B 2006 National Wool Growers Association, Personal Communication

Luft B J & Remington J S 1992 Toxoplasmatic encephalitis in AIDS. *Clinical Infectious Disease* 15: 211-222

MacAllister M M 2005 A decade of discoveries in veterinary protozoology changes our concept of “subclinical” toxoplasmosis. *Veterinary Parasitology* 132 (3-4): 241-247

Mainar R C, De La Cruz C, Asensio A, Dominguez L & Vazquez-Boland J A 1996 Prevalence of agglutination to *Toxoplasma gondii* antibodies in small ruminants of the Madrid region, Spain, and identification of factors influencing seropositivity by multivariate analysis. *Veterinary Research Communications* 20: 153-159

Malik M A, Dreesen D W & De la Cruz A 1990 Toxoplasmosis in sheep in northeastern United States. *Journal of the American Veterinary Medical Association* 196 (2): 263-265

McCrindle C M E 2005, Personal Communication

McCrindle C M E 2006, Community-based Animal Health: Veterinary Communication and Extension, Course CAH 812, University of Pretoria, South Africa

McCrindle C M E, Veary C M & Thomas J 2006 (unpublished data) Section Veterinary Public Health, Dept. Paraclinical Sciences, University of Pretotria, South Africa

Miller M A, Gardner I A, Kreuder C, Paradies D M, Worcester K R, Jessup D A, Dodd E, Harris M D, Ames J A, Packham A E & Conrad P A 2002 Coastal freshwater runoff is a risk factor for *Toxoplasma gondii* infection of southern sea otters (*Enhydra lutris nereis*). *International Journal for Parasitology* 32 (8): 997-1006

Motloung T, Myers M, Venter F, Delany S & Rees H 2004 Identifying acute HIV infection- a major new public health challenge. *South African Medical Journal* 94 (7): 531

Munday BL 1975 Prevalence of toxoplasmosis in Tasmanian meat animals. *Australian Veterinary Journal* 51: 315-316

National Department of Agriculture (NDA) 2003 Animal production-livestock numbers. Directorate Agricultural statistics, URL www.nda.gov.za

Negash T, Tilahun G, Patton S, Prevot F & Dorchies P H 2004 Serological survey on toxoplasmosis in sheep and goats in Nazareth, Ethiopia. *Revue de Medecine Veterinaire* 155 (10): 486-487

Nesbit J W, Lourens D C & Williams M C 1981 Spastic paresis in two littermate pups caused by *Toxoplasma gondii*. *Journal of the South African Veterinary Association* 52: 243-246

O'Donoghue P J, Riley M J & Clarke J F 1987 Serological survey for *Toxoplasma* infections in sheep. *Australian Veterinary Journal* 64 (2): 40-45

Pandey V S van Knapen F 1992 The seroprevalence of toxoplasmosis in sheep, goats and pigs in Zimbabwe. *Annals of Tropical Medicine and Parasitology* 86: 313-315

Penzhorn B L, Stylianides E, Van Vuuren M, Alexander K, Meltzer D G A & Mukarati N 2002 Seroprevalence of *Toxoplasma gondii* in free-ranging lion and leopard populations in southern Africa. *South African Journal of Wildlife Research* 32 (2): 163-165

Pereira-Bueno J, Quintanilla-Gozalo A, Perez-Perez V, Alvarez-Garcia G, Collantes-Fernandez E & Ortega-Mora L.M. 2004 Evaluation of ovine abortion associated with *Toxoplasma gondii* in Spain by different diagnostic techniques. *Veterinary Parasitology* 121 (1-2): 33-43

Plant J W, Richardson N & Moyle G G 1974 *Toxoplasma* infection and abortion in sheep associated with feeding of grain contaminated with cat faeces. *Australian Veterinary Journal* 50: 19-21

Plant J W, Freeman P & Saunders E 1982 Serological survey of the prevalence of *Toxoplasma gondii* antibodies in rams in sheep flocks in New South Wales. *Australian Veterinary Journal* 59: 87-89

Price R W 1996 Neurological complications of HIV infection. *Lancet* 348 (9025): 445-452

Riemann H P, Willadsen C M, Berry L J, Behymer D E, Garcia Z V, Franti C E & Ruppanner R 1977 Survey for *Toxoplasma* antibodies among sheep in Western United States. *Journal of the American Veterinary Medical Association* 171 (12): 1260-1264

Sekokotla J 2005, State Vet of the Moretele District, North West Province, South Africa: Personal communication

Sevgili M, Babür C, Nalbantoglu S, Karas G & Vatansever Z 2005 Determination of seropositivity for *Toxoplasma gondii* in sheep from Sanlurfa Province. *Türk Veterinerlik ve Hayvanclk Dergisi* 29 (1): 107-111

Skjerve E, Waldeland H, Nesbakken T & Kapperud G 1998 Risk factors for the presence of antibodies to *Toxoplasma gondii* in Norwegian slaughter lambs. *Preventive Veterinary Medicien* 35, Issue 3: 219-227

Smit J D 1961 Toxoplasmosis in dogs in South Africa: seven case reports. *Journal of the South African Veterinary Medical Association* 32 (3): 339-344

South African national biodiversity Institute SANBI 2007, http://www.sanbi.org

Sukthana Y, Chintana T & Lekkla A 2000 *Toxoplasma gondii* antibody in HIV-infected persons. *Journal of the Medical Association* 83 (6): 681-684

Tenter A M, Heckeroth A R & Weiss L M 2000 *Toxoplasma gondii*: from animals to humans. *International Journal of Parasitology* 30 (12-13): 1217-1258

Thrusfield M V 2005 *Veterinary Epidemiology* 3rd Edition. Blackwell Science Ltd., Oxford, UK. pp: 214-284

Torrey E F & Yolken R H 2003 *Toxoplasma gondii* and schizophrenia. *Emerging Infectious Disease* 9 (11): 1375-1380

Tyson P D 1986 *Climatic change and variability in southern Africa*. Oxford University Press, Cape Town, South Africa. pp: 56-134

Trees A J, Crozier S J, Buxton D & Blewett D A 1989 Serodiagnosis of ovine toxoplasmosis: an assessment of latex agglutination test and the value of IgM specific titres after experimental oocyst-induced infections. *Research in Veterinarian Science* 46 (1): 67-72

Turner G V S 1976 Toxoplasmosis as a public health hazard. *Journal of the South African Veterinary Association* 47 (3): 227-231

Uggla A & Hjort M 1981 A serological study on the prevalence of *Toxoplasma gondii* in meat-producing animals in Sweden. *Acta Veterinaria Scandinavica* 25: 567-576

Uneke C J, Duhlinska D D, Njoku M O & Ngwu B A 2005 Seroprevalence of acquired toxoplasmosis in HIV-infected and apparently healthy individuals in Jos, Nigeria. *Parasitology* 47 (2): 233-236

Van Heerden J & Van Rensburg I B J 1979 Toxoplasmosis in a dog. *Journal of the South African Veterinary Association* 50 (3): 211-214

Van der Puije W N A, Bosompem K M, Canacoo E A, Wastling J M & Akanmori B D 2000 The prevalence of anti-*Toxoplasma gondii* antibodies in Ghanaian sheep and goats. *Acta Tropica* 76 (1): 21-26

Van Rensburg I B J & Silkstone M A 1984 Concomitant feline infectious peritonitis and toxoplasmosis in cheetah *(Acinonyx jubatus). Journal of the South African Veterinary Association* 55: 205-207

Waldeland H 1976 Toxoplasmosis in sheep. The reliability of a microtitre system in Sabin and Feldman's Dye Test. *Acta Veterinaria Scandinvica* 17: 426-431

Walls K W 1988 Toxoplasmosis. *Laboratory diagnosis of infectious disease*. Balows A, Hausler W J, Ohashi J M & Turano A (Ed) Volume 1: 146-156

Weinman D & Chandler A H 1956 Toxoplasmosis in man and swine - An investigation of the possible relationship. *Journal of the American Medical Association* 161: 229

Wilson M, Ware D A & Juranek D D 1990 Serologic aspects of toxoplasmosis. *Journal of the American Veterinarian Association* 196 (2): 277-281

Wolf A & Cowen D 1937 Granulomatous encephalomyelitis due to an encephalitozoon (encephalitozic encephalomyelitis). A new protozoan disease of man. *Bulletin of the Neurological Institut of New York* 6: 306

World Health Organization (WHO) 2005> Acessed 10 May, 2005. URL http://www.who.org

Zain Eldin E A, Elkhawad S E & Kheir H S M 1985 A serologic survey of *Toxoplasma* antibodies in cattle, sheep, goats and camels (*Camelus dromedarius*) in the Sudan. *Revue d'Elevage et de Medecine Veterinaire des Pays Tropicaux* 38: 247-249

APPENDIX 1

QUESTIONNAIRE

Expert opinion questionnaire completed by medical doctors considered as experts on HIV/AIDS

QUESTIONNAIRE: **CODE**
EXPERT SURVEY ON TOXOPLASMOSIS IN HIV PATIENTS

QUESTION 1
Would you please give us a short CV (about one paragraph) to explain why your colleagues would regard you as having a special knowledge or expertise in HIV/AIDS prevention and treatment?

QUESTION 2
In your opinion, how significant is Toxoplasmosis as a pathogen related to human HIV infection? (Circle 1)

Very significant	Significant	Moderately significant	Not very significant	No significance at all

QUESTION 3
What is/are the main syndrome or syndromes associated with patients suffering from Toxoplasmosis as a complication of HIV?

4.1
4.2
4.3

Comments

QUESTION 4
In your opinion, what percentage of HIV/AIDS patients suffer from Toxoplasmosis as a complication?

QUESTION 5
In your opinion, what percentage of HIV/AIDS related deaths/ mortalities are linked to Toxoplasmosis as a complication?

QUESTION 6
In your opinion, what is the treatment of choice for Toxoplasmosis as a complication of HIV/AIDS?

QUESTION 7

In your opinion, how successful is the treatment of Toxoplasmosis as a complication of HIV/AIDS? (Circle 1)

Very successful	Successful	Moderately successful	Not very successful	Never successful

Comments

QUESTION 8

Any further comments or observations? Please write them below – use another sheet if required.

May we contact you for further information? Yes/ No (please circle).
YES | **NO**

Thank you very much for your time and responses.