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The prevalence of *Leptospira* serovars causing infection in dogs in South Africa

By

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Summary

Leptospirosis is a disease of global importance with a changing epidemiology in both humans and animals. It is also a significant zoonosis particularly in the developing world. To date there is limited knowledge of the incidence of leptospirosis in dogs in South Africa. This study was undertaken on a subset of dogs in South Africa to determine the presence of leptospiral antibodies to serovars known to infect dogs. Serum samples from both stray and owned dogs from various parts of South Africa were collected and tested against fifteen serovars of *Leptospira*. Five hundred and thirty samples were tested and twenty-five tested positive to seven different serovars. Nine of the 25 samples tested positive to more than one serovar. The two serovars that were most frequently represented were *L. Canicola*, which reacted to seventeen sera, and *L. Pyrogenes,* which reacted to nine sera in all. Currently the only vaccines available in South Africa in different combinations contain either *L. Canicola,* L. *Icterohaemorrhagiae,* L. *Pomona* or *L. Grippotyphosa*. The results show that the use of vaccines containing *L. Canicola* is still justifiable in certain regions of the country. However, the presence of antibodies to *L. Pyrogenes* in several dogs indicates that there is a need to investigate for the presence of antibodies in a larger group of dogs. This would allow vaccine manufacturers to tailor the *Leptospira* antigens present in vaccines to include those that are prevalent in a particular region or country.

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LIST OF ABBREVIATIONS

PCR	Polymerase chain reaction
SPCA	Society for the Prevention of Cruelty to Animals
PBS	phosphate buffered saline

TABLE OF CONTENTS

SUMMARY **II**

ACKNOWLEDGEMENTS **III**

LIST OF ABBREVIATIONS **III**

TABLE OF CONTENTS **IV**

LIST OF TABLES **V**

CHAPTER 1 **1**

INTRODUCTION 1

LITERATURE REVIEW 2

1. Classification and characteristics of the organism. *2*

2. The epidemiology of leptospirosis in domestic dogs *3*

3. Leptospirosis in humans *8*

4. The changing and emerging nature of Leptospira infections *9*

6. Control of leptospirosis in dogs *13*

CHAPTER 2 **15**

MATERIALS AND METHODS 15

1. Sample population *15*

2. Serum samples *16*

3. Leptospiral organisms *17*

4. Microscopic agglutination test *18*

5. Data Analysis *19*

CHAPTER 3 **20**

RESULTS 20

CHAPTER 4 **26**

DISCUSSION 26

CONCLUSION 34

REFERENCES **35**

ANNEXURES **44**

LIST OF TABLES

Table 1: The organisms used as capture antigens in the MAT represented eleven different serogroups. 17

Table 2: Numbers, status and distribution of dogs tested. 20

Table 3: Profiles of the dogs that yielded positive results with the MAT and test results. 22

Table 4: Percentages of dogs which yielded positive results expressed as a percentage of the total number tested per province. 23

Table 5: A 2x2 contingency table to compare the seropositivity in stray compared with owned dogs in all the Provinces. 25

Annexure A: Samples from stray dogs in KwaZulu Natal. 44

Annexure B: Samples from dogs with owners in KwaZulu Natal. 49

Annexure C: Samples from stray dogs in Western Cape. 52

Annexure D: Samples from dogs with owners in Western Cape. 54

Annexure D: Samples from stray dogs in Eastern Cape. 58

Annexure E: Samples from dogs with owners in Eastern Cape. 59

Annexure F: Samples from stray dogs in Gauteng. 60

CHAPTER 1

Introduction

Leptospirosis is a disease of global importance with a changing epidemiology in both humans and animals. Although many prevalence studies on dogs have been done in other countries, no recent work has been documented in South Africa. Two previous studies were confined to limited geographical areas and thus the prevalence of leptospirosis in the dog population of South Africa is at this time unknown (Beyers 1965; Malherbe& Kaschlua 1953). Many dogs are vaccinated with the current available bacterin vaccines against either serovars Canicola and Icterohaemorrhagiae or serovars Canicola, Icterohaemorrhagiae, Pomona and Grippotyphosa. In South Africa, there is no concrete knowledge of the degree of risk of leptospiral infection to dogs or the relevance of the above-mentioned serovars. Leptospirosis, as well as causing severe disease in dogs, is a zoonosis.

This project was therefore undertaken to determine the prevalence of leptospiral antibodies in serum samples collected from dogs from various regions in the country as well as to determine the serovars present. Data pertaining to the serum samples was recorded with regard to age, gender, breed and geographical area. The results were examined in order to make recommendations regarding the importance of leptospirosis in dogs in South Africa and the appropriateness of the current vaccines and whether the vaccines should be considered as primary vaccines or just be adMinistered to dogs at risk of contracting leptospirosis.

Literature Review

1. Classification and characteristics of the organism.

The *Leptospira* genus was initially divided into two species with *L. interrogans* containing the pathogenic strains and *L. biflexa* the saprophytic strains. These two species are then further subdivided into serovars based on an agglutination test using homologous antiserum. Related serovars are grouped into serogroups (Levett 2001a; 2004; Ahmed, Devi, Valverde Mde, Vijayachari, Machangu, Ellis & Hartskeerl 2006). This phenotypic / serological classification, which relies on the use of antisera to establish antigenic relationships between isolates, is now being replaced by a genotypic classification reliant on DNA-based identification methods (Levett 2001a; Bharti, Nally, Ricaldi, Matthias, Diaz, Lovett, Levett, Gilman, Willig, Gotuzzo & Vinetz 2003; Levett, Morey, Galloway, Turner, Steigerwalt & Mayer 2005). Until the DNA based identification methods are more freely available both methods continue to be used with the serological classification being used largely for prevalence studies.

Leptospires are tightly coiled, highly motile, obligate aerobic spirochaetes, which grow optimally at 28-30 ^{0}C. They are approximately 0.1 µm by 6 to 0.1 µm by 20 µm and show characteristics of both Gram positive and Gram negative bacteria. Leptospires take up stain poorly and phase contrast or dark field microscopy is therefore required for direct visualisation of the organism. A variety of media have been developed to culture them the most common of these being Ellinghausen-McCullough-Johnson-Harris (EMJH) medium. Culture from primary isolation is often slow and cultures may need to be retained for up to 13 weeks before discarding but pure subcultures on liquid media grow rapidly often within 10-14 days. (Levett 2001a; Bharti *et al.* 2003). Leptospirosis occurs more frequently in warm climates, in the wet seasons, when the

organism can survive outside the host species. The leptospiral organism can survive 180 days in wet soil and many months in surface water. The ideal environment for survival of the organism is wet and warm conditions where water accumulates to create marshy and muddy areas (McDonough 2001).

2. The epidemiology of leptospirosis in domestic dogs

Clinical cases of canine leptospirosis have historically been associated with *L. Icterohaemorrhagiae,* where the rat acts as the primary reservoir host, and *L. Canicola* where the dog is the maintenance host. It is suggested that the incidence of canine leptospiral disease is also under-reported due to lack of awareness, difficulty in making a specific diagnosis and in many cases subclinical infection (McDonough 2001).

a) Distribution of canine leptospirosis in South Africa.

There has unfortunately been very few prevalence studies conducted in RSA for canine leptospirosis. The first published report documenting the presence of leptospirosis in dogs in Cape Town, South Africa was published in 1953. Serovars Canicola and Sejroe were implicated. It was suggested in the article that leptospirosis also occurred in Durban although this was not confirmed serologically (Malherbe & Kaschula 1953). In Cape Town a serological study was carried out to determine the presence of leptospiral antibodies in dogs in 1965. One hundred dogs from a mostly poor community were tested. Forty-six of the dogs tested were negative, eleven had low titres and the remaining forty-three had high titres. They all reacted to either serovar Canicola, Icterohaemorrhagiae or both (Beyers 1965). A later study evaluated the prevalence of leptospirosis in dogs in the Pretoria area in 1993. Samples were collected from stray dogs and

tested for Canicola, Copenhageni, Grippotyphosa, Hardjo, Mini, Pomona, Pyrogenes and Tarassovi. Only 1, 5% of dogs tested were found to be positive and these reacted against serovars Tarassovi and Pyrogenes. It was suggested that leptospirosis is not of clinical significance in the Pretoria area, possibly as a result of the dry climate (Myburgh, Posnett & Lawrence 1993). In 1973, a report of serovar Canicola causing illness and death in dogs and abortions in pigs in the Stellenbosch district was published (van Rensburg 1973). There are also reports of leptospirosis amongst pigs, cattle and game animals in South Africa (Te Brugge & Dreyer 1985; Hunter, Flamand, Myburgh & van der Merwe 1988; Potts, Lotter & Robinson 1995; Gummow, Myburgh, Thompson, van der Lugt & Spencer 1999; Myburgh, Bengis, Bester & Chaparro 1990a; Myburgh & Otto 1990b). A recent study conducted on cattle from rural communities in Kwazulu Natal identified Pomona as the most common serovar although Tarassovi, Bratislava, Hardjo, Canicola and Icterohaemorrhagiae were also identified (Hesterberg, Bagnall, Bosch, Perrett, Horner & Gummow 2009).

b) Distribution of canine Leptospirosis worldwide

In the United States of America an increase in the number of leptospirosis cases diagnosed in veterinary teaching hospitals since 1990 has been reported with serovar Grippotyphosa being identified as the most commonly implicated serovar (Ward, Glickman & Guptill 2002; Ward, Guptill, Prahl & Wu 2004). In a much larger study where 23,005 dogs were tested using the microscopic agglutination test (MAT) during 2002-2004 a significant increase in positive titres was found. In the latter study, serovars Autumnalis and Grippotyphosa were the most prevalent (Moore, Guptill, Glickman, Caldanaro, Aucoin and Glickman 2006). It was suggested that the high number of positive reactions to serovar Autumnalis may have been due to a cross reaction

with Pomona (Prescott, McEwen, Taylor, Woods, Abrams-ogg & Wilcock 2002). There has been an emergence of infection associated with serovars Grippotyphosa and Pomona identified in Eastern North America (Ward *et al.* 2002; Cachay & Vinetz 2005). On the West coast serovars Bratislava and Pomona have predominated (Greene 2003). A recent study carried out in Washington state reported a seropositivity rate of 17,1% with Autumnalis being the most commonly detected serovar (Davis, Evermann, Peterson, VanderSchalie, Besser, Huckabee, Daniels, Hancock, Leslie & Baer 2008). In Canada a similar trend is seen with increasing numbers of cases being identified. In these studies, Grippotyphosa and Pomona were the most commonly implicated serovars (Chernesky 1970; Prescott *et al.* 2002).

In a serological survey of stray dogs in the tropics of Yucatan Mexico the overall prevalence was found to be 35%. Canicola was found to be the most common accounting for 65 % of the seropositive cases, with Icterohaemorrhagiae at 11,4 %, Panama at 9,3%, Pyrogenes at 7,9% and other serovars accounting for the remainder (Jiminez-Coello, Vado-Solis, Cardenas-Marrufo, Rodriguez-Beunfil & Ortega-Pacheco 2008). In France, the dog has been identified as the maintenance host for Canicola and the rat for Icterohaemorrhagiae. The seroprevalence of Icterohaemorrhagiae is the greatest, followed by Canicola. However, serogroups Australis and Sejroe are the next most prevalent preceding Grippotyphosa and Autumnalis (André-Fontaine 2006). In southern Germany Grippotyphosa, Saxkoebing, Bratislava and Sejroe have been identified as the predominant serovars, listed in decreasing order (Greene 2003; Geisen, Stengel, Brem, Muller, Greene & Hartmann 2007). In contrast, a survey conducted in Greece involving farm and domestic animals identified Copenhageni as the most prevalent serovar in the dog (Burriel, Dalley & Woodward 2003). In 2002 a survey was conducted in Italy, in which

leptospiral antibodies were found to be highest in kennelled dogs (between 13,8 % to 49,2 %) as opposed to owned healthy dogs presented for routine check-ups (3-4 %). The most commonly identified serovars were Bratislava and Grippotyphosa (Scanziani, Origgi, Giusti, Iacchia, Vasino, Pirovano, Scarpa & Tagliabue 2002). The current situation in the UK is unknown as there has been no recent serological survey published (Burr, Lunn & Yam 2009). A survey of dogs in the lower north island of New Zealand identified Copenhageni as the predominant serovar (O'Keefe, Jenner, Sandifer, Antony & Williamson 2002). A study of shelter dogs in Australia identified seropositive dogs in all mainland states, except South Australia. The prevalence ranged from 1-2,8 % with *Copenhageni* being the most commonly identified (Zwijnenberg, Smythe, Symonds, Dohnt & Toribio 2008). In Mongolia, two surveys conducted in two geographically distinct areas identified Cynopteri followed by Saxkoebing as the predominant serovars (Odontsetseg, Sakoda & Kida 2005). In Thailand Batavia and Canicola were the most commonly identified serovars (Meeyam, Tablerk, Petchanok, Pichpol & Padungtod 2006). In a survey of non-vaccinated dogs in Teheran, serovars Canicola, Icterohaemorrhagiae and Grippotyphosa were found to be the most prevalent (Rad, Zeinali, Yousofi, Tabatabayi & Bokaie 2004).

A recent study conducted in three ecologically distinct areas of Southwestern Nigeria identified Grippotyphosa as the most widespread infecting serovar with Bratislava and Pomona also being identified in one of the areas (Okewole & Ayoola 2009). In urban areas contact with reservoir animals is more likely to be limited to rats and dogs resulting in a limited variety of serovars whereas in tropical, more rural environments a wide variety of serovars are more likely to be isolated (Bharti et al 2003).

c) Hosts/reservoirs

Mammals can be classified as either maintenance or incidental/accidental hosts of the various leptospiral serovars. Maintenance hosts maintain the infection in their renal tubules and thus in the environment with little or no clinical signs or harm to themselves. Different species of mammals act as maintenance hosts for different serovars and a maintenance host may become an incidental host for another serovar and develop clinical illness as a result thereof. In general, certain hosts are associated with specific serovars, e.g. rats with serogroup Icterohaemorrhagiae, dairy cattle with Hardjo and Pomona, pigs with Pomona, Tarassovi or Bratislava and dogs with Canicola. Variations in the hosts and their associated serovars do occur throughout the world (Levett 2001a; Bharti *et al.* 2003). It is therefore important to have knowledge of the serovars present in an area and their associated maintenance hosts in order to assess risk to humans and animals and institute control measures if needed.

d) Transmission

The source of infection in the environment is the carrier animal. The organism adheres to the epithelial cell border of the renal tubular epithelial cells and is excreted into the environment. Infection occurs from either direct contact with urine of infected animals or via contaminated soil or water. The organism is taken up either through the mucous membranes, or through abrasions or cuts in the skin. Prolonged immersion in water has been suggested to allow penetration of the organism through the intact skin. The infection is passed between maintenance hosts by direct contact and may be acquired at a young age. Infection of an incidental host is usually by indirect contact with the maintenance host i.e. through contact with a urine contaminated environment and often results in clinical illness.

3. Leptospirosis in humans

In humans, leptospirosis may manifest in a wide variety of ways, ranging from mild febrile or even subclinical illness to the development of multi-organ involvement and death (Levett 2001a). The mortality rate in humans is variably reported as between < 5 to 30 % in different parts of the world (Cachay & Vinetz 2005; Spotts Whitney, Ailes, Myers, Saliki & Berkelman 2009). Leptospirosis is now recognised as an emerging disease of global importance (Bharti *et al* 2003; Levett 2004; Cachay & Vinetz 2005; Hartskeerl 2006). It is also suggested that the incidence of leptospirosis in humans is underestimated to a considerable degree because of a lack of clinical suspicion and awareness and resultant under diagnosis (Ahmed *et al* 2006; Hartskeerl 2006).

Humans are infected by direct or indirect contact with urine from an infected animal (Levett, Branch, Whittington, Edwards & Paxton 2001b; Bharti *et al*. 2003). Infection is usually through abrasions or cuts in the skin or through the conjunctiva (Levett 2001a). Previously leptospirosis was seen as largely an occupational disease associated with high-risk occupations such as farming, abattoir work, veterinary science, Mining and sewerage work (Bharti *et al*. 2003). Increasingly it is now recognised that leptospirosis is also a risk for urban dwellers and adventure travellers. A further epidemiological change experienced is the occurrence of outbreaks associated with heavy rainfall and flooding. According to ProMED- mail[1], a program for the International Society for Infectious Diseases, 680 cases are reported annually in the Philippines with an average of 40 fatal cases. They also reported that in October 2009 after the Ondoy floods, 167 fatalities and 2158 positive cases had occurred, indicating the potential for this

[1] promed@promed.isid.harvard.edu

disease to result in massive outbreaks. In the urban environment rats are the most likely source of infection but dogs are also important host species (Cachay & Vinetz 2005). In a survey conducted during 1998-2000 by Leptonet[2], which is a World Health Organisation (WHO) initiative financed by the International Leptospirosis Society (ILS), 40% of infections in humans were related to transmission from farm and domestic animals. Just over 9% of the latter were as a result of contact with infected dogs (Hartskeerl 2005).

4. The changing and emerging nature of *Leptospira* infections

There is a general trend in dogs, away from Canicola and Icterohaemorrhagiae being the predominant serovars (Greene 2003). In fact Canicola does not occur in dogs in Barbados and was reported to be absent from Australia as well (Weekes, Everard & Levett 1997). In an unanticipated finding, a study in Australia conducted in 2008 identified two dogs that reacted to Canicola and one that reacted to Ballum. Both of these serovars had not been identified in Australia before (Zwijnenberg *et al* 2008). In Trinidad, a survey identified Canicola, Icterohaemorrhagiae and Hebdomadis as predominant in 1979, yet in 2005, a subsequent survey failed to identify Canicola at all. Serovars Mankarso followed by Autumnalis and then Icterohaemorrhagiae were found to be the current dominant serovars (Adesiyun, Hull-Jackson, Mootoo, Halsall, Bennett, Clarke, Whittington & Seepersadingh 2006). The decrease in prevalence of serovars Canicola and Icterohaemorrhagiae has been ascribed to widespread vaccination against both these serovars, since the sixties, while the increase in other serovars is suggested to be as a result of the encroachment of dogs into the environment of wildlife reservoir hosts of other serovars (Greene 2003).

[2] http://www.leptonet.net/

5. Laboratory diagnosis

a) *Serological tests*

Serological tests used to diagnose either current or past exposure to leptospirosis include the microscopic agglutination test (MAT) and the enzyme linked immunoassay (ELISA). Other antibody tests including immunofluorescence, radio-immunoassay (RIA) and macroscopic slide agglutination tests are not widely available (Levett 2001a; Greene 2006.)

The MAT is the most commonly used serological test both in prevalence studies and as a diagnostic tool. It is the gold standard against which other serological tests are compared. The MAT also has a good specificity in that antibodies to other bacterial diseases do not cross-react with leptospiral antigens to any significant extent (McDonough 2001; Office International des Epizooties 2004). A suspension of a known live serovar is mixed with serial dilutions of the serum to be tested and incubated after which the sample is assessed for agglutination and the titres determined. The test is read using dark field microscopy and the end-point is taken as the highest dilution of serum at which 50% of the serovars are agglutinated when compared to a positive control serum (Levett 2001a). The test is used to detect antibodies to leptospiral organisms with both IgM and IgG antibodies being detected (Terpstra 2003). The humoral immune response in leptospirosis is to a large extent serogroup-specific and this characteristic is used to differentiate between serogroups. There is some serological cross-reactivity between different serogroups so that animals infected with one may have some antibodies which cross-

react with related serogroups. There is a greater cross-reactivity found in the acute phase of the disease and more serogroup specificity in the chronic or convalescent phase because of the presence of IgM in the acute phase (Levett 2001a). In performing a serological survey, screening for a wide variety of serogroups and not only ones that have been previously identified in the area, is recommended to avoid missing any unexpected types (Terpstra 2003). The test is unable to differentiate between vaccine-induced antibodies and infection-induced antibodies but vaccine titres are rarely higher than 1: 400 and only last 1-2 months post vaccination (Greene 2006). A titre of more than or equal to 100 is taken as evidence of past exposure to the disease or recent vaccination (Levett 2001a). There are limitations to the test and it is best used to give a broad idea of the serogroups present within a population rather than to accurately identify the infecting serovar in a particular animal (Levett 2001a; Levett 2003). In the case of acute illness, circulating antibodies are only detected after 8-10 days. This factor limits the usefulness of the test as a diagnostic tool in acute illness as it is desirable to institute treatment early, before the test would provide confirmation of the diagnosis. In general, titres of 1:800 in the presence of typical clinical signs are accepted to indicate recent or active infection and in cases with lower initial titres, a four-fold or greater increase in a second sample would be required to confirm active disease. (Greene 2006). The complexity of the MAT and the need for live organisms limits its availability and other serological tests have been attempted.

The ELISA can detect both IgM and IgG and may be positive before the MAT in an acute infection (Greene 2006).

b) *Isolation of the organism.*

Samples taken in the first few days after infection, particularly during the febrile stage of the disease may yield live isolates. These samples include blood, urine and CSF although after 5-7 days only samples from the brain, anterior chamber of the eye or renal tubules are likely to yield organisms. Tissue samples are usually only obtained from aborted foetuses and other products of abortion and may also yield organisms. The fastidious nature of the organism, susceptibility to a wide range of antimicrobials, limited numbers of laboratories stocking the culture medium and prolonged time required for culture however limits the usefulness of organism isolation as a method of diagnosis (Faine, Adler, Bolin & Perolat 1999).

The use of dark-field microscopy to directly visualise the leptospires in body fluids is not useful as it has low specificity and sensitivity and is technically demanding (Sessions & Greene 2004a).

c) *Molecular detection methods*

Polymerase chain reaction (PCR) assays are available at certain laboratories and have been validated for use in dogs, cattle and humans but they are not serogroup specific. The polymerase chain reaction can be performed on blood, urine, CSF, aqueous humour and semen. The advantage of PCR is that it can be used to detect organisms before the rise of antibodies, the presence of which are required for the ELISA or MAT. This would facilitate early diagnosis and thereby early treatment that has important implications for patient care and survival. The PCR could also in time become a useful tool in the identification of subclinical shedders of the organism (Sessions & Greene 2004a; Greene 2006).

6. Control of leptospirosis in dogs

a) *Vaccination*

In most countries, canine leptospira vaccines contain both serovars Canicola and Icterohaemorrhagiae although newer vaccines including Grippotyphosa and Pomona are also available. In Australia, vaccines against Copenhageni and Australis are available. Vaccines do not provide cross-protection against other serovars. Canine leptospira vaccines are whole cell bacterin vaccines (McDonough 2001). The vaccine induces an increase in IgG titres that subside after approximately 2 months although they induce a protective immunity for up to 1 year (Sessions & Greene 2004b). All of the current manufacturers' recommendations is to vaccinate annually. These include vaccines marketed in South Africa by Pfizer, Intervet/Schering –Plough, Virbac, Novartis and Afrivet/Fort Dodge. Because of the zoonotic potential of leptospirosis it must be remembered that vaccination is not only aimed at protection of the dog but also indirectly the owner by eliminating the development of a subclinical carrier that sheds bacteria.

Current recommendations made by the South African Veterinary Council, the World Small Animal Veterinary Association and the American Animal Hospital Association are as follows: In the case of puppies, the vaccine is given at 12 and again14-16 weeks of age. It is not advisable to vaccinate puppies younger than 12 weeks to ensure optimal response. In the case of the initial vaccination of adult dogs, two doses should be given 2 to 4 weeks apart. These vaccines should be boosted every 6-9 months where dogs are deemed to be subject to reasonable risk. Routine vaccination of toy breeds and puppies less than 12 weeks of age is not advised, as the incidence of adverse reactions is higher in this group. Only in cases where there is a high risk of exposure is vaccination of toy breeds recommended starting at 12 weeks and only as long as the risk is

present. It is acknowledged that disease prevalence varies for each serovar and according to the area. Therefore, recommendations that are more specific are not made.

b) *Chemotherapy*

Treatment must be directed against the leptospiraemia as well as preventing the development of a carrier state with resultant shedding of the organism. In the initial stages of acute illness, penicillins and their derivatives are used for 2 weeks. In this phase, they are administered parentally and once oral alimentation is feasible they are given orally. Supportive treatment to manage the acute renal failure is also necessary. These antibiotics are not useful in eliminating the carrier stage and are most commonly followed by doxycycline for a further 2 weeks to achieve this end although aminoglycosides, erythromycin derivates and tetracyclines may also be used (Lobetti 2007). In dogs that are not severely ill and can tolerate oral medication from the start it is acceptable to use doxycycline as the initial antibiotic. At the current time, the recommendation remains to use penicillin or its derivatives in acutely ill dogs followed by doxycycline for 2 weeks (Sessions & Greene 2004b).

Chapter 2

Materials and Methods

1. Sample population

Blood samples were collected by various branches of the Society for the Prevention of Cruelty to Animals (SPCA) throughout the country as well as by various private practices. The samples from the SPCAs were collected just prior to euthanasia from healthy stray dogs with unknown vaccination and medical histories. Samples from private practices were collected from dogs presented for routine checks, pre-anaesthetic profiles or for ill health where leptospirosis had been excluded as a differential. Every attempt was made to bleed unvaccinated dogs but in some cases the vaccination histories of the dogs were unavailable. A sample bias was included in that coastal regions were over-represented due to the fact that leptospirosis is known to be more common in warm, wet areas such as are found along the South African coast (McDonough 2001).

The following formula was used to calculate the minimum required sample size (Fosgate 2008):

n (sample size) $= Z^2P(1-P)/ d^2$

where:

Z is the statistic for a level of confidence

P is the expected prevalence, and

d is the precision

Estimating the expected prevalence to be 5 % and utilising a confidence interval of 95 % and precision of P/2 i.e. 0,0025, a sample size of 219.84 was calculated. Allowing for incomplete

data on some of the samples it was elected to over-sample by at least 100%. It was therefore decided to collect 440 blood samples.

2. Serum samples

Blood was collected by venupuncture from each dog and collected into either gel or plain serum tubes manufactured by Becton, Dickinson and Company. Those samples collected into plain tubes were allowed to settle out or were centrifuged to separate the serum prior to freezing. Those samples collected into gel tubes were separated out by centrifugation and the serum decanted into sterile, plain tubes and subsequently frozen at -16 °C. The samples from KwaZulu-Natal were stored at Vetdiagnostix Laboratory in Pietermaritzburg or at the Hilton Veterinary Clinic. The samples from the Western and Eastern Cape were stored at Idexx-GoldenVet laboratories in Cape Town and Port Elizabeth respectively and then forwarded on ice to the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, Onderstepoort, when required. Sera from the Gauteng region were stored at The Department of Veterinary Tropical diseases, Onderstepoort.

Serum samples were identified and numbered and the data (*i.e.* gender, age, breed and geographical region) pertaining to each recorded. The samples from the Western and Eastern Cape were collected in the first half of the year i.e. autumn going into winter whereas the samples from KZN were collected over a period of one year throughout the year.

3. Leptospiral organisms

Live leptospiral organisms representing fifteen serovars and 11 serogroups (see Table 1) were imported from the Royal Tropical Institute (KIT Biomedical Research Laboratory) in Amsterdam, the Netherlands.

Table 1: The organisms used as capture antigens in the MAT represented eleven different serogroups.

Serogroup Australis	*L. interrogans* serovar Australis strain Ballico *L. interrogans* serovar Australis strain Ballico var Bratislava jez Bratislava
Serogroup Autumnalis	*L. interrogans* serovar Autumnalis strain Akiyami A.
Serogroup Bataviae	*L. interrogans* serovar Bataviae strain Swart.
Serogroup Canicola	*L. interrogans* serovar Canicola strain Hond Utrecht IV.
Serogroup Grippotyphosa	*L. kirschneri* serovar Grippotyphosa strain Moskva V.
Serogroup Icterohaemorrhagiae	*L. interrogans* serovar Copenhageni strain M20 *L. interrogans* serovar Icterohaemorrhagiae strain RGA.
Serogroup Mini	*L. interrogans* serovar Szwajizak strain Szwajizak.
Serogroup Pomona	*L. interrogans* serovar Pomona strain Pomona.
Serogroup Pyrogenes	*L. interrogans* serovar Pyrogenes strain Salinem.
Serogroup Sejroe	*L. interrogans* serovar Hardjo strain Hardjoprajitno, *L. interrogans* serovar Wolfii strain 3705 *L. borgpetersenii* serovar Sejroe strain M84.
Serogroup Tarassovi	*L. borgpetersenii* serovar Tarassovi strain Perepelitsin.

The organisms were received in Difco™ Leptospira Enrichment EMJH broth. They were maintained at 29 ^{o}C in EMJH medium and subcultured in 5 ml quantities at 7 day intervals until

required. The viability of the cultures was checked by dark-field microscopy. Cultures were used for the serological tests between the 4th and 10th day of growth of that culture cycle. Once the serological tests had been completed the isolates were stored in liquid nitrogen in 1.2 mL cryovials containing EMBJ broth.

4. Microscopic agglutination test

The MAT was carried out as described in the Leptospirosis: Manual of diagnostic tests and Vaccines for Terrestrial Animals as published by the OIE (World Organisation for Animal Health, 2008). Sera were thawed and diluted 1:25 with phosphate buffered saline (PBS), after which they were heated to 56 ^{o}C for 30 minutes to inactivate Complement. This was done in order to prevent non-specific agglutination. The diluted sera were mixed with equal volumes (50 µl) of each of the different antigens in flat-bottomed microtitre plates to obtain a final dilution of 1:50 and incubated at 30 ^{o}C for 2 hours in a Labcon Low Temperature Incubator. The suspension in each well was examined microscopically for agglutination using dark field microscopy by means of a Leitz Ortholux 2 microscope with a Leitz Wetzlar objective. Positive and negative controls were run concurrently. Positive control sera were provided by the Onderstepoort Veterinary Institute that in turn obtained the control sera from the National Veterinary Services Laboratories, United States Department of Agriculture, Ames, Iowa, USA. In the case of the negative controls, an equal (50 µl) volume of PBS buffer was mixed with the different antigens.

Where agglutination was identified during the screening of the 1:50 dilutions, the relevant sample numbers were recorded and these sera were further diluted to determine an end-point titre for each. The agglutinating sera were further tested against the appropriate strains at two-fold

dilutions of the sera ranging from 1:100 to 1:6 400. The titre was taken as the highest dilution where 50 % agglutination was recorded compared to a negative control. For clarification purposes, when I did the agglutination tests I always looked at positive and negative controls and choose samples that agglutinated more than halfway between. A titre of ≥100 was accepted as evidence of past exposure to leptospires.

5. Data Analysis

Point estimates of prevalence and their 95% binomial exact confidence intervals (95% CI) were calculated. Prevalences were compared using Fisher's exact test. The seroprevalence in stray dogs compared to owned dogs was statistically evaluated using the odds ratio, Yate's and Pearson's Chi^2 tests. The calculator is found at http://faculty.vassar.edu/lowry/odds2x2.html.

Chapter 3

Results

A total of 530 sera were collected from the Kwazulu Natal, Eastern Cape, Western Cape and Gauteng regions (Table 2). Of these samples, 25 sera reacted positively to one or more serovars i.e. 4.7% of the total number tested.

Table 2: Numbers, status and distribution of dogs tested.

Province		**Total**	**Strays**	**Owned**
KwaZulu Natal		255	157	98
	M		63	42
	F		94	56
Eastern Cape		55	27	28
	M		13	13
	F		14	11
	O			4
Western Cape		195	53	142
	M		23	63
	F		30	79
Gauteng		25	25	
Total		**530**	**262**	**268**

M – male; F – female; O – other

The greatest numbers of samples (n=255) were collected from the Kwazulu Natal region with the majority of these collected in the Ethekwini and the Msunduzi municipal areas. Samples from 55 dogs were collected in the Eastern Cape, namely in the Port Elizabeth and Jeffrey's bay /Humansdorp areas. Samples (n=195) from the Western Cape were largely from the Cape Town

area (n=170) with a smaller number (n=25) from George. All the samples from the Gauteng region (n=25) were collected in the Pretoria / Johannesburg area.

In Kwazulu Natal province 40 % of stray dogs tested (n = 63) were male and 60 % (n = 94) were female. Of owned dogs 43% (n=42) were male and 57% (n=56) were female. In total 63 % of positive dogs (n = 8) were male. Only 3 out of the 13 positive dogs were owned. In the Western Cape 43.39% of stray dogs tested (n = 23) were male and 56.6 % (n = 30) were female. There were only two dogs which tested positive, one male and one female and both of these were strays. Thus 4.34 % (n=1) male strays were positive and 3.33 % (n=1) of females. In the Eastern Cape 49% of the stray dogs tested (n = 13) were male and 51% (n = 14) female. Of these 7.69 % (n=3) of males were positive and 7.14 % (n=3) of females. Amongst owned dogs tested in the Eastern Cape 46.43 % were male (n=13) and 39.28 % female (n=11) with the remainder unknown. Only 2 owned dogs tested positive and these were both females. In total in the Eastern Cape 14% (n=8) of the dogs tested positive 3 males and 5 females. In Gauteng all the dogs were strays from the SPCA. One female tested positive and the particulars of the other positive reactor were unknown. Thus 8% (n=2) of dogs tested positive.

The highest percentage of positive dogs tested was in the Eastern Cape followed by Gauteng, Kwazulu-Natal and the Western Cape in descending order. In all areas where both stray and owned dogs were tested, the percentage of positive strays was higher than that of owned dogs. In both the Western Cape and Kwazulu-Natal the percentages of positive male dogs was greater than females.

Table 3: Profiles of the dogs that yielded positive results with the MAT and test results.

Age	Breed	Gender	Area	Serovar	Titre
KwaZulu Natal					
Adult	Cross Breed	M	Kloof	Icterohaemorrhagiae	800
				Copenhageni	400
				Bratislava	200
Adult	Staffordshire Bull Terrier	M	Pinetown	Bratislava	200
				Pyrogenes	400
Adult	Cross Breed	F	Northdene	Pyrogenes	100
				Canicola	400
Adult	Pug	M	Gillits	Pyrogenes	200
				Canicola	200
				Bataviae	200
6y	German Shepherd Dog	M	Westville	Australis	800
2y	Spaniel	F	Dundee	Canicola	100
3y	Cross Breed	M	Pietermaritzburg	Canicola	200
9y	Greyhound	F	Pietermaritzburg	Canicola	100
10y	Fox Terrier	F	Hilton	Canicola	200
5y	German Shepherd Dog Cross	M	Pietermaritzburg	Canicola	400
3y	Cross Breed	F	Northdale	Canicola	800
				Icterohaemorrhagiae	100
5y	Labrador	M	Pietermaritzburg	Canicola	800
2y	Cross Breed	M	Napierville	Canicola	100
				Pyrogenes	100
Eastern Cape					
4y	Cross Breed	M	Port Elizabeth	Canicola	200
1y	Cross Breed	F	Port Elizabeth	Autumnalis	400
3y	Cross Breed	F	Port Elizabeth	Pyrogenes	200
10y	Cross Breed	M	Port Elizabeth	Pyrogenes	100
3y	Cross Breed	M	Port Elizabeth	Pyrogenes	200
				Canicola	800
4y	Cross Breed	F	Port Elizabeth	Canicola	800
Adult	Jack Russell	F	Humansdorp	Canicola	1600
6m	Border Collie	F	Humansdorp	Canicola	200
Western Cape					
2y	Cross Breed	M	Grassy Park	Pyrogenes	200
12y	Cross Breed	F	Grassy Park	Canicola	400
Gauteng					
	Sample 13		Gauteng	Canicola	100
				Pyrogenes	100
1.5y	Africanis	F	Gauteng	Canicola	1600
				Pyrogenes	400

Table 4: Percentages of dogs which yielded positive results expressed as a percentage of the total number tested per province.

	Strays	Owned	**Total**
KZN	7.00%	2.04%	**5.1%**
W Cape	3.70%	Nil	**2.1%**
E Cape	22.00%	7.14%	**14.50%**
Gauteng	8.00%	Nil	**8.00%**

Statistical significance of gender

KZN: Total: 13/255 = 5.1% [95% CI: 2.7, 8.6]

Male: 8/105 = 7.6% [3.3, 14.5]

Female: 5/150 = 3.3% [1.1, 7.6]

Comparison between males & females (Fisher's exact test): $P = 0.15$ (not statistically significant)

EC: Total: 8/55 = 14.5% [6.5, 26.7]

Male: 3/26 = 11.5% [2.4, 30.2]

Female: 5/25 = 20.0% [6.8, 40.7]

Comparison between males & females: $P = 0.47$ (not statistically significant)

WC: Total: 2/195 = 1.0% [0.1, 3.7]

Male: 1/86 = 1.2% [0.03, 6.3]

Female: 1/109 = 0.9% [0.02, 5.0]

Comparison between males & females: $P = 1.00$ (not statistically significant)

Gauteng: Total: 1/25 = 4.0% [0.1, 20.4]

Male: 0/11 = 0.0% [0.0, 23.8]

Female: 1/5 = 20.0% [0.5, 71.6]

Comparison between males & females: $P = 0.31$ (not statistically significant)

OVERALL: Total: 24/530 = 4.5% [2.9, 6.7]

Male: 12/228 = 5.3% [2.7, 9.0] (ditto)

Female: 12/289 = 4.2% [2.2, 7.1] (ditto)

Comparison between males & females: $P = 0.68$ (not statistically significant)

Therefore, there was not statistical difference in the prevalence of leptospirosis in the different genders of dogs.

Comparison of seroprevalance of *Leptospira* antibodies between provinces (Fisher's exact test):

KZN vs EC: $P = 0.02$ (significant)

KZN vs WC: $P = 0.02$ (significant)

KZN vs Gauteng: $P = 1.00$

EC vs WC: $P < 0.001$ (significant)

EC vs Gauteng: $P = 0.26$

WC vs Gauteng: $P = 0.31$

The results show that there were statistical differences in the prevalence of leptospiral antibodies between the coastal provinces, namely KwaZulu-Natal, Eastern Cape and Western Cape Provinces. However, due to the small sample size of dogs from Gauteng, no statistical differences could be shown when comparing the other Provinces to this one.

Statistical significance of seropositivity in stray dogs compared with owned dogs.

The 2x2 Chi^2 test was used as the sample size was greater than 100.

Table 5: A 2x2 contingency table to compare the seropositivity in stray compared with owned dogs in all the Provinces.

	Positive	Negative	**Total**
Stray	20	242	262
Owned	5	263	268
Total	25	505	530

The risk ratio was 4.0916 and the odds ratio was 4.3471 at a confidence interval of 95% that stray dogs were more likely to have antibodies to *Leptospira* spp. The probability that stray dogs were more likely to have antibodies to *Leptospira* spp. was statistically significant (<0.05): P = 0.003417 (Yate's Chi^2 test); and P = 0.001736 (Pearson Chi^2 test).

Chapter 4

Discussion

The aim of this project was to collect samples from dogs from various regions in the country, record the relevant data pertaining to each sample and test the samples for antibodies against a variety of *Leptospira* serovars. The findings of the study documented the presence of leptospiral antibodies to both the more commonly identified serovars Canicola and Icterohaemorrhagiae as well as to a number of the less commonly identified serovars. Previous studies in South Africa have documented the presence of leptospiral antibodies in dogs as well as in production animals and wildlife.

The serological results of this study revealed a preponderance of reactions to serovar Canicola that has been traditionally the serovar most closely associated with dogs. This was followed by serovar Pyrogenes. In the study of Malherbe and Kaschula in 1953, which was based on clinical cases identified at the small animal clinic of the Onderstepoort Faculty of Veterinary Science, reactions to both Canicola and Sejroe serovars were identified. The results of the study were presented at the 48th Annual Conference of the South African Veterinary Medical Association where in the discussion following it was reported that both Canicola and Icterohaemorrhagiae had been identified in dogs in Cape Town by a Dr. Brownlie. Subsequently in 1965, Beyers reported that dogs from the Cape Town region reacted to either one or both of Canicola and. Icterohaemorrhagiae. Van Rensburg, in 1973, identified antibodies against serovar Canicola in a number of ill dogs while investigating an outbreak of abortions, perinatal mortality, and infertility in a piggery in the Stellenbosch district. Thirty-seven serum samples from dogs were tested for antibodies and 32 reacted positively. Serovar Canicola was also isolated from the

kidney of one dog that died of the disease. In a study conducted in 1993 on sera collected from 400 dogs in the greater Pretoria area, Myburgh *et al* identified antibodies against Tarassovi (n= 5) and Pyrogenes (n=2).

A number of studies relating to leptospiral antibodies in southern Africa in animals other than dogs have been published. Of the serovars identified in this study both Copenhageni and Bratislava have been identified previously in South Africa in production animals and Copenhageni in game animals (Potts *et al* 1995; Hunter *et al* 1998; Gummow *et al* 1999; Hesterberg *et al* 2009). Antibodies to serovars Batavia and Autumnalis have also been detected in game in Zimbabwe and in a serological and bacteriological study conducted on cattle in Zimbabwe all the serovars identified in the current survey, except Bratislava and Copenhageni, were identified (Feresu 1990; Anderson & Rowe 1998). It is therefore feasible for dogs to have been exposed to these serovars in large parts of southern Africa, as there is considerable movement of game, dogs and humans across the borders and throughout the country.

Results from this study confirmed that serovars Canicola and Icterohaemorrhagiae are still present in South Africa but there are other serovars that are not included in any of the current generation of canine vaccines available in South Africa. This may become a problem over time. These are (with the number of positive samples in brackets) Pyrogenes (10), Bratislava (2), Batavia (1), Copenhageni (1), Australis (1) and Autumnalis (1). Serovar Pyrogenes was found in all of the provinces where samples were taken. Previous to this study, of the positive serovars only Canicola, Icterohaemorrhagiae and Pyrogenes had been identified in dogs in South Africa. Serovars Bratislava and Copenhageni had been identified in production animals and

Copenhageni in game animals. Serovars Autumnalis, Australis and Bataviae although identified in cattle and game in southern Africa had not been reported in South Africa.

Currently vaccine recommendations for companion animals are undergoing close scrutiny the world over as there is a trend away from routine annual vaccinations to more individually designed vaccination protocols which assess patient risk in that particular environment. According to the guidelines laid down by the South African Veterinary Council, the World Small Animal Veterinary Association and the American Animal Hospital Association vaccines for companion animals such as dogs are today classified as core and non-core vaccines. The former are administered routinely to all dogs and cats, and protect against severe life-threatening diseases with a global distribution, whereas the latter are given strategically following risk assessment. *Leptospira* vaccines are classified by the aforementioned associations as non-core vaccines. They also report that adverse reactions in dogs to leptospiral vaccines are reported with greater frequency than to other vaccines making it of more importance to assess relative risk for each patient rather than administering the vaccine routinely.

Various studies have been carried out in the USA and Canada to identify risk factors for acquiring leptospiral infection. Ward *et al* (2002) in a retrospective study from 1970-1998 found that hounds, working and herding dogs as well as sexually intact males were at a greater risk. Hartmann in 1984 reported that working and sporting dogs had a higher incidence than other breeds whereas in 2007, Ghneim *et al* found no significant breed differences. Ward *et al* (2004) also reported that dogs between 4 and 9 years of age were more likely to acquire infection than dogs less than 1 year of age in contrast to Ghneim *et al* (2007) who found a higher incidence

amongst dogs less than 1 year of age or older than 8 years. In contrast to these studies Harkin and Gartrell in 2002 identified more infected females than males and more German Shepherd Dog or German Shepherd Dog crosses while in 1992 Rentko *et al* found no age or breed predilection but more infected males than females. The latter two studies involved small (n=17) numbers of dogs.

In Australia in 2007 Miller *et al* found young male dogs more frequently infected whereas in a cross-sectional study in 2008, Zwijnenberg *et al* identified more positive female dogs less than 5 years of age, the latter findings were however deemed to be statistically insignificant.

In the current study, there was no statistically significant difference in numbers of positive males and females. Ages of stray dogs were unknown and could only be estimated at the time of sample collection and therefore the prevalence in different age groups was not determined. The link between breed and/or age and risk of disease is ambiguous.

The ages of dogs in this study with *Leptospira* antibodies ranged from 6 months to 12 years although the strays were of an undetermined age and only classified as adult i.e. estimated to be above 1 year, or an estimate of age was made. Of the eighteen dogs whose ages were known with some accuracy only four were above 6 years of age. Of the stray dogs bled in KZN, only 19 were estimated to be over 6 years of age. The preponderance of younger positive dogs may indicate that younger age is a risk factor or is more likely because many of the stray dogs came from extremely impoverished areas and are unlikely to reach the age of six or more and therefore mainly dogs were bled. Interestingly, only one of the seropositive dogs was 6 months of age. This was an owned female working dog and could therefore be exposed to possible risk factors.

Therefore, it seems likely that mature dogs are more likely to become infected. This could be due to the fact that mature dogs are more likely to roam, especially when they are sexually active.

It is well known that warm damp weather is conducive to the survival of leptospiral organisms in the environment and certain studies have documented a higher incidence of cases in the warmer and/or wetter months (Hartmann 1984; Adin & Cowgill 2000; Ghneim *et al* 2007; Miller, Ross, Sullivan & Perkins 2007). Ghneim *et al* in 2007, although not able to establish a correlation between rainfall and incidence, did find a correlation between disease incidence and the distance lived from open water. In this study it was found that a statistically significant higher prevalence of positive samples was found in Kwazulu Natal than in the Eastern and Western Cape and a higher prevalence in the Eastern than in the Western Cape. This may be as a result of the warm wet summers experienced in these regions.

In Italy in 2002, Scanziani *et al* observed that a higher prevalence was found in kennelled dogs, which they attributed to overcrowding and poor hygiene. A further important factor to consider regarding *Leptospira* is that in studies from various countries the historically important serovars i.e. Canicola and Icterohaemorrhagiae are becoming less frequently seen and are being replaced by other emerging serovars. In the United States of America and Canada, serovars Pomona and Grippotyphosa have been implicated as the most commonly found serovars currently causing leptospirosis (Prescott, Key & Ousch 1999; Ribotta, Fortin, Higgins & Beading 2000), while more recent studies have identified Autumnalis as a potentially emerging serovar (Prescott *et al* 2002; Moore et al 2006; Davis et al 2008). The concurrent finding of Autumnalis and Pomona in sick and injured racoons again emphasises the importance of non-domesticated animals as a

potential reservoir of infection (Davis *et al* 2008). Scanziani et al found Bratislava and Grippotyphosa to be the most common infecting serovars in a study in Italy while Geisen et al in Germany found Grippotyphosa followed by Saxkoebing to be the most commonly identified serovars. Zwijnenberg et al identified Copenhageni as the most prevalent serovar in their study of shelter dogs in mainland Australia.

Clearly stray dogs were 4.3471 times more likely ($P = 0.001736$) to have antibodies to *Leptospira* species. Although the vaccination status of these dogs was unknown, most of the dogs originated from informal settlements where there are no resources to vaccinate these dogs. Therefore it is most likely that the antibodies present are due to exposure to infection with *Leptospira* species. It is well known that free-roaming dogs compared to dogs restricted to properties are more likely to come into contact with known sources of the bacterium: either by drinking or swimming in contaminated stagnant freshwater; contact with rodents or rodent urine contaminated foods; as well as unrestricted mating with infected dogs. The fact that serovar Canicola was the most predominant serovar indicates that the latter is most likely.

Six of the thirteen positive dogs from KZN were cross breeds, whereas all the positive dogs in the Western Cape and six of the eight positive dogs in the Eastern Cape were cross breeds. Most strays in the study were crossbreeds, so it was thought that the fact that they were strays or free roaming was more likely to be a risk factor rather than the fact that there was a specific breed predilection.

Further evidence that the seropositivity was unlikely to be due to vaccination was the fact that, leptospiral bacterins for use in dogs in South Africa contains both serovars Canicola and Icterohaemorrhagiae. Therefore, one would expect a similar number of positive reactors to both serovars if the titres were indeed vaccine induced. This was not the case. It is therefore reasonable to assume that Canicola currently represents the biggest risk for infection in dogs in South Africa.

Serovar Pyrogenes has previously been identified in dogs in South Africa by Myburgh *et al* in 1993 but was not a commonly identified serovar in other studies. Similarly, in a survey conducted on 820 dogs in Japan over a three year period, ten positive samples were recorded (Ryu 1975). In the current survey, it does assume some importance as it was found in every region tested and was the second most commonly identified serovar.

Although it is well known that cross-reactive antibodies occur within a serogroup, it is generally considered that this is less prevalent when serovars belong to different serogroups (Table 1). Therefore the presence of antibodies to serovars Icterohaemorrhagiae and Copenhageni is most likely as a result of a cross reaction, with Icterohaemorrhagiae having the highest antibody titre being the most likely infecting serovar.

The results of this study support results from previous studies in South Africa that leptospirosis is a disease that still occurs in dogs in South Africa, and that vaccination should be recommended for dogs at risk. When doing a risk assessment during a consultation with a dog owner several risk factors should be considered.

1. The lifestyle of the dog. Does it roam freely particularly in rural or semi urban areas where it could be exposed to open water or wetlands?
2. Is it likely to come into direct or indirect contact with potential wild life reservoirs in particular rodents?
3. Is it a dog breed that is more likely to spend time in water if given access e.g. sporting or hunting dogs?
4. Is the climate in which the dog resides conducive to the survival of the *Leptospira* organism e.g. wet and warm?

If one or more of these questions are answered in the affirmative, vaccination with leptospira bacterins should be considered favourably.

Personal communication with veterinarians in both Kwazulu Natal and the Eastern Cape revealed that many believed that leptospirosis was present but was not substantiated with laboratory or necropsy results.

Areas where further research is required in South Africa:

1. Identification of emerging serovars in each region that may provide information to international vaccine manufacturers when contemplating the updating of leptospiral antigens used in the vaccines.
2. Investigate any possible cross-reactive antibodies when performing serosurveys
3. Laboratory diagnosis of suspected leptospirosis in dogs
4. The identification of the possible carrier or maintenance host of serovar Pyrogenes.

Conclusion

Although further surveys in other areas of the country, especially the inland provinces, still need to be undertaken, the results from this study have shown that leptospirosis still represents a risk, albeit a moderate one to domestic dogs. It also points out that the use of leptospira vaccines containing the serovars Canicola and Icterohaemorrhagiae should be continued particularly in the warm coastal regions of the country and for groups of dogs that are considered to be at risk.

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ANNEXURES

Annexure A: Samples from stray dogs in KwaZulu Natal.

Sample Number	Approximate Age - Years	Gender	Breed	Area
1	1	F	Cross Breed	Pietermaritzburg
2	1	F	Cross Breed	Imbali
3	1	F	Cross Breed	Imbali
4	1	F	Cross Breed	Hillcrest
5	1	F	Cross Breed	New Germany
6	1	F	Cross Breed	Edendale
7	1	F	Cross Breed	Pinetown
8	1	F	Cross Collie	Sobantu
9	1	F	German Shepherd	Pietermaritzburg
10	1	F	Labrador	Shallcross
11	2	F	Cross Boerbul	Pietermaritzburg
12	2	F	Cross Breed	Northdale
13	2	F	Cross Breed	Pietermaritzburg
14	2	F	Cross Breed	Pietermaritzburg
15	2	F	Cross Collie	Pietermaritzburg
16	2	F	Cross German Shepherd	Pietermaritzburg
17	2	F	German Shepherd	Pietermaritzburg
18	2	F	Great Dane	Hillcrest
19	2	F	Great Dane	Pietermaritzburg
20	2	F	Mastiff	Northdale
21	3	F	Cross Breed	Northdale
22	3	F	Cross Breed	Pietermaritzburg
23	3	F	Cross Breed	Pietermaritzburg
24	3	F	Cross German Shepherd	Edendale
25	3	F	Cross German Shepherd	Pietermaritzburg
26	3	F	Cross German Shepherd	Pietermaritzburg
27	3	F	Cross German Shepherd	Northdale
28	3	F	Cross Rottweiler	Northdale
29	4	F	Boerbul	Northdale

Sample Number	Approximate Age - Years	Gender	Breed	Area
30	4	F	Boerbul	Pietermaritzburg
31	4	F	Cross Breed	Dambuza
32	4	F	Cross Breed	Nqabeni
33	4	F	Cross Breed	Pietermaritzburg
34	4	F	Cross Jack Russell	Bisley
35	4	F	Cross Labrador	Sobantu
36	4	F	Maltese	Durban
37	5	F	Cross Breed	Imbali
38	5	F	Cross Breed	Pietermaritzburg
39	5	F	Cross Breed	Pietermaritzburg
40	5	F	Cross Bull Mastiff	Northdale
41	5	F	Cross Fox Terrier	Pietermaritzburg
42	5	F	Retriever	Kloof
43	6	F	Bull Dog	Dargle
44	6	F	Cross Breed	Pietermaritzburg
45	6	F	Cross German Shepherd	Durban
46	6	F	Cross Husky	Pietermaritzburg
47	6	F	German Shepherd	Pietermaritzburg
48	6	F	German Shepherd	Woodlands
49	6	F	Pomeranian	Pietermaritzburg
50	6	F	Retriever	Cowies hill
51	8	F	Cross Fox Terrier	Northdale
52	8	F	Fox Terrier	Pietermaritzburg
53	9	F	Greyhound	Pietermaritzburg
54	10	F	Cross Labrador	Hilton
55	10	F	Fox Terrier	Hilton
56	10	F	Schnauzer	Pietermaritzburg
57	12	F	Australian Cattle Dog	Pietermaritzburg
58	12	F	Min Pinscher	Hilton
59	13	F	German Shepherd	Pietermaritzburg
60	3m	F	Cross Breed	Pietermaritzburg
61	3m	F	Cross Breed	Pietermaritzburg
62	4m	F	Greyhound	Sobantu
63	4m	F	Pyrenean Mountain Dog	Pietermaritzburg
64	9m	F	German Shepherd	Dambuza
65	Adult	F	Boerbul	Crestholme
66	Adult	F	Boerbul	New Germany

Sample Number	Approximate Age - Years	Gender	Breed	Area
67	Adult	F	Boerbul	Crestholme
68	Adult	F	Bull Terrier	Camperdown
69	Adult	F	Cross Border Collie	Sarnia
70	Adult	F	Cross Boxer	Waterfall
71	Adult	F	Cross Breed	Bloemfontein
72	Adult	F	Cross Breed	Kloof
73	Adult	F	Cross Breed	Kwandingezi
74	Adult	F	Cross Breed	Pinetown
75	Adult	F	Cross Breed	Hillcrest
76	Adult	F	Cross Breed	Hammarsdale
77	Adult	F	Cross Breed	Hillcrest
78	Adult	F	Cross Breed	Pinetown
79	Adult	F	Cross Breed	Pinetown
80	Adult	F	Cross Breed	Wyebank
81	Adult	F	Cross Breed	Claremont
82	Adult	F	Cross Breed	New Germany
83	Adult	F	Cross Breed	Malvern
84	Adult	F	Cross Breed	Northdene
85	Adult	F	Cross Breed	Mpumalanga
86	Adult	F	Cross Breed	Kloof
87	Adult	F	Cross Breed	Reservoir Hills
88	Adult	F	Cross Chihuahua	Hillcrest
89	Adult	F	Cross German Shepherd	Northdene
90	Adult	F	Dachshund	Waterfall
91	Adult	F	Maltese	Dawncliff
92	Adult	F	Pit Bull Terrier	Westville
93	Adult	F	Spaniel	Kloof
94	Juvenile	F	Cross Breed	Kloof
95	2	M	Cross Breed	Napierville
96	2	M	Cross Breed	Pietermaritzburg
97	2	M	Cross Breed	Pietermaritzburg
98	3	M	Cross Breed	Pietermaritzburg
99	3	M	Cross Breed	Durban
100	3	M	Cross Breed	Wartburg
101	3	M	Cross Breed	Imbali
102	3	M	Cross German Shepherd	Northdale
103	4	M	Cross Breed	Imbali

Sample Number	Approximate Age - Years	Gender	Breed	Area
104	4	M	Cross Breed	Pietermaritzburg
105	4	M	Cross Breed	Pietermaritzburg
106	4	M	Jack Russell	Pietermaritzburg
107	5	M	Cross Breed	Pietermaritzburg
108	5	M	Cross German Shepherd	Pietermaritzburg
109	5	M	Dachshund	Pinetown
110	5	M	Labrador	Pietermaritzburg
111	6	M	Boerbul	Pietermaritzburg
112	6	M	Cross Breed	Edendale
113	6	M	German Shepherd	Northdale
114	10	M	Cross Breed	Pietermaritzburg
115	3m	M	Cross Breed	Pietermaritzburg
116	6m	M	Cross Breed	Pietermaritzburg
117	6m	M	Great Dane	Pietermaritzburg
118	Adult	M	Boerbul	Kloof
119	Adult	M	Border Collie	Highland hills
120	Adult	M	Bull Terrier	Hillcrest
121	Adult	M	Cross Breed	Kloof
122	Adult	M	Cross Breed	Kwadabeka
123	Adult	M	Cross Breed	Kwadabeka
124	Adult	M	Cross Breed	Pinetown
125	Adult	M	Cross Breed	Pinetown
126	Adult	M	Cross Breed	Pinetown
127	Adult	M	Cross Breed	Wyebank
128	Adult	M	Cross Breed	Dassenhoek
129	Adult	M	Cross Doberman	New Germany
130	Adult	M	Cross German Shepherd	Pinetown
131	Adult	M	Cross German Shepherd	Westmead
132	Adult	M	Cross German Shepherd	Shallcross
133	Adult	M	Cross German Shepherd	Cowies hill
134	Adult	M	Cross German Shepherd	Kloof
135	Adult	M	Cross Jack Russell	New Germany
136	Adult	M	Cross Jack Russell	New Germany
137	Adult	M	Cross Staffordshire Bull Terrier	Westville N
138	Adult	M	Cross Staffordshire Bull Terrier	Bothas Hill
139	Adult	M	Fox Terrier	Pinetown
140	Adult	M	Great dane	Everton

Sample Number	Approximate Age - Years	Gender	Breed	Area
141	Adult	M	Greyhound	Northdene
142	Adult	M	Jack Russell	Malvern
143	Adult	M	Jack Russell	New Germany
144	Adult	M	Jack Russell	Pinetown
145	Adult	M	Maltese	Hillcrest
146	Adult	M	Mastiff	Westville
147	Adult	M	Pug	Gillits
148	Adult	M	Rhodesian Ridgeback	Kloof
149	Adult	M	Rhodesian Ridgeback	New Germany
150	Adult	M	Rottweiler	Westville
151	Adult	M	Rottweiler	Reservoir Hills
152	Adult	M	Staffordshire Bull Terrier	Pinetown
153	Juvenile	M	Cross Breed	Westville
154	Juvenile	M	Cross Breed	Nagina
155	Juvenile	M	Cross Breed	Claremont
156	Juvenile	M	Cross Breed	Kloof
157	Juvenile	M	Dachshund	Bothas Hill

Annexure B: Samples from dogs with owners in KwaZulu Natal.

Sample Number	Approximate Age - Years	Gender	Breed	Area
158	1	F	Cross breed	Cowies Hill
159	1	F	Cross breed	Edendale
160	1	F	Fox Terrier	Summerveld
161	2	F	Cross breed	Underberg
162	2	F	Cross Labrador	Durban
163	2	F	Dachshund	Hilton
164	2	F	Husky	Hillcrest
165	2	F	Min pin	Hilton
166	2	F	Pug	Hilton
167	2	F	Rhodesian Ridgeback	Hilton
168	2	F	Spaniel	Dundee
169	2	F	Staffordshire Bull Terrier	Ashburton
170	3	F	Boerbul	Howick
171	3	F	Boxer	Westville
172	3	F	Bull Mastiff	Hilton
173	3	F	Cross breed	Hilton
174	3	F	Cross breed	Underberg
175	3	F	Cross breed	Hilton
176	3	F	Fox Terrier	Hilton
177	3	F	Pug	Underberg
178	3	F	Rhodesian Ridgeback	Hilton
179	4	F	Boxer	Hilton
180	4	F	Cross breed	Hilton
181	4	F	Cross Labrador	Hilton
182	4	F	German Shepherd	Hilton
183	4	F	Jack Russell	Hilton
184	5	F	Belgian Shepherd	Howick
185	5	F	Boerbul	Malvern
186	5	F	Boerbul	Howick
187	5	F	Husky	Westville
188	5	F	Rottweiler	Hilton

Sample Number	Approximate Age - Years	Gender	Breed	Area
189	6	F	Australian Cattle Dog	Hilton
190	6	F	Bull Mastiff	Pietermaritzburg
191	6	F	German Shepherd	Westville
192	6	F	German Shepherd	Hilton
193	6	F	Labrador	Hilton
194	7	F	German Shepherd	Hilton
195	7	F	Spaniel	Hilton
196	8	F	Dachshund	Underberg
197	8	F	Retriever	Hilton
198	9	F	Australian Cattle Dog	Midlands
199	10	F	Cross German Shepherd	Westville
200	10	F	German Shepherd	Merrivale
201	10	F	Labrador	Hilton
202	10	F	Labrador	Hilton
203	10	F	Pug	Hilton
204	10	F	Rhodesian Ridgeback	Midlands
205	10	F	Rottweiler	Howick
206	11	F	Belgian Shepherd	Hilton
207	12	F	Rottweiler	Hilton
208	12	F	Spaniel	Hilton
209	13	F	Staffordshire Bull Terrier	Hilton
210	15	F	Staffordshire Bull Terrier	Pinetown
211	3m	F	Boerbul	Dargle
212	3m	F	German Shepherd	Howick
213	4m	F	Cross breed	Pietermaritzburg
214	1	M	Cross breed	Westville
215	1	M	Dachshund	Kloof
216	2	M	Boerbul	Hilton
217	2	M	Boerbul	Hilton
218	2	M	Cross breed	Dargle
219	2	M	Dalmatian	Westville
220	2	M	Miniature Pinscher	New Germ
221	3	M	Maltese	Pinetown
222	4	M	Labrador	Hilton
223	4	M	Labrador	Pietermaritzburg
224	4	M	Labrador	Midlands
225	4	M	Rottweiler	Hilton
226	5	M	Cross breed	Howick

Sample Number	Approximate Age - Years	Gender	Breed	Area
227	5	M	Cross breed	Howick
228	5	M	Cross German Shepherd	Pietermaritzburg
229	5	M	Jack Russell	Howick
230	5	M	Rhodesian Ridgeback	Hilton
231	5	M	Rhodesian Ridgeback	Hilton
232	6	M	German Shepherd	Westville
233	6	M	Maltese	Howick
234	7	M	Dachshund	Underberg
235	7	M	Staffordshire Bull Terrier	Hilton
236	8	M	Rhodesian Ridgeback	Hilton
237	9	M	German Shepherd	Durban
238	10	M	Boerbul	Westville
239	10	M	Boxer	Hilton
240	10	M	Cross collie	Underberg
241	10	M	German Shepherd	Howick
242	10	M	Labrador	Hilton
243	10	M	Rhodesian Ridgeback	Hilton
244	10	M	Rhodesian Ridgeback	Midlands
245	10	M	Rhodesian Ridgeback	Hilton
246	11	M	Jack Russell	Hilton
247	11	M	Poodle	Hilton
248	12	M	Jack Russell	Hilton
249	12	M	Rottweiler	Howick
250	13	M	Rottweiler	Wartburg
251	16	M	Staffordshire Bull Terrier	Durban
252	3m	M	Cross breed	Merrivale
253	6m	M	Boerbul	Queensborough
254	6m	M	Boxer	Pietermaritzburg
255	8m	M	Labrador	Pietermaritzburg

Annexure C: Samples from stray dogs in Western Cape.

Sample Number	Approximate Age - Years	Gender	Breed	Area
256	8w	F	Cross Breed	Grassy Park
257	2m	F	Cross Boerbul	Grassy Park
258	2m	F	Cross Breed	Grassy Park
259	2m	F	Cross Breed	Grassy Park
260	3m	F	Cross Breed	Grassy Park
261	3m	F	Cross Breed	Grassy Park
262	3m	F	Cross Breed	Grassy Park
263	3m	F	Cross Breed	Grassy Park
264	6m	F	Australian Cattle Dog	Grassy Park
265	10m	F	Cross Breed	Grassy Park
266	1	F	Cross Breed	Grassy Park
267	1	F	Cross Breed	Grassy Park
268	1	F	Cross Breed	Grassy Park
269	2	F	Cross Breed	Grassy Park
270	2	F	Cross Breed	Grassy Park
271	2	F	Cross Breed	Grassy Park
272	2	F	Cross Breed	Grassy Park
273	2	F	Cross Breed	Grassy Park
274	3	F	Cross Breed	Grassy Park
275	3	F	Cross Breed	Grassy Park
276	4	F	Cross Breed	Grassy Park
277	4	F	Cross Breed	Grassy Park
278	4	F	Cross Breed	Grassy Park
279	4	F	Cross Breed	Grassy Park
280	4	F	Pomeranian	Grassy Park
281	6	F	Bull Terrier	Grassy Park
282	6	F	Cross Breed	Grassy Park
283	7	F	Cross Labrador	Grassy Park
284	8	F	Cross Breed	Grassy Park
285	12	F	Cross Breed	Grassy Park
286	2m	M	Cross Breed	Grassy Park
287	3m	M	Bull Dog	Grassy Park
288	3m	M	Cross Breed	Grassy Park
289	8m	M	Cross Breed	Grassy Park
290	9m	M	Bull Mastiff	Grassy Park
291	1	M	Cross Breed	Grassy Park

Sample Number	Approximate Age - Years	Gender	Breed	Area
292	2	M	Cross Breed	Grassy Park
293	2	M	Cross Breed	Grassy Park
294	3	M	Cross Breed	Grassy Park
295	3	M	Cross Breed	Grassy Park
296	3	M	Cross Breed	Grassy Park
297	3	M	Cross Breed	Grassy Park
298	3	M	Cross Breed	Grassy Park
299	3	M	Cross Breed	Grassy Park
300	4	M	Cross Breed	Grassy Park
301	6	M	Cross Breed	Grassy Park
302	6	M	Cross Breed	Grassy Park
303	7	M	Cross Fox Terrier	Grassy Park
304	8	M	Cross Breed	Grassy Park
305	9	M	Min Pinscher	Grassy Park
306	10	M	Cross Husky	Grassy Park
307	10	M	Pyrenean Mountain Dog	Grassy Park
308	19	M	Cross Breed	Grassy Park

Annexure D: Samples from dogs with owners in Western Cape.

Sample Number	Approximate Age - Years	Gender	Breed	Area
309	3m	F	Border Collie	Cape Town
310	6m	F	Rhodesian Ridgeback	Cape Town
311	6m	F	Unknown	George
312	6m	F	Unknown	George
313	7m	F	Cross Staffordshire Bull Terrier	Cape Town
314	7m	F	Unknown	George
315	8m	F	Labrador	George
316	8m	F	Unknown	George
317	11m	F	Cross Staffordshire Bull Terrier	Cape Town
318	1	F	Dachshund	Cape Town
319	1	F	Unknown	Uniondale
320	2	F	Border Collie	Cape Town
321	2	F	Cross Rottweiler	Cape Town
322	2	F	Unknown	George
323	3	F	Basset	Cape Town
324	3	F	Bouvier	Cape Town
325	3	F	Pekingese	Cape Town
326	3	F	Pointer	Cape Town
327	4	F	Boerbul	Cape Town
328	4	F	Cross Breed	George
329	4	F	Jack Russell	Cape Town
330	4	F	Labrador	Cape Town
331	4	F	Unknown	George
332	4	F	Unknown	George
333	5	F	Boerbul	Cape Town
334	5	F	Dachshund	Cape Town
335	5	F	Jack Russell	Cape Town
336	5	F	Labrador	Cape Town
337	5	F	Miniature Pinscher	Cape Town
338	5	F	Rottweiler	Cape Town
339	6	F	Border Collie	Cape Town
340	6	F	Bull Terrier	Cape Town
341	6	F	German Shepherd	Cape Town
342	6	F	Jack Russell	Cape Town
343	6	F	Rottweiler	Cape Town
344	6	F	Staffordshire Bull Terrier	Cape Town

Sample Number	Approximate Age - Years	Gender	Breed	Area
345	6	F	Staffordshire Bull Terrier	Cape Town
346	7	F	Cross Breed	Cape Town
347	7	F	Dachshund	Cape Town
348	7	F	Dalmatian	Cape Town
349	8	F	Dalmatian	Cape Town
350	8	F	Fox Terrier	Cape Town
351	8	F	Jack Russell	Cape Town
352	8	F	Retriever	Cape Town
353	9	F	Dachshund	Cape Town
354	9	F	Jack Russell	Cape Town
355	9	F	Maltese	Cape Town
356	9	F	Maltese	Cape Town
357	9	F	Miniature Pinscher	Cape Town
358	9	F	Miniature Pinscher	Cape Town
359	9	F	Rhodesian Ridgeback	Cape Town
360	9	F	Rottweiler	Cape Town
361	9	F	Unknown	George
362	10	F	Border Collie	Cape Town
363	10	F	Boxer	Cape Town
364	11	F	Cross Doberman	Cape Town
365	11	F	Jack Russell	Cape Town
366	11	F	Labrador	Cape Town
367	11	F	Spaniel	Cape Town
368	11	F	Staffordshire Bull Terrier	Cape Town
369	12	F	Border Collie	Cape Town
370	12	F	Border Collie	Cape Town
371	12	F	Cross Bull Mastiff	Cape Town
372	12	F	Dachshund	Cape Town
373	12	F	Maltese	Cape Town
374	12	F	Maltese	Cape Town
375	12	F	Maltese	George
376	12	F	Pit Bull Terrier	Cape Town
377	12	F	Poodle	Cape Town
378	12	F	Retriever	Cape Town
379	13	F	Cross Boerbul	Cape Town
380	13	F	Dachshund	Cape Town
381	14	F	Cross German Shepherd	Cape Town
382	14	F	Unknown	George

Sample Number	Approximate Age - Years	Gender	Breed	Area
383	15	F	Cross Fox Terrier	Cape Town
384	15	F	Staffordshire Bull Terrier	Cape Town
385	16	F	Border Collie	Cape Town
386		F	Border Collie	Cape Town
387		F	Cross Collie	Cape Town
388	2m	M	Yorkshire Terrier	Cape Town
389	3m	M	Doberman	Cape Town
390	3m	M	Pyrenean Mountain Dog	Cape Town
391	3m	M	Yorkshire Terrier	Cape Town
392	4m	M	Pit bull	Cape Town
393	6m	M	German Shepherd	Cape Town
394	6m	M	Unknown	George
395	7m	M	Unknown	George
396	9m	M	Border Collie	Cape Town
397	1	M	Rottweiler	Cape Town
398	1	M	Spaniel	Cape Town
399	1	M	Unknown	George
400	1	M	Unknown	George
401	2	M	Bull Terrier	Cape Town
402	2	M	Jack Russell	Cape Town
403	2	M	Pekingese	Cape Town
404	2	M	Pit Bull Terrier	Cape Town
405	2	M	Staffordshire Bull Terrier	Cape Town
406	3	M	Bull Terrier	Cape Town
407	3	M	Fox Terrier	Cape Town
408	3	M	Great Dane	Cape Town
409	3	M	Jack Russell	Cape Town
410	4	M	Boerbul	Cape Town
411	4	M	Chihuahua	Cape Town
412	4	M	Chow Chow	Cape Town
413	4	M	Dachshund	Cape Town
414	5	M	Cross Breed	George
415	5	M	Rhodesian Ridgeback	Cape Town
416	6	M	Boxer	Cape Town
417	6	M	Doberman	Cape Town
418	6	M	Doberman	Cape Town
419	6	M	Labrador	Cape Town
420	6	M	Rottweiler	Cape Town

Sample Number	Approximate Age - Years	Gender	Breed	Area
421	6	M	Rottweiler	Cape Town
422	6	M	Rottweiler	Cape Town
423	6	M	Unknown	George
424	7	M	Beagle	Cape Town
425	7	M	Bull Mastiff	Cape Town
426	7	M	Rottweiler	Cape Town
427	7	M	Unknown	George
428	7	M	Unknown	George
429	8	M	Bull Mastiff	Cape Town
430	8	M	Bull Mastiff	Cape Town
431	8	M	Dachshund	Cape Town
432	8	M	German Shepherd	Cape Town
433	8	M	Poodle	Cape Town
434	8	M	Unknown	George
435	8	M	Unknown	George
436	10	M	Maltese	Cape Town
437	10	M	Poodle	Cape Town
438	11	M	Fox Terrier	Cape Town
439	11	M	Irish Setter	Cape Town
440	11	M	Maltese	Cape Town
441	11	M	Whippet	Cape Town
442	12	M	German Shepherd	Cape Town
443	12	M	Maltese	George
444	12	M	Poodle	Cape Town
445	12	M	Schnauzer	Cape Town
446	12	M	Unknown	George
447	13	M	Fox Terrier	Cape Town
448	13	M	Spaniel	Cape Town
449	13	M	Unknown	George
450	17	M	Maltese	Cape Town

Annexure D: Samples from stray dogs in Eastern Cape.

Sample Number	Approximate Age - Years	Gender	Breed	Area
451	2m	F	Cross Breed	Port Elizabeth
452	1	F	Cross Breed	Port Elizabeth
453	1	F	Cross Breed	Port Elizabeth
454	2	F	Cross Breed	Port Elizabeth
455	3	F	Cross Breed	Port Elizabeth
456	3	F	Cross Breed	Port Elizabeth
457	3	F	Cross Breed	Port Elizabeth
458	3	F	Cross Breed	Port Elizabeth
459	4	F	Cross Breed	Port Elizabeth
460	4	F	Cross Breed	Port Elizabeth
461	4	F	Cross Breed	Port Elizabeth
462	5	F	Cross Breed	Port Elizabeth
463	5	F	German Shepherd	Port Elizabeth
464	5	F	Husky	Port Elizabeth
465	2	M	Cross Breed	Port Elizabeth
466	2	M	Cross Breed	Port Elizabeth
467	2	M	Cross Breed	Port Elizabeth
468	3	M	Cross Breed	Port Elizabeth
469	3	M	Cross Breed	Port Elizabeth
470	3	M	Cross Breed	Port Elizabeth
471	3	M	Cross Breed	Port Elizabeth
472	4	M	Cross Breed	Port Elizabeth
473	4	M	Cross Breed	Port Elizabeth
474	5	M	Cross Breed	Port Elizabeth
475	6	M	Cross Breed	Port Elizabeth
476	8	M	Cross Breed	Port Elizabeth
477	10	M	Cross Breed	Port Elizabeth

Annexure E: Samples from dogs with owners in Eastern Cape.

Sample Number	Approximate Age - Years	Gender	Breed	Area
478	6m	F	Border Collie	Humansdorp
479	3	F	Cross Breed	Humansdorp
480	3	F	Cross Breed	Humansdorp
481	3	F	Cross Breed	ST Francis
482	4	F	Cross Breed	ST Francis
483	4	F	Labrador	Humansdorp
484	5	F	Cross Fox Terrier	Humansdorp
485	5	F	Jack Russell	Humansdorp
486	6	F	Maltese	Humansdorp
487		F	Cross Breed	Humansdorp
488		F	Jack Russell	Humansdorp
489	1	M	Cross Breed	Humansdorp
490	1	M	Cross Breed	Humansdorp
491	2	M	Cross Breed	Humansdorp
492	3	M	Cross Breed	Humansdorp
493	3	M	Pointer	Jeffery's Bay
494	5	M	Toy pom	Humansdorp
495	6	M	Cross Breed	ST Francis
496	6	M	Spaniel	Jeffery's Bay
497	6	M	Spaniel	Humansdorp
498	8	M	Boerbul	Humansdorp
499	13	M	Cross Terrier	ST Francis
500	14	M	Staffordshire Bull Terrier	Humansdorp
501		M	Cross Breed	Humansdorp
502			Cross Breed	Humansdorp
503			Cross Breed	Humansdorp
504			Cross Breed	Humansdorp
505			Cross Breed	Humansdorp

Annexure F: Samples from stray dogs in Gauteng.

Sample Number	Approximate Age - Years	Gender	Breed	Area
506	2	M	German shepherd cross	Mamelodi
507	8w	M	Border Collie	Rayton
508	8w	M	Border Collie	Rayton
510	8w	F	Border Collie	Rayton
511	18m	M	German Shepherd Cross	Hammanskraal
512	18m	F	Africanis	Stinkwater
513	3m	F	Africanis	Tshipo St Extension
514	8w	M	Border Collie	Rayton
515	8w	M	Border Collie	Rayton
516	8w	M	Border Collie	Rayton
517	8w	F	Border Collie	Rayton
518	8w	F	Border Collie	Rayton
519	4m	M	Jack Russel	Tshipo St Extension
520	3m	M	Jack Russel	Moregleed
521	6m	M	Dachshund	Tramshed
522	2	M	Africanis	Winterveld

523- 530 stray dogs from Gauteng with no additional information available.