

## A serological survey of antibodies to *Leptospira* species in dogs in South Africa

J M Roach<sup>a,b\*</sup>, M van Vuuren<sup>a</sup> and J A Picard<sup>a</sup>

### ABSTRACT

Leptospirosis, a disease more common in the tropics, can cause a life-threatening multisystemic syndrome in humans and animals. Immunity, whether natural or vaccine-induced, is serogroup-specific with the infecting serovars varying according to geographical locality. In South Africa, in spite of the fact that the bacterin vaccine for some *Leptospira* serovars is often used, there is no recent information on the incidence of canine leptospirosis as well as the infecting serovar/s. The aim of this study, which was undertaken on sera collected in 2008 and 2009 from both strays and owned dogs predominantly in the coastal regions of South Africa, was to determine the presence of leptospiral antibodies to 15 serovars known to infect dogs. Of the 530 samples tested, 25 tested positive to 7 different serovars with the microscopic agglutination test (MAT). Nine of the 25 samples tested positive to more than one serovar. The 2 serovars most frequently represented were Canicola, which reacted to 17 sera, and Pyrogenes, which reacted to 10 sera. Currently the only vaccines available in South Africa in different combinations contain serovars Canicola, Icterohaemorrhagiae, Pomona and Grippotyphosa. The results showed that the use of vaccines containing serovar Canicola is still justifiable in certain regions of the country. However, the presence of antibodies to serovar Pyrogenes in several dogs, pending a broader investigation, indicates that this serovar should also be included in the range of *Leptospira* vaccines for use in South Africa.

**Keywords:** dogs, *Leptospira* serovars, microscopic agglutination test, South Africa, vaccine.

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### INTRODUCTION

Clinical cases of canine leptospirosis have historically been associated with *Leptospira* serovar Icterohaemorrhagiae, where rats act as the primary reservoir host and serovar Canicola where dogs are the maintenance host. In recent years the decrease in prevalence of these serovars in many 1st-world countries has been ascribed to widespread vaccination since the 1960s with homologous serovars<sup>7</sup>. A concomitant increase in other serovars, which is associated with the geographical region, is suggested to be the result of the encroachment of dogs into the environment of wildlife reservoir hosts of other serovars<sup>7</sup>.

The 1st published report documenting the presence of leptospirosis in dogs in Cape Town, South Africa was published

in 1953 where serovars Canicola and Sejroe were implicated<sup>13</sup>. In 1965, in Cape Town, sera of 100 dogs from a mostly impoverished community were tested for antibodies to *Leptospira* species. Of these, 43 had positive titres to serovar Canicola or serovar Icterohaemorrhagiae or both<sup>3</sup>. A later study evaluated the prevalence of leptospirosis in dogs in the Pretoria area in 1993. Sera collected from stray dogs were tested for serovars Canicola, Copenhageni, Grippotyphosa, Hardjo, Mini, Pomona, Pyrogenes and Tarassovi. Only 1.5 % of dogs tested had antibodies to 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<sup>17</sup>. In 1973, a report of serovar Canicola causing illness and death in dogs and abortions in pigs in the Stellenbosch district was published<sup>22</sup>. There are also reports of leptospirosis affecting pigs, cattle and wild animals in South Africa<sup>8,10,15,16,19,21</sup>. A recent study conducted on cattle from rural communities in KwaZulu-Natal identified Pomona as the most common serovar

although serovars Tarassovi, Bratislava, Hardjo, Canicola and Icterohaemorrhagiae were also identified<sup>9</sup>.

Even though veterinary practitioners in the subtropical coastal areas of South Africa have a perception that leptospirosis is present in dogs and immunise them against the disease, diagnoses of leptospirosis are lacking. It was the aim of this study to collect sera from healthy dogs to determine whether antibodies to any of the 15 different serovars could be demonstrated.

### MATERIALS AND METHODS

Clotted blood samples were collected by branches of the Society for Prevention of Cruelty to Animals (SPCA) and 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. Every attempt was made to bleed unvaccinated dogs but in some cases the vaccination histories of the dogs were unavailable. Estimating the expected disease 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 (Epi Info™ 3.5.1). Allowing for incomplete data in some of the samples it was elected to over-sample by at least 100 %. It was therefore decided to collect at least 440 blood samples.

Live leptospiral organisms representing 15 serovars and 11 serogroups (Table 1) were imported from the Royal Tropical Institute (KIT Biomedical Research Laboratory) in Amsterdam, the Netherlands.

They were maintained at 29 °C in Difco™ *Leptospira* Enrichment EMJH broth and subcultured in 5 ml quantities at 7-day intervals until required. The viability and purity of the cultures were checked by dark-field microscopy. Cultures were used for the serological tests between the 4th and 10th day of growth of that culture cycle<sup>4</sup>.

Serum samples were identified and numbered and the data (*i.e.* sex, age, breed and geographical region) pertaining

<sup>a</sup>Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa.

<sup>b</sup>Midlands Veterinary Clinic, 103a Main Street, Howick, 3290 South Africa.

\*Author for correspondence.  
E-mail: moritz.vanvuuren@up.ac.za

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to each recorded. Sera were frozen and stored prior to testing. The Microscopic Agglutination Test (MAT) was carried out as described in the Office International des Épizooties (OIE) Manual of Diagnostic Tests and Vaccines for Terrestrial Animals<sup>18</sup> using a 1:50 dilution of sera. All those sera showing agglutination underwent further 10-fold dilutions in a range of 1:100 to 1:6400 and were tested using serovars to which they had shown agglutination. The antibody titre was taken as the highest dilution where 50 % agglutination was recorded compared with a negative control. A titre of  $\geq 1:100$  was accepted as evidence of past exposure to leptospires.

## RESULTS

In total 530 sera were collected from the KwaZulu-Natal, Eastern Cape, Western Cape and Gauteng regions, where 25 sera revealed antibodies to one or more serovars *i.e.* 4.7 % of the total number tested (Table 2).

It was found that dogs from the Eastern

Table 1: The organisms used as capture antigens in the MAT represent 11 different serogroups.

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

Cape ( $P = 0.02$ ) followed by KwaZulu-Natal ( $P = 0.02$ ) were more likely to have leptospiral antibodies than dogs from the Western Cape. As a consequence of the small number of samples from Gauteng,

an inland province, no statistically significant differences were noted when the other provinces were compared with this one (Table 3).

At a confidence level of 95 %, it was

Table 2: Profiles of the dogs that were seropositive with the MAT.

Age	Breed	Sex	Area	Serovar	Titre
<b>KwaZulu-Natal</b>					
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
6 yr	German shepherd	M	Westville	Australis	800
2 yr	Spaniel	F	Dundee	Canicola	100
3 yr	Cross-breed	M	Pietermaritzburg	Canicola	200
9 yr	Greyhound	F	Pietermaritzburg	Canicola	100
10 yr	Fox terrier	F	Hilton	Canicola	200
5 yr	German shepherd cross	M	Pietermaritzburg	Canicola	400
3 yr	Cross-breed	F	Northdale	Canicola	800
				Icterohaemorrhagiae	100
5 yr	Labrador	M	Pietermaritzburg	Canicola	800
2 yr	Cross-breed	M	Napierville	Canicola	100
				Pyrogenes	100
<b>Eastern Cape</b>					
4 yr	Cross-breed	M	Port Elizabeth	Canicola	200
1 yr	Cross-breed	F	Port Elizabeth	Autumnalis	400
3 yr	Cross-breed	F	Port Elizabeth	Pyrogenes	200
10 yr	Cross-breed	M	Port Elizabeth	Pyrogenes	100
3 yr	Cross-breed	M	Port Elizabeth	Pyrogenes	200
				Canicola	800
4 yr	Cross-breed	F	Port Elizabeth	Canicola	800
Adult	Jack Russell	F	Humansdorp	Canicola	1600
6 m	Border collie	F	Humansdorp	Canicola	200
<b>Western Cape</b>					
2 yr	Cross-breed	M	Grassy Park	Pyrogenes	200
12 yr	Cross-breed	F	Grassy Park	Canicola	400
<b>Gauteng</b>					
Adult	Sample 13	Unknown	Gauteng	Canicola	100
				Pyrogenes	100
1.5 yr	Africanus	F	Gauteng	Canicola	1600
				Pyrogenes	400

Table 3: A statistical comparison of the seroprevalance of *Leptospira* antibodies between provinces (Fisher's exact test).

Province	Number of positive animals/Total	KwaZulu-Natal	Eastern Cape	Western Cape
KwaZulu-Natal	13/255			
Eastern Cape	8/55	0.02		
Western Cape	2/195	0.02	<0.001	
Gauteng	1/25	1.00	0.26	0.31

found that male (confidence interval (CI) 2.7, 9.0) and female (CI 2.2, 7.1) animals were equally likely to have antibodies to *Leptospira* serovars ( $P = 0.68$ ). This was true even when the sexual differences within provinces were examined (results not shown). Of the 25 positive dogs, 12 were cross-breeds. Using the Pearson  $2 \times 2$  chi-square test, it was found at a 95 % confidence level that stray dogs were more 4.09 (risk ratio) times more likely to have leptospiral antibodies than owned dogs ( $P = 0.0017$ ).

## 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. 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<sup>14</sup>.

Clearly stray dogs were 4.347 times more likely ( $P = 0.0017$ ) 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 dogs. It is therefore most likely that the antibodies present are due to exposure to infection with *Leptospira* species. It is known that free-roaming dogs compared with 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, by contact with rodents or rodent urine-contaminated foods, as well as unrestricted mating with possibly infected dogs. In a study in the USA it was found that dogs that developed leptospirosis had statistically significant higher odds of having swum in or drunk open water in the 2 weeks preceding their illness than those which did not become ill<sup>6</sup>. The fact that the canine serovar *Canicola* predominated in this study indicates that unrestricted mating or the close contact with urine aerosols from other dogs is the most likely method of transmission<sup>4</sup>.

Six of the 13 seropositive dogs from KwaZulu-Natal, all the seropositive dogs in the Western Cape and 6 of the 8 seropositive dogs in the Eastern Cape were cross-breeds. Other than the cross-breeds, no other breed predominated. It was thought that since the cross-breeds in the study were mainly strays, the fact that they were able to roam freely and were able to breed was more of a risk factor than the fact that there was a specific breed predilection.

There was no significant difference found in prevalence between the sexes in this study; however, in a retrospective study conducted in the USA between 1997 and 2000, male dogs were found to be at increased risk of leptospirosis than females<sup>23</sup>. The same was true for Japan where twice as many male as female dogs had leptospiral antibodies<sup>20</sup>. In a study conducted in Australia, although there was a trend for more female dogs to be positive than male, it was not found to be statistically significant<sup>24</sup>. There appear, therefore, to be other risk factors that are specific to a particular region or country, *i.e.* the proportion of breeding animals in a dog population and whether dogs are able to roam freely.

Antibodies to serovar *Canicola* predominated and were identified in all the provinces tested. As this serovar is present in the vaccine, the possibility that immunisation responses were being detected must be considered. In South Africa, all the leptospiral bacterins for use in dogs contain both serovars *Canicola* and *Icterohaemorrhagiae*. One would therefore expect a similar number of positive reactors to both serovars if the titres were indeed vaccine-induced. With the exception of 1 owned dog, this was not the case. In this dog the antibody titre to serovar *Canicola* was high at 1:800, indicating that the antibody response to at least serovar *Canicola* was due to natural infection.

Serovar *Pyrogenes* was previously identified in dogs in South Africa by Myburgh *et al.* in 1993<sup>17</sup> but was not a commonly identified serovar in other studies conducted in South Africa<sup>3,13,22</sup>. Similarly, in a survey conducted on 820 dogs in Japan over a 3-year period, the 3rd most common serovar to which anti-

bodies was detected after *Icterohaemorrhagiae* (57 dogs) and *Canicola* (53 dogs) was *Pyrogenes* (10 dogs)<sup>20</sup>. In Brazil, antibodies to this serovar were found in 12 % of the dogs tested<sup>1</sup>. In the current survey, it does assume some importance as it was found in every region tested and was the 2nd most commonly identified serovar.

Of the 4 regions represented, 3 (*i.e.* Western and Eastern Cape and Gauteng) only had positives to serovars *Canicola* and *Pyrogenes* apart from 1 positive reactor to serovar *Autumnalis* in the Eastern Cape. By contrast, the dogs tested in KwaZulu Natal reacted to 7 different serovars including some not previously identified in South Africa. The climate in KwaZulu-Natal, particularly in the coastal areas, is warm and wet and ideal for the environmental survival of the organism<sup>4</sup>.

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)<sup>11,12</sup>. Therefore the presence of antibodies to serovars *Icterohaemorrhagiae* and *Copenhageni* is most likely the result of a cross-reaction, with serovar *Icterohaemorrhagiae* being the most likely infecting serovar since it had the highest antibody titre. However, in most cases antibodies were found to different serogroups, the most common combination being serovars *Canicola* and *Pyrogenes*. This seems to imply that those dogs have been exposed to several serovars.

A number of studies relating to *Leptospira* 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 serovar *Copenhageni* in wild animals<sup>8,9,10,19</sup>. Antibodies to serovars *Batavia* and *Autumnalis* have also been detected in game in Zimbabwe. 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<sup>2,5</sup>. It is therefore feasible for free-ranging dogs which may at times be in the same habitat as livestock and wild animals to have been exposed to any of the *Leptospira* serovars found in southern Africa.

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) serovars 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. Prior 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 serovar Copenhageni in wild animals. Serovars Autumnalis, Australis and Bataviae although identified in cattle and game in southern Africa have not been reported in South Africa.

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 is suggested 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. It is also motivated that serovar Pyrogenes be included in any new vaccines.

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