

Serological survey for antibodies reactive with *Ehrlichia canis* and *E. chaffeensis* in dogs from the Bloemfontein area, South Africa

A-M Pretorius^a and P J Kelly^b

ABSTRACT

Sera from 161 dogs in the Bloemfontein area in South Africa were tested for the presence of antibodies reactive with *Ehrlichia canis* and *E. chaffeensis* by indirect fluorescent antibody testing. Overall, 68 (42 %) of the dogs had significant antibody titres ($\geq 1/64$) against *E. canis* and 61 (38 %) had significant titres ($\geq 1/64$) against *E. chaffeensis*. Seven (11 %) dogs had higher titres to *E. chaffeensis* than *E. canis* (1/2048 and 1/1024 (2 dogs); 1/1024 and 1/512 (2 dogs); 1/2048 and 1/512; 1/512 and 1/256 and 1/512 and $< 1/64$, respectively). The remaining seropositive dogs had equal ($n = 26$; 42 %) or 2- ($n = 17$; 25 %), 3- ($n = 13$; 2 %) or 4-fold ($n = 5$; 7 %) higher titres against *E. canis*. Dogs from economically depressed, high-density suburbs (60/112; 48 %) had significantly higher prevalences of antibodies against *E. canis* than those from more affluent, low-density suburbs (8/49; 14 %) ($\chi^2 = 19.38, p < 0.001$). Higher titres to *E. chaffeensis* than *E. canis* were found in dogs from affluent, low-density suburbs (3/49) and in dogs from economically depressed, high-density suburbs (4/112).

Key words: Bloemfontein, dogs, *Ehrlichia*, serological survey, South Africa.

Pretorius A-M, Kelly P J **Serological survey for antibodies reactive with *Ehrlichia canis* and *E. chaffeensis* in dogs from the Bloemfontein area, South Africa.** *Journal of the South African Veterinary Association* (1998) 69(4): 126–128 (En.) Department of Medical Microbiology, Internal Box G4, Faculty of Health Sciences, University of the Orange Free State, Bloemfontein, 9300 South Africa.

INTRODUCTION

Ehrlichia canis is the aetiological agent of canine tropical pancytopenia that was first described in dogs from Algeria in 1935⁸ and has subsequently been reported to occur worldwide⁹. Previous serological surveys have indicated that *E. canis* infections are common in dogs in countries in North Africa⁴, Egypt³, Senegal¹⁷ and Zimbabwe^{13,14,15}. In South Africa, clinical cases of canine tropical thrombocytopenia are apparently common²¹ but no supportive serological data are available. *E. chaffeensis* is the agent of human monocytic ehrlichiosis, which was first described in the United States of America (USA) in 1987¹. Subsequent studies have indicated that human infections also occur in Europe¹⁶ and Africa^{5,20}, and in the USA dogs are regarded as potential reservoirs of infection, as they are susceptible to natural⁷ and experimental⁶ infections with *E. chaffeensis*.

To provide serological data on the prevalences of *E. canis* and *E. chaffeensis* infections in dogs in South Africa, we tested

sera collected in the Bloemfontein area for antibodies reactive with the organisms.

MATERIALS AND METHODS

Sera

Whole blood was collected from 161 apparently healthy dogs in the Bloemfontein area that were presented to veterinary clinics for sterilisation. Sera were separated and stored at 70 °C until indirect fluorescent antibody (IFA) testing was performed.

Serology

Each serum was tested for antibodies to *E. canis* (Oklahoma strain) and *E. chaffeensis* (Arkansas strain) grown in DH82 continuous cell cultures as described previously¹⁴. Sera were screened at the recommended 1/64 dilution⁷ in phosphate-buffered saline (PBS; pH 7.4) and reactive antibodies detected with an optimised dilution (1:50) of fluorescein isothiocyanate-labelled protein A conjugate (Biogenesis Inc., Sandown, USA) in PBS and a fluorescence light microscope using $\times 400$ magnification. Protein A conjugate reacts with IgG of a wide range of mammalian species, including dogs¹², and is used in our laboratory to avoid the expense of purchasing specific antisera.

Serial 2-fold dilutions of sera positive at 1/64 were made to determine end titres.

RESULTS

Sera from 161 dogs were tested for the presence of antibodies reactive with *E. canis* and *E. chaffeensis* by IFA (Table 1). The Botshabelo, Thaba Nchu and Heidedal areas are high-density, economically depressed suburbs situated within a 65 km radius of Bloemfontein, while the Bloemfontein residential areas are more affluent, low-density suburbs situated within the city itself. Of the dogs tested, 68 (42 %) had significant antibody titres ($\geq 1/64$) against *E. canis* and 61 (38 %) had significant titres ($\geq 1/64$) against *E. chaffeensis*. Seven (11 %) dogs had higher titres to *E. chaffeensis* than *E. canis* (1/2048 and 1/1024 (2 dogs); 1/1024 and 1/512 (2 dogs); 1/2048 and 1/512; 1/512 and 1/256 and 1/512 and $< 1/64$, respectively). The remaining seropositive dogs had equal ($n = 26$; 42 %) or 2- ($n = 17$; 25 %), 3- ($n = 13$; 2 %), or 4-fold ($n = 5$; 7 %) higher titres against *E. canis*. Dogs from Botshabelo, Thaba Nchu and Heidedal (60/112; 48 %) had significantly higher prevalences of antibodies against *E. canis* than those from the Bloemfontein residential areas (8/49; 14 %) ($\chi^2 = 19.38, p < 0.001$). Of the dogs that were seropositive for antibodies against *E. chaffeensis*, higher titres to *E. chaffeensis* than *E. canis* were found in dogs from Bloemfontein residential areas (3/7; 6 %) and in dogs from Thaba Nchu (3/25; 12 %) and Heidedal (1/13; 8 %).

DISCUSSION

Our results show that sera from a high proportion of apparently healthy dogs in the Bloemfontein area of South Africa have antibodies reactive against *E. canis* and *E. chaffeensis*. The prevalence of antibodies against *E. canis* we detected was significantly higher in dogs from the economically depressed suburbs surveyed (Botshabelo, Thaba Nchu and Heidedal) than in dogs from the more affluent Bloemfontein residential areas. Since ticks are the principal vectors of *Ehrlichia* spp.⁹, it appears likely that the lack of effective tick control in economically depressed areas was responsible for

^aDepartment of Medical Microbiology (Internal Box G4), Faculty of Health Sciences, University of the Orange Free State, Bloemfontein, 9300 South Africa.

^bBiomedical Research and Training Institute, 17 Beveridge Road, Avondale, Harare, Zimbabwe.

Received: April 1998. Accepted: October 1998.

Table 1. Prevalences of antibodies reactive with *Ehrlichia chaffeensis* and *E. canis* in sera from dogs in the Bloemfontein area, South Africa.

Location	n	Reciprocal IFA titres to:					
		<i>E. chaffeensis</i>			<i>E. canis</i>		
		<64 n (%)	64–1024 n (%)	≥2048 n (%)	<64 n (%)	64–1024 n (%)	≥2048 n (%)
Bloemfontein	49	42 (86)	5 (10)	2 (4)	41 (84)	5 (10)	3 (6)
Botshabelo	47	31 (66)	9 (19)	7 (15)	30 (64)	9 (19)	8 (17)
Heidedal	32	19 (59)	10 (31)	3 (9)	17 (53)	9 (28)	6 (19)
Thaba Nchu	33	8 (24)	23 (70)	2 (6)	5 (15)	24 (73)	4 (12)
Total	161	100 (59)	47 (33)	14 (9)	93 (54)	47 (33)	21 (14)

the difference in prevalence rates.

Although we tested for antibodies reactive against *E. canis* and *E. chaffeensis*, serological cross-reactivity occurs between these organisms and other members of the tribe Ehrlichieae, some of which may infect dogs. These include *Cowdria ruminantium*¹¹, *E. ewingii*², *Neorickettsia helminthoeca*¹⁸, *E. equi*¹⁰ and *E. risticii*¹⁹. Of these, only *C. ruminantium* is known to occur in Africa, but the organism is not present in the Bloemfontein area where our samples were collected²². The previously reported serological cross-reactivity between *E. canis* and *E. chaffeensis*^{1,6} was also evident from our results, with most seropositive dogs having antibodies reactive against both organisms. The higher titres to *E. canis* in the majority of our positive sera indicate that most of the dogs we surveyed had been infected with *E. canis*. The seroprevalences we report to *E. canis* are similar to those recorded elsewhere in Africa, mainly Zimbabwe (33–68%)^{13,14,15}, countries in North Africa (47%)⁴, Senegal (13–78%)¹⁷ and Egypt (33%)³. Tropical canine pancytopenia is a difficult disease to diagnose as there are no pathognomonic signs and organisms are difficult to detect in peripheral blood smears^{13,14,15}. Serology remains the most effective means of diagnosing infections and the results of our study indicate a need for a routine *Ehrlichia* serological diagnostic facility to be established in South Africa.

Our finding that 7 seropositive dogs had higher titres to *E. chaffeensis* provides further evidence that the agent of human monocytic ehrlichiosis or a closely related species occurs in Africa. There are now over 400 reported cases of human monocytic ehrlichiosis in people, with fever, headache, malaise, myalgia, arthralgia, nausea and/or vomiting being the most common clinical signs¹. Experimental infections of dogs with *E. chaffeensis* result in only mild clinical signs, including low-grade transient fever and ocular discharges that are not associated with haematological abnormalities⁶.

Dogs may, however, harbour infections for up to 26 days, and such infections provide no protection against subsequent *E. canis* challenge⁶. Recent surveys in the USA have shown that natural infections of dogs with *E. chaffeensis* are more prevalent than *E. canis* infections in some areas⁶. With growing evidence for the presence of *E. chaffeensis* in Africa, further studies are required to determine the role of dogs in the epidemiology of human monocytic ehrlichiosis on the continent.

ACKNOWLEDGEMENTS

We thank Professor L J Fourie and Mr P Jacobs, Department of Zoology/Entomology, University of the Orange Free State, for their assistance with supplying sera, and J Dawson of the Centers for Disease Control, Atlanta, for the *E. canis* and *E. chaffeensis* isolates. Funding was provided by the Central Research Fund of the University of the Orange Free State, Bloemfontein, South Africa.

REFERENCES

- Anderson B E, Dawson J E, Jones D C, Wilson K H 1991 *Ehrlichia chaffeensis*, a new species associated with human ehrlichiosis. *Journal of Clinical Microbiology* 29: 2838–2842
- Anderson B E, Greene C E, Jones D C, Wilson D H 1992 *Ehrlichia ewingii* sp. nov., the etiologic agent of canine granulocytic ehrlichiosis. *International Journal of Systematic Bacteriology* 42: 299–302
- Botros B A, Elmolla M S, Salib A W, Calamaio C A, Dasch G A, Arthur R R 1995 Canine ehrlichiosis in Egypt: sero-epidemiological survey. *Onderstepoort Journal of Veterinary Research* 62: 41–43
- Brouqui P, Davoust B, Haddad S, Vidor E, Raoult D 1991 Serological evaluation of *Ehrlichia canis* infections of military dogs in Africa and Réunion Island. *Veterinary Microbiology* 26: 103–105
- Brouqui P, Le Cam C, Kelly P J, Laurens R, Tounkara A, Sawadogo S, Velo-Marcel, Gondao L, Faugere B, Delmont J, Bourgeade A, Raoult D 1994 Serologic evidence for human ehrlichiosis in Africa. *European Journal of Epidemiology* 10: 1–4
- Dawson J E, Ewing S A 1992 Susceptibility of dogs to infection with *Ehrlichia chaffeensis*, causative agent of human ehrlichiosis. *American Journal of Veterinary Research* 53: 1322–1327

- Dawson J E, Biggie K L, Warner C K, Cookson K, Jenkins S, Levine J F, Olson J G 1996 Polymerase chain reaction evidence of *Ehrlichia chaffeensis*, an etiologic agent of human ehrlichiosis, in dogs from southeast Virginia. *American Journal of Veterinary Research* 57: 1175–1179
- Donatien A, Lestoquard F 1935 Existence en Algérie d'une rickettsia du chien. *Bulletin de la Société de Pathologie Exotique* 28: 418–419
- Greene C E, Harvey J W 1984 Canine ehrlichiosis. In Greene C E (ed.) *Clinical microbiology and infectious diseases of the dog and the cat*. WB Saunders, Philadelphia: 545–561
- Johansson K-E, Pettersson B, Uhlen M, Gunnarsson A, Malmquist M, Olsson E 1995 Identification of the causative agent of granulocytic ehrlichiosis in Swedish dogs and horses by direct solid phase sequencing of PCR products from the 16S rRNA gene. *Research in Veterinary Science* 58: 109–112
- Kelly P J, Matthewman L A, Mahan S M, Semu S, Peter T, Mason P R, Brouqui P, Raoult D 1994 Serological evidence for antigenic relationships between *Ehrlichia canis* and *Cowdria ruminantium*. *Research in Veterinary Science* 56: 170–174
- Kelly P J, Tagwira M, Matthewman L, Mason P R, Wright E P 1993 Reactions of sera from laboratory, domestic and wild animals in Africa with protein A and a recombinant chimeric protein AG. *Comparative Immunology, Microbiology and Infectious Diseases* 16: 299–305
- Matthewman L A, Kelly P J, Bobade P A, Tagwira M, Majok A, Mason P R, Brouqui P, Raoult D 1993 Infections with *Babesia canis* and *Ehrlichia canis* in dogs in Zimbabwe. *The Veterinary Record* 133: 344–346
- Matthewman L A, Kelly P J, Mahan S M, Semu D, Tagwira M, Bobade P A, Brouqui P, Mason P R, Raoult D 1993 Western blot and indirect fluorescent antibody testing for antibodies reactive with *Ehrlichia canis* in sera from apparently healthy dogs in Zimbabwe. *Journal of the South African Veterinary Association* 64: 111–115
- Matthewman L A, Kelly P J, Mahan S M, Semu S M, Mason P R, Bruce D, Brouqui P, Raoult D 1994 Reactivity of sera collected from dogs in Mutare, Zimbabwe, to antigens of *E. canis* and *Cowdria ruminantium*. *The Veterinary Record* 134: 498–499
- Morais J D, Dawson J E, Greene C, Filipe A R, Galhardas L C, Bacellar F 1991 First European case of ehrlichiosis. *Lancet* 338: 633–634
- Parzy D, Davoust B, Raphenon G, Vidor E 1991 Canine ehrlichiosis in Senegal: human

- and canine seroepidemiological survey in Dakar. *Medicine Tropical* 5: 59–63
18. Rikisha Y 1991 Crossreacting antigens between *Neorickettsia helminthoeca* and *Ehrlichia* species shown by immunofluorescence and western immunoblotting. *Journal of Clinical Microbiology* 29: 2024–2029
 19. Ristic M, Dawson J, Holland C J, Jenny A 1988 Susceptibility of dogs to infection with *Ehrlichia risticii*, causative agent of equine monocytic ehrlichiosis (Potomac horse fever). *American Journal of Veterinary Research* 49: 1497–1500
 20. Uhaa I J, Maclean J D, Greene C R, Fishbein D B 1992 A case of human ehrlichiosis acquired in Mali: clinical and laboratory findings. *American Journal of Tropical Medicine and Hygiene* 46: 161–164
 21. Van Heerden J, Immelman A 1979 The use of doxycycline in the treatment of canine ehrlichiosis. *Journal of the South African Veterinary Association* 50: 241–244
 22. Walker, J B 1991 A review of the ixodid ticks (Acari, Ixodidae) occurring in southern Africa. *Onderstepoort Journal of Veterinary Research* 58: 81–105