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

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**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 (≥1/64) against *E. canis* and 61 (38 %) had significant titres (≥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-fold (n = 17; 25 %), 3-fold (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 %) (χ² = 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.

**INTRODUCTION**

*Ehrlichia canis* is the aetiological agent of canine tropical pancytopenia that was first described in dogs from Algeria in 1935 and has subsequently been reported to occur worldwide. Previous serological surveys have indicated that *E. canis* infections are common in dogs in countries in North Africa, Egypt, Senegal and Zimbabwe. In South Africa, clinical cases of canine tropical thrombocytopenia are apparently common 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 1987. Subsequent studies have indicated that human infections also occur in Europe and Africa, and in the USA dogs are regarded as potential reservoirs of infection, as they are susceptible to natural and experimental 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 previously. Sera were screened at the recommended 1/64 dilution 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 ×400 magnification. Protein A conjugate reacts with IgG of a wide range of mammalian species, including dogs, 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 Heidelberg 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 (≥1/64) against *E. canis* and 61 (38 %) had significant titres (≥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-fold (n = 17; 25 %), 3-fold (n = 13; 2 %) or 4-fold (n = 5; 7 %) higher titres against *E. canis*. Dogs from Botshabelo, Thaba Nchu and Heidelberg (60/112; 48 %) had significantly higher prevalences of antibodies against *E. canis* than those from the Bloemfontein residential areas (8/49; 14 %) (χ² = 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 Heidelberg (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 Heidelberg) than in dogs from the more affluent Bloemfontein residential areas. Since ticks are the principal vectors of *Ehrlichia* spp., it appears likely that the lack of effective tick control in economically depressed areas was responsible for...
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 Ehrlichiaceae, some of which may infect dogs. These include Cowdria ruminantium, E. ewingii, Neorickettsia helminthoeca, E. equi and E. risticii. 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 collected. The previously reported serological cross-reactivity between E. canis and E. chaffeensis 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%), countries in North Africa (47%), Senegal (13–78%) and Egypt (33%). 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. Serology remains the most effective means of diagnosing infections and the results of our study indicate a need for a 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 signs. 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 abnormalities.

Dogs may, however, harbour infections for up to 26 days, and such infections provide no protection against subsequent E. canis challenge. Recent surveys in the USA have shown that natural infections of dogs with E. chaffeensis are more prevalent than E. canis infections in some areas. 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.

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REFERENCES


From a personal and South African perspective there are some very interesting and useful contributions. The paper on central/national diagnostic laboratories by J E Pearson (coordinator of the compendium) makes the striking point that although the structure, organisation and reporting channels of the 24 laboratories reviewed varied considerably, almost all are responsible to and report to the chief veterinary officer (CVO) of the country concerned. South Africa, in effect, does not have a CVO, and the Agricultural Research Council (ARC) institutes at Onderstepoort that fulfil the role of a national laboratory have no direct link to the national or provincial Directorates of Animal Health and Veterinary Public Health. Conversely, the major problem of accreditation and funding of national and international reference laboratories (Edwards and Alexander) is something that the ARC laboratories at Onderstepoort share with equivalent institutions throughout the world. The paper on high-security laboratories (Murray), apart from listing and comparing major institutions around the world and outlining the fundamentals of biological containment, provides a most useful table comparing disease risk categories used by different international organisations and some industrialised nations.

This number of the OIE Scientific and Technical Review is essential reading for those involved in the management of veterinary laboratories in the public domain, particularly those that need to achieve national or international recognition. There are also chapters that will benefit laboratory scientists and veterinarians involved in serology, particularly those in which ELISA systems are being used routinely to enable international trade.

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