Antibodies reactive with *Bartonella henselae* and *Ehrlichia canis* in dogs from the communal lands of Zimbabwe

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ABSTRACT

The prevalences of antibodies against *Bartonella henselae* and *Ehrlichia canis* were determined in sera from 228 dogs in 5 communal lands of Zimbabwe, areas where traditional subsistence agro-pastoralism is practised. The sera were collected from apparently healthy dogs during routine rabies vaccination programmes and tested with indirect fluorescent antibody assays using *B. henselae* (Houston-I) and *E. canis* (Oklahoma) as antigens. We found reactive antibodies (≥1:80) against *B. henselae* in 14 % of the dogs tested. Seropositive animals were found in Bikita (41 %; 17/42), Omay (13 %; 6/48), Chinamora (5 %; 2/38) and Matusadona (15 %; 7/48). No seropositive dogs were found in Chiredzi (0 %; 0/52). Antibodies reactive with *E. canis* (≥1:80) were found in 34 % of the dogs tested, from Bikita (88 %; 37/42), Chiredzi (31 %; 16/52), Omay (17 %; 8/48), Chinamora (26 %; 10/38) and Matusadona (15 %; 7/48). Our survey shows dogs in the communal lands of Zimbabwe are frequently exposed to *E. canis* and *B. henselae* or closely related species. Further studies are indicated to determine the pathogenicity of the organisms infecting these dogs and their clinical significance.

Key words: *Bartonella,* communal lands, dogs, *Ehrlichia,* serosurvey, Zimbabwe.

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INTRODUCTION

Ehrlichia canis is a Gram-negative bacterium that is an agent of canine monocytic ehrlichiosis[29]. The organism is transmitted by *Rhipicephalus sanguineus* and, in the acute stage of infection, there is often fever, anorexia, lymphadenomegaly, splenomegaly and thrombocytopaenia. Most dogs survive the acute stage and enter the subclinical phase of the disease, which might last for years. During this phase, animals are apparently healthy although thrombocytopaenia is common. Dogs may spontaneously eliminate *E. canis* during the subclinical phase[6,21] or go on to develop the chronic phase of the disease in which there is marked weight loss and signs resulting from pancytopaenia. While serosurveys have shown that high percentages of urban dogs in Zimbabwe have antibodies reactive with *E. canis*[36–38], there are no published data on infections in dogs in the rural communal lands. These are areas where traditional subsistence agro-pastoralism is practised and which contain 70 % of the national dog population[10].

Bartonella henselae is a Gram-negative bacterium that is an emerging human and veterinary pathogen worldwide[23]. The domestic cat is the natural host of *B. henselae* and high percentages of cats have asymptomatic bacteraemia which may persist for years[32]. Seropositive animals have been found in Zimbabwe (24 %; 28/119) and South Africa (21 %; 11/52)[27] and *B. henselae* has been isolated from the blood of domestic cats in Zimbabwe (10 %; 3/30)[28] and South Africa (3 %; 1/31)[44].

In humans, *B. henselae* is an important emerging zoonotic agent that is often associated with contact with cats and their fleas[23]. The organism has been implicated as an agent of an ever-increasing spectrum of diseases including cat-scratch disease, bacillary angiomatosis, endocarditis, bacteraemia, encephalopathy, neuroretinitis, osteomyelitis and peliosis hepatis[27]. Immunosuppressed people are often at particular risk of infection with *B. henselae* and a recent study in South Africa found 10 % of outpatients attending HIV clinics in Johannesburg had *Bartonella* bacteraemia[19].

Bartonella henselae is transmitted between cats by the cat flea, *Ctenocephalides felis*[14], which has a broad host range and is also commonly found on dogs[49]. Recent studies have shown that *B. henselae* can infect dogs and cause clinical disease[20,31,39]. *Ctenocephalides felis* is a common ectoparasite of dogs in southern Africa[12], particularly in dogs in the communal lands of Zimbabwe where ectoparasite control is seldom used.

To determine if antibodies to *B. henselae* were present in dogs in Zimbabwe, a serosurvey on dogs from 5 widely separated communal lands was conducted. The dogs were also tested for antibodies to *E. canis* to provide data on the prevalence of canine ehrlichiosis in the rural areas of Zimbabwe. The results of our surveys are described in this report.

MATERIALS AND METHODS

Sera

Blood samples were obtained from apparently healthy dogs (≥20 weeks of age) during rabies vaccination programmes in communal lands in central (Chinamora, –17.58, 31.25), southeast (Chiredzi, –21.00, 31.50; Bikita, –20.07, 31.60) and northern (Matusadona, –16.43, 28.58; Omay, –17.08, 28.25) Zimbabwe. Sera were separated and stored at –20°C until indirect fluorescent antibody assays (IFA) were performed. Negative and positive control sera for the IFAs were identified from studies performed previously in our laboratories[27,37].

Indirect fluorescent antibody assays

Bartonella henselae (Houston-I; ATCC 49882) was grown in Vero cells as described previously[27]. When 60–90 % of the cells were infected they were pelleted, washed and resuspended in phosphate buffered saline, applied (5$\mu\ell$ aliquots) to the wells of 32 well Teflon slides and air-dried. *E. canis* (Oklahoma) was grown in DH82 continuous cell cultures, harvested and applied to the wells of Teflon-coated slides as described previously[38].

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Table 1: **Ages of dogs sampled in 5 communal lands in Zimbabwe.**

	n	≤1 year	>1 to ≤2 years	>2 to ≤3 years	>3 to ≤4 years	>4 years
Bikita	42	14 (33%)	17 (41%)	4 (10%)	5 (12%)	2 (5%)
Chinamora	38	16 (42%)	5 (13%)	9 (24%)	5 (13%)	3 (8%)
Chiredzi	52	15 (29%)	21 (40%)	9 (17%)	3 (6%)	4 (8%)
Omay	48	15 (31%)	12 (28%)	14 (29%)	4 (8%)	3 (6%)
Matusadona	48	14 (29%)	13 (27%)	13 (27%)	5 (10%)	3 (6%)
Total	228	74 (33%)	68 (30%)	49 (22%)	22 (10%)	15 (7%)

n = sample size.

Table 2: **Ages and percentages of dogs from communal lands in Zimbabwe with indirect fluorescent antibody titres of ≥1:80 against *Ehrlichia canis*.**

	Overall	≤1 year	>1 to ≤2 years	>2 to ≤3 years	>3 to ≤4 years	>4 years
Bikita	88% (37/42)	86% (12/14)	88% (15/17)	100% (4/4)	100% (5/5)	50% (1/2)
Chiredzi	31% (16/52)	20% (3/15)	38% (8/21)	33% (3/9)	33% (1/3)	25% (1/4)
Omay	17% (8/48)	33% (5/15)	0% (0/12)	7% (1/14)	25% (1/4)	33% (1/3)
Chinamora	26% (10/38)	38% (6/16)	40% (2/5)	11% (1/9)	20% (1/5)	0% (0/3)
Matusadona	15% (7/48)	36% (5/14)	0% (0/13)	8% (1/13)	20% (1/5)	0% (0/3)
Total	34% (78/228)	42% (31/74)	37% (25/68)	20% (10/49)	41% (9/22)	20% (3/15)

Reactive antibodies were detected against *B. henselae* and *E. canis* using previously reported IFA procedures[27,37] and fluorescein isothiocyanate-labelled protein G conjugate (Biogenesis Inc, Sandown, NH, USA). Based on the results of previous studies, sera with IFA titres of ≥1:80 were regarded as positive for previous exposure to *Bartonella* spp.[24,27] or to *Ehrlichia* spp.[37,38].

RESULTS

Sera

Sera were obtained from 228 dogs in the 5 communal lands surveyed. Over half of the dogs sampled (142/228; 62 %) were 2 years of age or younger (Table 1).

Indirect fluorescent antibody assays

Dogs with antibodies against *E. canis* were found in all the communal lands sampled (Table 2) and seroprevalences varied from 15 % (7/48 in Matusadona) to 88 % (37/42 in Bikita). There were no significant differences between the overall seroprevalences in the different age groups of dogs studied. High antibody titres (arbitrarily defined as ≥1:640) were only found in dogs 3 years of age or younger from Chinamora (5; 50 % of the positive dogs), Chiredzi (11; 69 % of positive dogs) and Bikita (23; 63 % of the positive dogs).

Dogs with antibodies against *B. henselae* were found in all communal lands apart from Chiredzi (Table 3). There was no obvious correlation between age and seropositivity. The highest seroprevalence was in dogs from Bikita (41 %) where the only high titres against *B. henselae,* arbitrarily defined as ≥1:640, were found in 4 dogs which were 2 years of age or younger.

DISCUSSION

In 1990, Brooks[10] reported that dogs under a year of age constituted 33 % of the population in Manicaland Province in the east of Zimbabwe and that only 11 % of dogs were over 4 years of age. A similar age distribution was reported subsequently in 2000[13] and has now also been found in this study (Table 1). It appears that there has been little improvement in the very rapid turnover of dogs in the communal lands of Zimbabwe in the past decade.

This study is the 1st to show that rural dogs in southern Africa have high prevalences of antibodies to *E. canis.* The percentage of communal land dogs we found seropositive against *E. canis* (34 %) is similar to that described previously for urban dogs in Zimbabwe and South Africa (33–42 %)[36–38,43]. Although local experience indicates infections with *E. canis* are frequent in southern Africa[29,53], there is little direct supporting evidence. There are no African isolates of *E. canis* and only 3 reports identifying *E. canis* or a closely related species on the continent. In a recent report, only a few positive results were obtained when dogs suspected of having canine ehrlichiosis in South Africa were tested for *E. canis* with a highly sensitive and specific PCR assay[2]. In a study using PCR and sequencing, organisms closely related to *E. canis* have been identified in a sheep in South Africa[1]. Using similar methods, organisms closely related to *E. canis* have been identified in cattle ticks from Mali and Niger, but DNA of *Ehrlichia* spp. was not found in 86 *R. sanguineus* from dogs in Mali and Sudan[42].

Although antibodies to *E. canis* were detected, there is serological cross-reactivity between members of the genus and we could not, then, determine the

Table 3: **Ages and percentages of dogs from communal lands in Zimbabwe with indirect fluorescent antibody titres of ≥1:80 against *Bartonella henselae*.**

	Overall	1 year	>1 to ≤2 years	>2 to ≤3 years	>3 to ≤4 years	>4 years
Bikita	41% (17/42)	7% (1/14)	53% (9/17)	100% (4/4)	40% (2/5)	50% (1/2)
Chiredzi	0% (0/52)	0% (0/30)	0% (0/21)	0% (0/9)	0% (0/3)	0% (0/4)
Omay	13% (6/48)	20% (3/15)	0% (0/12)	7% (1/14)	0% (0/4)	66% (2/3)
Chinamora	5% (2/38)	13% (2/16)	0% (0/5)	0% (0/9)	0% (0/5)	0% (0/3)
Matusadona	15% (7/48)	29% (4/14)	0% (0/13)	8% (1/13)	0% (0/5)	66% (2/3)
Total	14% (32/228)	14% (10/74)	13% (9/68)	12% (6/49)	9% (2/22)	33% (5/15)

Ehrlichia spp. that had infected the dogs we studied. There is extensive serological cross-reactivity between *E. canis* and *E. chaffeensis*[47], the agent of human monocytic ehrlichiosis. The organism can also infect dogs and cause clinical and pathological signs indistinguishable from those caused by *E. canis*[7]. Although antibodies to *E. chaffeensis* have been found in both dogs[43] and people in southern Africa[11,45], all available information indicates that *E. chaffeensis* only occurs in the USA and it is thus unlikely that infections with the organism influenced our results.

Ehrlichia ruminantium (formerly *Cowdria ruminantium*) is the agent of heartwater, a disease of domestic ruminants that occurs widely in Africa and is transmitted by *Amblyomma* spp. Experimentally infected dogs do not show clinical signs or laboratory abnormalities but become bacteraemic for up to 3 weeks and seroconvert against *E. ruminantium*[26]. There is extensive antigenic cross-reactivity between *E. canis* and *E. ruminantium* with dogs experimentally infected with *E. ruminantium* becoming positive in IFA and immunoblots against *E. canis*[26,38]. Recently, PCR and sequencing studies identified the DNA of an *E. ruminantium* in dogs in South Africa[2] and *Amblyomma* spp. have been found on dogs in the region[12]. Although we did not determine the ticks on the dogs in our study, *Amblyomma* spp. occur widely in Zimbabwe[51] and it would appear likely that at least some of the seropositive dogs had been infected with an *E. ruminantium* or a closely related organism.

Other recognised *Ehrlichia* spp. that are closely related to *E. canis* and have serological cross-reactivity are *E. muris* which infects mice, and perhaps people, in Japan[25] and *E. ewingii* which is an agent of canine granulocytic ehrlichiosis that has only been reported in the USA[33]. Although it is very unlikely that these organisms influenced our results, there are other incompletely characterised *Ehrlichia* and *Anaplasma* spp. that have been described in southern Africa[1,2,51] and their role as pathogens in dogs is yet to be determined. With the apparent high exposure of dogs in southern Africa to *Ehrlichia* spp. and/or closely related organisms, further studies are indicated to determine the organisms involved and their role in the rapid turnover of dogs in the region.

Bartonella henselae is known to infect both cats[27,28,44] and people[19] in southern Africa and our study now provides evidence that infections also occur in dogs. We found a significant prevalence (14 %) of antibodies against *B. henselae* in dogs from widely separated communal lands in Zimbabwe. Similar seroprevalences have been found in dogs from the United Kingdom (6.5 %)[3], Hawaii (3 %)[18] and Japan (8 %)[52]. These findings are consistent with the reported widespread distribution of *B. henselae*[27] and its vector, the cat flea[49].

There are only limited data on the effects of *B. henselae* in dogs. Experimental infections cause no detectable clinical signs or result in short-lived bacteraemia[31]. Natural infections have been associated with peliosis hepatis[31], a vasculoproliferative disorder characterised by cystic, blood-filled spaces in the liver, and pyogranulomatous hepatitis[20]. *Bartonella henselae* has also been found in 3 dogs suffering from various conditions with a wide variety of historical, clinical, haematological, and biochemical abnormalities[39].

While there is no serological cross-reactivity between *Bartonella* spp. and *E. canis* and spotted fever group rickettsiae[9], serological cross-reactivity has been reported between *B. henselae* and other members of the genus. There is extensive cross-reactivity with *B. quintana*[22] which causes trench fever, bacillary angiomatosis and endocarditis in people and is transmitted by the human body louse (*Pediculus humanus*). Although *B. quintana* occurs in people in Africa[46], there are no reports of infections occurring in dogs and it would appear unlikely, then, that the organism was responsible for the antibodies we detected.

Serological cross-reactivity has also been described between *B. henselae* and *B. clarridgeiae*[50]. The cat flea is the presumed vector of *B. clarridgeiae*[48] and the natural reservoir is the cat, in which the organism causes a chronic asymptomatic bacteraemia[34]. *Bartonella clarridgeiae* also infects dogs and has been associated with vegetative endocarditis[15] and Doberman hepatopathy[20]. Although there are no reports of *B. clarridgeiae* in Africa, the organism occurs very widely[30] in North and South America, Europe, South East Asia and New Zealand. It would appear likely, then, that *B. clarridgeiae* occurs in Africa and that infections with the organism occurred in at least some of the dogs that were studied.

Other *Bartonella* spp. are known pathogens of dogs but appear unlikely to have influenced our results. *Bartonella vinsonii* subspecies *berkhoffii* was the 1st *Bartonella* spp. to be identified as a pathogen in dogs[5] and has been reported in dogs with arrhythmias and endocarditis[8], granulomatous lymphadenitis, granulomatous rhinitis[41] and anterior uveitis and choroiditis[40]. Although an IFA study using *B. vinsonii* subspecies *berkhoffii* as antigen found 65 % of dogs from the Sudan to be seropositive[17], antibodies to *B. vinsonii* subspecies *berkhoffii* do not react with *B. henselae*[9] and would not thus have been detected in our study. Further experiments using specific serological and/or molecular tests for *B. vinsonii* subspecies *berkhoffii* are required to determine if the organism occurs in southern Africa. *Bartonella elizabethae* has been identified in a dog with a wide variety of clinical and laboratory abnormalities[39] but the organism has only been found in the Americas[4] and there appears to be only minor serological cross-reactivity between *B. henselae* and *B. elizabethae*[35]. *Bartonella washoensis* has been found in a dog with mitral valve endocarditis[16] and, although serological cross-reactivity with *B. henselae* has not been determined, it would appear unlikely that we detected antibodies against *B. washoensis* as the organism has only been described in the United States.

In summary, our study has shown that dogs in widely separated rural areas of Zimbabwe, and hence probably the region, are not uncommonly infected with *E. canis* and *B. henselae* or closely related species. Veterinarians should be aware that these organisms might cause disease in their canine patients and laboratories should offer appropriate diagnostic tests. Further studies are indicated to determine the *Ehrlichia* spp. and *Bartonella* spp. that occur in southern Africa and the role they play as pathogenic agents.

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