Association between polyarthritis and thrombocytopenia and increased prevalence of vectorborne pathogens in Californian dogs

J. FOLEY, N. DRAZENOVICH, C. M. LEUTENEGGER, B. B. CHOMEL

TICKBORNE diseases are regionally common problems in dogs, with almost undetectable to fatal clinical manifestations, including fever, weakness, icterus, epistaxis and lameness (May and others 1990, Greig and others 1996, Harrus and others 1997, Pappalardo and others 1997, Greene and Breitschwerdt 1998, Foley and others 2001). In California, important tickborne diseases include Rocky Mountain spotted fever (RMSF), caused by *Rickettsia rickettsii*, canine monocytic ehrlichiosis, caused by *Ehrlichia canis*, canine granulocytic anaplasmosis (GA), caused by *Anaplasma phagocytophilum* (formerly *Ehrlichia equi*), and borreliosis, caused by *Borrelia burgdorferi* sensu stricto. Bartonellosis, caused by *Bartonella vinsonii berkhoffii*, has an unconfirmed arthropod vector but is suspected to be transmitted by ticks (Pappalardo and others 1997, Breitschwerdt and others 1998, Kordick and others 1999b, Chang and others 2001, MacDonald and others 2004).

Tickborne diseases are a significant risk in California, with the northern coast mountain range ranked by the Centers for Disease Control and Prevention (CDC) as a 'moderate risk' for Lyme disease in human beings (Brown and Lane 1992, CDC 2001). In particular, GA, transmitted by *Ixodes pacificus* ticks, is a very important threat to dogs, horses and human beings (Madigan and Gribble 1987, Gewirtz and others 1996, Foley and others 1999, 2001, 2004a). Signs of the disease can include pyrexia, lethargy, myalgia, nausea, arthralgia, ataxia, thrombocytopenia and neutropenia (Greig and others 1996, Foley and others 2001). *E canis* is transmitted primarily by *Rhipicephalus sanguineus*, a tick that occurs sporadically among dogs in California. Infected dogs may have thrombocytopenia, vomiting, oculonasal discharge, peripheral oedema, arthritis, glomerulonephritis, ataxia, dyspnoea and splenomegaly (Neer 1998). *B vinsonii berkhoffii* causes endocarditis, myocarditis, granulomatous lymphadenitis, immune-mediated polyarthritis, thrombocytopenia and granulomatous rhinitis in dogs (Pappalardo and others 2000, MacDonald and others 2004, Henn and others 2005). RMSF, transmitted in California by *Dermacentor variabilis* and *Dermacentor andersoni*, can induce thrombocytopenia, septic neutrophilic vasculitis, coagulation defects, fever, oedema in various sites, ocular and nasal discharge, and scleral injection, petechiae, epistaxis and melaena, lymphadenopathy, neurological abnormalities and limb necrosis (Greene and Breitschwerdt 1988).

Possibly the two most important and common sequelae of tickborne infection in many dogs are polyarthritis and thrombocytopenia. However, both of these problems can also occur as a result of many other diseases. The mechanism for polyarthritis in tickborne disease may include immune-complex deposition, which can occur with ehrlichiosis, RMSF, anaplasmosis and borreliosis; borreliosis is also associated with primary infectious synovitis (Greene 1998). Thrombocytopenia may occur due to ineffective haematopoiesis, immune-mediated platelet destruction, infectious or immune-mediated vasculitis, and sequestration or margination of platelets in infection, with *E canis*, RMSF, bartonellosis and possibly anaplasmosis (Dumler and Bakken 1995, Wong and Thomas 1998, Harrus and others 1999).

At the University of California Davis Veterinary Medical Teaching Hospital, the two most common indications for testing with a 'tick panel' are thrombocytopenia and polyarthritis. The aim of this study was to determine whether the prevalence of tickborne pathogens would be increased in dogs with polyarthritis and/or thrombocytopenia.

A case-control design was used to evaluate this hypothesis. A secondary goal of the study was to describe regional and demographic risks for serological and PCR-positive results. A panel testing for all suspected canine tickborne diseases was administered to 110 dogs seen at the hospital with signs of thrombocytopenia, polyarthritis, or both, and to 110 control dogs randomly chosen from dogs seen at the hospital on the same day as the case dog.

The location of the dog's residence, based on the owner's address, was grouped as follows: coast range mountains (a set of mountain ranges from 304 to 1700 m, with a climate varying from temperate rainforest to Mediterranean, extending from the coast to 50 km inland, from the Oregon border southwards to near the Los Angeles area), the hot dry Central Valley (extending north to south between the coast ranges and the Sierra Nevada mountains), the Sierra Nevada mountains (along the eastern state boundary), and out-of-state regions (including two dogs from Nevada and one from Oregon). Differences in haematological values were assessed by a *t* test and location by a chi-squared test. P values for odds ratios were obtained by Fisher's exact test. For all statistical tests P≤0·05.

TABLE 1: Distribution of 110 dogs with polyarthritis and/or thrombocytopenia (cases) and 110 control dogs, by American Kennel Club breed group or related category, location and demographic characteristics

	Cases	Controls
Breed		
Herding dogs	15	10
Hounds	9	14
Non-sporting dogs	8	9
Retrievers	29	26
Sled dogs*	3	2
Sporting dogs	8	6
Terriers (and Jack Russell terriers)	13	19
Toy breeds	5	8
Working dogs (and Australian shepherd dogs)	20	16
Chi-squared P value	P=0·75	
Location†		
Central Valley	34	34
Coast ranges	43	48
Sierra Nevada	29	17
Out of state	3	0
Chi-squared P value	P=0·12	

* Huskies, malamutes, American eskimo dogs and crosses
† Geographical location was not recorded for one case dog and 11 controls

TABLE 2: Differences in mean (sd) haematological parameters between case dogs (with polyarthritis and/or thrombocytopenia) and control dogs

	Cases	Controls	P
Platelets	285,769 (185,859)	320,155 (182,364)	0·05
PCV (%)	36·8 (10·4)	40·6 (8·9)	0·003
Leucocytes	15,297 (10,119)	16,673 (8454)	0·21
Protein (g/l)	66 (111)	67 (92)	0·13

PCV Packed-cell volume

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J. Foley, DVM, PhD,
N. Drazenovich, MS,
Center for Vector-Borne Diseases,
C. M. Leutenegger, DrVetMed, PhD, FVH,
Department of Medicine and Epidemiology,
B. B. Chomel, DVM, PhD,
Department of Population, Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA 95616, USA

Dr Foley is also at the Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA 95616, USA

TABLE 3: Prevalence and 95 per cent confidence intervals (CI) for the pathogens tested in dogs with polyarthritis and/or thrombocytopenia (cases) and control dogs, with odds ratios and P values

Pathogen	Prevalence (95% CI) Cases	Prevalence (95% CI) Controls	Overall prevalence (95% CI)	Odds ratio	P
Anaplasma phagocytophilum (serology)	7·3 (3·42-14·26)	2·0 (0·35-7·74)	4·76 (2·44-8·84)	3·84	0·11
A phagocytophilum (PCR)	1·82 (0·32-7·06)	0	0·9 (0·16-3·76)	1	0·25
Ehrlichia canis (serology)	1·82 (0·32-7·06)	0	0·9 (0·16-3·76)	1	0·50
E canis (PCR)	0·9 (0·04-5·59)	0	0·47 (0·02-3·0)	1	0·9
Rickettsia rickettsii (serology)	4·55 (1·69-10·8)	3·0 (0·78-9·15)	3·81 (1·78-7·64)	1·54	0·72
Bartonella vinsonii berkhoffii (serology)	0·9 (0·04-5·59)	2·0 (0·35-7·74)	1·42 (0·37-4·46)	0·45	0·61
Borrelia burgdorferi (serology)	3·64 (1·17-9·59)	1·0 (0·05-6·22)	2·38 (0·80-5·77)	3·74	0·37
B burgdorferi (PCR)	0	0 (0-4·6)	0	1	0·9

Complete blood counts were performed using an automated instrument (Serono Baker 9000; Biochemical Immunosystems). Differential cell counts were performed manually from thin Wright-stained blood smears. Serology for *A phagocytophilum* and *B vinsonii berkhoffii* was performed as described by Dumler and others (1995) and Chang and others (2000). Antibodies to *E canis* and *R rickettsii* were assayed by an immunofluorescence assay (IFA) with commercial slides (Protatek) as for *A phagocytophilum*. IFA for *B burgdorferi* was performed using commercial slides (VMRD); positive sera were confirmed by Western blot, and interpreted according to CDC guidelines (CDC 1995), except that three or more diagnostic bands were considered positive and the presence of bands at 31 and 34 kDa was considered evidence of vaccine exposure.

DNA was extracted from whole blood (DNeasy Tissue kit; Qiagen), and TaqMan PCR systems for *A phagocytophilum* and *B burgdorferi* were run as described previously (Leutenegger and others 1999, Pusterla and others 1999). The PCR for *E canis* used primers Ec.139f 5′-ATGGCTATTCCG TACTACTAGGTAGATTC-3′, Ec.32r 5′-CATGCAAGTCGAACGGACAAT-3′ and an internal, fluorescent-labelled TaqMan probe Ec.61p 5′-TCTGCCACTAACAATTTCCTATAGCCAGAGGC-6-carboxy-tetramethyl-rhodamine. The results of all TaqMan PCR assays were considered positive if the C_t value was less than or equal to 25. For *B vinsonii berkhoffii* and *R rickettsii*, PCR was performed

TABLE 4: Summary of presentation and diagnostic findings in dogs with polyarthritis and/or thrombocytopenia, or dogs chosen as controls that had a serological or PCR-based indication of tickborne disease (see Table 3)

Dog	Case/ control	Signalment	Location	Clinical/cytological findings	Diagnosis	Serology	PCR	Diagnosed at time of management?
1	Case	5 y, FN, GSD	Coast range (Marin)	138 x 10^6/ml platelets	GA	*A phag*=5120	*A phag* +ve	No
2	Case	10 y, MN, Bernese mountain dog	Coast range (San Francisco)	Lethargy, heartworm, fever, anaemia, morulae in PMNs	GA	*A phag*=6400	*A phag* +ve	Yes
3	Case	10 y, MI, GSD	Coast range (Sonoma)	Lethargy, fever, diarrhoea, 114 x 10^6/ml platelets	GA	*A phag*=160	*A phag* +ve	Yes
4	Case	12 y, MI, Walker hound	Coast range (Sonoma)	GN, lameness, concurrent neoplasia	*E canis*	*A phag=E canis*=80; *R rick*=320	*E canis* +ve	No
5	Case	3 y, FI, Shar pei	Not known (stray)	Diarrhoea/melaena, cachexia, 43 x 10^6/ml platelets	Suspect *E canis*	*A phag=E canis*=1560	–ve	No
6	Case	6 y, MI, Plott hound	Sierra (Chico)	Lethargy, fever, pleural effusion, PA (attacked by bear)	Suspect *E canis*	*E canis*=160	–ve	No
7	Case	4 y, FN, weimaraner	Sierra	Soreness, reluctance to move; IM PA treated with steroids	Lyme disease	WB +ve for Lyme disease	–ve	No
8	Case	1 y, FI, weimaraner	Sierra	Bone pain, 144 x 10^6/ml platelets	Lyme disease	WB +ve for Lyme disease	–ve	No
9	Control	5 y, MN, great Dane	Coast range (San Francisco)	Osteosarcoma	Lyme disease	WB +ve for Lyme disease	–ve	No
10	Control	3 y, MN, German wirehair pointer	Coast range (central)	AV block; mitral valve insufficiency	Lyme disease	WB +ve for Lyme disease	–ve	No
11	Case	5 y, FN, cocker spaniel	Coast range (northern travel outside state)	Melaena, epistaxis, fever, thrombocytopenia	RMSF	*R rick*=1280	–ve	Yes
12	Case	11 y, MN, golden retriever	Coast range (Santa Cruz)	Acute and chronic renal insufficiency, leptospirosis, GN	Suspect RMSF	*R rick* >120	–ve	No
13	Case	6 y, FN, labrador retriever	Travel to/from Texas	Acute and chronic renal insufficiency, heartworm, GN	Suspect RMSF	*R rick* >120	–ve	No
14	Control	2 y, FN, miniature schnauzer	Coast range (San Francisco)	Liver shunt	Incidental	*R rick* >120	–ve	No
15	Control	10 y, FN, miniature schnauzer	Central valley	GI bleeding, sick sinus syndrome	Incidental	*R rick* >120	–ve	No
16	Case	10 y, MN, beagle	Sierra	Intraocular mass, glaucoma; history of heartworm	Suspect bartonellosis	*A phag*=160; *B vinsonii*=128	–ve	No
17	Control	6 y, FN, St Bernard	Sierra	Seizures, ataxia	Suspect bartonellosis	*A phag*=240; *B vinsonii*=64	–ve	No
18	Control	9 y, MI, St Bernard	Coast range (northern)	Meningitis, vomiting, megaoesophagus	Bartonellosis	*B vinsonii*=256	–ve	No

y Years, FN Female neutered, GSD German shepherd dog, GA Granulocytic anaplasmosis, *A phag Anaplasma phagocytophilum*, +ve Positive, MN Male neutered, PMN Polymorphonuclear cell, MI Male intact, GN Glomerulonephritis, *E canis Ehrlichia canis*, *R rick Rickettsia rickettsii*, FI Female intact, –ve Negative, PA Polyarthritis, WB Western blot, IM Immune-mediated, AV Atrioventicular, RMSF Rocky Mountain spotted fever, GI Gastrointestinal, *B vinsonii Bartonella vinsonii berkhoffii*

as described previously (Regnery and others 1991, Chang and others 2000, Chomel and others 2001).

The breeds and locations of the dogs are given in Table 1. There were 56 dogs with polyarthritis and 54 with thrombocytopenia, with an overall prevalence of tickborne disease in the two groups of 26·8 per cent and 25·9 per cent, respectively. The prevalence of tickborne disease in the controls was 6 per cent. The difference between the overall prevalence in dogs with thrombocytopenia and those with polyarthritis was not statistically significant, but when grouped together, the case dogs had significantly more exposure than the controls (P=0·002). Packed-cell volume differed significantly between the cases and controls (P=0·003), but location, serum protein and total leucocytes did not (Tables 1, 2). Serology for *A phagocytophilum* and *E canis* exposure documented prevalences of 5 per cent and 1 per cent, respectively, in all the dogs (Table 3). Three *A phagocytophilum* and one *E canis* PCR-positive dogs were detected in the case group. No dogs were *B vinsonii berkhoffii* or *R rickettsii* PCR-positive. The seroprevalence for RMSF, *B burgdorferi* (both vaccine positive and due to natural exposure), and *B vinsonii berkhoffii* ranged from 1 to 4 per cent. Regional seroprevalence and odds ratios of serological tests and case status were not statistically significant. Nevertheless, 12 probable and six possible cases of tickborne disease were identified retrospectively based on clinical signs, serology and PCR, including seven that had been overlooked at the time of treatment (Table 4).

Despite a relatively high serological and PCR prevalence of tickborne disease among dogs tested in this study, neither PCR nor serology for any single pathogen was significantly associated with polyarthritis or thrombocytopenia. The lack of a statistical association between case and specific test status in this study was due to the frequent presence of active infection in the absence of clinical signs (test-positive controls), as well as the high proportion of case dogs with other aetiologies (test-negative cases, aetiologies often not determined) that accounted for the clinical signs. In addition, the relatively small sample size and other factors may have been important, since a weak association of these cases may have been discovered with a sufficiently large sample size. Importantly, the diagnosis of tickborne disease was overlooked in many dogs, in part because of their failure to show clinical signs and also because of the initial choice by the clinicians to apply no or few diagnostic tests for tickborne disease rather than a comprehensive panel. The results of this study suggest that comprehensive testing for tickborne disease in certain cases is very important; however, there remains a high likelihood that other, non-tick-transmitted diseases will be the cause of polyarthritis or thrombocytopenia in many cases.

Because odds ratios were not significant, the overall prevalence for each pathogen was calculated, grouping cases and controls in order to describe regional and demographic trends for risk of infection. Breed and residence location were not risk factors, although both can be important determinants of tickborne disease. In California, the northern coast ranges and western foothills of the Sierra Nevada mountains are the most affected by tickborne disease, with desert and valley locations being typically too hot and dry for *I pacificus*, therefore providing a low risk for infection (CDC 2001, Foley and others 2001, 2004b, 2005, Hoar and others 2003). Cases of Lyme disease have been reported in dogs from northern California (Foley and others 2004b), although confirming a diagnosis may be difficult. Evaluating Western blots, in particular the vaccine-associated 31 and 34 kDa bands, is helpful in this regard (Barthold and others 1995).

The three dogs in the present study with possible monocytic ehrlichiosis included one suspect exposure based only on serology, one probable case with a high titre and consistent clinical signs of late-stage ehrlichiosis, and one confirmed case with glomerulonephritis, lameness, lymphadenopathy and anaemia, but an anomalously low titre despite being PCR positive. Confirming a diagnosis of this disease can be critically important because it is treatable in the early stages but can result in chronic and fatal progressive glomerulonephritis in some cases (Neer 1999). Little information exists regarding the overall prevalence and spatial distribution of this disease in California. The seroprevalence of *B vinsonii berkhoffii*, reported previously as less than 3 per cent (Henn and others 2005), was also low in the present study, although rates as high as 93 per cent have been described for a heavily tick-infested kennel population of dogs in North Carolina (Kordick and others 1999a). Despite low exposure risk, this disease remains a very important cause of cardiac disease in dogs (Henn and others 2005).

In summary, a minimum estimate of 20 per cent of dogs with thrombocytopenia or polyarthritis in a population from California had evidence of exposure to tickborne diseases. The data presented in this study should assist clinicians considering vectorborne diseases as a differential diagnosis of canine health problems, and, in general, should help to broaden knowledge of the scope of sequelae of infection with *B burgdorferi*, *A phagocytophilum*, *R rickettsii* and *B vinsonii berkhoffii*. Because these diseases may produce severe clinical manifestations, ongoing evaluations are warranted, particularly in dogs in known enzootic regions.

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