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An unmatched case controlled study of clinicopathologic abnormalities in dogs with *Bartonella* infection

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ABSTRACT

We compared clinicopathologic findings in dogs with *Bartonella* infection to *Bartonella* spp. negative dogs suspected of a vector-borne disease. Cases ($n = 47$) and controls ($n = 93$) were selected on the basis of positive or negative enrichment culture PCR results, respectively. Signalment, clinicopathologic findings and treatments were extracted from medical records. DNA sequencing identified *Bartonella henselae* ($n = 28$, 59.6%), *Bartonella vinsonii* subsp. *berkhoffii* ($n = 20$, 42.6%), *Bartonella koehlerae* ($n = 3$, 6.4%), *Bartonella volans*-like ($n = 3$, 6.4%) and *Bartonella bovis* ($n = 1$, 2.1%). There were no significant differences in age, breed, size, sex or neuter status between cases and controls. Dogs infected with *Bartonella* sp. often had a history of weight loss [OR = 2.82; 95% CI: 1.08–7.56] and were hypoglobulinemic [OR = 4.26; 95% CI: 1.31–14.41]. With the exception of weight loss and hypoglobulinemia, clinicopathologic abnormalities in *Bartonella*-infected dogs in this study were similar to dogs suspected of other vector-borne infections.

1. Introduction

During the last decade, infection with several *Bartonella* species has been reported in dogs [1,2]. However, hematological, biochemical and urinalysis findings that could assist clinicians in the selection of cases for testing have rarely been reported in dogs following diagnostic confirmation of *Bartonella* sp. infection by bacterial isolation or polymerase chain reaction (PCR). Previous descriptions of clinicopathological findings in *Bartonella*-infected dogs were based primarily on serological test results and have generally involved individual cases or small case series. Also, only a limited number of studies have described clinicopathological findings in dogs experimentally infected with *Bartonella* spp. [3,4]. In people, pathological lesions associated with *Bartonella* infections are highly variable and are influenced by the immunological status of the host as well as differences in virulence among *Bartonella* species and strains [5,6]. In dogs, histopathological findings that have been reported with *Bartonella* infections include endocarditis, myocarditis, granulomatous lymphadenitis and hepatitis, cutaneous panniculitis, bacillary angiomatosis (BA) and peliosis hepatis [1,7–9]. Importantly, naturally-infected dogs and human patients infected with *Bartonella* spp. share many similar disease manifestations [1].

In a study in which dogs were tested for *Bartonella vinsonii* subsp. *berkhoffii* (*Bvb*) antibodies, thrombocytopenia, anemia, neutrophilia and eosinophilia were the most frequently reported hematological abnormalities in *Bvb* seroreactive dogs [10]. Another study reported

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thrombocytopenia in 44% of *Bartonella henselae* (*Bh*) seroreactive dogs [11]. A seroepidemiological study from the University of California at Davis found highly variable clinicopathological abnormalities among individual dogs and only eosinophilia was significantly associated with *Bartonella* seroreactivity [12]. Because similar clinicopathologic abnormalities occur frequently in dogs infected with other vector-borne pathogens [13,14], dogs suspected to have a tick borne infection were selected as a control group for this study.

Confirmation of *Bartonella* infection in dogs and human patients is notably challenging. Conventional diagnostic tests such as blood culture, serology and PCR often lack specificity, sensitivity or both [2,15–17]. Previously, we reported that only 25% of *Bh* and 50% of *Bvb*-infected dogs were seroreactive by IFA testing [2]. Utilization of a novel diagnostic platform based upon PCR amplification and DNA sequencing of *Bartonella* DNA from clinical specimens prior to or after enrichment culture in *Bartonella* alpha-*Proteobacteria* Growth Medium (BAPGM), hereafter called "enrichment PCR", has facilitated documentation of *Bartonella* spp. infections in blood, lymph node, tissue samples and a variety of diagnostic fluid specimens, including cerebrospinal (CSF), pleural, abdominal, seroma and joint fluids [2,17,18].

For this study, medical records of *Bartonella*-infected dogs described in a previous study from our laboratory [2] were retrospectively reviewed and the extracted data was compared with a control group of clinically-ill dogs suspected by the attending veterinarian to have a vector-borne infectious disease. *Bartonella* DNA was not identified by enrichment PCR in the control dogs. Our objective was to further identify potential risk factors and clinicopathological abnormalities associated with *Bartonella* infection in dogs that would assist clinicians in the prioritization of diagnostic testing.

2. Materials and methods

2.1. Case and control dogs

As a component of a previous study [2] that spanned July 1, 2003 through July 1, 2009, 924 diagnostic blood samples obtained from 663 dogs suspected of having a vector-borne infection were concurrently tested in the NCSU-CVM-VBDDL by BAPGM enrichment blood culture PCR (enrichment PCR). Of those, 61 dogs (9.2%) were *Bartonella* PCR positive at one or more testing steps. A total of 47 cases and 93 retrospectively selected controls tested during the same time frame met the inclusion criteria for this study. The case definition for *Bartonella* sp. infection in this study was based on DNA sequence confirmation of a specific *Bartonella* sp. amplified from one or more clinical specimens prior to or after enrichment culture. Only clinically-ill case and control dogs with adequately detailed medical records were included in this study. The control group was comprised of dogs suspected by the attending veterinarian to be infected with one or more of the following vector-borne organisms for which serological or PCR testing was available in the NCSU-CVM-VBDDL: *Anaplasma phagocytophilum*, *Anaplasma platys*, *Babesia canis*, *Babesia gibsonii*, *Babesia conradae*, *Borrelia burdgorferi*, *Ehrlichia canis*, *Ehrlichia chaffeensis*, *Ehrlichia ewingii*, *Bartonella* spp. or spotted fever group *Rickettsia*. Therefore, control group dogs were categorized as "suspected vector-borne disease infections", which in this study was defined as sick dogs whose blood samples were submitted to the NCSU-CVM-VBDDL for testing for one or more vector-borne organisms and concurrent BAPGM enrichment culture for *Bartonella* spp. Diagnostic tests requested for individual dogs were determined by the attending veterinarian and did not include testing for all of the organisms listed above. All dogs in the control group were negative for *Bartonella* spp. DNA using the enrichment PCR protocol. Despite being clinically-ill, serological evidence supporting infection with one or more vector-borne organisms was not documented diagnostically in all control dogs. No attempt was made to match case and control dogs on the basis of age, sex, or disease process.

2.2. Data collection

Attending clinicians were contacted by telephone, fax or electronic mail to retrieve the medical records, which were reviewed in detail by one of the authors (CP). Data in each medical record was considered adequate when complete signalment (age, sex, breed, neuter status and weight), demographic information (zip code, state), results of physical examinations, complete blood cell count (CBC) and serum chemistry panel (which included at least blood glucose, total proteins, liver enzymes and serum creatinine) were available for review. The same dataset was extracted from the medical records of case and control dogs.

Specific information abstracted from each medical record included:

2.2.1. Signalment and medical history

The age, breed, size (defined as small $\leq$10 kg, medium 11–25 kg, and large >25 kg), sex and neuter status were documented. Information regarding travel history outside the state and outside the United States, as well as environment (presence of other animals in the household, history of ectoparasite exposure) was recorded. The duration of clinical signs was categorized as less than 1 month, one month to a year, or longer than one year. In those cases where weight loss was reported by the owner, the historical weight data was not consistently provided, and therefore, the degree of weight loss was not investigated.

2.2.2. Clinical findings

Physical examination abnormalities were summarized. Often there were multiple physical examinations performed during the dog's illness; therefore, results from the examination temporally closest to the sample(s) tested by enrichment PCR were used for data extraction. Fever was defined as a body temperature above 39.4 °C, and hypothermia was defined as a body temperature below 37.8 °C.

2.2.3. Laboratory findings

Complete blood count (CBC), serum biochemistry and urinalysis results (when performed) were reviewed. When there was >1 test result available in the patient record,