Typical and Atypical Manifestations of Anaplasma phagocytophilum Infection in Dogs

Matthew D. Eberts, DVM, Pedro Paulo Vissotto de Paiva Diniz, PhD, DVM*, Melissa J. Beall, DVM, Brett A. Stillman, PhD, Ramaswamy Chandrashekar, PhD, Edward B. Breitschwerdt, DVM

ABSTRACT

Eighteen clinically ill dogs, naturally infected with Anaplasma phagocytophilum, were examined at a veterinary practice in Baxter, Minnesota. A clinical examination, complete blood cell count, enzyme-linked immunosorbent assay (ELISA) for A phagocytophilum, Borrelia burgdorferi, and Ehrlichia canis antibodies and Dirofilaria immitis antigen, and a polymerase chain reaction test for A phagocytophilum DNA were obtained for all dogs. Physical examination findings included fever, arthropathy, lymphadenopathy, epistaxis, acute gastritis, cervical hyperpathia, and central nervous system dysfunction. Complete blood cell count abnormalities included thrombocytopenia, morulae in neutrophils, anemia, leukopenia, eosinopenia, lymphopenia, and monocytosis. Seroreactivity to A phagocytophilum was found in 61%, B burgdorferi antibodies in 17%, and D immitis antigen in 5% of the dogs. Fever, arthropathy, neurologic dysfunction, and epistaxis are clinical syndromes that can be associated with A phagocytophilum infection. Treatment with doxycycline resulted in rapid resolution of clinical signs in all dogs. (J Am Anim Hosp Assoc 2011; 47:e86-e94. DOI 10.5326/JAAHA-MS-5578)

Introduction

Anaplasma phagocytophilum is a gram-negative intracellular bacterium transmitted by Ixodes species ticks and is the causative agent of granulocytic anaplasmosis. In the United States, Ixodes scapularis is the vector for A phagocytophilum in the Northeast and Midwest, whereas Ixodes pacificus vectors the organism on the west coast. Surveys identified a wide distribution in seroprevalence among dogs in the United States, and high rates of exposure corresponded to the distribution of I scapularis and I pacificus. In recent years, there were dynamic changes in the range of these tick vectors and their associated diseases. Climate change and changes in wildlife distribution enabled expansion of I scapularis in North America, and this trend is predicted to continue.

A phagocytophilum has been documented to cause disease in a wide variety of species, including dogs, cats, horses, ruminants, and humans. Canine granulocytic anaplasmosis was first described as an acute febrile disease in Minnesota and Wisconsin dogs in 1996. Granulocytic anaplasmosis can cause hematologic abnormalities, most typically thrombocytopenia, leukopenia, and anemia. The mechanism of thrombocytopenia is not fully understood, but based on mouse experimental models and the presence of antiplatelet antibodies in naturally infected dogs, both consumptive- and immune-mediated mechanisms appear to be involved. Granulocytic intracytoplasmic morulae, which are clusters of reproducing bacteria located in phagosomes, can be visualized during blood smear evaluation. Serum biochemical abnormalities can include increased serum alkaline phosphatase activity, hypoalbuminemia, elevated serum alanine transferase, and elevated total bilirubin.

Both enzyme-linked immunosorbent assay (ELISA) and indirect fluorescent antibody tests are commercially available, and documentation of seroconversion can be used to confirm the diagnosis of granulocytic anaplasmosis. Experimental evidence demonstrated that dogs might seroconvert within 10 days of infection.
exposure. Clinical illness usually occurs within 1–2 wk after tick transmission of A phagocytophilum; however, based on the high seroprevalence in historically healthy dogs in endemic regions, most exposed dogs will not develop overt clinical disease. Due to the acute nature of this infection, approximately 40% of clinically ill dogs will not produce a detectable level of A phagocytophilum antibodies at the time of presentation when using current serologic tests.\(^5\) In contrast, A phagocytophilum polymerase chain reaction (PCR) assays can be used to detect organism-specific DNA sequences in the blood during the early stages of illness.\(^6\) With proper primer selection and validation, PCR testing provides a sensitive and specific modality for the diagnosis of acute anaplasmosis in animals and human patients, and a positive PCR result is considered indicative of infection.\(^10\) However, it is important to note that A phagocytophilum PCR can be positive in clinically normal dogs and periodically negative in actively infected dogs, possibly due to variations in numbers of circulating organisms during infection.\(^8\)\(^,\)\(^11\)\(^,\)\(^12\)

As a vector-borne infectious pathogen, A phagocytophilum can induce a variety of disease manifestations in dogs, which presents a challenge to practitioners attempting to accurately diagnose and determine effective treatment of their patients. Canine anaplasmosis is expanding geographically across North America. With increasing number of dogs exposed to this pathogen, natural infection studies need to be disseminated to provide the veterinary community with a more complete definition of the spectrum of disease. It is important that practitioners be aware of potential clinical signs and the tests available to diagnose anaplasmosis. The purpose of this study is to describe clinical, hematologic, and serologic findings and treatment outcomes for 18 dogs naturally infected with A phagocytophilum in an I scapularis–endemic region.

### Materials and Methods

#### Dogs

Cases were retrospectively selected from a previous study involving 731 dogs, 81% of which were purebred (Eberts, unpublished data). These dogs were examined between July 2004 and January 2006 at a companion animal hospital located in Baxter, Minnesota, an area endemic for I scapularis.\(^12\) Based on resources available at the time of the study, retrospective PCR testing was performed at North Carolina State University (NCSU) on 273 dogs, of which 51 had clinical signs consistent with anaplasmosis and/or borreliosis.\(^12\) Of these 51 dogs, 19 tested PCR positive for A phagocytophilum DNA.\(^12\) Further retrospective PCR testing was performed at IDEXX Laboratories on eight additional clinical cases (four already PCR tested at NCSU and four not previously tested at NCSU). Cases were selected from 23 clinically ill dogs PCR tested at NCSU and IDEXX Laboratories. Inclusion criteria for the cases selected for this study included molecular confirmation of A phagocytophilum infection using PCR in conjunction with the availability of a medical history, physical examination findings, a complete blood count, an in-clinic serologic assay\(^8\), and access to the clinical response to doxycycline, as follow-up data. Eighteen dogs fulfilled these criteria (three dogs were excluded due to lack of complete blood count at initial presentation, and two dogs were excluded for lack of follow-up information). Anaplasmosis was diagnosed in all 18 cases between April and November 2005.

#### Complete Blood Counts

Complete blood counts were performed at the time the dog presented for diagnostic evaluation using an in-clinic laser flow cytometry automated unit.\(^6\) Certified veterinary technicians visually examined a blood smear to evaluate cell morphology, to perform differential cell counts, and to determine whether morulae were present.

#### Serology

All dogs were tested at the time of presentation with a qualitative ELISA assay that detects Ehrlichia canis and Borrelia burgdorferi antibodies, and Dirofilaria immitis antigen.\(^5\) Retrospectively, all dogs were also tested with a qualitative ELISA assay detecting E canis, B burgdorferi, and A phagocytophilum antibodies and D immitis antigen.\(^6\) The latter testing platform detects antibodies directed against a synthetic A phagocytophilum peptide from the major surface protein (p44/MSP2) and reportedly has 99.1% sensitivity and 100% specificity compared with an immunofluorescent assay (IFA) test.\(^13\) The B burgdorferi C6 peptide used in this assay does not cross-react with vaccinal antibodies and is considered to indicate active as opposed to previous infection.\(^14\)

#### Molecular Analysis

PCR testing was performed either by personnel in the Vector Borne Disease Diagnostic Laboratory at NCSU or at IDEXX Laboratories in Westbrook, Maine. Eighteen ethylenediaminetetraacetic acid–anticoagulated blood samples collected between April 2005 and November of 2005 were processed for genomic DNA and analyzed by PCR to detect A phagocytophilum infection. NCSU tested a total of 14 samples, and IDEXX tested a total of 8 samples. Four of the 18 samples were tested by both laboratories. DNA was extracted from 200 µL of canine ethylenediaminetetraacetic acid whole-blood samples that were stored frozen at −70°C with a commercially available kit.\(^4\) The final eluted volume was 200 µL per sample. The DNA concentration was
quantified by spectrophotometry, and the absence of PCR inhibitors was demonstrated by the amplification of a fragment of the glyceraldehyde-3-phosphate dehydrogenase gene, as previously described.15

North Carolina State University Protocol
PCR was performed using 16S rRNA oligonucleotide primers that were designed to amplify all Anaplasma and Ehrlichia species. Subsequently, the Anaplasma platys GroEL gene and the A phagocytophilum AnkA gene were targeted as a secondary confirmation of the initial PCR result and to determine the infecting Anaplasma species (A phagocytophilum vs A platys). The oligonucleotide primers were described elsewhere.12 The 16S rRNA gene amplifications were performed in a 25-μL final volume reaction containing 1× buffer with 2 mM magnesium chloride, 0.625 U of Takara Ex Taq DNA polymerase, 12.5 pmol of each primer, 200 μM (each) of deoxyadenosine triphosphate, deoxycytidine 5'-triphosphate, deoxyguanosine triphosphate, and 1–5 μL of DNA template, according to the DNA concentration determined for each sample (50–200 ng/reaction).

The GroEL and AnkA gene amplifications were performed as previously described, with 7.5 pmol of each primer added in each reaction. Conventional PCR was performed under the following conditions: a single hot-start cycle at 95°C for 2 min followed by 55 cycles of denaturation at 94°C for 15 sec, annealing temperature 62°C for 15 sec, and extension at 72°C for 15 sec. Amplification was completed by an additional cycle at 72°C for 1 min, and products were analyzed by 2% agarose gel electrophoresis containing 0.2 μg of ethidium bromide/mL under ultraviolet light.

IDEXX Protocol
A conventional PCR for A phagocytophilum was performed according to a published protocol and used the following msp2 primer pair: msp2-3f (5’-CCAGCGTGGGCGATAAGAG) and msp2-3r (5’-GCCGTAACATCATAAGC).16 Amplification was performed in a 25 μL volume reaction, containing a 1× buffer mix of 1.5 mM magnesium chloride, 200 μM each of deoxyadenosine triphosphate, deoxycytidine 5'-triphosphate, deoxyguanosine 5'-triphosphate, and deoxyguanosine triphosphate, 2.5 U of Taq DNA polymerase, 2 μL DNA template, and 0.5 μM of each the forward and reverse msp2 primer. The conventional testing was performed in an Applied Biosystems Thermocycler® with the following program: a single 4 min cycle at 94°C followed by 40 cycles of denaturing at 94°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 1 min. Once the 40 cycles were complete, the samples were held for 5 min at 72°C. The amplified PCR products were then evaluated on a 2% agarose gel stained with ethidium bromide.

Canine DNA from a healthy dog was used as a PCR negative control. Plasmid clones of partial DNA sequences identical to the following accession numbers were also used as positive controls: A platys 16S rRNA (AY821826), A platys GroEL (AF478129), A phagocytophilum 16S rRNA (AF507941), A phagocytophilum AnkA (CP000235), and A phagocytophilum msp2 (AY151054). The DNA concentration of the purified plasmid was determined by restriction enzyme digestion and agarose gel electrophoresis (2%). Plasmid clones were diluted 10-fold in purified dog DNA, ranging from 1 million to 1 plasmid/μL. The limit of detection observed in PCR amplifications was 16S rRNA = 10 copies per reaction, AnkA gene = 25 copies per reaction, and GroEL gene = 5 copies per reaction. The limit of detection was reached in 100% of tests. To prevent PCR amplicon contamination, sample extraction, reaction setup, PCR amplification, and amplicon detection were performed in separated areas.

Results
Dogs
Fifteen of the 18 dogs (83%) were purebred, and the median age of dogs was 6 yr (range, 3 mo to 14 yr). There were three intact females, seven spayed females, three intact males, and five castrated males. The most common presenting complaints were lethargy (13 dogs) and lameness (10 dogs), with circling, vomiting, and epistaxis reported in 1 dog each. Median duration of clinical signs before presentation was 3 days (range, 1–14 days) (Table 1).

Physical Examination
Sixteen of 18 dogs (89%) were febrile (rectal temperature >39.2°C), and the median temperature of febrile dogs was 40.2°C (range, 39.4–40.8°C). Ten of 18 dogs (55%) had arthropathy, which was defined clinically as pain and swelling localized to joints on physical examination. Nine dogs (50%) had polyarthropathy, and one dog (5%) had monoarthropathy involving the right radiocarpal joint. Lymphadenopathy, left cerebral dysfunction, cervical hyperpathia, epistaxis, and acute gastritis were found in one dog each (Table 1).

Complete Blood Count
Seventeen of 18 dogs (94%) were thrombocytopenic (normal range defined as 175,000/μL to 500,000/μL), with platelet counts ranging from 40,000/μL to 120,000/μL (mean 70,941/μL). Morulae were identified in neutrophils during blood smear examination in 17 of 18 dogs (94%). Twelve dogs (67%) had a nonregenerative normochromic normocytic anemia (normal range defined as hematocrit
37–55%), with hematocrits ranging from 25.7% to 36.9% (mean 32.5%). Ten dogs (55%) were leukopenic (normal range defined as 5,500–16,900/μL), with total white blood cell counts ranging from 3,670/μL to 5,420/μL (mean 4,432/μL). Eight dogs (50%) were eosinopenic (normal eosinophil count defined as 100–1,490/μL), with eosinophil counts ranging from 20/μL to 80/μL (mean 55/μL), of which one dog had concurrent heartworm disease. Seven dogs (39%) were lymphopenic (normal lymphocyte count defined as 700–4,900/μL), with lymphocyte counts ranging from 200/μL to 680/μL (mean 512/μL), and one dog that presented for lameness and lethargy had a monocytosis (normal monocyte count defined as 100–1,400/μL) of 1,430/μL. This dog was concurrently infected with *D. immitis*.

Serology

Of the 18 dogs in this study, *A. phagocytophilum* antibodies were found in 11 (61%) dogs at the time of initial presentation; *B. burgdorferi* antibodies were found in 3 (17%); *D. immitis* antigens were found in 1 (5%); and no dog was *E. canis* seroreactive (Table 2). Four dogs, three of which were *A. phagocytophilum* seroreactive by SNAP 4Dx at the time of initial presentation, had follow-up testing using the in-clinic ELISA test in subsequent years. Dog 1 initially tested positive for *A. phagocytophilum* but tested negative for all analytes in August 2007 (28 mo later). Dog 2 initially tested negative to *A. phagocytophilum* (but morulae were observed in neutrophils), and then tested negative to all analytes in June 2007 (22 mo later) and June 2008 (34 mo later). Dog 6 initially tested positive for *A. phagocytophilum*, and then tested positive for *A. phagocytophilum* in May 2008 (36 mo later). Dog 10 initially tested positive to *A. phagocytophilum* but tested negative to all analytes in June 2007 (25 mo later) and tested negative again in June 2008 (37 mo later) (Table 2).

### Molecular Analysis

As part of the retrospective evaluation, blood samples from all dogs obtained at the time of presentation tested positive for *A. phagocytophilum* DNA, targeting either the AnkA gene (n=14) or the msp-2 gene (n=8). Molecular evidence of infection correlated with the presence of morulae in all but one case where morulae were not detected. Of the samples tested by both laboratories (n=4), complete agreement was obtained. No molecular evidence of *A. platys* infection was detected in the 14 samples tested by NCSU.

### Treatment

Treatment with doxycycline at a dose ranging from 6 to 8 mg/kg PO q 12–24 hr for 14–30 days resulted in a positive therapeutic response in all dogs. Three dogs were treated for 14 days, 1 dog for 21 days,
and 14 dogs for 28–30 days. Treatment duration did not appear to influence outcome, as disease manifestations resolved within 24 hr in three dogs, including the dog with central nervous system dysfunction, 48 hr in seven dogs, 72 hr in four dogs, and 4–6 days in the remaining four dogs (Table 3).

Discussion

For the dogs described in this study, fever accompanied by arthropathy was the most common clinical presentation associated with *A. phagocytophilum* infection. On physical examination, 10 of the 18 dogs had joint pain and effusion. Lyme disease, which is caused by *B. burgdorferi* and transmitted by the same *Ixodes* ticks, also often manifests as a febrile arthropathy.14 One challenge when trying to define the clinical disease that is associated with canine anaplasmosis is concurrent or sequential transmission of *A. phagocytophilum* and *B. burgdorferi*. Although it is clear that dogs in nature can experience frequent and repeated tick infestations, only a small percentage of infected ticks contain these two pathogens, yet individual ticks may be infected with one or both organisms. For example, depending on location, studies from the Midwest identify *B. burgdorferi* in 16.5–57% and *A. phagocytophilum* in 3.8–14% of collected *I. scapularis*.17–20 In the study from which these dogs were selected, disease manifestations were more often observed in dogs that were coinfected with *A. phagocytophilum* and *B. burgdorferi*.12 The timing of the *B. burgdorferi* infection could not be determined in these dogs, but it might represent a prior transmission event, because up to 6 wk is generally required for the dog to develop a detectable antibody response after tick attachment.21

In mouse studies, coinfection with *A. phagocytophilum* and *B. burgdorferi* alters the host immune response, which can lead to increased severity of Lyme arthritis.22,23 In the original serosurvey, coinfected dogs were more likely to have lameness, joint pain, and joint effusion than dogs with single infections.12 In this study, 3 of the 18 dogs were coinfected with *B. burgdorferi* (dogs 3, 7, and 14) (Table 2). All three presented with arthropathy (Table 1), and the severity of clinical signs did not appear to be worse than the other dogs with arthropathy. The coinfected dogs were thrombocytopenic; however, the degree of thrombocytopenia was not more severe than dogs without borreliosis. When looking at response to doxycycline, 4 of the 18 dogs in this study took >3 days for clinical signs to completely resolve (Table 3). It is interesting to note that, of those four dogs, three were coinfected with *B. burgdorferi*. The limited number of coinfected dogs makes it difficult to determine whether this has true clinical relevance.

Although neurologic signs were reported for other rickettsial infections in the dog, such as Rocky Mountain Spotted Fever and

### TABLE 2

Enzyme-Linked Immunosorbent Assay (ELISA) Results

<table>
<thead>
<tr>
<th>Dog no.</th>
<th><em>Anaplasma phagocytophilum</em> antibodies</th>
<th><em>Borrelia burgdorferi</em> antibodies</th>
<th><em>Ehrlichia canis</em> antibodies</th>
<th><em>Dirofilaria immitis</em> antigen</th>
<th>Long-term follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>28 mo later tested negative to all analytes</td>
</tr>
<tr>
<td>2</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>22 and 34 mo later tested negative to all analytes</td>
</tr>
<tr>
<td>3</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>36 mo later tested positive to <em>Anaplasma</em></td>
</tr>
<tr>
<td>5</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>25 and 37 mo later tested negative to all analytes</td>
</tr>
<tr>
<td>6</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>36 mo later tested positive to <em>Anaplasma</em></td>
</tr>
<tr>
<td>7</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>25 and 37 mo later tested negative to all analytes</td>
</tr>
<tr>
<td>8</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>25 and 37 mo later tested negative to all analytes</td>
</tr>
<tr>
<td>9</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>25 and 37 mo later tested negative to all analytes</td>
</tr>
<tr>
<td>10</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>25 and 37 mo later tested negative to all analytes</td>
</tr>
<tr>
<td>11</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>25 and 37 mo later tested negative to all analytes</td>
</tr>
<tr>
<td>12</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>25 and 37 mo later tested negative to all analytes</td>
</tr>
<tr>
<td>13</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>25 and 37 mo later tested negative to all analytes</td>
</tr>
<tr>
<td>14</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>25 and 37 mo later tested negative to all analytes</td>
</tr>
<tr>
<td>15</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>25 and 37 mo later tested negative to all analytes</td>
</tr>
<tr>
<td>16</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>25 and 37 mo later tested negative to all analytes</td>
</tr>
<tr>
<td>17</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>25 and 37 mo later tested negative to all analytes</td>
</tr>
<tr>
<td>18</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>25 and 37 mo later tested negative to all analytes</td>
</tr>
</tbody>
</table>

No. positive (%) | 11 (61%) | 3 (17%) | 0 (0%) | 1 (5%) |
A retrospective Swedish study did not show an association between *A. phagocytophilum* antibodies and the incidence of neurologic disease; however, because numerous tick exposed dogs in endemic regions become infected without developing clinically apparent illness, seroprevalence studies might have less utility than DNA-based testing modalities for establishing disease associations.25

Dog 11 presented with a history of acute-onset lameness; cervical hyperpathia was the only abnormality identified during physical and neurologic examinations. The anatomic source of the neck pain was unclear, but meningitis was considered a diagnostic possibility. As morulae were observed on the blood smear, cerebrospinal fluid was not sampled for analysis; thus, pain could have originated from the cervical musculature, cervical joints, or the intervertebral discs. The rapid resolution of pain and lameness after initiation of doxycycline, with no further problems or recurrence of disease reported, indicated that anaplasmosis could cause acute cervical pain in dogs.

Bleeding problems were not reported with granulocytic anaplasmosis but were described for other rickettsial infections in dogs, such as monocytic ehrlichiosis (*E. canis*), cyclic thrombocytopenia (*A. platys*), and Rocky Mountain Spotted Fever (*Rickettsia rickettsii*).1,2,6,27 In addition to rickettsial organisms, other infections were associated with canine epistaxis, including aspergillosis, bartonellosis, and leishmaniasis.28–30 Dog 18 presented with epistaxis, accompanied by fever, joint pain, and thrombocytopenia. The platelet count was 101,000/μL, which would suggest that factors other than thrombocytopenia were associated with spontaneous bleeding in this dog. Although the mechanism of epistaxis was not determined, rapid and sustained resolution of bleeding occurred after initiation of doxycycline therapy. Further diagnostic testing was not performed. In the dog, it appears that polymicrobial infections might be an important factor to consider diagnostically when examining infectious causes of epistaxis.24,28 Because of the atypical presentation in this dog, it was an intriguing possibility that multiple vector borne infections might have been involved, but in the absence of further diagnostic testing, this conclusion remained purely speculative.

The medical history describing a rapid onset illness, in conjunction with the temporal relationship of the clinical presentation with *I. scapularis* activity in central Minnesota, supported an acute disease process rather than acute decompensation after chronic *A. phagocytophilum* infection. Dogs most often presented in the spring and fall with clinical disease at a time that coincided with the highest numbers of *I. scapularis* in the environment.31 Of the 273 dogs tested by PCR in the original

### TABLE 3

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Doxycycline</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6 mg/kg q 12 hr for 21 days</td>
<td>Resolution in 2 days</td>
</tr>
<tr>
<td>2</td>
<td>7 mg/kg q 12 hr for 28 days</td>
<td>Resolution in 1 day</td>
</tr>
<tr>
<td>3</td>
<td>7 mg/kg q 12 hr for 28 days</td>
<td>Resolution in 4 days</td>
</tr>
<tr>
<td>4</td>
<td>7 mg/kg q 12 hr for 14 days</td>
<td>Resolution in 2 days</td>
</tr>
<tr>
<td>5</td>
<td>5 mg/kg q 12 hr for 14 days</td>
<td>Resolution in 2 days</td>
</tr>
<tr>
<td>6</td>
<td>6 mg/kg q 12 hr for 14 days</td>
<td>Resolution in 3 days</td>
</tr>
<tr>
<td>7</td>
<td>7 mg/kg q 12 hr for 28 days</td>
<td>Resolution in 5 days</td>
</tr>
<tr>
<td>8</td>
<td>7 mg/kg q 12 hr for 28 days</td>
<td>Resolution in 1 day, with no further neurologic dysfunction</td>
</tr>
<tr>
<td>9</td>
<td>8 mg/kg q 24 hr for 28 days</td>
<td>Resolution in 2 days</td>
</tr>
<tr>
<td>10</td>
<td>8 mg/kg q 24 hr for 28 days</td>
<td>Resolution in 6 days</td>
</tr>
<tr>
<td>11</td>
<td>8 mg/kg q 12 hr for 28 days</td>
<td>Resolution in 3 days</td>
</tr>
<tr>
<td>12</td>
<td>7 mg/kg q 24 hr for 30 days</td>
<td>Resolution in 2 days</td>
</tr>
<tr>
<td>13</td>
<td>8 mg/kg q 24 hr for 28 days</td>
<td>Resolution in 3 days</td>
</tr>
<tr>
<td>14</td>
<td>8 mg/kg q 12 hr for 28 days</td>
<td>Resolution in 5 days</td>
</tr>
<tr>
<td>15</td>
<td>6 mg/kg q 12 hr for 28 days</td>
<td>Resolution in 1 day</td>
</tr>
<tr>
<td>16</td>
<td>7 mg/kg q 12 hr for 28 days</td>
<td>Resolution in 2 days</td>
</tr>
<tr>
<td>17</td>
<td>6 mg/kg q 12 hr for 28 days</td>
<td>Resolution in 2 days</td>
</tr>
<tr>
<td>18</td>
<td>8 mg/kg q 12 hr for 28 days</td>
<td>Resolution in 3 days</td>
</tr>
</tbody>
</table>
serosurvey, no dog tested *A phagocytophilum* positive during the months of December, January, February, and March.12

All of the dogs in this study responded clinically to doxycycline therapy, and no dog developed subsequent illness consistent with granulocytic anaplasmosis. To date, there have been no clinical reports that document chronic infection with *A phagocytophilum*; however, there was experimental evidence that supported this possibility. Two experimental studies demonstrated presence of *A phagocytophilum* DNA months after initial infection and even after doxycycline therapy.32,33 Importantly, notable clinical signs other than thrombocytopenia did not occur after the acute phase of infection, and viable organisms could not be transmitted by blood transfusion from a PCR positive dog to a naïve dog. In addition to chronic infection, the possibility of reinfection exists for dogs in highly endemic areas where dogs may be re-exposed to anaplasmosis over months and years. There is evidence to support *A phagocytophilum* strain variation in various regions of the United States and internationally.34,35 There are no data to define whether a dog can be reinfected with homologous or heterologous *A phagocytophilum* strains. As in previous studies, doxycycline was an effective treatment of canine anaplasmosis.5–8 The ideal duration of therapy has not been established, although a 28 day course of doxycycline at 10 mg/kg q 24 hr PO has been recommended.36 In human medicine, current recommendations call for a 10 day treatment of doxycycline for granulocytic anaplasmosis.37 In this case series, 3 dogs were treated for 14 days, 1 dog for 21 days, 13 dogs for 28 days, and 1 dog for 30 days. All dogs responded to treatment in a similar manner. In experimentally infected dogs, *A phagocytophilum* DNA persisted after doxycycline treatment of 14 and 28 days.33,38 Because dogs were not followed with sequential PCR testing, chronic or reinfection in asymptomatic recovered dogs in this study could not be determined.

Three puppies, dog 1 (5 mo of age), dog 12 (6 mo of age), and dog 15 (3 mo of age) were described in this case series. Dogs 1, 12, and 15 were treated with doxycycline for 21, 30, and 28 days, respectively, with no evidence of tooth discoloration. It was interesting to note that dog 15, an offspring of dog 6, was born 2 wk before the onset of illness in the dam. None of the other puppies in that litter had problems during nursing, weaning, or when placed in new homes. Dog 6 remained in the same household as the dam and developed clinical disease 14 wk after the dam’s illness. Perinatal transmission of *A phagocytophilum* was reported in a human infant who developed symptoms 9 days after birth.39 A recent study involving a bitch naturally infected just before whelping failed to demonstrate perinatal transmission to the puppies.40 In a bovine experimental infection study, an infected cow gave birth to an infected calf, which had clinical signs consistent with anaplasmosis 13 days after birth.41 The 14 wk time frame between illness of dog 6 and dog 15 made tick transmission more likely than perinatal transmission.

The complete blood count is a useful diagnostic test for granulocytic anaplasmosis. Thrombocytopenia is a common hematologic abnormality and evaluation of stained blood smears facilitates visualization of granulocyte morulae in acutely infected dogs. For veterinary clinics that obtain in-house automated complete blood counts, having a trained technician visually evaluate blood smears is critically important for the diagnosis of anaplasmosis, as well as other tick-borne infections, including babesiosis, cytazoonosis, ehrlichiosis, and hepatazoonosis.32 Morulae provide rapid diagnostic information but are not present in every infection. Another problem with morulae is that based on microscopy alone *A phagocytophilum* morulae are indistinguishable from *E ewingii* morulae.10 Dependent on the skill level and experience of the technician, other granulocyte changes or artifacts (e.g., other cytoplasmic structures or stain precipitate) have the potential to be misdiagnosed as *A phagocytophilum* inclusions.

In cases where no morulae are seen, PCR testing can facilitate an accurate diagnosis, which offers the advantage of differentiating between other infectious and noninfectious diseases that have similar hematologic and clinical presentations. In this study, a PCR positive test was part of the inclusion criteria to insure that the dogs had active infection at the time of presentation. This introduced a bias, because in the original study from which the cases were selected, not all suspected cases of anaplasmosis were PCR positive. For example, there were 16 morulae positive dogs tested by PCR and 14 were positive.12 The two PCR-negative, morulae-positive dogs might have represented false-negative PCR results or the morulae might have been misdiagnosed. Another potential pitfall is that clinically normal dogs can test *A phagocytophilum* PCR positive.5,12 In the original serosurvey, seven asymptomatic dogs were PCR positive, four of which were also seropositive.12 These dogs likely represented asymptomatic acute infections; however, in the four seropositive PCR positive dogs, asymptomatic chronic infection was a possibility. These data suggested that an ill PCR positive dog might have clinical disease unrelated to *A phagocytophilum* infection. Failure to achieve a prompt treatment response to doxycycline would tend to rule out canine anaplasmosis or could suggest cointection with organisms that were not doxycycline responsive.

Seven dogs in this study had negative *A phagocytophilum* serologic test results at the time of diagnosis, which was not surprising because acute infection with vector-borne organisms, including *Anaplasma, Ehrlichia,* and *Rickettsia* spp., can cause
clinical signs before the dog has a measureable antibody response. Therefore, treatment decisions should not rely solely on antibody testing, whether the results are negative or positive. The seronegative dog might have been recently infected, whereas the seropositive dog might have been exposed months earlier and already eliminated the organism through innate immunity. Serology results must be considered in the context of physical examination, hematologic abnormalities, and potentially PCR results. Long-term serological follow up using the in-clinic serologic screening test was available for four dogs, of which three tested negative at subsequent testing intervals up to 3 yr. One dog had a repeatable A phagocytophilum antibody response, which might have been due to persistent antibody production or due to re-exposure to A phagocytophilum infected ticks and an amnestic response after repeated transmission events. With the limited number of dogs and testing time points in this study, it was not possible to draw conclusions as to how long after infection an individual dog would retain an A phagocytophilum antibody titer using the ELISA-based screening test. After natural infection in a group of 14 Swedish dogs, IFA titers decreased below the diagnostic cutoff in some dogs by 4–12 mo after infection; however, variations in immune response in conjunction with the possibility of reinfection made predictions for clinical practice problematic.7

Conclusion

As diagnostic capabilities available to practicing veterinarians continue to increase, the spectrum of clinical disease associated with canine granulocytic anaplasmosis will be further refined. Although an acute febrile illness accompanied by lameness or joint effusion is seemingly the most typical presentation of canine anaplasmosis, clinicians should be aware of other infrequent or atypical presentations. Neurologic disease, lymphadenopathy, vomiting, and epistaxis were the predominant abnormalities in some dogs in this study. Prompt treatment was associated with a rapid clinical response and the long-term prognosis appeared to be excellent. In cases where anaplasmosis is suspected but the dog does not respond to doxycycline, other disease processes should be considered.

The costs of molecular analysis and retrospective serologic testing were paid for by IDEXX Laboratories.

FOOTNOTES

a SNAP 4Dx Test; IDEXX Laboratories, Inc, Westbrook, ME
b LaserCyte Hematology Analyzer; IDEXX Laboratories, Inc, Westbrook, ME
c SNAP 3Dx Test; IDEXX Laboratories, Inc, Westbrook, ME
d DNeasy Blood & Tissue Kit; Qiagen, Inc, Valencia, CA
e Applied Biosystems Thermocycler; Life Technologies Corp., Carlsbad, CA

REFERENCES


