CHAPTER 29

Anaplasmosis

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Overview of Anaplasmosis

First Described: First reports of *Anaplasma phagocytophilum* came from sheep in 1951 (Scotland).[1] Canine infection was first reported in the United States (California) in 1982.[2] *Anaplasma platys* was first reported in the United States (Florida) in 1978.[3]

Cause: *Anaplasma phagocytophilum* (granulocytic anaplasmosis), *Anaplasma platys* (thrombocytotropic anaplasmosis)

Affected Hosts: *A. phagocytophilum* causes disease in dogs, cats, humans, ruminants (European strains), horses, and camelids. *A. platys* causes thrombocytopenia in dogs.

Geographic Distribution: In the United States, *A. phagocytophilum* is most prevalent in the upper Midwest, northeast and western states. Infection also occurs throughout continental Europe and the United Kingdom, Asia, and Russia. The organism has been detected in dogs from Africa and South America. *A. platys* occurs throughout the Americas, Europe, Asia, Australia, the Middle East, and Africa.

Mode of Transmission: Tick vectors, primarily *Ixodes ricinus-persulcatus* complex ticks transmit *A. phagocytophilum*. Although unconfirmed, *Rhipicephalus sanguineus* is suspected to be the major vector of *A. platys.*

Major Clinical Signs: The major clinical signs of *A. phagocytophilum* infection are fever, lethargy, inappetence, and lameness due to polyarthritis, although vomiting, diarrhea, cough, and neck pain may occur. *A. platys* usually causes no signs, but fever and lethargy are possible.

Differential Diagnoses: Major differential diagnoses include other tick-borne diseases (such as the ehrlichioses, rickettsioses, Lyme borreliosis and babesiosis), bartonellosis, leptospirosis, primary immune-mediated disease, and lymphoma.

Human Health Significance: *A. phagocytophilum* causes human granulocytic anaplasmosis. Dogs act as a sentinel for human infection and may carry unfed ticks to humans on their coats.

Canine anaplasmosis is caused by *Anaplasma phagocytophilum* (formerly *Ehrlichia equi*, *Ehrlichia phagocytophila*, and, in humans, the human granulocytic ehrlichiosis [HGE] agent) and *Anaplasma platys*. These are tick-borne, gram-negative, obligately intracellular bacteria that belong to the family Anaplasmataceae. *A. phagocytophilum* predominantly infects neutrophils but also eosinophils, where it forms host membrane-enclosed morulae. Ticks that belong to the *Ixodes ricinus-persulcatus* complex are the major vectors for *A. phagocytophilum*. *Anaplasma platys* forms morulae within platelets. The vector of *A. platys* is probably *Rhipicephalus sanguineus*. As with the ehrlichioses, co-infections with other pathogens that are transmitted by the same or other tick species may occur and influence the clinical manifestations of disease.

Anaplasma phagocytophilum Infection

Etiology and Epidemiology

A. phagocytophilum causes granulocytic anaplasmosis in dogs, humans, horses, and, in Europe, domestic ruminants that include sheep, cattle, goats, and deer. Cats and camelids may also be affected. A variety of wild animal species, which include rodents and deer, act as reservoir hosts. For the midwestern and eastern United States, white-footed mice *(Peromyscus leucopus)* and eastern chipmunks may act as reservoirs, whereas in the western states, dusky-footed woodrats, gray squirrels, and chipmunks have been implicated. In Europe, bank voles, wood mice, shrews, and deer are likely reservoirs. Many strains of *A. phagocytophilum* exist that differ in pathogenicity and host tropism. For example, strains that infect domestic ruminants in Europe and white-tailed deer in the United States appear to be distinct from those that infect horses, humans, and dogs. Dogs, cats, and humans are accidental hosts and are not important in the transmission of infection to other host species.

The geographic distribution of the disease follows that of the tick vectors (Figure 29-1). In North America, the tick vectors of *A. phagocytophilum* are *Ixodes scapularis* in the northeastern and upper midwestern states, and *Ixodes pacificus* in the West. In Europe, the primary vector is *Ixodes ricinus*, and the disease has been described in dogs throughout continental Europe and in the UK. *Ixodes persulcatus* and *Dermacentor silvarum* ticks transmit the organism in Asia and Russia. Other *Ixodes* spp. ticks have also been implicated in transmission. Evidence of *A. phagocytophilum* infection has been found in dogs from Brazil and Tunisia, and a closely related organism was found in dogs from South Africa.[4-6] In humans, rare reports exist of direct transmission that followed close contact with blood or respiratory secretions, transplacental spread, or transmission through blood transfusion.

Many seroprevalence studies of dogs have been reported worldwide.[7] The seroprevalence varies with geographic location and whether the dogs studied were sick or healthy. The prevalence of positive antibody titers in dogs from some regions of Europe and North America exceeds 50%. A study that used a commercially available ELISA assay to determine the prevalence

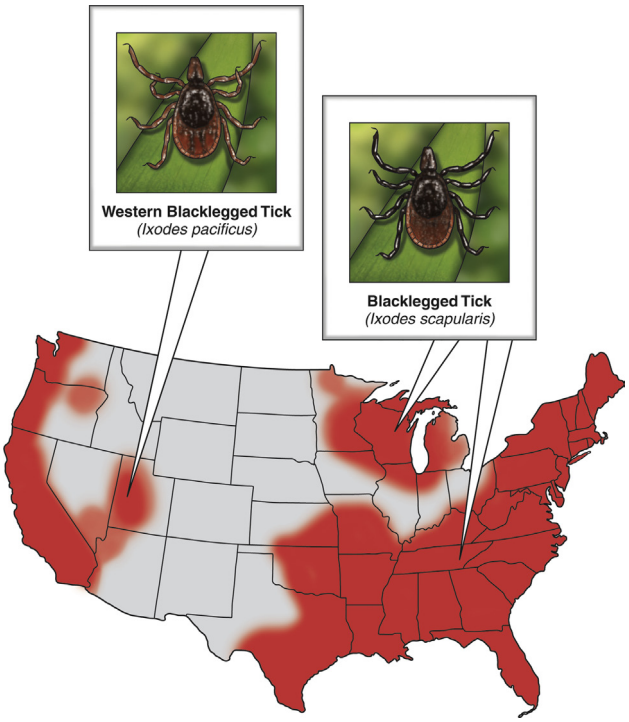

FIGURE 29-1 Distribution of *Ixodes scapularis* and *Ixodes pacificus*, the vectors of *Anaplasma phagocytophilum*, in the United States. The distribution of granulocytic anaplasmosis follows that of the tick vectors.

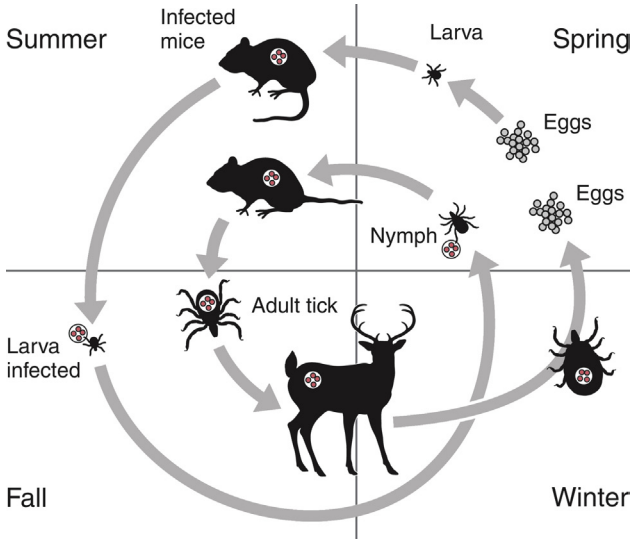

FIGURE 29-2 Life cycle of *Ixodes scapularis* ticks and *Anaplasma phagocytophilum* infection. Uninfected larvae (top right) hatch in the late spring and acquire infection from small rodents in the summer. They then over-winter (often in protected mouse burrows) and then molt into nymphs the following spring. The nymphs feed in the late spring or early summer on a variety of animal species including rodents, humans, deer, and dogs. Nymphs molt into adults in the late summer to fall, which subsequently feed on large mammals such as deer, where they mate and drop off. The females then lay eggs and die. Dogs, cats and humans become infected by nymphs or adult ticks.

of seroreactivity to *A. phagocytophilum* in more than 400,000 dogs from the United States revealed wide variation in seroprevalences. Some counties in the upper Midwest and northeastern United States had seroprevalences that exceeded 40%, although the overall seroprevalence in these regions were 6.7% and 5.5%, respectively.[8] Serologic cross-reactivity with *A. platys* may also influence these data.

The seasonal pattern of disease reflects times of peak nymphal and adult tick activities, as well as periods when humans and their dogs are active outdoors. In the western United States, *A. phagocytophilum* infection occurs most frequently in dogs between April and July, when nymphal ticks are abundant. Some infections occur in October, during early questing of adult ticks. In Minnesota and Wisconsin, most cases are diagnosed in late spring (May, June) and fall (October, November).[9-11] In a study from Berlin, most cases occurred between April and September.[12] The median age of clinically affected dogs is 6 to 8 years (range, 6 months to 14 years).[9,10,12-14] In the upper Midwest of the United States, a bimodal age distribution has been recognized, with 25% of dogs 1 year of age or less, and 50% of dogs at least 8 years of age.[10] Affected cats in the northeastern United States have ranged from 4 months to 13 years of age (mean, 3.7 years).[15] Although no breed predispositions are recognized, in one study, golden retrievers comprised almost half of affected dogs; this may reflect the popularity of these dogs for outdoor activities.[14] Infection with other tick-borne pathogens is a risk factor for *A. phagocytophilum* infection. Co-infection with *Borrelia burgdorferi,* the cause of Lyme disease, is common because *B. burgdorferi* is transmitted by the same *Ixodes* tick species.[8,10,11] In northern California, dogs seroreacted to *A. phagocytophilum* were 18 times more likely to be seropositive for *Bartonella vinsonii* subspecies *berkhoffii* than dogs that were seronegative.[16]

Clinical Features

Signs and Their Pathogenesis

A. phagocytophilum is transmitted transstadially within the tick (i.e., from larva to nymph to adult), and not transovarially (from adult to egg). Dogs and cats thus become infected after exposure to infected nymphs or adult ticks, which acquire infection when they feed on wild animal reservoir hosts as larvae or nymphs (Figure 29-2). Ticks must attach for 36 to 48 hours for transmission to occur. Once *A. phagocytophilum* enters the bloodstream, it attaches to the sialylated ligands on the surface of neutrophils, such as P-selectin glycoprotein ligand-1.[7] The organism then enters the neutrophil through caveolae-mediated endocytosis. *A. phagocytophilum* survives in the harsh neutrophil environment through dysregulation of neutrophil function and by bypassing phagolysosomal pathways. It inhibits neutrophil superoxide production and can reduce neutrophil motility and phagocytosis. *A. phagocytophilum* also reduces neutrophil adherence to endothelium and inhibits neutrophil transmigration into tissues, possibly through downregulation of selectin molecule expression.[7] This may promote its survival in peripheral blood.

Normally, neutrophils circulate for 10 to 12 hours before they enter tissues and undergo death through apoptosis. *A. phagocytophilum* delays neutrophil apoptosis, which allows it to survive longer periods of time within the neutrophil.[7] *A. phagocytophilum* may also infect other cell types, such as bone marrow cells, endothelial cells, and megakaryocytes, although the importance of these cells in the pathogenesis of infection is not clear.

The clinical signs and laboratory abnormalities that occur in dogs and cats with granulocytic anaplasmosis probably vary somewhat in different geographic locations as a result of local strain variation. The vast majority of dogs infected with

A. phagocytophilum show no clinical signs. Some dogs and cats develop a self-limiting febrile illness, which in dogs occurs after an incubation period of 1 to 2 weeks. Lethargy occurs in almost all clinically affected cats and dogs. Fever and inappetence are also common findings. Lameness, reluctance to move, polydipsia, vomiting, diarrhea, and a soft cough can also occur. Generalized lymphadenopathy and splenomegaly develop as a result of reactive lymphoid hyperplasia and, in the spleen, concurrent extramedullary hematopoiesis.[17] Uncommonly, hemorrhage, manifested as mucosal petechiae, melena, or epistaxis, has been reported in naturally infected dogs, but co-infections with other tick-borne pathogens may contribute to these signs in some dogs.[9,12,13,18] Neurologic signs such as seizures, circling, cervical pain, and decreased placing reactions have been described in a few dogs from the upper Midwest with granulocytic anaplasmosis, but one dog with seizures had a history of idiopathic epilepsy.[9,18] Neurologic signs and detection of the organism in the CSF have been uncommonly reported in humans.[19]

Infection with *A. phagocytophilum* results in mild to moderate thrombocytopenia, although other cytopenias may also occur (Table 29-1). The mechanism(s) of these hematologic abnormalities remain unclear. Anti-platelet antibodies occur in serum from humans and dogs with granulocytic anaplasmosis,[12,20] and so immune-mediated mechanisms may contribute to thrombocytopenia. However, thrombocytopenia occurs in acute disease, before antibodies are noted, so other mechanisms may be important. The bone marrow of infected dogs shows megakaryocyte hyperplasia, so platelet destruction may be involved.[21]

Impaired neutrophil function as a result of *A. phagocytophilum* infection may predispose to development of secondary opportunistic infections or influence the outcome of co-infections with other tick-borne pathogens such as *B. burgdorferi*. Although uncommon, opportunistic infections have been occasionally documented in humans and dogs with granulocytic anaplasmosis and are well in small ruminants.[22]

Infection with *A. phagocytophilum* may be self-limiting in dogs and cats, with minimal fatality or chronic disease manifestations. The extent to which *A. phagocytophilum* can persist in tissues and contribute to chronic disease manifestations in humans and dogs has been controversial, and may be dependent on the infecting strain and host immune response to infection. In one study, treatment of dogs that had been experimentally inoculated with *A. phagocytophilum* with prednisolone up to 6 months after infection was followed by the development of positive PCR results for the organism and, in some dogs, thrombocytopenia and reappearance of morulae on blood smears.[23] One dog infected with a California strain of *A. phagocytophilum* was persistently PCR-positive through day 60 postinfection, the last time point evaluated.[23]

Physical Examination Findings

The most common findings on physical examination in dogs include lethargy, fever (up to 106.7°F [41.5°C]), dehydration, tachypnea, mild peripheral lymphadenopathy, and splenomegaly.[7,10] Scleral injection may be noted. Lameness, reluctance to move, swollen joints, and pain on joint manipulation may also be detected in some dogs. Increased lung sounds, abdominal pain, epistaxis, petechial hemorrhages, and neurologic signs such as circling, cervical pain, and decreased placing reactions occur uncommonly.

Physical examination findings reported in cats have been similar to those described in dogs, and include fever, lethargy, tachypnea, increased lung sounds, mild abdominal pain, hepatomegaly, splenomegaly, vomiting, ataxia, hyperesthesia, muscle and joint pain, lameness, conjunctivitis, and ocular discharge.[15,24,25]

Diagnosis

Granulocytic anaplasmosis should be suspected in dogs and cats with acute febrile illness and thrombocytopenia that reside in endemic areas, regardless of tick exposure history. Diagnosis relies on detection of morulae within granulocytes, results of acute and convalescent serology, or molecular testing using PCR assays. The diagnostic criteria for confirmed human granulocytic anaplasmosis are clinical signs and laboratory findings suggestive of granulocytic anaplasmosis together with (1) detection of morulae within neutrophils combined with a single positive reciprocal antibody titer to *A. phagocytophilum* of at least 80; (2) a fourfold increase or decrease in the antibody

TABLE 29-1

Hematologic Abnormalities in Nine Dogs with Granulocytic Anaplasmosis in Northern California*

Test	Reference Range	Number below the Reference Range	Number within the Reference Range	Number above the Reference Range	Range for dogs with Granulocytic Anaplasmosis
Hematocrit (%)	40-55	6	3	0	19-45
Neutrophils (cells/μL)	3000-10,500	0	6	3	3513-18,592
Band neutrophils (cells/μL)	Rare	0	1	8	0-1282
Monocytes (cells/μL)	150-1200	1	7	1	142-1346
Lymphocytes (cells/μL)	1,000-4000	7	2	0	71-2693
Eosinophils (cells/μL)	0-1500	0	9	0	0-328
Platelets (cells/μL)†	150,000-400,000	6	2	0	37,000-262,000

*Diagnosis was based on compatible clinical signs and either morulae within circulating neutrophils and/or a positive real-time PCR assay result for *A. phagocytophilum*.

†Platelets were clumped for one dog.

titer within 4 weeks; (3) a positive PCR test result using specific *A. phagocytophilum* primers; or (4) isolation of *A. phagocytophilum* from blood.[22] These criteria could also be applied to dogs. The use of multiple diagnostic modalities may be needed to confirm the diagnosis of granulocytic anaplasmosis in some dogs.

Laboratory Abnormalities

Complete Blood Count

Thrombocytopenia occurs in approximately 90% of dogs with granulocytic anaplasmosis.[9,10,12,13] The platelet count in thrombocytopenic dogs may occasionally be as low as 5000 platelets/μL, although more often it is mild to moderately decreased (see Table 29-1).[9,10,12-14] The majority of affected dogs are lymphopenic, but lymphocytosis can occur.[9,12,13] Circulating reactive lymphocytes may be present. Anemia is common and typically mild and nonregenerative. Both neutrophilia and neutropenia occur, but most dogs have neutrophil counts that lie in the lower half of the reference range. Low numbers of band neutrophils, as well as mild neutrophil toxicity, are often present. Monocytopenia or monocytosis can occur.[9,12,13] Cytologic examination of blood smears often reveals morulae within granulocytes (Figure 29-3). Morulae were detected in neutrophils from 36%, 56%, 67%, and 100% of dogs in four respective case series.[9,12-14] Morulae appear as early as 4 days after experimental inoculation of dogs and persist for 4 to 8 days.[17] The morulae are indistinguishable from those of *Ehrlichia ewingii*, so serology or PCR assays are needed to confirm *A. phagocytophilum* infection.

In contrast to dogs, thrombocytopenia appears uncommon in cats infected with *A. phagocytophilum*. None of 15 sick, PCR-positive cats from the northeastern United States were thrombocytopenic.[15] The most common hematologic abnormality in cats has been lymphopenia. Morulae have been detected in some cats with granulocytic anaplasmosis.

Serum Biochemical Tests

The most frequent serum biochemistry finding in dogs with granulocytic anaplasmosis is mild to moderate hypoalbuminemia (Table 29-2). Mild hyperglobulinemia, mild electrolyte abnormalities (hypokalemia, hyponatremia, and metabolic acidosis), and a mild increase in the activities of serum ALP and to a lesser extent ALT may occur.[9,10,13,14]

Urinalysis

Urinalysis in dogs with granulocytic anaplasmosis may reveal isosthenuria, hyposthenuria, and proteinuria. Urine protein-to-creatinine ratios in two affected dogs from the United States were

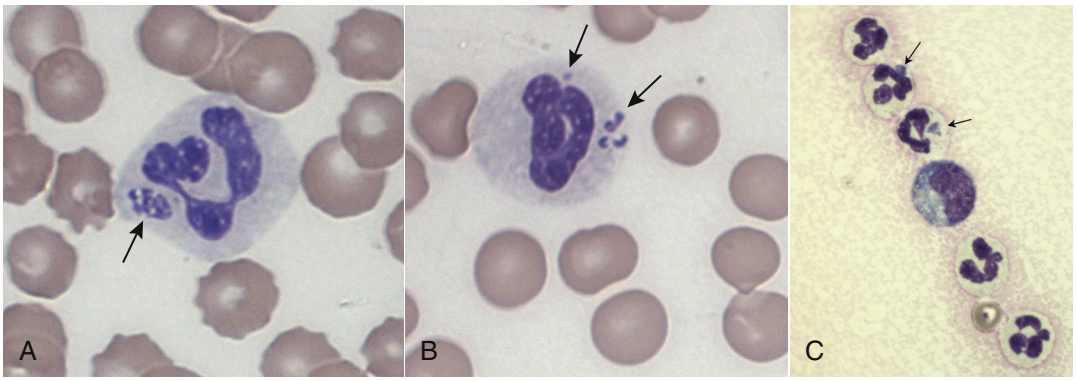

FIGURE 29-3 Intracytoplasmic morula **(A)** and cocci **(B)** of *Anaplasma phagocytophilum (arrows)* within neutrophils of an 8-year-old male neutered golden retriever with granulocytic anaplasmosis; in **(C)**, morulae are present in neutrophils within the synovial fluid of a 5-year-old curly-coated retriever that had polyarthritis.

TABLE 29-2

Findings on Serum Biochemistry Analysis in Nine Dogs with Granulocytic Anaplasmosis in Northern California*

Test	Reference Range	Number below the Reference Range	Number within the Reference Range	Number above the Reference Range	Range for dogs with Granulocytic Anaplasmosis
Sodium (mmol/L)	145-154	4	5	0	142-147
Potassium (mmol/L)	4.1-5.3	2	7	0	3.3-4.9
Bicarbonate (mmol/L)	16-26	3	6	0	11-18
Albumin (g/dL)	2.9-4.2	6	3	0	1.5-3.4
Globulin (g/dL)	2.3-4.4	0	8	1	2.7-6.0
Total bilirubin (mg/dL)	0-0.4	0	8	1	0-1.0
Alanine aminotransferase (U/L)	19-70	0	8	1	26-122
Alkaline phosphatase (U/L)	15-127	0	7	2	26-653

*Diagnosis was based on compatible clinical signs and either morulae within circulating neutrophils and/or a positive real-time PCR assay result for *A. phagocytophilum*.

1.5 and 2.2,[10] but there is no evidence that *A. phagocytophilum* infection causes severe glomerulonephritis in dogs.

Synovial Fluid Analysis

Dogs with granulocytic anaplasmosis can develop neutrophilic polyarthritis. Cytologic examination of synovial fluid in these dogs reveals increased numbers of nondegenerate neutrophils. In some affected dogs, morulae are found within these cells and the synovial fluid can be PCR positive (Figure 29-3,*C*).

Diagnostic Imaging

Plain Radiography

Thoracic radiographs are usually normal in dogs with granulocytic anaplasmosis but may show a mild interstitial infiltrate. Focal alveolar infiltrates occur as well.[26]

Sonographic Findings

Abdominal ultrasound may reveal splenomegaly, with a hypoechoic spleen, or mild abdominal lymphadenopathy.

Microbiologic Tests

Diagnostic assays available for anaplasmosis in dogs and cats are shown in Table 29-3.

Serologic Diagnosis

Diagnosis of granulocytic anaplasmosis can be accomplished with acute and convalescent serology. Many veterinary laboratories perform serologic testing using immunofluorescent antibody (IFA) techniques. IgG antibodies are first detectable approximately 8 days after exposure, 2 to 5 days after morulae appear. As a result, early in the course of illness, antibodies may be undetectable, so PCR assays may be more useful for diagnosis of acute infection in the absence of detectable morulae. Because positive titers can reflect previous exposure, demonstration of a fourfold rise in titer is required. Antibody titers may persist for many months.[14,23] In some human patients, antibody titers have persisted as long as 3 years.[22] In the United States, a silicon disc-based ELISA assay is available commercially for detection of antibodies to *A. phagocytophilum* (Accuplex 4, Antech Diagnostics, Irvine, CA). The performance of this assay when compared with IFA requires further study. An in-clinic lateral-flow ELISA device (IDEXX SNAP 4Dx Plus), which uses a recombinant Msp2/p44 protein, is also available for the detection of antibodies to *Anaplasma* species in dog serum. Positive results obtained with these ELISA assays do not imply that *A. phagocytophilum* is the cause of illness, and as with IFA testing, negative results can occur in dogs with acute illness due to the lag in antibody production relative to the onset of clinical signs.

Serologic cross-reactivity between *Anaplasma* species occurs. Dogs infected with *A. platys* also seroreact to *A. phagocytophilum* antigen; this includes the recombinant Msp2 assay.[8] Serologic cross-reactivity between *A. phagocytophilum* and *E. canis* also occurs, but is relatively uncommon and minor.[13,26,27] Dogs diagnosed with granulocytic anaplasmosis that have antibodies to *A. phagocytophilum* generally lack antibodies to *E. canis* using IFA testing.[7,10]

Molecular Diagnosis Using the Polymerase Chain Reaction

Several conventional and real-time PCR assays for *A. phagocytophilum* exist for detection of *A. phagocytophilum*

TABLE 29-3

Diagnostic Assays Available for Anaplasmosis in Dogs and Cats

Assay	Specimen Type	Target	Performance
Cell culture	Whole blood	*Anaplasma phagocytophilum*; *Anaplasma platys* cannot be cultured	Not widely offered or utilized for routine diagnostic purposes. Requires several weeks' incubation.
Cytology	Whole blood, buffy coat smears, body fluids, tissue aspirates	*A. phagocytophilum* or *A. platys* morulae	Low sensitivity (especially for *A. platys* infection). Morulae of *A. phagocytophilum* cannot be distinguished from those of *Ehrlichia ewingii*. Morulae may be confused with cytoplasmic granules or stain precipitate.
IFA serology	Serum	Antibodies to *Anaplasma* spp.	Acute and convalescent serology is required for diagnosis of acute infection, because initial results may be negative in dogs with acute disease and positive results may reflect previous exposure rather than active infection. Cross-reactivity occurs between *Anaplasma* spp.
ELISA serology	Serum	Antibodies to *Anaplasma* spp. antigens	Rapid, inexpensive, can be performed as an in-practice test. Similar limitations as IFA. Lack of quantitation limits ability to document seroconversion.
PCR	Whole blood, splenic or lymph node aspirates, bone marrow aspirates, buffy coat specimens, tissue specimens	*A. phagocytophilum* or *A. platys* DNA	Confirms active infection. Sensitivity and specificity may vary depending on assay design and specimen type. Because healthy animals may be PCR positive, positive PCR results must be interpreted in light of the clinical signs.

IFA, Immunofluorescent antibody.

DNA in peripheral blood, buffy coat, bone marrow, or splenic tissue. These can be useful for early diagnosis of granulocytic anaplasmosis in dogs and cats. The sensitivity and specificity of PCR assays may vary depending on the assay design and the laboratory used. Most, but not all, assays detect either the 16S rRNA gene or the outer surface protein gene *msp2* (p44). Assays that detect the *msp2* gene are usually specific for *A. phagocytophilum*, whereas assays that detect the 16S rRNA gene may detect other *Anaplasma* species, and even other bacteria. *A. phagocytophilum* DNA has occasionally been amplified from healthy dogs, so results must be interpreted in light of the clinical signs.[11] In experimentally infected dogs, whole-blood PCR becomes positive several days before and after morulae appear on blood smears.[17,23]

Culture

A. phagocytophilum can be isolated in human promyelocytic leukemia cell lines (HL-60) and tick embryo cell lines. Culture is highly sensitive for diagnosis of acute infection in human patients,[28] but it is not routinely used in dogs for diagnostic purposes because it requires special facilities, technical expertise, and prolonged incubation.

Pathologic Findings

Little information is available regarding the pathology of granulocytic anaplasmosis in dogs. Tissue injury appears to result from the host inflammatory response, rather than the bacterial infection itself.[29] In people, splenic lymphoid depletion, macrophage aggregates and apoptosis within the liver, paracortical lymphoid hyperplasia, and hemophagocytic cells within reticuloendothelial tissues have been described.[30]

Treatment and Prognosis

The treatment of choice for granulocytic anaplasmosis in dogs is doxycycline (5 mg/kg PO q12h). The optimum duration is unknown, but 2 weeks may be sufficient. The prognosis is excellent. Most dogs show clinical improvement within 24 to 48 hours of treatment, although some dogs require more than 1 week of treatment before clinical signs resolve.[10,13] Platelet counts normalize 2 to 14 days after treatment is initiated.[10] In one study, 30% of 23 owners reported that their dog returned to normal activities in 1 to 2 days after treatment was initiated, 30% reported this took 3 to 5 days, 21% 1 to 3 weeks, and 17% reported that it took a month or longer for their dogs to return to normal activity.[10] Death due to granulocytic anaplasmosis has not been described in dogs. Intravenous crystalloid fluids and antiemetics may be required for supportive care until clinical signs improve.

Immunity and Vaccination

The immune response to *A. phagocytophilum* infection is not fully characterized. Both humoral and cell-mediated immune responses appear to be important in clearance of infection. Host cytokines such as IFN-γ may play a role in the initial control of *A. phagocytophilum* infection[31,32] but may also contribute to the inflammatory process associated with disease.[33] In lambs, *A. phagocytophilum* evades the host immune response through differential expression of MSP2, an outer surface protein involved in immune recognition.[34] Variation in MSP2 also occurs in chronically infected woodrats.[35] Further research is required to document whether persistence occurs through similar mechanisms in other host species. It is possible that recovery from natural infection confers long-term protection against development of disease. Reinfection has not been reported in dogs, but was described in one human patient.[36] A vaccine for granulocytic anaplasmosis is not available for companion animals.

Prevention

Infection may be prevented by avoidance of tick exposure, prompt tick removal, and use of topical ectoparasiticides (see Chapter 28). Although not a guaranteed protection, combinations of topical imidacloprid and permethrin, or fipronil, amitraz and (*S*)-methoprene, prevent transmission of *A. phagocytophilum* to dogs from infected ticks.[37,37a]

Public Health Aspects

A. phagocytophilum infection was first recognized in humans in the upper Midwest of the United States in the early 1990s and has since been increasingly recognized in humans from the United States, Europe, and Asia. Human granulocytic anaplasmosis closely resembles the disease in dogs, which has been described as an "influenza-like illness after a tick bite."[38] Men are affected slightly more frequently than women. In Europe, the disease is most commonly reported in from Sweden and Slovenia, where it was first reported in 1997.[39] Morulae are observed less often in humans with granulocytic anaplasmosis in Europe, and the disease may be milder than that described in humans from the United States.

The most common clinical signs reported in human patients are myalgia, headache (which is often severe), malaise, and chills. Anorexia, nausea, arthralgias, and cough may also occur.[22,38,40] The disease typically resolves within 2 months in the absence of appropriate antibiotic treatment. Occasionally more severe disease may occur. In one study, up to 17% of affected humans required admission to an intensive care unit.[38] Death occurs in 1% or fewer clinically affected humans, usually as a result of complications such as a septic or toxic shock–like syndrome, respiratory insufficiency, opportunistic fungal or viral infections, rhabdomyolysis, acute renal failure, hemorrhage, or neurologic disease.[22] Severe illness tends to occur in humans of advanced age or with concurrent immunosuppressive illness or drug therapy. Laboratory testing of peripheral blood typically reveals normal or slightly decreased white blood cell and platelet counts, sometimes with a neutrophilic left shift, and mild to moderate elevation of hepatic transaminase activities. In the United States, the disease is reportable to the Centers for Disease Control and Prevention (CDC). Dogs act as sentinels for human exposure and may be a source of infection through mechanical carriage of infected ticks to humans. Blood from affected dogs should be handled with caution, and needle-stick injuries should be avoided.

Anaplasma platys Infection

Etiologic Agent and Epidemiology

Anaplasma platys infects and forms inclusions within platelets and is the cause of canine cyclic thrombocytopenia, or thrombocytotropic anaplasmosis. It does not appear to infect cats. The organism is very widespread, and infections occur throughout the Americas, Europe, Asia, Australia, the Middle East, and

Africa. *R. sanguineus* ticks are believed to transmit *A. platys*, because the DNA of *A. platys* has frequently been found in *R. sanguineus* ticks worldwide, and dogs infected with *A. platys* are often co-infected with *E. canis*. However, in a single attempt, experimental transmission of *A. platys* by *R. sanguineus* ticks failed.[41] *A. platys* DNA has been found in other tick species, such as *Dermacentor auratus* ticks in Thailand,[42] *Rhipicephalus turanicus* ticks from Israel,[43] and *Haemaphysalis* spp. and *Ixodes nipponensis* ticks from Korea.[44] Different strains of *A. platys* exist that appear to vary in pathogenicity. The organism has never been isolated in cell culture.

Clinical Features

Signs and Their Pathogenesis

Anaplasma platys causes thrombocytopenia in dogs, most often in the absence of other clinical signs, although fever, lethargy, lymphadenopathy, uveitis, pallor, and mucosal hemorrhages have been described.[45,46] Co-infections with other vector-borne pathogens may contribute to clinical signs in some dogs. Thrombocytopenia occurs 1 to 2 weeks after experimental inoculation. This initial episode is associated with the highest number of organisms in platelets, as detected using light microscopic examination of stained blood smears.[47] The platelet count nadir occurs 2 to 3 weeks postinfection and in some dogs may be lower than 20,000 platelets/μL. Visible organisms then disappear from platelets, and the platelet count returns to normal or near-normal limits within 3 to 4 days. This also corresponds with a decrease in organism load and failure to detect the organism in peripheral blood with real-time PCR, although bone marrow and splenic aspirates may remain positive.[48] Cycles of thrombocytopenia and bacteremia then occur at intervals of 7 to 14 days, after which infection and thrombocytopenia persist in some dogs but morulae or inclusions become more difficult to detect within platelets.[47] The mechanism of thrombocytopenia is unknown, but direct damage to platelets, sequestration of platelets in the spleen, and immune-mediated destruction have been hypothesized to play a role.

Co-infection of dogs with *A. platys* and *E. canis* may lead to more severe anemia than occurs with isolated *E. canis* infection.[49] The duration of *A. platys* infection may also be prolonged.

Physical Examination Findings

Most dogs with *A. platys* infection show no clinical signs. Fever, petechial hemorrhages, and uveitis have been reported, but co-infections with other vector-borne pathogens may have contributed to these signs.

Diagnosis

Laboratory Abnormalities

Usually, the only abnormality present on the CBC, biochemistry panel, and urinalysis in dogs with *A. platys* infection is thrombocytopenia. A mild, nonregenerative anemia may be found. The diagnosis can be made when morulae are seen within platelets on blood smears, but this is insensitive, and other inclusions or stain precipitate may be mistaken for organisms.

Microbiologic Tests

Direct Fluorescent Antibody

Direct fluorescent antibody techniques can be used to identify *A. platys* in platelets, but these are not widely available for routine diagnostic purposes. Molecular diagnostic assays have overcome the need for direct fluorescent antibody or immunocytochemical staining.

Serologic Diagnosis

Recent infection with an *Anaplasma* species can be detected through the use of acute and convalescent serology with IFA. Because antibodies to *A. phagocytophilum* bind to *A. platys* antigens it is not possible to distinguish the immune response to each of these pathogens with currently available assays. This can be problematic in geographic regions where both *A. platys* and *A. phagocytophilum* are found. A positive result on commercially available ELISA assays also only indicates previous exposure to an *Anaplasma* species. Dogs with recent infection may be seronegative, because antibodies to *A. platys* are not detectable for 1 to 2 weeks after inoculation. Because a single positive test result only indicates previous exposure, and seroprevalence rates in endemic areas are high, thrombocytopenia in a seropositive dog may be due to an etiology other than *Anaplasma* infection. Cross-reactivity to *E. canis* antigens does not occur in dogs with *A. platys* infections, so positive titers to *E. canis* and *A. platys* may represent previous exposure to both pathogens, or to other *Ehrlichia* or *Anaplasma* species.

Molecular Diagnosis Using the Polymerase Chain Reaction

Several conventional and real-time PCR assays have been described for detection of *A. platys* DNA. In the absence of clearly identifiable morulae within platelets, molecular diagnostic testing with reliable PCR assays that specifically detect *A. platys* DNA (as opposed to *A. phagocytophilum* DNA or *Anaplasma* species DNA) is the only way to confirm active infection. Real-time PCR assays that detect *A. platys* DNA are available commercially and in combination with assays for other vector-borne pathogens. Suitable specimens include whole blood, buffy coats, or bone marrow and splenic aspirates,[48] although it is not clear which of these specimens is optimal. When buffy coats were used, PCR assays for *A. platys* became positive as early 3 to 5 days after experimental infection, but by day 21, negative results occurred in some dogs.[48,49] The use of splenic or bone marrow aspirates may yield positive results when whole blood or buffy coat PCR for *A. platys* is negative yet infection is still suspected.

Pathologic Findings

Pathologic findings have been described in a small number of dogs that were experimentally infected with *A. platys*, and necropsied early in the course of infection.[50] The only gross necropsy finding was generalized lymphadenomegaly. Lesions consisted of reactive lymphoid hyperplasia and erythrophagocytosis by sinusoidal macrophages within lymph nodes and the spleen, crescent-shaped regions of perifollicular hemorrhage in the spleen, mild lymphoplasmacytic infiltrates in the renal interstitium, and multifocal hyperplasia of Kupffer's cells in the liver. Megakaryocyte numbers in the bone marrow were increased in some dogs.

Treatment and Prognosis

The recommended treatment for thrombocytopenic dogs infected with *A. platys* is doxycycline. The optimum dose and duration of treatment is unknown, but it is apparently eliminated using regimens effective for treatment of *E. canis* infection.

Infection could not be detected with PCR for a 3-week follow-up period after just 8 days of doxycycline treatment (10 mg/kg PO q24h), which was initiated in the acute phase of infection.[51] However, one dog remained infected after 2 weeks of treatment with tetracycline. In another study, doxycycline treatment was administered for 28 days, and infection could not be detected after this time point, even after pharmacologic immunosuppression with dexamethasone several months later.[49] Whether the duration of infection at the time of treatment affects treatment efficacy is unknown.

Prevention

See the previous discussion of *Anaplasma phagocytophilum* infection.

Public Health Aspects

Infection with *A. platys* has not been confirmed in humans.

CASE EXAMPLE

Signalment: "Copper," an 8-year old male neutered Labrador retriever from Napa, CA

History: Copper was brought to the University of California, Davis, VMTH emergency service for the problems of lethargy, inappetence, apparent neck pain of 1 week's duration, and fever. The dog had been taken to a local veterinary clinic on the day the illness began, and thrombocytopenia (113,000 platelets/μL) was detected on a CBC. ELISA serology for antibodies to *E. canis*, *B. burgdorferi*, and *A. phagocytophilum* and *Dirofilaria immitis* antigen (4Dx SNAP test, IDEXX Laboratories) was negative. Treatment with enrofloxacin (2.4 mg/kg PO q12h) and metronidazole was instituted, during which time the dog improved clinically, but when the metronidazole was discontinued after 5 days, lethargy, inappetence, and signs of neck pain returned 2 days later. A CBC at that time showed no clinically significant abnormalities. Treatment with enrofloxacin was continued, and methocarbamol (11.6 mg/kg PO q8h) and meloxicam (0.1 mg/kg PO q24h) were instituted for the neck pain. The owner took Copper's rectal temperature at home on the morning he was brought to the VMTH, and it was 105.6°F (40.9°C). Copper lived on a 20-acre winery with a reservoir and had access to wildlife and frequent tick exposure. There had been no known exposure to toxins and no travel history. The dog received monthly heartworm preventative, and flea and tick preventative was used, but only when the owner found ticks. He was normally fed a commercially available dry dog food.

Current Medications: Enrofloxacin (2.4 mg/kg PO q12h), methocarbamol (11.6 mg/kg PO q8h), and meloxicam (0.1 mg/kg PO q24h).

Other Medical History: Nine months previously, Copper was seen by the local veterinary clinic for neck pain. The dog was treated with methocarbamol, a nonsteroidal antiinflammatory drug, and rest, and the pain resolved after 7 days. The owners also reported that Copper had exhibited some weakness of his left pelvic limb for several years.

Physical Examination:

Body weight: 43.1 kg

General: Quiet, alert, responsive, adequately hydrated. T = 103.7°F (39.8°C), HR = 110 beats/min, panting, mucous membranes pink, CRT <2 s.

Integument: No clinically significant abnormalities were identified. No ectoparasites were noted.

Eyes, Ears, Nose, and Throat: Mild gingivitis and dental calculus were present. No other significant abnormalities were noted.

Musculoskeletal: Body condition score 4/9. The dog was ambulatory but had a slightly stiff thoracic limb gait. A decreased range of motion of coxofemoral joints was detected, with the left worst than the right. There was no evidence of joint pain or effusion.

Cardiovascular, Respiratory Systems, Gastrointestinal and Urogenital Systems: No clinically significant findings were identified. Rectal examination revealed no significant abnormalities.

Lymph Nodes: All peripheral lymph nodes were normal sized but slightly firm on palpation.

Neurologic Examination: Mentation and cranial nerve function were within normal limits. The dog was reluctant to move his neck on manipulation, especially to the right side, and palpation of the cervical spine elicited pain. Decreased placing reactions were noted in the left pelvic limb.

Laboratory Findings:

CBC:

HCT 35.8% (40%-55%)
MCV 64 fL (65-75 fL)
MCHC 35.8 g/dL (33-36 g/dL)
Reticulocyte count 10,200 cells/μL (7000-65,000 cells/μL)
WBC 7100 cells/μL (6000-13,000 cells/μL)
Neutrophils 6603 cells/μL (3000-10,500 cells/μL)
Band neutrophils 284 cells/μL
Lymphocytes 71 cells/μL (1000-4000 cells/μL)
Monocytes 142 cells/μL (150-1200 cells/μL)
Platelets 37,000/μL (150,000-400,000 platelets/μL)
MPV 17.2 fL (7-13 fL).

Slight toxicity was detected in neutrophils and band neutrophils, and multiple neutrophils contained basophilic intracytoplasmic inclusions (morulae) (see Figure 29-3).

Serum Chemistry Profile:

Anion gap 23 mmol/L (10-24 mmol/L)
Sodium 145 mmol/L (145-154 mmol/L)
Potassium 3.7 mmol/L (3.6-5.3 mmol/L)
Chloride 115 mmol/L (108-118 mmol/L)
Bicarbonate 11 mmol/L (16-26 mmol/L)
Phosphorus 3.9 mg/dL (3.0-6.2 mg/dL)
Calcium 10.2 mg/dL (9.7-11.5 mg/dl)
BUN 12 mg/dL (5-21 mg/dL)
Creatinine 0.7 mg/dL (0.3-1.2 mg/dL)
Glucose 61 mg/dL (64-123 mg/dL)
Total protein 6.8 g/dL (5.4-7.6 g/dL)

Albumin 3.1 g/dL (3.0-4.4 g/dL)
Globulin 3.7 g/dL (1.8-3.9 g/dL)
ALT 54 U/L (19-67 U/L)
AST 26 U/L (19-42 U/L)
ALP 106 U/L (21-170 U/L)
Creatine kinase 79 U/L (51-399 U/L)
GGT < 3 U/L (0-6 U/L)
Cholesterol 255 mg/dL (135-361 mg/dL)
Total bilirubin 0.1 mg/dL (0-0.2 mg/dL)
Magnesium 2.1 mg/dL (1.5-2.6 mg/dL).

Urinalysis: SGr 1.031; pH 8.0, 25 mg/dL protein, no bilirubin, 25 erythrocytes/μL hemoprotein, no glucose, 0-3 WBC/HPF, 15-25 RBC/HPF, no other significant abnormalities were detected.

Imaging Findings:

Spinal Radiographs: Multiple right lateral projections of the spine were reviewed. There were multiple sites of mild ventral spondylosis deformans throughout the thoracolumbar spine. Severe spondylosis was seen at the lumbosacral space with sclerosis of the endplates either side, most likely due to degenerative change. There was severe osseous remodeling of the lumbar articular facets, which was consistent with osteoarthrosis. Small areas of intervertebral disc mineralization were seen at the L2-3 and L4-5 spaces.

Thoracic Radiographs: The cardiovascular and pulmonary structures were within normal limits. There was slightly increased soft tissue opacity in the region of the sternal lymph node on the right lateral projection.

Abdominal Ultrasound: The liver was mildly hypoechoic but normal in size. The spleen was markedly enlarged but had normal echogenicity. The rest of the abdominal organs were within normal limits.

Microbiologic Testing: Aerobic bacterial urine culture: No growth

ELISA serology for vector-borne pathogens (IDEXX 4Dx SNAP test): Positive for antibodies to *A. phagocytophilum*. Negative for antibodies to *E. canis* and *B. burgdorferi*. Negative for *D. immitis* antigen.

IFA serology for vector-borne pathogens: Positive for antibody to *A. phagocytophilum* (1:2560). Negative for antibodies to *R. rickettsia* at <1:40. Negative for antibodies to *Babesia canis* at <1:40. Weak positive for antibody to *E. canis* at 1:40.

Vector-borne real-time PCR panel (whole blood): Positive for *A. phagocytophilum* (Msp gene PCR). Negative for *Anaplasma platys*, *Bartonella* spp., *Borrelia burgdorferi*, *Ehrlichia canis*, and *Rickettsia* spp. DNA.

Diagnosis: Granulocytic anaplasmosis

Treatment: Copper was treated with doxycycline (5 mg/kg PO q12h) the day after admission, when results of the CBC were available. In the interim, pain was managed with meloxicam and hydromorphone, and IV crystalloid fluids were administered. A day later, the fever had resolved and the dog's appetite and neck pain had improved, but thrombocytopenia persisted (36,000 platelets/μL). Two days after initiation of doxycycline treatment, the platelet count was 302,000 platelets/μL, and all other hematologic variables that were abnormal had returned to the reference range. At a recheck examination, 2 weeks after discharge, Copper was doing well, with no evidence of pain or stiffness. A CBC showed a platelet count of 487,000 platelets/μL.

Comments: This was an unusual case of granulocytic anaplasmosis in a dog that developed apparent cervical pain in association with infection. The dog also had osteoarthritis, but clinical signs resolved almost completely once doxycycline was instituted. Although the dog's joints did not appear painful or swollen on physical examination, it is possible that polyarthritis may have contributed to the clinical signs of stiffness. Serology was initially negative because infection was too recent for an antibody response to have developed. The diagnosis of granulocytic anaplasmosis was confirmed with molecular diagnostic testing and supported by seroconversion to *A. phagocytophilum* and identification of morulae within neutrophils. The weak positive IFA test result to *E. canis* may have reflected serologic cross-reactivity. Treatment with enrofloxacin was apparently ineffective. The need for more consistent application of tick preventatives was discussed with the owners.

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Carrade DD, Foley JE, Borjesson DL, et al. Canine granulocytic anaplasmosis: a review. J Vet Intern Med. 2009;23:1129-1141.

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