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Two groups in Anaplasmataceae: Group 1- infects red blood cells; Group 2 - infects other blood cells

Animal Group(s) Affected	Transmission	Clinical Signs	Severity	Treatment	Prevention and Control	Zoonotic
Group 1 – Ruminants Group 2 - Wide range of mammals (including humans)	Group 1+2 - Biological transmission via ticks; mechanical transmission by infected blood cell transfer; transplacental Group 1 mechanical transmission (e.g. biting flies)	Group 1 Anemia, lethargy, pale mucous membranes Group 2 Headache, pyrexia, chills, myalgia, Anemia.	Group 1 - Severity increases with age Group 2 - typically mild, more severe in mature or immuno-suppressed animals, or with co-infections	Group 1 – Oxytetra-cycline, chlortetra-cycline (in feed) Group 2 - doxycycline	Group 1 - Control ticks and biting flies; prevent entry of carriers; vaccination; prophylactic antibiotics Group 2 - control tick exposure	Group 1 - no Group 2 - yes

Fact Sheet compiled by: Dawn Zimmerman; updated by Dorothy Geale

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Fact Sheet Reviewed by: Katherine Kocan; Alvin Gajadhar

Susceptible animal groups:
Group 1 - Cattle, sheep, goats, mule deer and other ruminants.
Group 2 - Mammals: humans, cattle, sheep, goats, pigs, domestic dogs and cats, small mammals; birds and many wildlife species.

Causative organism: Obligate intracellular bacteria: Order Rickettsiales, Family Anaplasmataceae, Genus *Anaplasma*.
Group 1 - infect red blood cells (*A. marginale, A. ovis*)
Group 2 – infects white blood cells and platelets (*A. phagocytophilum, A. bovis, A. platys*)
Biological transmission by ticks occurs in both groups. Iatrogenic mechanical transmission by transfer of infected blood cells occurs in Group 1 by contaminated scalpels, needles or tattooing, dehorning and castration equipment and in Group 2, in humans by blood transfusions or organ transplants. Natural mechanical transmission is effected in Group 1 by biting flies (most commonly horse and stable flies). For Group 1, transplacental transmission is reported especially with acute infection in the 2nd or 3rd trimester. It also occurs in Group 2 but its epidemiological role is not well characterized for either group.

Group 1
● *A. marginale* infects cattle and is the agent of bovine anaplasmosis. Major reservoirs are cattle and ticks. Less pathogenic is *A. marginale* subspecies *centrale* which is never reported in North America and used as a live vaccine strain in South Africa, Israel and some South American countries.
● *A. ovis* infects sheep, goats, deer (mule deer and reindeer) primarily in North America.
Group 2

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● *A. phagocytophilum* (previously *Ehrlichia phagocytophila*, *E. equi*, and the human granulocytic ehrlichiosis agent) infects a wide variety of mammals including ruminants (cattle, white tailed deer), horses, rabbits, pigs and small rodents (e.g., white-footed mice, wood rats, gray squirrels).
● *Anaplasma bovis* (previously *Ehrlichia bovis*) infects cattle, deer which are reservoirs in Asia and Africa.
● *Anaplasma platys* (previously *Ehrlichia platys*) is reported in dogs and rarely in cats, impala, and sheep in Asia, Europe and South America and is the only *Anaplasma* to infect platelets.

Zoonotic potential: Group 1 *Anaplasma* sp. are not infective for humans and therefore not zoonotic. *A. phagocytophilum* of Group 2, first described in sheep in Europe, has become an emerging pathogen of humans in both Europe and the U.S. It is transmitted by *Ixodes* ticks as biological vector. Blood transfusions or organ transplants pose a risk as the organism infects blood cells.

Distribution:
Group 1 – *A. marginale* occurs worldwide and in all states of US, except Hawaii. It is endemic throughout the Gulf Coast states and several of the Midwestern and Western states. Outbreaks are often seasonal and coincide with the emergence of arthropod vectors in warmer months (spring, early summer, and early fall).
Group 2 - *A. phagocytophilum* also occurs worldwide and in US primarily in the west, upper Mid-west and northeast but future distribution may change with tick vectors. States reporting the highest incidence in 2010 included Minnesota, Wisconsin, New York, New Jersey, Rhode Island, and Connecticut. Norway, UK, Sweden, Switzerland, and Germany have reported infections in ruminants, dogs, and people. *A. phagocytophilum* is less frequently reported in Asia and South America.

Incubation period:
Group 1 - ranges from 7 to 60 days (*A. marginale*, *A. ovis*, *A. marginale* subsp. *centrale*) depending on dose. Rickettsemia doubles every 24 hours with acute clinical disease in 7-10 days in susceptible species.
Group 2 – ranges from 7 to 14 days in humans, sheep and dogs.

Clinical signs:
Group 1 – Clinical signs are highly variable, ranging from subclinical infection in calves under a year to severe peracute disease in adult naïve cattle, characterized by significant production losses (milk or weight gain), bull infertility, severe anemia, icterus, inappetance, dehydration, constipation, dark yellow urine, weight loss, pyrexia, abortion and death. The acute form generally occurs in cattle from 1-3 years old, with similar but more moderate clinical signs. All recovered animals become persistent carriers and reservoirs of infection for life.
Group 2 – Humans (*A. phagocytophilum*): Clinical disease is associated with acute parasitemia of which the duration and severity is variable. Co-infection with other pathogens results in greater severity of symptoms. Genetic variants of *A. phagocytophilum* and be associated with mild or flu-like clinical signs or rash in 10% of patients. The aged and immunosuppressed show more severe signs. These signs include headache, pyrexia, chills, myalgia, nausea, ataxia, organ failure, susceptibility to opportunistic infections, neuritis or respiratory complications. The US case fatality rate from 2000-2010 was 1% with a rise in incidence from 1.4 to 6.1 cases per million.
Dogs (*A. platys*; *A. phagocytophilum*): Most often disease is subclinical or a mild, flu-like presentation that is self-limiting. Animals with an acute infection often have vague signs including pyrexia, malaise, lethargy, anorexia, and general muscle pain resulting in reluctance to move. *A. phagocytophilum* most commonly causes clinical disease in dogs older than 8 years with joint pain and lameness so it must be distinguished from Lyme disease. Gastrointestinal, respiratory and neurological signs may also occur. Infections may be subclinical or in a carrier state. In endemic areas, over 40% of dogs may be

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seropositive, while morbidity is low. Dogs co-infected with *Borrelia burgdorferi* and *A. phagocytophilum* are nearly twice as likely to develop clinical disease. *A. platys* produces clinical disease related to a cyclic thrombocytopenia (typically <20,000/µl for 1-2 days then repeats in 1-2 weeks). Although usually mild, more severe clinical signs including pyrexia, lethargy, pale mucus membranes, petechial hemorrhages, epistaxis, and lymphadenopathy occur.

Other animals *(A. phagocytophilum*): Sheep may have mild clinical signs of inappetance, lethargy with abortions in ewes. Similarly in endemic areas, dairy cattle exhibit abortions, drop in milk production and respiratory disease. Complications occur due to secondary bacterial infections (e.g., pastuerellosis, listeriosis). Horses may have acute onset with older animals developing fever, lethargy, inappetance, limb edema while young animals typically have mild disease. A report in maned wolves (*Chrysocyon brachyurus*) describes coughing and tachypnea due to severe pulmonary congestion, splenomegaly, ataxia, anorexia, lethargy, dehydration; mild jaundice, petechiation; leukocytosis, anemia, hyperfibrinogenemia.

Post mortem, gross, or histologic findings:

Group 1 - include anemia, jaundice, splenomegaly and the liver may be enlarged with a deep orange color. Hepatic and mediastinal lymph nodes may appear brown and the gall bladder distended with thick brown or green bile. Serous effusions may occur in body cavities, edema, petechial hemorrhages in the epi- and endocardium often accompanied by severe gastrointestinal stasis. Reticuloendothelial phagocytosis of erythrocytes may be evident microscopically in various organs, most notably in the spleen.

Group 2 - *A. phagocytophilum* is only one of four human neutrophil intracellular organisms forming morulae (cytoplasmic vacuole containing multiple coccoid to ellipsoid basophilic rickettsia) approximately 1.5 µm to 2.5 µm in diameter (reported up to 6 µm). During acute rickettsemia, the organism has been demonstrated in the alveolar macrophages, Kupffer cells, and other tissue macrophages. Sites of persistence in-between recurrent rickettsemia remain to be established.

Diagnosis:

Hematology

Group 1 - Initially based on clinical signs, history of tick exposure and clinical pathology (lymphopenia, mild to severe thrombocytopenia, mild to moderate nonregenerative anemia, elevated ALP, mild to moderate hypoalbuminemia and hyperfirbrinogenemia may occur). In the acute phase, the presence of characteristic intracellular inclusions (marginal bodies) on Giemsa/Wright's/Diff-Q-stained blood smears (buffy coat recommended) along the margins of the erythrocyte (*A. marginale*) or more centrally (*A. marginale* subsp. *centrale).*

Group 2 - Neutrophils infected by *A. phagocytophilum* (1-27%) contain distinctive granulocytic morulae which appear in the peripheral blood at 4-14 days and persist up to 8 days. In animals with polyarthritis, synovial fluid may exhibit decreased viscosity and an increased leukocyte count (>3000 cells/µl; predominantly neutrophils but ≤1% may contain morula). *A. platys* morulae may be found in circulating platelets. Hematology is not reliable for pre-symptomatic or carriers.

Antibody serology

Group 1 - Enzyme-linked immunosorbent assay (ELISA) marketed by VMRD will not differentiate between *Anaplasma* species as the test is based on major surface protein 5 (MSP5) which is highly conserved in the genus. The reported *A. marginale* sensitivity 95% and specificity 98% is limited by cross-reactivity, low early sensitivity and low specificity for true negative cattle after oral chlortetracycline treatment. The complement fixation (CF) and card agglutination tests (CAT) are no longer considered to be valid and thus not used for diagnosis of bovine anaplasmosis.

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Group 2 - ELISA (IDEXX SNAP 4Dx) for *A. phagocytophilum* (sensitivity 99.4%, specificity ~100%), reportedly detects as early as 8 days post-inoculation (dogs with *Ehrlichia ewingii)* do not likely cross-react, some cross-reactivity with *A. platys*). The indirect immunofluorescence assay (IFA) detects a 4-fold increase in IgG-specific antibody titer to *A. phagocytophilum* antigen in paired serum samples (taken the 1st week of illness and 2-4 weeks later). Note: IgM tests are not always specific, and that the IgM response may be persistent. Seroconversion in dogs may occur as soon as 2-5 days after morulae appear in the peripheral blood. Positive titer ≥1:80, most will have titer ≥1:320.

Antigen detection

Group 1 + 2 - polymerase chain reaction (PCR) is the most specific method (nested PCR theoretically detects 0.0000001% rickettsemia or 30 infected red blood cells per ml); can distinguish between species, but poses problems due to non-specific amplification, requiring confirmation of the amplified fragment (sequencing). Should be repeated if negative in suspect carriers (*A. marginale*).

Other means of diagnosis

Group 1 + 2 – Immunohistochemistry can demonstrate *Anaplasma* antigen in a biopsy/necropsy sample. The organism can be isolated in cell culture (mainly research as it is impractical for clinical cases). The gold standard for *A. marginale* is the demonstration of the organism 4-8 weeks after inoculation of suspect blood into splenectomized calves.

Material required for laboratory analysis: For both Group 1 + 2:
Anticoagulant blood, thin and thick blood films.
At necropsy: thin blood films of liver, kidney, spleen, lungs; and peripheral blood.
PCR: whole blood (EDTA)
Sample blood prior to starting antimicrobials to avoid false negative test results.

Relevant diagnostic laboratories: *Anaplasma* sp. can be diagnosed at most accredited diagnostic laboratories using ELISA (in-house SNAP 4Dx, IDEXX for *A. phagocytophilum*); cELISA for *A. marginale* (VMRD, Antech (FastPanel™ PCR Canine Ehrlichiosis/Anaplasmosis Profile) for *A. platys* and *A. phagocytophilum*, cross-reacts with *Ehrlichia*. Zoologix PCR (*A. platys*) also offers tickborne disease PCR panel, that includes *A. platys*.

Treatment:

Group 1 – In acute outbreaks, parenteral oxytetracycline (cattle) is used as recommended by extension specialists. The survival rate is high in the early stages of the disease (PCV >15%). Blood transfusions, electrolyte solutions, and hematinic drugs may be beneficial in later stages of the disease. Convalescent period of up to 3 months. Cattle remain immune for life but become persistent carriers.

Group 2 - Treatment may be difficult as clinical signs often do not appear until the disease has progressed. Testing for co-infections with other tick-borne organisms is recommended. Tetracycline antibiotics (usually doxycycline in humans and dogs) for 10-14 days (or at least until 3 days after fever subsides). A marked improvement is usually seen in 24-48 hours.

Prevention and control: Group 1 + 2 organisms need control of tick vectors. Additionally for Group 1, strict sanitation with needles and surgical/dehorning instruments; remove carriers from herd; and chlortetracycline during vector season (medicated salt-mineral blocks or feed blocks). In South Africa, Australia, Israel, and South America, live *A. centrale* is used as a vaccine. In the US, USDA approved killed vaccines (Anaplaz®, Plazvax®) are no longer available. A conditional killed vaccine made from a Mississippi strain is available in some southern states. In California, a modified live vaccine (Anavac®) is available for cattle ≤11 months but lacks efficacy and is rarely used.

Suggested disinfectant for housing facilities: No disinfectant. Application of acaricides and removal

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of leaf litter/brush (tick habitat) can be effective.

Notification: *A. marginale* is a reportable disease in ~ 30 states and tracked nationally through National Animal Health Reporting System (USDA) in ~ 48 states. *A. phagocytophilum* is monitored through National Notifiable Disease System (CDC).

Measures required for introducing animals to infected animal: Infected animals become carriers and act as a reservoir of infection for life. In endemic areas, early infection or vaccination in cattle promotes life-long immunity. With no killed vaccines available in the US, separation of carriers and non-infected introduced mature animals is essential.

Conditions for restoring disease-free status after an animal outbreak:

Group 1 - Carriers may relapse when immunosuppressed (e.g. corticosteroids), when infected with other pathogens, or after splenectomy. As a lifelong reservoir of infection, they should be removed. No anti-microbials are approved in the US for eliminating *A. marginale* infections in cattle. Allegedly, the carrier state may be eliminated with a long-acting oxytetracycline treatment, but experimental work at Kansas State University in the mid -2000s found chemosterilization inconsistent. Although, long term oral tetracycline at high extra-label doses had greater success, parenteral administration was less so, and conflicting results suggests differences in susceptibility of *A. marginale* strains. Chemosterilized animals are fully susceptible to re-infection.

Experts who may be consulted:

Katherine M. Kocan
Oklahoma State University
Veterinary Pathobiology
004 McElroy Hall
Stillwater, OK 74078
Phone: (405) 744-7271
Fax: (405) 744-5275
Katherine.Kocan@okstate.edu

References:

1. Aubry, P., and D.W. Geale. 2011. A review of bovine anaplasmosis. Trans. Emerg. Dis. 58: 1-30.
2. CDC. Anaplasmosis. www.cdc.gov/ticks/diseases/anaplasmosis/ Accessed 13 September 2013.
3. Dumler, J. S., A. F. Barbet, C. P. Bekker, G. A. Dasch, G. H. Palmer, S. C. Ray, Y. Rikihisa, and F. R. Rurangirwa. 2001. Reorganization of genera in the Families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila*. Int. J. Syst. Evol. Microbiol. 51: 2145-2165.
4. Dumler, J.S., J.E. Madigan, N. Pusterla, and J.S. Bakken. 2007. Ehrlichioses in humans: epidemiology, clinical presentation, diagnosis, and treatment. Clin. Inf. Dis. 45 (Suppl): S45-51.
5. Foley, J. 2000. Human ehrlichiosis: a review of clinical disease and epidemiology for the physician. Inf. Dis. Clin. Pract. 9: 93-98.
6. Iowa State University. 2013. Ehrlichiosis and anaplasmosis: zoonotic species. The Center for Food Security & Public Health and the Institute for International Cooperation in Animal Biologics. http://www.cfsph.iastate.edu/Factsheets /pdfs/ehrlichiosis.pdf. Accessed 11 September 2013.
7. Kocan, K.M., J. de la Fuente, A.A. Guglielmone, and R.D. Melendéz. 2003. Antigens and

alternatives for control of *Anaplasma marginale* infection in cattle. Clin. Microbiol. Rev. 16: 698-712.

8. Kocan, K.M., J. de la Fuente, D.L. Step, E.F. Blouin, J.F. Coetzee, K.M. Simpson, S.G. Genova, and M.J. Boileau. 2010. Current challenges of the management and epidemiology of bovine anaplasmosis. Bov. Pract. 44(2): 93-102.
9. Kocan, K.M., J.F. Coetzee, D.L. Step, J. de la Fuente, E.F. Blouin, E. Reppert, K.M. Simpson, M.J. Boileau. 2012. Current challenges in the diagnosis and control of bovine anaplasmosis. Bov. Pract. 46: 67-77.
10. Merck Veterinary Manual. Anaplasmosis. http://www.merckmanuals.com/vet/index.html Accessed 11 September 2013.
11. Padilla, L.R., A. Bratthauer, L.H. Ware, V. Acosta, K.L. Hope, J. Siegal-Willott, and S. Murray. 2010. *Anaplasma phagocytophilum* infection in captive maned wolves (*Chrysocyon brachyurus*) at the Smithsonian Conservation Biology Institute. Proc. AAZV AAWV Joint Conference, South Padre Island, Texas: 162.
12. Reinbold, J.B., J.F. Coetzee, L.C. Hollis, J.S. Nickell, C. Riegel, K.C. Olson, and R.R. Ganta. 2010: The efficacy of three chlortetracycline regimens in the treatment of persistent *Anaplasma marginale* infection. Vet. Microbiol. 145(1-2): 69-75.
13. Woldehiwet, Z. 2010. The natural history of *Anaplasma phagocytophilum*. Vet. Parasitol. 167: 108- 122.