Network for wildlife health surveillance in Europe

Diagnosis Card

Anaplasmosis

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Etiology

The genus *Anaplasma* (Rickettsiales: Anaplasmataceae), reorganized by Dumler et al., (2001), currently includes 3 groups of obligate intracellular pathogens: (1) 3 species that infect erythrocytes of ruminants (*A. marginale*, *A. centrale*, and *A. ovis*); (2) 3 species that infect leukocytes (*A. phagocytophilum, A. bovis* and *A. platys*) and (3) one species, *Aegyptianella,* which infects erythrocytes of birds. This fact sheet will focus on *A. marginale* and *A. phagocytophilum* because these pathogens cause diseases in humans and domestic animals (cattle and sheep) or wild animals with economic impact. *A. marginale* is transmitted biologically by ticks (most notably *Dermacentor* and *Rhipicephalus*), as well as mechanically by transfer of infective blood on fomites or the mouthparts of biting insects, and, less commonly by transplacental transmission from dams to their calves. While *A. marginale* is host-specific for ruminants, *A. phagocytophilum,* which has a wide host range, is transmitted by *Ixodes* sp., with *I. ricinus* are the primary tick vector of *A. phagocytophilum* in Europe.

Affected species (wildlife, domestic animals, humans)

A. marginale: *(the type species)* is host-specific for ruminants, causes disease primarily in cattle (bovine anaplasmosis), but also infects bison. *A. centrale* is a less pathogenic species that also infects cattle, which is used as a live vaccine in Israel and South Africa. *A. ovis* is a pathogen of wild and domestic sheep, goats and some wild deer species (mule deer in the U.S.). Some species of deer are also reported to become infected without known pathology.

A. phagocytophilum: has a wide host range including humans (human granulocytic anaplasmosis, HGA), dogs (canine granulocytic anaplasmosis, CGA), horses (equine granulocytic anaplasmosis, EGA), sheep (tick-borne fever, TBF). This pathogen is also infective for other species including, deer, pigs, rabbits, rodents, dogs, cats and birds without known pathology. HGA is an emerging disease of humans in the United States and is also becoming recognized in humans in Europe.

Epidemiological characteristics and disease course

A. marginale: Bovine anaplasmosis occurs worldwide in tropical and subtropical areas and the disease is a major constraint to cattle production in many countries. Infected erythrocytes are removed from the host reticuloendothelial system, and the decrease in erythrocytes often results in anemia which is associated with the clinical symptoms and pathology of the disease. Within bovine erythrocytes, membrane bound inclusions, commonly referred to as initial or marginal bodies, which contain from 4-8 rickettsiae, and as many as 70% or more of the erythrocytes may be parasitized during acute infections. Notably, the incubation period of infection (prepatent period) varies with the infective dose and can range from 7 to 60 days, with an average of 28 days. After being detected in the blood, the number of parasitized erythrocytes increases geometrically. Cattle that survive acute infection develop life-long persistent infections characterized by five to six week cycles of low-level rickettsemia. Persistently infected or “carrier” cattle are considered to be infected for life, have life- long immunity and upon challenge-exposure, do not exhibit clinical disease. Calves infected by transplacental transmission also become persistent carriers and are protected against challenge-exposure. Importantly, cattle persistently infected with *A. marginale* are the most important reservoir of the pathogen for infection of ticks or for mechanical transmission after transfer of infected blood to susceptible cattle.

The authors are responsible for the final contents of the card. Please refer to this card when you publish a study for which the APHAEA protocol has been applied. Reference suggestion: «This method is recommended by the EWDA Wildlife Disease Network (www.ewda.org)»; citation: Authors, Year, APHAEA/EWDA Diagnosis card: [name of disease], www.ewda.org

A. phagocytophilum: *A. phagocytophilum* has been detected in mammals and ticks throughout the world, and has been reported to be the most widespread tick-borne infection in animals in Europe. Most notably *A. phagocytophilum* is a well established pathogen of small ruminants, especially sheep in which it causes tick borne fever (TBF). Clinical presentations of *A. phagocytophilum* infection have been documented in sheep, goats, cattle, horses, dogs, cats, roe deer, reindeer, and in humans and, recently, human granulocytic anaplasmosis (HGA) was considered as an emerging tick-borne disease of humans in the United States, Europe and Asia. Strains or variants of *A. phagocytophilum* and the resulting diseases vary with strain, host and geographic location. In contrast to *A. marginale*, *A. phagocytophilum* has a wide host range that coincides with the extensive distribution of the *Ixodes* vectors, and an increase in the number of reservoir hosts will likely contribute to the spread of the disease. Infection of animals with *A. phagocytophilum* may cause immunosuppression, increasing susceptibility to secondary infections which may contribute to the clinical signs.

Clinical signs

A. marginale: The clinical signs of bovine anaplasmosis are directly related to the erythrocytic parasitemia, which results in mild to severe anemia due to extravascular hemolysis and icterus without hemoglobinemia or hemoglobinuria. Clinical signs are more pronounced in cattle over 2 years and may include fever, weight loss, abortion, lethargy, pale mucous membranes, icterus, and often death. Cattle that survive acute infection develop life-long persistent infections characterized by five to six week cycles of low level rickettsemia.

A. phagocytophilum: *A. phagocytophilum* infects neutrophils in the vertebrate host where the pathogen multiplies within a parasitophorous a vacuole called a “morulae” that is approximately 1.5 µm to 2.5 µm in diameter. A prepatent period of 4-7 days occurs between transmission from the tick bite and development of rickettsemia. During the rickettsemia, the main targets for *A. phagocytophilum* infection include the eosinophils, monocytes, and primarily neutrophils. In *A. phagocytophilum* infection in sheep, goats and cattle, as many as 90% of the granulocytes may become infected, but the severity of infection and febrile reaction is dependent on the strain and the susceptibility and immune status of the host. In sheep, cattle and horses rickettsemia is accompanied by fever of an approximate 7 days of duration. Notably, clinical signs are related to the variant and host and both infection and clinical signs may not be apparent. While fever is the main clinical sign, pyaemia in lambs, respiratory signs in cattle and secondary infections that appear some days after being introduced to tick-infested pastures are indicators with TBF. Dairy cattle may experience reduced milk production. The severe leukopenia and especially the prolonged neutropenia that accompanies the disease are also are evident with TBF. In some cases abortions may occur, especially when pregnant ewes or cows are moved to tick-infested pastures during the third trimester. Equine and canine infections are characterized by fever, depression, anorexia, leukopenia, and thrombocytopenia. The symptoms of human patients are most commonly fever; chills, headache and myalgias.

Gross lesions

A. marginal: Lesions of bovine anaplasmosis are typical of those occurring in animals with anemia due to erythrophagocytosis. The carcasses of cattle that die from anaplasmosis are generally markedly anemic and jaundiced and the blood is thin and watery. The spleen is characteristically enlarged and soft, with prominent follicles, and the liver may be mottled and yellow-orange; the gallbladder is often distended and contains thick brown or green bile. Hepatic and mediastinal lymph nodes appear brown and serous effusions can be found in the body cavities. Pulmonary edema, petechial hemorrhages in the epi- and endocardium are notable, and often evidence of severe GI stasis.

A. phagocytophilum: Infection with *A. phagocytophilum* ranges from the absence of clinical signs or pathologic changes to severe leukopenia due to lymphocytopenia, neutropenia and thrombocytopenia. Anemia has been reported as a feature of infection in cattle. The main pathologic findings are also those of lymphoid depletion of the spleen, with little effect on the bone marrow. Therefore, the leukopenia is likely to be due to the sequestration of infected granulocytes; the bone marrow of infected humans was reported to be normocellular or hypercellular. The pathologic changes in humans include perivascular lymphohistiocytic inflammatory infiltrates in multiple organs, hepatitis with infrequent apoptoses, normocellular bone marrow, mild lymphoid depletion, mononuclear phagocyte hyperplasia in spleen and lymph nodes, and, rarely, splenic necrosis.

Histological lesions

A. marginale: Widespread phagocytosis of erythrocytes is evident on microscopic examination of the reticuloendothelial organs. A significant proportion of erythrocytes are usually found to be parasitized after death due to acute infection.

A. phagocytophilum: The host cells for *A. phagocytophilum* are those of neutrophil lineage. During the rickettsemia, the main targets for *A. phagocytophilum* infection include the eosinophils, monocytes, and primarily neutrophils, and infection of these cells occurs by the end of the initial rickettsemia. in

sheep, goats and cattle, as many as 90% of the granulocytes may become infected, but the severity of infection and the febrile reaction is influenced by the *A. phagocytophilum* strain, the susceptibility and immune status of the host. Hemophagocytosis is observed in bone marrow, liver, and spleen. *A. phagocytophilum* is rarely identified immunohistochemical tests in histologic lesions.

Differential diagnosis

A. marginale: Icterus and anemia of different causes, anthrax, leptospirosis, emaciation caused by parasitism, babesiosis and malignant lymphoma. Microscopic examination of Giemsa-stained thin and thick blood films is critical to distinguish anaplasmosis from babesiosis and other conditions that result in anemia and jaundice, such as leptospirosis and theileriosis. Blood in anticoagulant should also be obtained for hematologic testing.

A. phagocytophilum*:* The differential diagnosis of fever following tick exposure should include other tick-borne infectious diseases (depending on the species and geographic area) such as monocytic ehrlichiosis, babesiosis, ehrlichiosis and other rickettsial infections.

Criteria for diagnosis

A. marginale: Microscopic examination of Giemsa-stained thin and thick blood films is critical to distinguish anaplasmosis from babesiosis and other conditions that result in anemia and jaundice. Blood in anticoagulant should also be obtained for hematologic testing. In Giemsa-stained thin blood films, *Anaplasma* spp appear as dense, homogeneously staining blue-purple inclusions 0.3-1.0 m in diameter.

A. phagocytophilum*:* In sheep and cattle, especially after tick exposure, high fever is the main signal which accompanies bacteremia and usually lasts for approximately 7 days, with possible recurrences of short duration. The febrile reaction may be higher than 41^0 C, and subsequent recurrent fever may also occur.

Recommended diagnostic method(s) and preferred samples (incl. recommended amount and appropriate storage)

A. marginale*:*

Microscopy: Blood in anticoagulant should also be obtained for hematologic testing. In Giemsa-stained thin blood films, *A. marginale* inclusion bodies in erythrocytes appear as dense, homogeneously staining blue-purple inclusions 0.3-1.0 m in diameter.

Culture: Not available for diagnosis.

Nucleic-acid-based tests: Blood samples have been tested experimentally by polymerase chain reaction (PCR), and these DNA-based tests, most notably *msp4* assays, are capable of detecting the presence of low-level infection in carrier cattle and tick vectors. A nested reaction is necessary to identify low-level carriers using conventional PCR and nonspecific amplification can occur. Recently, real-time PCR assays with analytical sensitivity equivalent to nested conventional PCR have been developed. However, PCR assays have not been used for routine diagnosis.

Serology: The potentially long prepatent periods and the cyclic rickettsemias of carrier cattle pose challenges to the diagnosis of *A. marginale* infected cattle because of fluctuating antibody levels, and therefore often require repeated serologic testing in order to confirm infection (in-depth information will be presented in a follow-up paper). A competitive enzyme-linked immunosorbent assay (cELISA) is currently the recommended serologic test for *Anaplasma* sp. Cross reactivity between *Anaplasma* spp. occurs because the cELISA is based on the highly conserved major surface protein (MSP)5 with cross-reactivity described between *A. marginale*, *A. centrale and A.ovis*.

A. phagocytophilum:

Microscopy: Intracellular inclusions (morulae) can often be visualized in granulocytes on Wrights- or Giemsa- stained blood smears, but with some host/variant combinations, detection of infected granulocytes may be difficult.

Culture: Diagnosis of *A. phagocytophilum* can be done by cultivation in HL-60 cells inoculated with acute-phase blood. However, tests done using blood samples must be conducted before antibiotic therapy because treatment will rapidly reduce the rickettsemias. Culture may not prove to be an efficient diagnostic method.

Serology: Indirect immunofluorescence assay is the principal test used to detect infection. The acute and convalescent phase serum samples can be evaluated to look for a four-fold change in antibody titer to *A. phagocytophilum*. A cELISA is also commercially available in some countries. A positive ELISA is an indicator that the host was exposed to *A. phagocytophilum* but does not confirm active infection. Cross-reactivity in cELISA and IFA assays can occur with some *Anaplasma* and *Ehrlichia* infections.

Nucleic-acid-based tests**:** PCR assays have been developed for experimental studies and used to detect infection in blood and tissue samples but PCR assays are currently not used routinely in diagnostic laboratories.

APHAEA protocol (for harmonization at large scale)

Examination of Giemsa stained blood film combined with PCR.

Laboratories that can be contacted for diagnostic support

Instituto Zooprofilattico Sperimentale della Sicilia, Palermo, Sicily, Italy

SaBio, Instituto de Investigación en Recursos Cinegéticos IREC-CSIC-UCLM-JCCM, Ciudad Real, Spain.

Recommended literature

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