

College of Agriculture and Natural Resources Veterinary Medical Diagnostic Laboratory

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Connecticut

Anaplasma phagocytophila -anaplasmosis : a versatile and highly prevalent tick borne disease in Connecticut and neighboring states

With this early spring, adult ticks of various species have already emerged and got active in southern New England. Lets take this opportunity to re-examine the current knowledge and diagnostic criteria on one of these important tick borne diseases:

Anaplasmosis affects horses, dogs, cats, ruminants and humans in Connecticut and neighboring states. The nomenclature of this bacterium that infects granulocytes has been reviewed. The former Ehrlichia equi and phagocytophila denominations have been unified under a single Anaplasma phagocytophila specie. Therefore, the disease is now called equine or canine anaplasmosis for horses and dogs (formerly granulocytic ehrlichiosis). For ruminants, it is still called bovine or ovine ehrlichiosis, because "ruminant anaplasmosis" refers to infection with Anaplasma marginale or ovis, intracellular bacteria that infect erythrocytes and not granulocytes (Fig 1).

In our region, *A. phagocytophila* is predominantly transmitted by Ixodes scapularis (Fig 2) as is the case with *Borrelia burgdorferi*. At CVMDL we found 3% of our tick submissions that were positive for both agents over the past 3 years.



Fig. 1 A. Anaplasma phagocytophila infects granulocytes , B. Anaplasma marginale infects erythrocytes . (j. com. med.)

Anaplasmosis is well recognized in **horses** throughout our region. Horses older than 2-years, exhibiting acute fever, lethargy, anorexia and eventually affected by limb swelling or mucous membrane petechiation during tick season are good candidates for testing. A leukopenic (granulocytes and lymphocytes) and thrombocytopenic hemogram reinforces the diagnosis, but definite confirmation requires specific testing (see section below)¹. In 2011, CVMDL tested 119 horses by serology, and found **53%** with positive serological titers (>1/80).

Anaplasmosis in dogs and cats is a recently described disease (1995 & 2004)². Animals have similar clinical signs to those in horses. Dogs additionally may show coughing and lameness.

The most consistent clinicopathologic finding in dogs with anaplasmosis is a moderate to severe thrombocytopenia. The platelet depletion is excacerbated by coinfection with Lyme disease, but even then the lower level of 20.000 pt/ul is not reached. Therefore spontaneous bleeding does not occur typically in dogs³. In 2011, CVMDL tested 6 dogs by serology and all had positive titers.



Fig 2, Graph of real-time PCR for detection of *A. phagocytophila*. Typical specific amplification occurs after 24 cycles.

Ehrlichiosis in ruminants has been well characterized in Europe, in sheep and is also **emerging in cattle**⁴. A serologic survey in Connecticut has demonstrated the serological signature of the agent in cattle⁶.

Laboratory confirmation: Regardless of the species, the good news is that laboratory confirmation is available with **one or two blood tubes**. Two tools are being currently used:

- PCR is the best technique in the acute phase of disease (Fig 2). It is very sensitive and detects *A. phagocytophila* as early as 3 days post-infection in dogs, and lasts for about 2 weeks ⁵. It has made the historical blood smear examination for morulae (Fig 1) obsolete in a diagnostic setting.
- Antibody detection by the serological method of IFA is not as early and as specific as PCR, but it has the advantage of lasting longer (several months in dogs). Dogs seroconvert typically 10 to 20 days after inoculation ^{2,5}. A rise in titer between paired samples is considered the gold standard for serological diagnosis in humans, and it is recommended in low titer results: in horses a 4-fold increase cut-off value has been proposed ¹.

Special offer: We are in the process of validating our PCR testing and will offer **free PCR testing** to samples taken from animals with clinical signs compatible with anaplasmosis during tick season and submitted for serology (until we get enough data for validation). Don't forget therefore to add a purple top tube (EDTA) and request for this on your submission !

Testing for the prevalent tick borne diseases of New England at CVMDL

Pathogen	Species	Method	Sample	turn around time	price
A. phagocytophila	Any	PCR	Blood 2ml in EDTA	3-5 days	\$50.00
A. phagocytophila	Equine, canine, bovine	IFA	Serum 2ml	2-3 days	\$28.00
Lyme Disease	Canine, Equine	ELISA	Serum 2ml	2-3 days	\$17.00
Lyme Disease	Bovine, Caprine, Feline, Ovine, Porcine	IFA	Serum 2ml	2-3 days	\$17.00
Lyme Disease	Bovine, Canine, Equine, Feline	Western Blot	Serum 2ml	5-7 days	\$45.00
Lyme Disease bundle	see above	ELISA/IFA and Western Blot combination	Serum 2ml	5-7 days	\$60.00

These diseases can also be tested directly on ticks by PCR

References

1. Bradford Smith, Large animal internal medicine, 4th edition 2. Green, Infectious diseases of dogs & cats, 3rd ed 3. Mazepa et al, J Am Anim Hosp Assoc. 2010, 405-12

4. Woldehiwet, Ann NY Acad Sci, 2006, 446-60

5. Egenvall et al, Vet Rec, 1998, 10, 412-17

6. Magnarelli et al, ,J Med Microbiol, 2002, 51, 326-331

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