

# Selection and interpretation of diagnostic testing

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## Abstract

It has been said that making a diagnosis is the most difficult undertaking of humans. It can be easy with an overwhelming case to run a battery of diagnostic tests searching for an answer in a challenging case. Further, it can be tempting to enter into a herd-level diagnostic undertaking without a real plan for what to do with the results. As veterinarians, it is our responsibility to perform diagnostic tests with strategy and a plan for each of the possible outcomes. This is what separates us from laypersons who submit samples directly to a diagnostic lab expecting a printout with a plan.

**Key words:** anaplasmosis, leukemia, Johne's, bovine, diagnostics

## Diagnostic discipline

When deciding to run a diagnostic test on an individual animal or a herd, we should answer the following questions prior to submission:

1. What is your diagnostic question?
2. Does this test make significant progress toward answering that question?
3. Are you (or the client) going to take action based on the result/answer?

## Bovine leukemia virus

Bovine leukemia virus (BLV) is an oncogenic retrovirus that causes neoplastic disease (lymphosarcoma) in adult beef and dairy cattle. Tumors may arise in the lymph nodes, abomasum, heart, spinal column and other organs, resulting in eventual death. A veterinarian presented with an individual animal whose physical exam suggests lymphosarcoma may choose from a few diagnostic tests:

ELISA serology detects antibodies to infection by BLV. As it is a retrovirus, a positive antibody test indicates infection. It can be easy, then, in a nonspecifically ill animal, to obtain a positive BLV ELISA and diagnose the animal with lymphosarcoma. We know, however, that likely less than 2% of BLV-positive animals will develop a tumor in their lifetime. For this reason, BLV serology should not be applied generically to a nonspecifically ill animal and used to diagnose lymphosarcoma. All that is confirmed with the positive test is infection. Now that, coupled with multiple palpably enlarged lymph nodes and evidence of masses in other target organs, increases the value of the positive test.

Polymerase chain reaction (PCR) testing is also available on whole blood and may be positive earlier than serology, which is usually not necessary in clinical cases of lymphosarcoma. Demonstration of neoplastic cells provides definitive diagnosis in individual cases. Fine needle aspiration of enlarged lymph nodes has been shown to only be 41% sensitive<sup>1</sup> and, as such, suspected neoplastic tissues should have a biopsy sample obtained.

The first diagnosis of a case on lymphosarcoma on an operation will often result in a knee-jerk response by the owner wanting to test and cull all of their infected cows. This is where the veterinarian's understanding of the disease, along with their discipline to ask the diagnostic accountability questions becomes critical. What is the goal of the test for this producer? To find all the infected cows. Does this test (ELISA) get you down the road to that goal? Yes. Are they willing to take action based on the results? Here's usually where the hang up is. What if 20% of the cows come back positive? Are they going to cull them? Are they going to maintain separate positive and negative herds? If the answer to this is yes, then a strategy for identifying positive cattle and preventing transmission through control of vectors and iatrogenic means should be initiated. If the answer is no, then testing the herd doesn't make sense. That producer's time is better used preventing transmission in the herd. For most commercial producers, once they understand the low prevalence of tumors in infected cattle, they learn to live with the disease rather than put themselves in a position of having to decide whether or not to cull a significant percentage of the herd.

If the decision is made to move forward and screen a group of animals, generally the ELISA is the preferred test. The PCR may be able to detect infection earlier than ELISA but it has a higher cost and has been shown to be unreliable for routine detection of BLV in high prevalence herds,<sup>2</sup> due to low numbers of infected cells at the time of collection. ELISA is not without its challenges either; false negative results may occur in periparturient cows or cattle infected in recent months. The test is also of limited use in calves less than 4 months of age due to maternal antibody presence.

## Johne's disease

Johne's disease diagnosis is made more complicated than that for other diseases by some inherent characteristics of the disease: infection with *Mycobacterium avium* ssp. paratuberculosis (MAP) is usually established in young calves, but then it remains latent for years as a silent, undiagnosable state. Some animals may never develop antibodies to the organism and fecal shedding of the organism may be intermittent and there is individual variability in shedding.

Fecal culture is the most sensitive test available for MAP but suffers from the significant drawbacks of the need for prolonged incubation and associated cost. Incubation times observed by laboratories may be 2 weeks up to 16 weeks, making this test impractical for the individual suspected case. For this reason, the fecal PCR has gained favor in most situations with its high sensitivity and specificity and speed to diagnosis. Both culture and PCR are quantitative tests, making it possible to identify high shedders for removal from the herd. It is possible, however, to have pass-through organisms from contamination of feed by MAP and this is a possible source of false positive tests. The CT threshold with rtPCR, or the number of PCR cycles required to

detect sufficient nucleic acid to make the test positive, has been shown to correlate to fecal shedding. The less cycles required, the more organism present in the original sample.

Antibody testing is also available, with a serum or milk ELISA the most frequently used test. Results are rapid, but animals may shed organism prior to seroconversion and some animals may never seroconvert. ELISA positivity has been shown to correlate to shedding level. Complement fixation testing (CF) is another serum antibody test, but is usually reserved for export testing for countries that consider it an official test.

For the individual animal presented with clinical signs suggestive of Johne's disease that is the first case seen in that herd, a fecal PCR followed up with confirmatory postmortem testing is an appropriate diagnostic strategy. ELISA and PCR have been shown to have good agreement so it is possible the ELISA alone could be diagnostic, but demonstration of the organism is ideal.

For asymptomatic cattle in a herd that has had an infected animal, a producer might elect to screen the herd with the ELISA test and use the magnitude of the ELISA positive to infer the shedding contribution of the individual to the herd to make isolation or culling decisions. For valuable animals, it may be elected to confirm with fecal PCR or culture prior to making a culling decision. Producers entering into Johne's herd-level testing should be informed up front that it is a years-long process that requires vigilance. Guidance can be obtained from the Voluntary Johne's Disease Control Program of USDA.

What about purchasing young replacements at less than 2 or 3 years old? Due to the long latency and silent nature of this disease, purchasing young stock is risky. Ideally, purchases would only come from herds participating in the VJDCP who have a long history of testing older adult animals. In situations where data on the adult cattle of the herd of origin is not available, a purchase should be tested using the ELISA and PCR, understanding that there is a high risk of missing the organism at this stage of life.

## Anaplasmosis

*Anaplasma marginale* is a red blood cell pathogen that can cause severe hemolytic anemia in cattle. Clinical signs may be seen when 15% of red blood cells are infected. Clinical cases are usually seen in the late summer or fall in endemic areas, with affected cattle presenting weak, pale, recumbent, febrile and inappetent.

Although physical exam findings of pallor and icterus are highly suggestive of anaplasmosis, the recent introduction of the red blood cell pathogens, *Theileria orientalis* Ikeda into the United States has created a stronger need for differentiation of the pathogen.

In the field, obtaining a blood sample can confirm very thin blood within the syringe. Blood smears created directly from the syringe may be of higher quality than those made after the blood has been mixed with anticoagulant. Well maintained Diff-Quik stain is adequate for initial examination of the smears in the clinic or unstained slides may be submitted to a reference laboratory. For *A. marginale*, morula may be seen intracellularly on the outer margin, but care should be taken to ensure stain artifact is not mistaken for the organism. *Theileria* may appear within the body of the red blood cell. Given that clinical signs may be seen with a very low percentage of red

blood cells infected, further testing should be performed if a blood smear is negative or there is any doubt.

Polymerase chain reaction testing is available on whole blood for both *A. marginale* (and *A. phagocytophilum*) and *Theileria orientalis* Ikeda and Chitose. It is highly sensitive and useful for differentiating between these 2 organisms as well as differentiating them from other species within their genera. The competitive ELISA (cELISA) test for *Anaplasma* antibodies reportedly has high sensitivity and specificity (98%/100%)<sup>3</sup> and is considered an official test for import/export. This test does, however, have an issue in that it may cross react with Maltose-binding protein that may be found in the serum of 40% in of cattle. Although the cELISA has been improved in this cross reactivity, this may serve as a source of false positive tests<sup>4</sup> and in situations where an animal's exact status must be known, PCR should be used as follow up.

*Anaplasma* is a regional disease, as is *Theileria*. When a positive animal is identified for *Anaplasma*, the question is raised as to whether attempts should be made to attempt to clear infected animals. This decision should be based on whether the herd is in an endemic area and/or the prevalence of infection in the herd. Because animals can become infected throughout life, clearing animals in endemic areas may simply return that animal to an immunologically naive status, making them susceptible to potentially serious reinfection. The cELISA test may also be used to screen herd to determine herd prevalence. If the herd prevalence is well below 50% and the herd is in a low-risk area or is using good biosecurity practices, the decision may be made to clear infected animals, perhaps in the situation of a seedstock herd providing breeding stock. If the herd prevalence is greater than 50%, the disease is considered endemic in that herd and good needle management and other biosecurity practices should be employed to limit transmission and maintain stability. In these herds, it is valuable to train owners to evaluate mucous membrane color to identify pallor or icterus. This will allow for early identification and treatment of clinical cases while reducing the stress of handling for evaluation by a veterinarian.

## References

1. Washburn KE, Streeter RN, Lehenbauer TW, et al. Comparison of core needle biopsy and fine-needle aspiration of enlarged peripheral lymph nodes for antemortem diagnosis of enzootic bovine lymphosarcoma in cattle. *J Am Vet Med Assoc* 2007;230(2):228-232.
2. Nagy DW, Tyler JW, Kleiboeker SB, Stoker A. Use of a polymerase chain reaction assay to detect bovine leukosis virus in dairy cattle. *J Am Vet Med Assoc* 2003;222(7):983-985.
3. Chung C, Wilson C, Bandaranayaka-Mudiyanselage CB, et al. Improved diagnostic performance of a commercial *Anaplasma* antibody competitive enzyme-linked immunosorbent assay using recombinant major surface protein 5-glutathione S-transferase fusion protein as antigen. *J Vet Diagn Invest* 2014;26(1):61-71.
4. Primo ME, Thompson CS, Valentini BS, et al. Development of a novel fusion protein with *Anaplasma marginale* and *A. centrale* MSP5 improved performance of *Anaplasma* antibody detection by cELISA in infected and vaccinated cattle. *PLoS ONE* 2019;14(1):1-13.

