Johne's Watch

The latest information concerning Johne's Disease. Vol 3 Issue 2

A New Look At Johne's Disease Progression

~Dr. Todd Byrem, Ph.D., Technical Specialist

You may have heard that diagnostic tests for Johne's disease don't work, or at least don't work well. Have you ever wondered why Johne's tests get such a bad reputation compared to other tests for diseases such as BVD, leukosis or even mastitis? This issue of *Johne's Watch* presents some of the particulars of Johne's, and currently available diagnostic tests, that make Johne's diagnosis uniquely difficult.

Since Johne's was identified over a century ago, breakthroughs in available diagnostics have been few and far between. While some advances have taken place recently with the introduction of genetic-based technologies, their utility has been limited due to the high cost of running tests and/or the difficulty of sample collection.

The Diagnostics

There are two primary types of diagnostic tests for Johne's disease, organism-based and antibody-based. Organism-based tests, like fecal culture, look for the presence of the actual organism. In the case of Johne's, organism can be readily found in the feces since the bacteria resides primarily in intestinal tissues. Antibody-based tests, such as the Enzyme Linked ImmunoSorbent Assay (ELISA) detect the antibodies that an animal produces in response to an immune challenge – for example infection by *Mycobacterium paratuberculosis*.

For a variety of reasons, including convenience, speed and cost, the ELISA is the most commonly used diagnostic test for Johne's disease. But for all the advantages, there are still a number of difficulties associated with its ability to reliably detect Johne's.

In fact, new research coming out of the NAHMS Dairy 2002 study shows that sensitivity of the ELISA *(its ability to appropriately categorize infected animals as positive)* was below 30 percent. This is even less encouraging than previous research, which has shown ELISA sensitivity to be around 50 percent of the sensitivity of fecal culture, which is considered the "gold standard" for Johne's detection.

But why? ELISA's are used worldwide to detect infection by viruses, bacteria, molds and their metabolites with high accuracy and reliability, so why is the effectiveness of the Johne's ELISA so low? The answer lies in how Johne's develops and the variable length of time it takes to progress to a level of infection that is widely accepted as clinical.

Before deciding all Johne's tests, especially ELISA's, aren't worth the effort it takes to collect the samples, you need to keep in mind a key point. All Johne's tests are at the mercy of the organism, or the animal's response to that organism, in terms of whether the target is present to be detected. For example, fecal culture will return a negative result if no organism is being shed in the feces. This is the

case regardless of whether no organism is present because the animal is truly not infected *or* if the disease is at a stage in its progression when no organism is being shed. So, while it is true that some degree of care needs to be taken when designing testing strategies, you can be confident that the testing you do is contributing to your disease management goals.

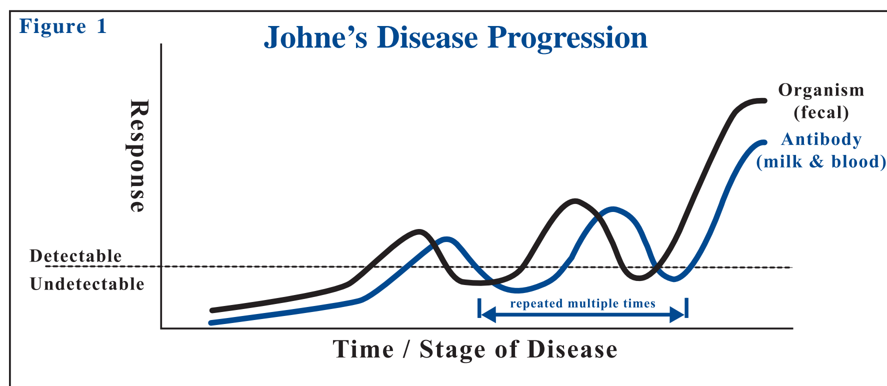
Disease Progression

There are some reasons that may clarify imperfect test performance, which is exemplified either by missed infected animals (false negatives) or by contradicting test results (an animal that tests fecal 'positive', but serum 'negative').

Let's recap what we know about how Johne's disease progresses. It is well documented that infection most often occurs in young calves when the immune system is not yet fully developed. It is generally understood that infection occurs via a fecal-oral route by ingestion of organism present in the environment because of fecal contamination by animals carrying the disease. It is also commonly accepted, that, *Mycobacterium paratuberculosis* takes a long time, typically years, to establish itself to the point where the infected animal begins to show clinical signs.

However, shedding of the organism, and transmission of the disease, occurs in animals which are not exhibiting clinical signs. The following series of graphs are based on hypothetical results that may be observed if Johne's disease progression was tracked in an individual animal.

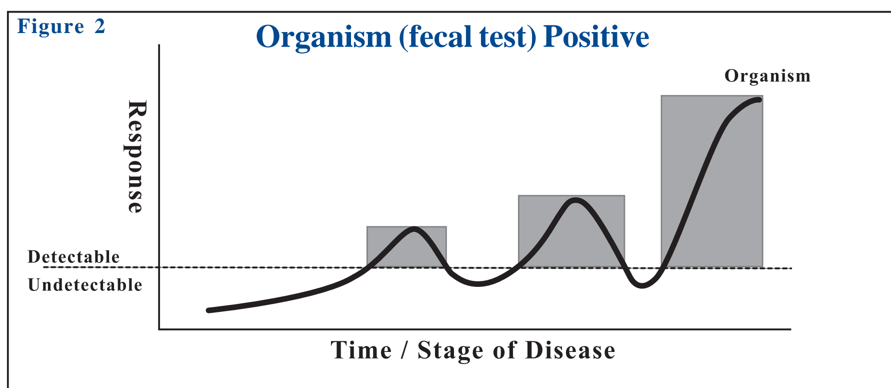
Figure 1 presents Johne's disease progression in an animal. The oscillating lines represent organism and antibody presence in the animal throughout its lifetime. The horizontal dashed line represents the threshold at which organism presence or antibody response can be detected by currently available diagnostics.

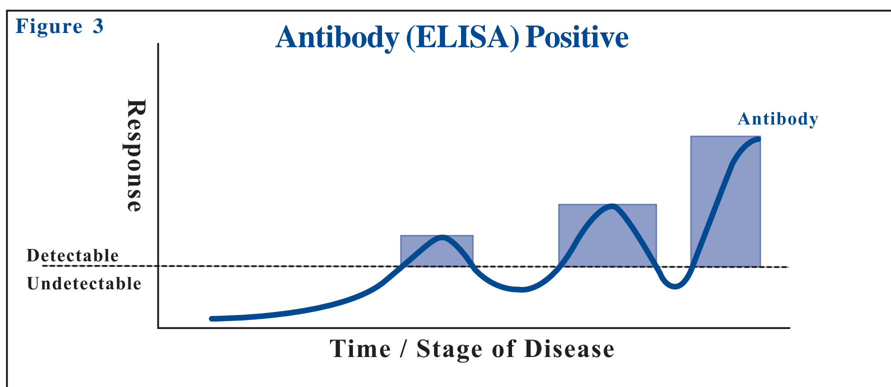


A few things are important to note from the graph: 1) organism response is usually detectable prior to the antibody response, 2) the center section of oscillations may repeat a number of times throughout the animal's lifespan, and 3) in general the final rise in both organism and antibody response corresponds with progression toward clinical stages of Johne's disease.

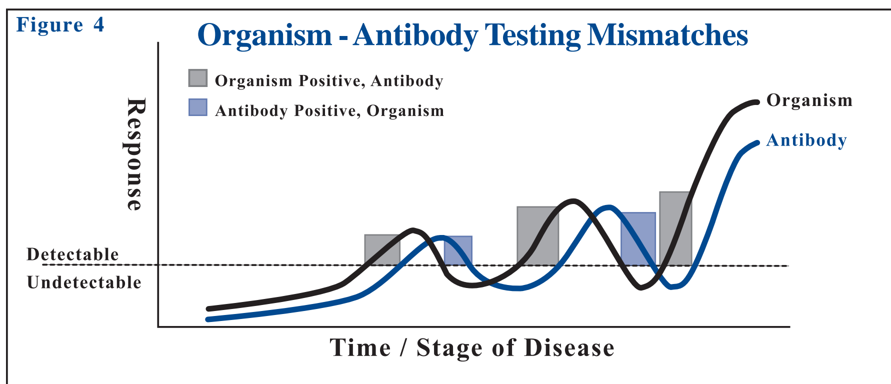
So how can this graphical representation of an animal's response to Johne's disease infection help explain the inability of either organism- or antibody-based tests to detect a response in infected animals? Let's break down the graph and take a closer look at each type of response individually.

In Figure 2, the boxes represent the periods during an animal's lifespan where the disease-causing organism is actually being shed at detectable levels. As you can see an organism-based test, such as fecal culture, will turn up positive a majority of the time, however there are certainly periods during which no organism can be detected in the feces. This may occur for a variety of reasons, for example low stress periods in the production cycle may enable the immune system to mount an effective counterattack, temporarily knocking back bacterial proliferation.





Likewise, in Figure 3, there are distinct periods of detectable antibody response. You may notice the duration of positive response, while similar to that of organism-based tests, is decidedly smaller. This helps to explain the relative effectiveness of each type of test. Since fecal shedding occurs a greater proportion of the time than antibody response, tests designed to detect organism in the feces *should* be more reliable.



By now it is probably clear that some of the inability of tests to detect Johne's disease can be explained by the periodic nature of organism shedding or antibody response. But what about conflicting test results?

By overlaying the organism and antibody curves once again we can identify periods of time that could produce conflicting results. The gray boxes in Figure 4 provide examples of when the analysis of simultaneously collected samples would result in a positive organism-based test, but a negative antibody-based test. Conversely, the blue boxes are times when antibody-based tests would return positive results, but organism-based tests would be negative. All of these opportunities provide plenty of chances for mismatched results.

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One Time is Not Enough

There is no doubt that developing an efficient and effective testing protocol and integrating the results into a Johne's control program is challenging and at times confusing. So what are you to do?

Incorporate an awareness of disease progression into herd goals for disease reduction or elimination, and ultimately into the disease testing strategy. It should be clear now why testing once for Johne's disease does not provide sufficient proof that a herd *does not* have a Johne's problem. Likewise, a single test cannot hope to provide an accurate picture of the disease in an animal.

Given the periodic nature of an animals' response to Johne's disease it makes sense that routine testing over time will effectively negate the variability of disease progression. Therefore, testing at regular intervals provides the best opportunity to detect infection early enough in the variable, pre-clinical stage to have a significant impact on disease transmission.

Annual testing is recommended in most control programs, however; optimal frequency should be determined by a herd's program goals, disease prevalence and management factors such as expansion. Consultation with an animal health professional will help to design the most effective Johne's testing program for each herd's unique situation.

While a "silver bullet" for detecting Johne's does not exist, the currently available diagnostics do provide sufficient tools for you to manage the disease. Much like a puzzle, the more individual pieces you can assemble, the better idea you will have of what the finished product will look like. Repeated, whole-herd Johne's testing provides the most pieces of information to help assemble the puzzle faster.

For assistance with Johne's testing or in designing a testing strategy contact me at byremt@antelbio.com or call ***1.800.631.3150****.*

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Antel BioSystems, Inc.
3655 Forest Road
Lansing MI, 48910
1.800.631.3510

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