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Navigating the Maze of Johne's Disease Diagnostic Tests

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One of the most challenging educational aspects of Johne's disease is explaining the complexities of diagnostic testing.

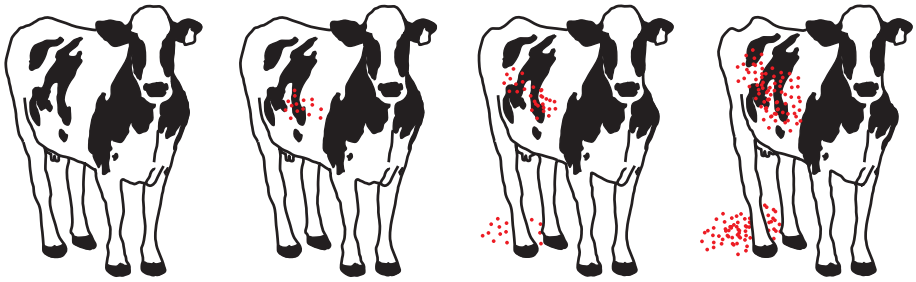
Despite years of significant effort, a single diagnostic test that is both rapid and accurate still eludes us. Probably the biggest factors influencing diagnostic test accuracy are that Johne's disease is very slow to develop; the organism causing Johne's disease, *Mycobacterium avium* ssp. *paratuberculosis* (*MAP*), is shed lightly or inconsistently early in the disease; and *MAP* is very effective at eluding the immune system.

Even with these limitations, current diagnostic tests are useful and a critical part of a Johne's disease control program. We just have to be very smart on how and when to use them. In any Johne's-infected herd or flock, animals can be theoretically classified into four different groups:

- **Uninfected**
- **Infected, but not shedding (not infectious)**
- **Infected and shedding**
- **Animals with visible disease.**

(See Figure 1). The first 3 groups are visibly indistinguishable, and current tests are not very accurate until after the animal reaches group 3 or 4. As animals go through each stage of Johne's disease status, each test performs differently. Based on this we have to be very specific about what we want to accomplish in order to appropriately select and interpret diagnostic tests.

Figure 1. *In a Johne's disease infected herd or flock, animals can be theoretically classified into 4 different groups. Diagnostic tests perform differently depending on the group of animals targeted.*



1) Uninfected
No test can accurately identify an un-infected animal.

2) Infected, not shedding
Animals can be infected for years without being detected.

3) Infected and shedding
Fecal tests are the most accurate, but convenience and cost may cause veterinarians to recommend blood or milk testing.

4) Clinical, visible disease
Blood, milk, and fecal tests are excellent at detecting clinical disease.

Most Common Diagnostic Tests

Let's briefly go over the most common diagnostic tests available.

Serum ELISA. This test, which has been around since the early 1990s, measures antibodies and, in general, animals do not make anti-*MAP* antibodies until the disease is fairly advanced. It is an excellent test for confirming clinical disease. In certain situations, such as a high prevalence rate, veterinarians may recommend this test in subclinical animals.

The literature suggests the sensitivity varies between 10 percent and 30 percent in subclinical animals and, while not perfect, there is a correlation between shedding levels and ELISA positivity. We expect around one in every 100 to 500 animals to have a false positive result with the ELISA.

Milk ELISA. While the accuracy of this assay is similar to the serum ELISA, this diagnostic test tests for antibodies excreted in the milk. It is very convenient for dairy producers to use and has been growing in popularity.

Having a milk ELISA result right along with milk production gives producers and veterinarians information that may help with culling decisions.

Fecal PCR. This assay has only been widely available for about eight years and can be used on individual, pooled or environmental feces. Fecal PCR detects *MAP* DNA, and results are reported out of the PCR machine as a Ct value. This simply means the number of cycles the PCR machine completed before the sample reached a signal threshold. The lower the

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Ct value, the more *MAP* DNA was in the sample at the beginning. If the machine cycles the maximum number of times—usually around 40 to 42—and the signal never reaches threshold, the result is reported as "undetermined" and interpreted as negative.

Laboratories should report out the Ct value as well as an interpretation/explanation.

It is important to remember that the fecal PCR measures DNA—not live organisms—so fecal culture which does measure live organisms and direct PCR may not always agree especially if there are very few organisms or a very small amount of DNA.

If the animal is in a heavily contaminated environment, the fecal sample may signal back with a Ct value, just from DNA in the environment and not because the animal is infected or shedding. This has been called pass-through, and it seems to be a much more severe problem with the fecal PCR test than with fecal culture. Your veterinarian in conjunction with the laboratory can help estimate the probability if pass-through DNA is causing the signal.

Generally, consideration should be given to the Ct value of the sample, the number of heavily shedding animals in the herd and the amount of feces in the environment.

Fecal culture. This is still the gold standard test for Johne's disease diagnostics. It also can be used on individual, pooled or environmental feces.

Laboratories will use either solid or liquid media. Liquid media is more sensitive and is currently the most accurate test, but it takes six to eight weeks to get a result. Because of this, it often makes sense to select a different test.

It is also important to note that routine fecal culture cannot culture sheep strains of *MAP*, and PCR is preferable to use in this species.

Stages of Johne's Disease, Test Performance

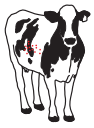
Now that we have covered the tests, let's go back and talk about the stages of Johne's disease and test performance in more detail.

Group 1: The uninfected animal

None of the commonly used tests can tell you with any confidence that an animal is uninfected. However, testing multiple animals over time can give information that the animals in the herd are not likely to be infected.

This may be confusing, but if an owner conducts surveillance testing on adult animals and only purchases animals from herds with similar negative surveillance testing, confidence can be built over time that the herd is not infected and consequently the animals are not infected.

The bottom line is don't test single animals for purchase and think a negative test means they don't have Johne's disease. The best way to evaluate the risk of infection is to look at the entire herd the animal comes from, not the individual animal itself.



Group 2: The infected animal that is not shedding

In any infected herd, a large number of animals are probably infected and not shedding. There is no good way to differentiate these animals from the uninfected animals. Occasionally one of these animals will have an uncharacteristic antibody response that is detectable with the ELISA, but not very often. These animals can live their lives productively and never shed the organism or break with clinical Johne's disease, but if an immune suppressive event occurs, such as bad batch of feed, or a move to a new herd, the animal could shed or break with disease.

There are occasional reports describing a closed herd that has tested negative for years suddenly breaking with clinical disease due to a severe acidotic event or something similar. It is cows in this stage that are responsible for this happening. A certain percentage of these animals will continue to progress with disease regardless of management.

Group 3: The infected non-clinical shedding animal

At this stage diagnostic tests start working more accurately. The level of disease in these normal-looking animals can still vary greatly, from low intermittent shedders to animals with severe disseminated disease that are contaminating the farm with massive numbers of organisms. It is important to understand that some normal-looking animals can shed as much as clinical animals.

Thankfully, diagnostics tests are pretty good at identifying heavily shedding cows. Sensitivity of the tests will be low in intermittent shedders and high in heavy shedders.

Fecal testing is the best direct measure for identifying the cows that are contaminating the environment. However, as mentioned previously, there is correlation with the ELISA.

Group 4: The clinical animal

These are the classic thin Johne's disease animals. All diagnostic tests described here are accurate at determining if an animal is thin or has diarrhea due to Johne's disease.

Notice that we did not say, "Accurate at determining whether or not they are infected." Animals could still be infected, but when we test an animal

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Before Moving Cattle, Know This

The Code of Federal Regulations—commonly referred to as simply CFR—prohibits moving animals across state lines if they have tested positive for Johne's disease on a PCR or culture.

The exceptions to the rule: 1) Animals going directly to slaughter that are not unloaded anywhere between the departure point and destination or to an approved facility for sale to slaughter. 2) Animals may be moved with the approval of—and under conditions prescribed by—the APHIS Administrator who will look at circumstances on a case-by-case basis.

In herds with a known client, veterinarians should not sign a certificate of veterinary inspection (CVI) for animals known to be infected. Veterinarians asked to sign a CVI for a herd they are not familiar with should question the producer and ask to see the Johne's disease test records signing the CVI.

Veterinarians and Producers: Please contact your State Designated Johne's Coordinator or State Animal Health Department to learn more about the movement of animals that have tested positive for Johne's disease or those suspected of being infected with Johne's disease.

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for Johne's disease that is thin or has diarrhea, our question is "Is Johne's disease causing the animal to be sick?" not "Is the animal infected?" Turnaround time in these situations is important, and most veterinarians will select the ELISA test.

In certain situations such as a previously test negative herd, ELISA results should be confirmed by an organism detection test such as fecal PCR or culture in the unlikely event of a false positive ELISA result.

Sampling, Laboratory Dos and Don'ts

The common saying "garbage in, garbage out" applies to diagnostic testing.

- Use clean needles, sleeves, and sample containers for every cow to avoid cross contamination or interference/inhibition of tests.
- Avoid exposing samples to extreme hot or cold temperatures, and ship to the laboratory as soon as possible.
- Freeze samples only according to the recommendations of the laboratory. For example freezing fecal samples at standard temperatures (-20°) is detrimental to our ability to detect *MAP* via culture.

The proficiency of the laboratory is also an important consideration. It is best to use a laboratory that has taken and passed the USDA's proficiency testing program. Approved laboratories can be found at ***http://www.aphis.usda.gov/animal_health/lab_info_services/approved_labs.shtml*** or use your favorite search engine and query "USDA NVSL approved laboratories".

Milk and serum ELISA approved laboratories are found under Johne's disease—Serology. Fecal PCR and culture approved laboratories are found under Johne's disease—organism-based methods, both individual testing and pooling.

Summary

In summary, there is no one "best test" for Johne's disease. They all have their uses, and their successful implementation depends on numerous factors including the reason for testing, the stage and prevalence of disease, the ability to collect quality samples, the cost of the testing, and others. Remember, diagnostic testing for Johne's disease can be successful if it is only a part of a comprehensive control program, and we are bound to be disappointed in the results of diagnostic tests unless they are carefully and appropriately used.

Colostrum, Milk Replacers, Waste Milk

Dairy producers have long been warned that colostrum from Johne's disease-positive cows can infect calves. Research by Dr. Patrick Pithua and coworkers of the University of Minnesota evaluated the risk of transmission of *Mycobacterium avium* subsp. *paratuberculosis* (*MAP*), the organism that causes Johne's disease, and reconfirmed this warning.

In the study, calves born on 12 dairies in Wisconsin and Minnesota were fed either maternal colostrum (n = 261) according to the normal management on the farm or were fed a commercial plasma-derived colostrum replacer (n = 236) according to label directions. After the initial 24 hours, calves were housed, managed, fed and raised similarly, then bred and entered the milking string after calving. At 30 months of age, 42 months and 54 months, each cow in the study was tested for *MAP* by ELISA for serum antibodies and fecal sampling.

Study results show that calves fed the colostrum replacer were significantly ($P < 0.06$) less likely to become infected with *MAP* compared to calves fed maternal colostrum.

The reduction of risk of infection with Johne's was 44 percent. Yes, calves fed the replacer were 44 percent less likely to get Johne's disease than calves fed colostrum.

To help prevent Johne's disease, calves should be given milk replacer or pasteurized waste milk. Colostrum should always be from a cow that has tested negative for Johne's disease.

The 'Why' Behind MAP-Infected Colostrum

Animals infected with *MAP* excrete the bacterium in their milk, and we're not talking just cows showing clinical signs of Johne's disease. Infected cows that appear healthy can also pass along the bacterium.

Dairy producers who opt to feed colostrum rather than milk replacer can minimize the transmission of Johne's disease from cow to calf by following three simple rules:

1. Use only colostrum from Johne's disease test-negative cows.
2. Do not pool colostrum from multiple animals.
3. Thoroughly clean the udder and teats before collecting colostrum.

Milk Replacers vs. Waste Milk

A safe, effective alternative to using milk replacers is to pasteurize waste milk on the farm, as pasteurization kills virtually all *MAP* that may contaminate raw milk as well as other viral and bacterial agents that could affect the health of dairy heifer replacements. One study found no difference in the number of new cases of Johne's disease arising in dairy herds between those that pasteurized and those that used milk replacer.

Recommended protocols for pasteurizing waste milk: 145°F (63°C) for 30 minutes for batch pasteurization or 162°F (72°C) for 15 seconds for flash pasteurization. The milk should be stirred or otherwise in motion to ensure even heat distribution.

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