



Johne's Disease – Beef

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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.

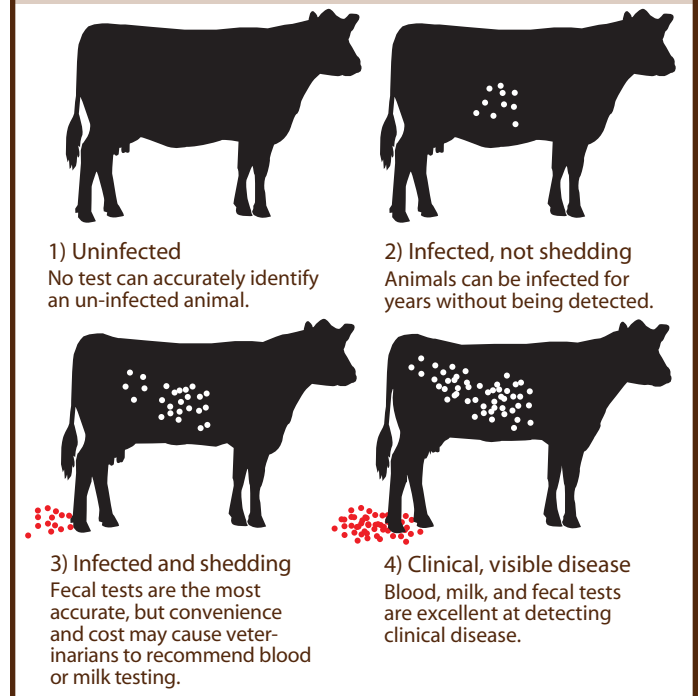
Most Common Diagnostic Tests

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

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**

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.



every 100 to 500 animals to have a false positive result with the ELISA. (This statement is not consistent with what we are seeing in North Dakota. Please visit with your veterinarian for details.)

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 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.

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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 (indicates presence of DNA), 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 (a stressful situation or concurrent immunosuppressant disease like BVD or Leukosis). It is in this stage cows 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 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. Not every animal with diarrhea has Johne's and not every animal with Johne's has diarrhea.

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

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Vaccine Project Progressing

The Johne's disease vaccine project—a collaboration effort of the Johne's Disease Integrated Program and USDA/APHIS—that is investigating vaccine efficacy, with the expectation of identifying one of more Johne's disease vaccine candidates for possible commercial development, is progressing on schedule. Initiated in 2009, the vaccine project will also serve to validate the goat Johne's disease experimental challenge model proposed in the 2007 AMSC manuscript "Experimental Challenge Models for Johne's Disease: A Review and Proposed International Guidelines" by Hines et. al.

During Phase I, the in vitro macrophage phase of the study, 18 knockout mutants were evaluated to identify those showing the best attenuation. These results, coupled with an apoptosis study in Dr. Paul Coussens' lab identified the top eight vaccine candidates that were moved into Phase II, the mouse vaccine efficacy trial.

This part of the study measured *MAP* colony-forming units in tissues after experimental infection to assess protection from the test vaccines. Samples were also retained for immunological monitoring of the mice to be performed at a separate lab.

Five mutant vaccines showing the best protection after *MAP* challenge were identified and moved forward into the final phase of the vaccine project, Phase III, the

goat challenge study that is currently in progress in the lab of Dr. Murray E. Hines II at the University of Georgia.

Five treatment groups and three control groups of 10 goat kids each are being evaluated. Goat kids were vaccinated in mid-September 2011 with the five test vaccines and a commercial control vaccine, then challenged four weeks later with a K10 strain of *MAP* following the parameters of the Goat Experimental Challenge Model proposed and published by the JDIP AMSC committee.

Monthly fecal cultures are being collected for HEY culture and PCR, and monthly serum samples are being collected for ELISA and AGID testing. Periodic comparative cervical intradermal skin testing is also being performed. Whole blood is being provided to Dr. Torsten Eckstein's lab at Colorado State University monthly for other immunologic testing with *MAP* cell wall lipids.

At necropsy, the gross and microscopic lesions detected will be graded for statistical analysis. Select tissues will be collected at necropsy, with PCR and HEY culture then performed on these samples. Limited amounts of goat serum, feces and tissues samples will be archived during the Phase III study.

It is anticipated that final results will be available in the spring of 2013.

Johne's Disease Diagnostic Tests

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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.

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AI, Breeding Season and Johne's Disease Transmission

If you AI your females, should you be concerned about the semen used transmitting Johne's disease to the females inseminated? The answer: No, not if you're using commercially processed semen.

"Although Johne's disease is worldwide and it has been reported that you can find the organism in semen, it has never been scientifically reported that the organism will be passed via semen that has been collected, frozen and thawed," states Dr. Kent Weigel, associate vice president, animal health, Genex Cooperative Inc.

"The organism is not known to survive the freeze and thaw process of semen."

More good news is that large reputable AI companies only purchase or lease bulls from herds that have a Johne's disease management

program in place. They also avoid seeking AI sires from herds that have a known history of Johne's disease. In addition, the AI companies routinely test bulls for Johne's disease.

Another precautionary measure taken by large AI companies: Before going into a stud, potential AI bulls are screened on the farm for Johne's disease using serology testing. Once in the stud, resident test-negative bulls are routinely tested using fecal cultures and, in some cases, PCR testing, once or twice a year.

Dr. Charles Brown II, head veterinarian with ABS Global Inc., points out that international trade requires Johne's disease fecal culture tests at least annually from donor bulls.

"CSS (Certified Semen Services) also mandates that we document that an animal is healthy the day of

collection," Weigel adds. "Healthy' means that tests indicate the animal is free from Johne's disease."

With such measures in place, using semen from these centers should not present a risk for the introduction of Johne's disease into a herd.

Natural Service Bulls

Although *Mycobacterium avium* ssp. *paratuberculosis*, commonly called *MAP*, has been found in the semen and feces of infected bulls, semen from Johne's disease-positive bulls used for natural service is not believed to pose a large risk for the spread of Johne's disease in typical production settings. On the other hand, manure from Johne's disease-infected bulls is an entire different situation.

In a nutshell, manure from a herdsire infected with Johne's disease can wreak potential havoc in a herd.

Transmission of Johne's disease between a herd bull and the herd can occur when *MAP*-infected manure is ingested by extremely young calves. Research shows that extremely young calves—those just a few weeks old—are highly susceptible to *MAP* infection. And it doesn't take a high concentration of the organism to infect a very young calf. The old Brylcream commercial slogan of "A little dab will do ya" applies.

Regarding transmitting *MAP* to older animals, well, research also indicates that, while animals greater than one year of age may acquire *MAP* infection, they are more resistant to infection than young calves.

To help prevent introducing or spreading Johne's disease in a herd, the smart move is to only purchase and/or lease bulls from herds that have tested for Johne's disease and are low-risk herds.



To help prevent Johne's disease, only herdsires from Johne's disease low-risk herds should be used. After all, it's young calves like these that can easily become infected when they ingest *MAP*-infected manure from herdsires that are carry Johne's disease.