



# Johne's Watch

*A monthly bulletin for livestock producers*

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## Testing Options and Diagnosis of Johne's Disease

### Developing a Testing Protocol

**C**ontrolling Johne's disease on the farm requires proven management practices coupled with an aggressive, routine testing program. The lack of a perfect Johne's disease diagnostic test requires producers and their veterinarians to work in tandem to achieve the best overall evaluation of the herd's status.

To date there is no single test sufficient for complete diagnosis of the disease; however, reasonably accurate and cost-effective tests are available for differing diagnostic and control needs. Determining which test to use requires an understanding of the testing methods available and their advantages and limitations.

**M**ost producers test for Johne's disease only after an animal begins to show clinical signs of Johne's disease. Unfortunately, by this time the producer may be several years behind in controlling the disease if proper management practices have not been put in place.

When an animal exhibits clinical signs, it is important to determine how long the animal has been in the herd. If the animal was born on the farm, it is likely that there are other infected animals in the herd. If the animal was brought into the herd, then the length of time the animal has been in the herd will influence the number of animals potentially infected.

Producers interested in determining whether or not they have Johne's disease in the herd have several testing options. A random sampling of the herd, approximately 30 animals, or a statistical subset of the herd - will give the herd owner a yes or no answer. If all animals test negative, it is possible the herd does not have Johne's disease. However, if any animal tests positive, the random sampling method will not tell the producer how widespread the disease is in the herd (herd prevalence).

The first step in a Johne's control program is to estimate the prevalence of *M. paratuberculosis* infection on the farm by conducting a whole-herd screening test on all animals three years of age or older, says Donald Sockett, DVM, MS, Ph.D., Wisconsin Veterinary Diagnostic Laboratory—Madison. I recommend the herd be tested with a whole-herd ELISA test followed by a fecal culture test within a 12 month period.

Whole-herd testing should be done within a narrow window of time, usually within four to six weeks depending on the size of the herd, to give a true picture of the disease prevalence in the herd. Todd Byrem, Ph.D., manager of production and technology for AntelBio, says, by testing the animals at only one point in their lactation cycle, for example at dry-off, producers do not get an accurate analysis of the herd. Byrem also explains, by waiting until the dry period (typically 36 months of age and older) to test, producers may miss detection in younger animals, important information in evaluating Johne's control programs.



## Selecting a Diagnostic Test

To better understand the accuracy of Johne's diagnostic tests, the four stages of Johne's disease as they relate to diagnosis must be understood. According to the U.S. Animal Health Association (USAHA) Stage I, the early infection, is not detectable by existing tests. In Stage II, the infected animal, with no visible signs of disease, can be detected with appropriate testing strategies. Stage III, the clinically diseased, and Stage IV, the final phase, are more readily detected by all testing procedures.

Like most diagnostic tests, those used in the detection of Johne's disease are evaluated in terms of sensitivity and specificity. Sensitivity is defined as the ability of a test to detect known infected animals. It is the percentage of infected animals that will test positive using the particular testing method. In an infected herd, the lower the sensitivity of a test, the more false-negative results will occur. Specificity, is the ability of a test to detect non-infected animals. Specificity is the percentage of animals free of the infection that will test negative using the particular testing method. The lower the specificity, the more false-positive results will occur.

Diagnostic tests commonly used in detecting Johne's disease fall into one of three categories: detection of antibodies to *M. paratuberculosis* (serology); detection of the bacterium that causes Johne's disease, *M. paratuberculosis*; and detection of a cell-mediated immune response to antigens of *M. paratuberculosis*.

## Serological Tests

A number of serological tests are currently available, they include the Agar Gel Immunodiffusion (AGID) assay, the Complement Fixation Test (CFT) and the Enzyme-Linked Immunosorbent Assay (ELISA).

Depending on the diagnostic tools available to the producer, most veterinarians recommend screening the herd with a serological test such as the ELISA. The test is useful in assessing the prevalence of Johne's disease infection in the herd. Subsequent testing, when necessary, generally involves the use of a fecal culture test or other more sensitive testing methods.

ELISA technology is inexpensive, fast, sensitive, easily automated and because results are expressed on a continuous scale, cutoffs can be changed to alter the sensitivity and specificity of the test. Most cutoff values in ELISA tests result in sensitivities approaching 40 percent and specificities greater than 90 percent. Whenever possible, ELISA results should be reported with a numerical value and not just positive or negative. This improves the interpretive value of the results because as the ELISA values increase; the probability or likelihood of infection and fecal shedding also increases.

The ELISA test is a good screening test because it is relatively inexpensive (usually \$4 to \$8) and results can be generated in a few days. Estimates of Johne's prevalence are most accurate when the ELISA is used on large populations of animals. Prevalence is the proportion of animals that have the disease. An ELISA test on only a few animals fails to address the fact that Johne's disease is a herd problem, rather than an individual cow problem.

There is a new ELISA in the animal health industry. A test using milk samples has been developed and is now available commercially. The AntelBio Milk ELISA has been shown to be extremely comparable in sensitivity and specificity to the serum ELISA. In fact, in a study conducted by AntelBio, when compared to fecal culture, no significant differences in milk

## Detecting *M. paratuberculosis*

and serum ELISA sensitivities and specificities were detected. In addition, the AntelBio Milk ELISA has the additional convenience of incorporating sample collection with the routine DHIA milk sampling system and the added benefit of easy integration with dairy herd management records.

Producers who have vaccinated animals for Johne's disease should not use tests, such as the ELISA, since the vaccination stimulates the production of antibodies and will therefore produce a positive result on the serum test.

The remaining serologic tests are much less commonly used. The AGID test has a sensitivity of 75 percent in cattle with clinical signs of Johne's disease. Infected cattle without clinical signs of Johne's disease have a much lower test sensitivity (25 percent or less). Due to the low sensitivity of the AGID, it is not recommended as a screening test to identify subclinically infected animals. The CFT, which is required by many countries for export or import of cattle, is considered by the USAHA as intermediate in sensitivity and specificity to AGID and ELISA. With a high rate of false positives and false negatives the CFT is not recommended in the United States for routine diagnostic use.

Once the whole herd has been evaluated with the ELISA or another screening test, animals that test positive, but do not exhibit clinical signs should be retested using a method that detects the *M. paratuberculosis* organism (like traditional fecal culture), to confirm the results of the ELISA test.

Fecal culture is the most common testing method used to detect the presence of the organism. It is traditionally viewed as the most effective method available; however, it can take up to four months to generate a result. Conducting the fecal culture requires the organism be grown in the laboratory. Because of the slow-growing nature of *M. paratuberculosis*, it can take eight to 16 weeks to achieve test results. Other methods are available with nearly the same effectiveness, but often have other limitations.

The USAHA reports that fecal culture sensitivity is considered to be 40 percent plus or minus 10 percent and its specificity to be 99.9 percent if done correctly. Fecal culture tests cannot detect Stage I or some Stage II animals; therefore a negative test result in known *M. paratuberculosis* infected herds, must be interpreted with caution.

DNA probe tests detect small pieces of DNA that are only found in *M. paratuberculosis*. It is not necessary to grow or culture the bacterium, thereby substantially reducing the time to obtain results. While commercial versions of these tests are not readily available from Johne's testing centers, AntelBio has developed a Rapid Fecal Test which utilizes DNA probe technology, to determine the presence of *M. paratuberculosis* in a fecal sample in less than three days. Like the fecal culture, DNA probe tests cannot detect animals in Stage I or some Stage II animals. Its sensitivity is about 40 percent and its specificity nearly 99.9 percent.

A radiometric culture test is available to detect the presence of *M. paratuberculosis*. While this method is faster than traditional fecal culture, seven weeks versus 16 weeks, the test is radioisotope-based, and because it involves the handling of radioisotopes, and the use of special equipment, the testing is also more expensive. Its sensitivity is about 40 percent and its specificity about 99 percent.



## Cell-Mediated Immune Response

The most definitive test for Johne's disease is to microscopically identify characteristic changes and identification of *M. paratuberculosis* in tissues. Microscopic evaluation requires specific tissues be collected by surgical procedure or by necropsy in addition to special tissue preparation prior to sending the sample to a diagnostic laboratory.

The use of a skin-response test, similar to that used in the detection of bovine tuberculosis is in crude form at this time. The sensitivity of the Johne's skin test is about 54 percent and the specificity is approximately 79 percent. A more sophisticated laboratory test for cellular response to *M. paratuberculosis* is the gamma interferon assay. Gamma interferon is a protein that is important in the regulation of the immune system. This test is conducted on blood samples and measures the release of gamma interferon from white blood cells exposed to antigens of *M. paratuberculosis*. The special care and handling of these samples make its use in the field limited.

Johne's Test Comparison

Test	Cost	Speed	Specificity	Sensitivity
AntelBio Milk ELISA	\$6	1-5 days	95-99%	30-40%
Serum ELISA	\$4-8	1-5 days	95-99%	30-40%
Culture	\$10-20	8-16 weeks	99%	40-50%
AntelBio Rapid Fecal	\$20-100	3-14 days	99%	35-45%
AGID	\$5-10	1-5 days	99%	25%
Necropsy	\$55-100	7 days	~ 100%	~100%

## Establishing a Management Plan

Based on the number of test-positive animals in the herd (prevalence), the producer and veterinarian should implement a management plan that includes routine testing of the herd. Sockett stresses that because testing methods available are imperfect, testing alone cannot control the disease. He adds that producers wanting to control the disease on their farm need to follow sound management practices, including: colostrum management, segregation of calves, good hygiene and a clean milk pool (not feeding waste milk).

If you would like additional information please contact AntelBio at 1.800.631.3510.

## References

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