



VCHIP Vermont Cattle Health Improvement Project

Johne's Disease in Cattle - Article 7

This is the seventh article in a series presenting current information regarding Johne's disease in cattle. It is directed toward helping veterinarians and their clients prevent or control this disease and was adapted by permission from the original 1999-2000 series presented by the AABP Food Safety Committee. Content was edited and reviewed by the National Johne's Working Group and endorsed by the USAHA.

Testing - Choosing the Right Test for the Right Purpose

Part 4 of 4 articles on the topic of Johne's Disease testing

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Have a strategy

Despite some limitations, testing is useful in most Johne's disease farm plans. However, if a plan is not in place for interpreting and using the test results, time and resources are wasted, confusion is created and confidence is eroded in efforts to prevent or control Johne's disease. To gain the most return from an investment in testing, veterinarians and cattle producers should discuss testing strategy issues.

These issues are outlined in several places: Addendum I of this article; Section C in the Johne's Disease Plan Manuals for Veterinarians in the May 1999 issue of *The Bovine Practitioner*; and the NYSCHAP Johne's Disease Herd Testing Reference Manual.

Using tests for different purposes

The choice of Johne's tests and strategies is client-specific. Some examples of objectives for testing and test choices are provided.

1. Diagnosis for a cow with clinical signs of Johne's disease.

Remember that establishing a diagnosis

is an imperfect process for most diseases. It is usually accomplished through a series of steps that rule out different disease possibilities. For example, a herd with no confirmed history of Johne's disease may have a 6-year-old cow with diarrhea, gradual weight loss, no fever and a good appetite. By thorough examination and preliminary testing, most diseases can be ruled out, and a 20 percent probability that the cow has Johne's disease can be estimated. Now, which test to use?

The most rapid and least costly tests to confirm a "suspected case of Johne's disease" are ELISA and AGID. When clinical signs are present, performance of AGID is similar to ELISA. The probability that a positive ELISA test is accurate (PVP) is near 82 percent. The probability that the result is a false positive is at least 18 percent. (See Table 1)

On the other hand, the best positive predictive values are with a fecal culture test. In this case, a positive culture test is virtually 100 percent accurate for positive identification of *M. avium subs. paratuberculosis*. In this example,

disadvantages of fecal culture are the delay in results (8 to 16 weeks) and higher cost.

If the serology test result was negative, confidence in its accuracy is 85 percent, leaving a 15 percent chance of a false-negative result. Regardless of the validity of a negative test result, a wise disease management decision is to isolate this cow and submit a fecal sample for culture. A fecal culture pursues a definitive status for the test-negative clinical suspect and is particularly important if Johne's disease was not previously confirmed in the herd.

Occasionally cows with clinical Johne's disease do not have detectable antibody by ELISA. This occurs less often by AGID. Alternatively, appropriate tissue samples can be collected at slaughter for histopathology and culture.

A second scenario may be a three-year-old cow, with the same signs, in a herd that culls 3 percent of cows per year because of confirmed Johne's infection. An impression may be that an estimated 50 percent of cows are infected. So the probability that this animal has Johne's is +50 percent. The PVP of a positive ELISA test is now +95 percent and may offer enough added confidence to cull the heifer immediately.

Table 1. Probabilities (Prob.) for correct ELISA and fecal culture results at various pre-test prevalences (Est.)

Est. Chance Before Testing	Prob. ELISA Positive is TRUE	Prob. ELISA Negative is TRUE	Prob. A culture Positive is TRUE	Prob. A culture Negative is TRUE
< 1%	15%	99%	100%	99%
10%	67%	92%	100%	94%
+20%	+82%	84%	100%	85%
+50%	95%	57%	100%	63%

2. Pursuing more definitive diagnosis after serology.

Testing positive ELISA animals with fecal culture or histology and culture of appropriate tissues is recommended in an individual animal or herd of uncertain Johne's status and for the Voluntary Johne's Disease Status Program. Follow-up testing with an organism-detection test is also recommended whenever a positive serology test is not consistent with clinical assessment or additional information. Greater confidence is placed in all diagnostic tests for Johne's disease in herds with culture-confirmed animals and/or animals with clinical signs of Johne's disease. Remember that tests for Johne's in mature infected, but non-clinical cattle, have diagnostic sensitivities between 25 and 40 percent. Consequently, repeated testing of herds and individuals is required to definitively establish negative or positive status.

All Tests and Laboratories are not the same

Methods used by laboratories for fecal culture, serology and other tests vary and thus test performance can also vary. To help diagnostic laboratories standardize performance for Johne's disease tests in cattle, the National Veterinary Services Laboratories (NVSL) coordinates an annual laboratory proficiency check-test for fecal culture, ELISA and AGID. Any laboratory can request to receive the check-test sample panels. Laboratories can use whatever method they choose, but must correctly identify the status in 90 percent of the samples. Since its beginning in 1997, a majority of labs in the US now participate in this program. Practitioners are strongly recommended to use a laboratory approved in the NVSL program. Approved labs are listed on the USAHA Web site at www.usaha.org.

3. Preliminary evaluation of the herd.

Some clients may not know the status of Johne's in their herd but would like information to help assess risk. This is an opportunity to suggest "targeted testing" to find infected animals. An option is to start with ELISA and to fecal culture any animals with S/P or OD values just below and above the positive cut-off. Mature animals that have a higher risk of being infected are also a good place to start, i.e., suspected exposure, poor performance or unknown source. A sample size of 10 to 20 percent of the herd, chosen according to existing knowledge of risk, can offer insights for a relatively inexpensive investment.

For a statistical estimate, randomly testing 30 animals over second lactation is another choice. If all the animals test negative, then prevalence in the herd is statistically estimated at 10 percent or less. This scheme is the basis of the Voluntary Johne's Disease Herd Status Program.

Targeted testing can identify infected animals and provide further insight into the extent of herd infection. It offers the chance to gain experience with Johne's tests and their interpretation. It also prompts discussions that advance understanding of Johne's disease, while considering whether larger scale herd testing is appropriate.

4. Estimating the herd prevalence.

An estimate of prevalence, based on history and optimally backed by testing, is important to help interpret test results, choose management strategies and monitor progress. One reasonably accurate and quick way to measure the herd's infection level is by ELISA testing

all mature cattle (two years and older). Results are most reliable for herds with confirmed isolation of the microbe from an animal(s).

Suspected and positive animals should be evaluated by fecal culture for confirmation of *M. paratuberculosis*. Fecal culture gives a more accurate estimate of prevalence in a herd. Because of its expense, it is only recommended for focused and aggressive management plans, or to more quickly achieve a higher level of negative herd status.

The relationship of test prevalence and true prevalence.

Remember that the apparent or test prevalence (AP) is the number of mature cattle that test positive, divided by the total number tested at a specified point or time period. Prevalence is also estimated by testing subsets of the herd over a period of time. At the end of the time period, divide the number of positives by the number of animals tested. From the AP, and estimates for SE and SP of the test, an estimate of the true prevalence (P) is calculated:

$$P = [AP + (Sp-1)] / [Se+(Sp-1)]$$

In Article 4, Part 1, the example was used where 5 percent (AP) of the herd tested ELISA positive, the SE equaled 25 percent and SP equaled 98 percent. (See Article 4, Part 2) An estimate of the true prevalence is calculated:

$$P = [.05 + (.98 - 1)] / [.25 + (.98 - 1)]$$
$$P = .13 \text{ or } 13\%$$

The estimate of the truly infected animals is 13 percent, versus the 5 percent that are test-positive.

If a second herd is tested by fecal-culture and 5 percent (AP) of the animals are

culture positive, a SE of 40 percent and a SP of 100 percent is used for the fecal culture test (Article 6) to calculate P:

$$P = (.05 + 1.0 - 1) / (.40 + 1.0 - 1)$$

$$P = .125 \text{ or } 12.5\%$$

An estimate of truly infected (P) animals in the second herd, based on culture results, is 12.5 percent versus the 5 percent of animals that are test-positive. The two tests obtained the same percent of test-positive animals, a similar estimate of true prevalence (P) and an AP value that doubled, but this is not always the case.

If the ELISA AP was 15 percent in a herd:

$$P = (.15 + .98 - 1) / (.25 + .98 - 1)$$

$$P = 57\%$$

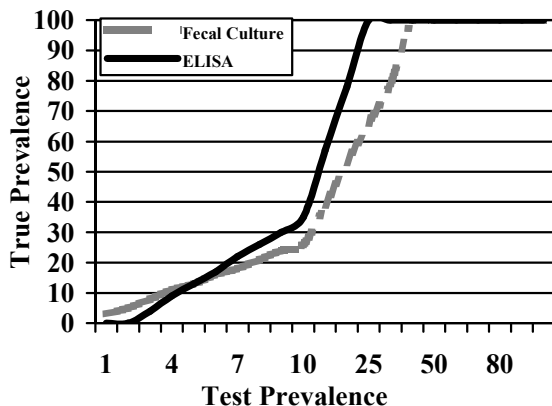
In this herd the estimate for the percent of animals actually infected is close to four-times the test prevalence.

If the fecal culture AP was 15 percent in a different herd:

$$P = (.15 + 1.00 - 1) / (.40 + 1.00 - 1)$$

$$P = .37 \text{ or } 37\%$$

When 15 percent test positive by culture the estimated P is slightly more than twice the AP. The differences in P shows how an estimate of the number of infected cattle depends on the SE and SP of the test used.



As crude rules-of-thumb go for Johne's tests, a reasonable estimate for the true prevalence is two to four times the AP, depending on the test used.

This relationship, however, fails for the ELISA test whenever the AP is close to the ELISA false positive rate, i.e. 2 percent. If the ELISA AP is 2 percent, then the estimate of P is close to 0 percent. See Addendum II for additional comments about P and AP.

5. Testing for control.

For clients interested in controlling or eliminating Johne's disease, critical management practices come first, followed by separating and/or culling high-risk, test-positive animals. Both elements are particularly important to reduce high rates of herd infection. An appropriate test strategy should be chosen by evaluating factors discussed in Addendum I of this article; Section C in the Johne's Disease Plan Manuals for Veterinarians in the May 1999 issue of The Bovine Practitioner; and the NYSCHAP Herd Testing for Johne's Disease Reference Manual.

Economics favor using ELISA as the primary test in a control program, with some degree of confirmation by culture, to be comfortable with how the test performs in the herd. More aggressive strategies can be used for more rigorous goals. For example, the most information about an individual's status is gained -- at a higher cost -- by testing all animals with ELISA and fecal culture. This provides the benefit of information from both tests, which overlap to a limited degree. Fecal culture detects animals in earlier infection that are missed by ELISA. Some cattle with elevated ELISA values are negative on fecal culture. In fact, on one set of tests

in subclinical cattle, no more than 50 percent of ELISA positive animals are positive on fecal culture. When results of both tests are the same, predictive values are improved. When the results are different, closer monitoring is triggered.

Another option is to alternate ELISA with fecal culture at six-month intervals. An intermediate approach can be to fecal culture only those animals that have marginally elevated ELISA values, in the range above “background” levels up to and around the positive cut-off value.

One more major option with control strategies is to test the whole herd at once, or by subsets, until all are tested in a period of time, i.e. in one year. “All at once” testing makes sense when owners need prevalence information to make significant management or culling decisions. Testing by subsets permits more strategic timing and efficient use of results in ongoing control programs.

If there is no compelling reason to know herd status quickly, animals can be tested at intervals by groups, and coordinated so that current results are available at the time critical control decisions are made. For example, in dairy herds, cohorts of mid-to-late gestation cows can be tested each month so the results are available by dry-off. Advantages are that testing and costs are distributed over time, and the frequent inflow of new results keeps Johne’s management and decisions an active part of the farm routine.

Timing is appropriate in beef herds where short interval or small group testing may not be appropriate. To accommodate management in cow-calf

herds, one thorough strategy is to test all cows once or twice a year. The test should be timed to have results available before calving or breeding.

6. Testing *immature* replacements.

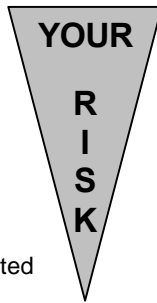
Unfortunately, because of the slow progression of *M. avium subs. paratuberculosis* infections from Stage I to Stage III, no current tests demonstrate sufficient sensitivity in young cattle, less than 20 months old and in Stages I or II, to warrant any general recommendation.

7. Testing herd additions.

Herds become infected with Johne’s disease by introducing infected, asymptomatic animals. The risk increases with each addition to the herd. Therefore, the best way for a herd to remain free of Johne’s is to remain closed. However, that is not always realistic. Obtaining cattle from a low-risk source is the only reliable way to know that any individual animal is low-risk. Thus, securing animals from herds that are one-time or repeatedly test-negative is the best way to reduce the risk of introducing Johne’s. Testing individuals from unknown status sources and introducing only those that are negative only reduces risk to a limited degree. The strategies listed below are ordered according to the degree that they can decrease the risk of Johne’s in new animals. “Low risk” means no history of Johne’s and critical management practices are in place.

Risk for introducing Johne's disease decreases as information about status of the source herd increases, including:

- No risk assessment
- Test additions before buying
- Test before and after buying
- Test repeatedly after buying
- Low risk source according to history
- Low risk, plus testing, even if low numbers of animals are positive
- Low risk and 30 random animals tested negative
- Low risk, less than 5 percent are AP, according to herd tests
- Low risk, negative herd tests
- Low risk, repeated negative tests



If clients purchase cattle, they are likely to buy animals infected with Johne's. It is a wise decision to establish a plan to prevent its spread.

8. The Voluntary Johne's Disease Herd Status Program for Cattle.

A task force of the Johne's Committee of the U.S. Animal Health Association developed guidelines for a voluntary Johne's disease herd-status program in 1998. This is a scientifically sound, affordable program that encourages

producers to identify their herds as low-risk.

The New York State Cattle Health Assurance Program has adopted most of the Voluntary Johne's Disease Herd Status Program (VJDHSP) guidelines as criteria for herd owners that are interested in pursuing Stage 3 test-negative status on the NYSCHAP Johne's disease program. New York has modified the VJDSP testing scheme to accommodate the recommended Johne's KELA ELISA and follow-up fecal culture testing strategies. As a result, herd status testing is slightly more comprehensive, provides more information to the producer and exceeds the criteria for the equivalent national herd status levels.

Consider participating in the New York State Cattle Health Assurance Program. The national VJDHSP guidelines can be accessed through the USAHA Web site at www.usaha.org.

More on Apparent and True Prevalence when Using Tests for Johne's Disease

These comments add to the discussion in Section 4 where the ELISA apparent prevalence (AP) was 15 percent:

$$P = (.15 + .98 - 1) / (.25 + .98 - 1)$$

$$P = .57 \text{ or } 57\%$$

Prevalence (P) is close to four-times the test prevalence, which makes intuitive sense with an ELISA test that is 25 percent sensitive. Recall, however, in the first herd with 5 percent AP, P was 13 percent, only 2.6 times greater. The reasons for this are:

1. When the sensitivity of a test is relatively low (SE equals 25 percent), then a relatively small number and percent of infected animals will test positive.
2. When relatively few animals in a herd are infected and the test also produces 2 percent false positives (SP equals 98 percent, not 100 percent), then a significant proportion of the test-positive results are likely false.

In the first case, five of 100 cows tested positive. According to sensitivity of the tests, approximately two of the five (40 percent) are likely false and three are likely correct. The three positives that are likely correct represent approximately 25 percent of the infected animals, so P is close to 12.

When the expected prevalence is low, false positive results influence identification of P, based on AP. This effect decreases as the number of infected animals (P) increases and minimizes the effect of the false positives.

In the second ELISA example, where AP was 15 percent, the two expected false positives are a much smaller proportion of the 15 total positives. Thirteen, nearly all, of the 15 positives are expected correct. The 13 represent approximately 25 percent of infected animals, thus the expected P is 57 percent, which is very close to four-times the total number of test positives. The calculation between AP and P adjusts for the expected number of true and false results for the test.

A rule-of-thumb with Johne's disease tests, therefore, is to speculate that the actual prevalence may be two to four times the AP, depending on the test used. The biggest difference for Johne's tests is due to the range of sensitivity -- from 25 percent to 40 percent.

The caveat to remember is that when true prevalence is less than predicted by sensitivity alone, the test causes false positives, such as occurs with ELISA. In most herd-testing circumstances, P is greater than the AP from testing. Low prevalence situations are an exception and P may be lower than the AP because a relatively high proportion of positives may be false. The fact that a positive ELISA result in a low-risk herd has a high chance of being false is why it also has a poor predictive value (PVP).

Points to Include in a Herd Testing Strategy

To get the most from an investment in testing, veterinarians and cattle producers should think about the following issues in advance, before taking any samples. The process helps planning, choosing the right test and strategy and putting results to work to make better decisions.

1. **Know the tests**, including their pluses and minuses toward helping meet the goals, and how to interpret and get the most from numeric results.
2. **Know the laboratory**. It should provide quantitative results and be approved by the NVSL Proficiency test for Johne's ELISA and/or fecal culture.
3. **Formulate an estimate of the prevalence** in the herd or the probability that the individual animal(s) being testing are infected. How does it influence the confidence in the positive or negative test results received and the decisions made?
3. **Agree with clients on what testing should accomplish** and in what time frame. What is the testing expected to do? A variety of possible objectives include:
 - Confirm a clinical case
 - Test animals to determine if infection is likely present in the herd
 - More accurately assess the herd prevalence
 - Embark on a serious control effort by identifying and taking action to segregate or remove infectious cattle
 - Establish a Johne's test-negative herd status
 - Gain initial information and experience with the tests and the disease in the herd
 - Other objectives

Which objective is appropriate depends on each client's situation.

Do not forget to review and anticipate how state regulations and potential legal or ethical responsibilities associated with testing for Johne's disease may impact the business economics of a clients operation and/or their ability to market cattle or genetics in short and long-term time frames.

5. **Design the test strategy to work hand-in-hand with the preventive management plan**. Testing is a tool to help management break the infection cycle. A strong management plan is essential for testing to permit aggressive progress toward the goal. Critically evaluate how comprehensive the preventive management practices are and the extent to which management and employees have the resources, ability and commitment to carry them out. Determine how using test results increase the effectiveness of preventive management efforts.
6. **Outline a decision plan for test results**. Determine what individual animal and management action(s) will be taken for the given result or combination of test

results. Include how to use other health and production criteria with test results to make individual animal decisions. Decide how to define the following categories of animals and what actions will be taken, if any, to reduce the spread of Johne's disease:

- High risk with clinical signs
- High risk test-positive, high PVP, with no signs
- Low risk on test with no signs
- Negative on test(s)
- Pregnant versus not pregnant for each of the above

Several types of decisions can be made for animals at risk of being infected. Each contributes to the total Johne's control effort to varying degrees. Consider which decisions will be effective and economically or otherwise justifiable for the farm. Some possibilities include:

Cull – immediately or when further justified by other performance criteria

Segregate – to separate group, physical location, facility, pasture or herd

Manage differently – colostrum, calving, calving area, replacement decisions, do not breed, do not treat or visibly identify

The aggressiveness of test-based decisions depends on the confidence in the test result, the impact of the potential false positives and negatives, cost of the test compared to the longer term expected return on the decision and each farm's situation, goals, and resources.

7. **Choose which animals to test to meet current objectives.** Get the most from the test dollar by verifying testing objectives. These objectives may include testing only individuals, target groups at risk, the entire herd at the same time or subgroups from the herd at separate times. Expect that objectives and testing will change as more is learned.
8. **Choose the timing of testing.** To make the best decisions, test results should be available and as "current" as possible when management and Johne's control decisions about tested individuals will be made: at calving, breeding, pasture allocation and replacement decisions. Take into account the lag time between submitting samples and getting results.