

*This is the fourth part of the fourth article in a series presenting the most current information and methods about Johne's disease in cattle. The series is directed toward veterinarians helping clients prevent or control this disease. Information was edited and reviewed by the National Johne's Working Group.*

---

## **Testing - Choosing the Right Test for the Right Purpose Part 4 of 4**

Prepared and edited by Christine Rossiter and Don Hansen  
Members of the AABP Food Safety Committee and the National Johne's Working Group

### **Have a strategy**

Despite some limitations, testing will be indicated and useful in most Johne's disease farm plans. However, if you and your client are lacking a plan for interpreting and using the tests, the results can prompt confusion, waste time, and erode confidence in efforts to prevent or control Johne's disease. To get the most from an investment in testing, veterinarians and cattle producers should discuss testing strategy issues as outlined in Section C in the Johne's Disease Plan Manuals for Veterinarians printed in the May 1999 issue of *The Bovine Practitioner*.

### **Using tests for different purposes**

The choice of Johne's test and strategy will be client-specific. Some examples of testing choices are provided.

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

Remember that for most diseases, establishing a diagnosis is an imperfect process usually accomplished through a series of steps that rule-out the different disease possibilities. For an example, you have a herd with no confirmed history of Johne's disease and a 6-year-old cow with diarrhea, gradual weight loss, no fever and a good appetite. There could be ten reasons why this

cow is showing these signs including Johne's disease. By thorough examination and preliminary testing, you could rule out most diseases and estimate that there is a 20% chance this cow has Johne's disease, i.e., your pretest probability for Johne's disease is 20%. Now, which test to use?

The most rapid and least costly tests to confirm this "suspected case of Johne's disease" are the ELISA and AGID. Since the AGID performs best in animals with clinical signs, in this case, the performance of the AGID and ELISA are similar. The probability of a positive test being true (PVP) is 82% and the probability of a false-positive is at least 18%. Table 1.

On the other hand, the best positive predictive values come with the fecal culture test. In this case a positive culture test is virtually 100% i.e., identification of *M. avium subs. paratuberculosis*. Disadvantages to fecal culture here are the delay in results (8 to 16 weeks) and higher cost.

If the serology test result was negative, we could be about 85% confident that the cow is not infected, leaving a 15% chance of a false-negative result. Regardless of a negative test result, it

would be a wise disease management decision to isolate this cow and submit a fecal sample for culture as a second test for this test-negative clinical suspect, particularly since Johne's disease has not been confirmed in the herd before. Occasional cows with clinical Johne's disease don't have detectable antibody. An alternative would be to collect appropriate tissue samples at slaughter for histological examination or culture.

A second scenario may be a 3 year-old cow with the same signs in a herd with an established Johne's infection and culling 3% of cows per year with Johne's. Your impression is that an estimated 50% of cows are infected. The probability that this animal has Johne's is +50%. The PVP of a positive ELISA test now is +95% and may offer enough added confidence to cull the heifer immediately. Table 1

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 s 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.

Following serology testing in a herd with uncertain status, or for the Voluntary Johne's Disease Status Program, it is recommended to retest positive ELISA animals with fecal culture, or histology and culture of appropriate tissues. Retesting with an organism-detection test is also recommended whenever a positive serology test is not consistent with your clinical assessment. Greater confidence can be placed in all

diagnostic tests for Johne's disease in herds that have had a culture-confirmed case and or animals with clinical signs compatible with Johne's disease. Remember that tests for Johne's in mature, infected but non-clinical cattle have diagnostic sensitivities between 25-40%. Consequently, repeated testing is required to definitively establish a negative or positive status.

## 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 their risk. This is an opportunity to suggest some "targeted testing". The object of target testing is to find infected animals. An option is to start with the ELISA, and fecal culture any animals with S/P or OD values just below or above the positive cut-off. Select mature animals to test that may have higher risk of being infected i.e., suspected exposure, poor performance, unknown source, etc. A 10-20 percent sample will offer insights for a relatively inexpensive investment.

For a statistical estimate, randomly testing 30 animals over 2<sup>nd</sup> lactation is another choice. If all are negative, prevalence in the herd is statistically estimated to be 10 % or less.

Target testing can discover infected animals that provide insight into the extent of infection in the herd. 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, better if 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 (2 years and older).

The apparent, or test prevalence (AP) is the number of mature cattle that test positive divided by the total number tested (at one point or over a specified period of time). You can then calculate an estimate of the true prevalence (P) from the AP, by using the test's Se and Sp values.

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

For example, when 5% (AP) of the herd tests ELISA positive, using the test's Se of 25% and Sp of 98% the true prevalence is calculated:

$$P = [.05 + (.98 - 1)] / [.25 + (.98 - 1)].$$

$P = .13$  or 13%. Our more accurate estimate of the truly infected animals is therefore 13% versus the 5% that were detected as test-positive. A crude rule-of-thumb for estimating the true prevalence from the test results is to double the AP value. In this case, the calculated value is close to the rule-of-thumb rule. However, this is not always the case.

If the ELISA AP was 15%, then the true prevalence is estimated to be  $(.15 + .98 - 1) / (.25 + .98 - 1) = 57\%$ . In this case, the percent of animals actually infected is estimated at close to 4X the test prevalence. Using fecal culture, test sensitivity = 40%, the true prevalence estimate would be  $(.15 + 1.00 - 1) / (.40 + 1.00 - 1) = .37$  or 37%. This difference indicates how the true prevalence estimate is affected by

the sensitivity of the test. As rules-of-thumb go, a reasonable estimate for the true prevalence may be 2-4X the AP. The relationship fails for the ELISA when the AP approaches the expected false positive rate, e.g. 2%. If the ELISA AP is 2%, P is 0%.

### **5. Testing for control.**

For clients interested in controlling or eliminating Johne's disease, critical management practices plus separating and or culling high risk test-positive animals are essential actions, particularly if herd infection is high. The appropriate strategy should be chosen by evaluating factors discussed in the Johne's disease Plan Manuals for Veterinarians in *The Bovine Practitioner*, May 1999.

Economics favor using the ELISA as the primary test in a control program. However, more aggressive strategies can be used for more rigorous goals.

For example, the most information about an individual's status is gained, for a higher cost, by testing all animals with ELISA and fecal culture. Fecal culture will detect animals in earlier infection and missed by ELISA. Occasionally, culture negative cattle have elevated ELISA values, which trigger closer monitoring.

Another option is to alternate ELISA with fecal culture at 6-month intervals. An intermediate approach can be to fecal culture animals that have marginally elevated ELISA values, in the range above "background" levels and below the positive cut-off value.

One more major decision in control strategies is to test the whole herd at

once, or by subsets eventually testing everyone in a period of time i.e. 1 yr. "All at once" makes sense when owners plan to make significant management or culling decisions in the near future.

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, cows in mid to late gestation can be tested monthly and results will be available by dry-off. The timing will depend on the lag time for results. Advantages are that testing and costs are distributed over time, and the frequent inflow of new results makes Johne's management a routine and active issue on the farm.

Where short interval or small group testing may not be appropriate in beef herds, timing is. To accommodate management in cow calf herds, one thorough strategy is to test all cows 1-2X per year, timed to have results available before calving or breeding.

#### **6. Testing *immature* replacements.**

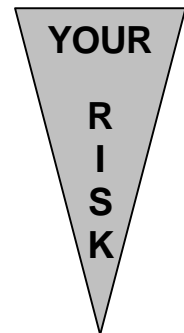
Unfortunately, because of the slow progression of *M. avium subs. paratuberculosis* infections from Stage I to Stage III, no current test has demonstrated sufficient sensitivity in young cattle, <20 months old and in Stages I or II, to warrant recommendation.

#### **7. Testing herd additions.**

Herds become infected with Johne's disease by introducing infected, asymptomatic animals. The risk increases with each addition. The best way for a herd to stay 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 is low risk. Thus, securing animals from herds that are one time or repeatedly test-negative reduces the risk of introducing Johne's to the minimum. Testing individuals and introducing only those that are negative can reduce risk to limited degrees only. The strategies listed below are in order of decreasing risk of Johne's in new animals. Low risk history means no history of Johne's and critical management practices are in place:

- No risk assessment
- Test additions before buy
- Test before and after buy
- Test repeatedly after buy
- Low risk herd history
- Low risk herd history plus testing, even if some are positive
- Low risk history and 30 random animals tested negative
- Low risk history, < 5% AP herd test
- Low risk history, negative herd test
- Low risk history, repeated negative tests



If you buy, you are likely to buy Johne's. Put a plan in place 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 a voluntary Johne's disease herd-status program in 1998. It is a scientifically sound, affordable program that encourages producers to identify their herds as low risk. It is the topic of Article 5 in the next newsletter.

**NOTE:** The National Johne's Working Group of the U.S. Animal Health Association has reviewed the information in this article. Some material has been adapted and edited with the kind permission from M.T. Collins, Johne's Information Center, web site at <http://www.vetmed.wisc.edu/pbs/johnes/>