Testing for Johne’s

Before you work with your clients to test herds for Johne’s disease, you need to understand the ins, outs and issues that surround testing.

By Geni Wren

One of your clients has some thin cows, maybe with or without diarrhea. You suspect there may be a Johne’s problem and think that testing for the disease might be a good idea. Or, your client may have a registered herd and thinks it’s important to verify the animals he is and will be selling are Johne’s-free. But before you jump into testing these individual animals or herds, stop at this point and ask yourself and your clients some hard questions.

“Before starting, I think a veterinarian should ask him or herself ‘how can I get current with the latest recommendations for prevention or control of the disease?’” says Don Hansen, DVM, Oregon State University. “The second is to ask yourself what you want to achieve with testing, such...”
Testing definitions

Many people have the impression that testing is useless and the tests for Johne’s just don’t work, but that isn’t true (see Tests chart). What has caused confusion, perhaps, is the terminology applied to the tests, such as sensitivity, specificity, prevalence, etc. These are terms veterinarians must understand if they are going to offer meaningful and accurate advice to clients concerning testing for Johne’s disease. What follows are some definitions offered by Mike Collins, DVM, PhD, and Don Hansen, DVM.

- **Prevalence (p)** is the percentage of the population with a given disease at a point in time. It is distinct from incidence which is the number of new cases of disease over a specified period of time.

  Prevalence is described in two ways:

  1. Apparent prevalence (test prevalence) is the number of animals testing positive for the disease divided by the number tested.

  2. True prevalence is an estimate of the actual percentage of the population with the disease. True prevalence is calculated from the sensitivity (Se) and specificity (Sp) of the diagnostic test used and the apparent prevalence (AP). The equation for true prevalence is:

     \[
     \text{TP} = \frac{\text{AP} \times \text{Sp} - 1}{\text{Se} \times \text{Sp} - 1}
     \]

     For the absorbed ELISA, a simple rule of thumb is that the true prevalence of Johne’s in a herd is double the percentage of those testing positive. If six cows in a herd of 60 test positive, the apparent prevalence is 10%, but the true prevalence is 20% since the test misses roughly half the infected cows.

- **Sensitivity (Se)**. The terms sensitivity and specificity describe the accuracy of a test. Sensitivity is the ability of a test to correctly identify truly diseased animals as test positive (true positives), the proportion of diseased animals that react to the test. The proportion of diseased animals that give false-negative results is 1-Se.

- **Specificity (Sp)** is the test’s ability to correctly classify truly non-diseased animals as test negative (true negatives). The proportion of non-diseased animals that give false-positive results is 1-Sp.

  These values are calculated by independently testing diseased and non-diseased animals. Because of the difficulty in studying large numbers of truly infected and truly non-diseased animals, the sensitivity and specificity of many tests are estimates. The accuracy of a test may be influenced by the population and the environment from which the tested animals come. The sensitivity of all tests for Johne’s disease increases as the infection in the tested animal progresses. In young or newly infected animals, it is extremely low, while in advanced clinical cases it will be close to 100%.

- **Herd sensitivity (HSe)** is defined as the percentage of truly infected herds that are detected by a herd test. This is a function of the diagnostic sensitivity (Se) of a given test, the average prevalence (p) of infection in infected herds and the number (n) of animals tested. Herd sensitivity of a test is always greater than the sensitivity of a test when applied to individual animals. Sensitivity for detection of individual animals is the number typically used to compare tests.

  Collins says using the simple equation: 

  \[
  \text{Hse} = 1-(1-\text{Se})^n
  \]

  you can calculate the herd sensitivity. “A one-time herd test gives you a lot of information about the probable infection status of the herd. Decision making based on whole herd or partial herd tests is a very powerful way to help clients make the right decisions, particularly when buying cattle and trying to avoid bringing this infection into their herd,” he says.
How do the tests measure up?

Mike Collins, DVM, PhD, offers this list of the currently available tests for Johne’s disease, accuracy estimates and the pros and cons of each test. Collins notes that sensitivity and specificity are relative, not absolute, numbers and estimation of these measures of test accuracy is heavily dependent on the infection level in the populations of animals tested.

### Tests for the organism, *Mycobacterium paratuberculosis*

<table>
<thead>
<tr>
<th>TEST</th>
<th>SENSITIVITY</th>
<th>SPECIFICITY</th>
<th>PROS</th>
<th>CONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>STANDARD FECAL CULTURE</td>
<td>45%</td>
<td>100%</td>
<td>Detects infectious (shedding) cattle Widely available</td>
<td>Slow (16 weeks)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Expensive ($10-15)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Not standardized; accuracy between labs varies widely</td>
</tr>
<tr>
<td>BACTEC FECAL</td>
<td>55%</td>
<td>100%</td>
<td>Detects infectious (shedding) cattle Faster than standard culture (8 weeks)</td>
<td>Expensive ($16)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Not widely available</td>
</tr>
<tr>
<td>GENE PROBE</td>
<td>35%</td>
<td>100%</td>
<td>Detects infectious cattle (heavy shedders) Fastest of detection tests (3 days) Available as a licensed diagnostic kit</td>
<td>Expensive ($25)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Laborious, not good for herd screening</td>
</tr>
</tbody>
</table>

### Tests for serum antibody

<table>
<thead>
<tr>
<th>TEST</th>
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<th>SPECIFICITY</th>
<th>PROS</th>
<th>CONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>COMPLEMENT FIXATION</td>
<td>25%</td>
<td>95%</td>
<td>Commonly used for international export Low cost ($2-4)</td>
<td>Not well-standardized, labs prepare several 'homemade' reagents causing variability</td>
</tr>
<tr>
<td>AGID</td>
<td>35%</td>
<td>100%</td>
<td>Simple and easy, can be done in a vet clinic Available as a licensed diagnostic kit</td>
<td>Only for clinical cases Not for herd screening</td>
</tr>
<tr>
<td>ELISA</td>
<td>55%</td>
<td>99%</td>
<td>Available as a licensed diagnostic kit Low cost ($4-6) Correlates with fecal culture results Quantitative, can rank cows for culling</td>
<td>Misses some fecal shedders May not be able to eliminate infected cattle from a herd without use of a culture-based test</td>
</tr>
</tbody>
</table>

### Tests for cellular immunity

<table>
<thead>
<tr>
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<th>SENSITIVITY</th>
<th>SPECIFICITY</th>
<th>PROS</th>
<th>CONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>SKIN TEST</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Detects early infections Can be used on heifers &lt;24 months old Simple and low cost</td>
<td>Bad reputation, has never been fairly evaluated Must handle animal twice, 1) injection and 2) reading</td>
</tr>
<tr>
<td>GAMMA INTERFERON</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Detects early infections Can be used on heifers &lt;24 months old Only have to handle animal one time</td>
<td>High cost ($25) Logistically difficult, blood to lab in 12 hours Accuracy not established Not licensed in the U.S.</td>
</tr>
</tbody>
</table>
are you willing to disclose that the herd is infected to the state veterinarian or a purchaser of the cattle? Alternatively, what is the liability associated with ignoring a potential Johne’s problem and doing nothing to try to solve it?”

Knowing a herd is infected but not disclosing that fact may expose the owner to charges of fraud or misrepresentation, so it is best to consult a lawyer in your state and check with your particular state’s regulations concerning Johne’s disease. “The practical approach involves making decisions based on the complete understanding of the consequences. People’s livelihoods are at stake and they should be aware of how our recommendations will affect them,” Roussel says.

Choosing tests
There are several tests available for *Mycobacterium paratuberculosis* (see sidebar), but they’re not all equal when it comes to accuracy, cost and the time involved.

Roussel says, “The classification plan proposed by the certification sub-committee of the National Johne’s Working Group calls for ELISA testing followed by confirmation by fecal culture of all ELISA positive animals.” This strategy is the most effective for confirming Johne’s in an infected herd.

Collins says veterinarians should also carefully select the lab they use. He says the USDA-NVSL runs check tests annually and only certifies for Johne’s testing those labs that pass the test; some are certified for fecal culture, some for ELISA and some for both. He suggests asking the lab if it is certified and for which tests.

“You must first base your testing on the overall goal for prevention or control, then the cost, then how accurate the test needs to be and what you’re trying to get out of it,” adds Hansen. “It may also depend on what tests your local lab runs, but cost is usually the underlying factor in your choice.”

Johne’s testing strategies
So now you have a management plan in place and you think you want to test a herd, but what animals do you test? Keep in mind that you can employ all the testing strategies that you want or that your client is willing to pay for, but you’ll be wasting time and money if you’re working with an open herd that brings replacements in from other sources without knowing the Johne’s status of the herd of origin.

Hansen offers some suggestions of what type of herds/animals should be tested:

- Dairies, seedstock producers and operations committed to a classification or control program.
- Herds where you want to establish an apparent prevalence of the disease before launching a control program.
- Herds that are committed to a test-negative status or strict control.
- Where clinical suspects are occurring at more than 3 percent as an annual incidence in older cows and 1 or 2 percent in first or second lactation cows.

If using the ELISA test, Collins says it is only slightly less sensitive than the tube test for brucellosis. He offers these suggestions for strategies based on using the ELISA test:

<table>
<thead>
<tr>
<th>ELISA* (S/P RATIO)</th>
<th>INTERPRETATION</th>
<th>RECOMMENDATION OR COW’S STATUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - .09</td>
<td>Negative</td>
<td>Do nothing</td>
</tr>
<tr>
<td>10 - .24</td>
<td>Suspect</td>
<td>Retest in 6 months</td>
</tr>
<tr>
<td>25 - .39</td>
<td>Low positive</td>
<td>Infected, early stage</td>
</tr>
<tr>
<td>40 - .99</td>
<td>Positive</td>
<td>Infected and shedding</td>
</tr>
<tr>
<td>1.00 - 10.00</td>
<td>Strong positive</td>
<td>Soon to go clinical</td>
</tr>
</tbody>
</table>

*Valid only for the USDA-licensed ELISA sold by IDEXX Laboratories, Inc.

**Target-testing**: An alternative to whole herd screening is target-testing. “Testing more cows improves the accuracy of the herd screen, but it
Mike Collins, DVM, PhD, says it’s the veterinarian’s responsibility to help clients decide when and how to confront a Johne’s disease problem in their herds.

is possible to do target testing of specific classes of cows to get the most for your money,” says Collins. For example, you could target test 30 cows in their second lactation or older, test all cows second lactation or older, test all milking cows, or test all milking and dry cows.

- Quarterly testing: Quarterly testing spreads out the cost to the producer. Some options include:
  - Option #1: Test dry cows. Do not calve the ELISA positives.
  - Option #2: Test all milking cows in their second lactation or older, test all milking cows.

How fast do you want to go?
If you and your client are committed to a Johne’s control and testing program, you can do it a slow and inexpensive way or be more aggressive and spend more money to get rid of the disease faster. Collins says there are six basic plans from the slow to the fast to accomplish these goals.

1. **Enviro-plan:** Management changes only, no testing costs (see “Johne’s Control,” *Bovine Veterinarian*, May/June 1998).
2. **Enterprise plan:** Stop raising heifers, use contract raisers.
3. **Standard plan:** ELISA test-and-cull plus management changes. ELISA test the herd one time per year and cull all positives.
4. **Accelerated plan:** Two tests plus management changes. ELISA test and fecal culture herd one time per year, cull all positives.
5. **Fast track plan:** Three tests plus management changes. ELISA test, fecal culture and gamma interferon test one time per year. Cull all positive cows and the last daughter born to positive cows.
6. **Ultimate plan:** Three tests on cows and heifers plus management changes. ELISA test, fecal culture and gamma interferon test two times per year on all animals over six months old. Cull all positive animals including the last daughter born to positive cows.

There is a Johne’s vaccine available, but while vaccination may be applicable in a moderate to high Johne’s-prevalent herd dedicated to getting out of the disease, says Hansen, veterinarians should bear in mind that vaccination of a whole herd will interfere with the ELISA test and has other negative aspects such as injection site lesions, regulatory paperwork and a human health hazard.

What’s your responsibility?
Johne’s disease is not always a quick, easy problem to solve, and some veterinarians and producers shy away from facing the problem for a variety of reasons — fear of finding the disease, lack of confidence in tests and ethical dilemmas. But does that mean you and your clients should turn a blind eye to it?

The first reason to want to prevent and control it comes down to economics. “If a veterinarian understands the economic impact of what the disease is costing his client he should raise the issue to avoid severe disease consequences,” Hansen says. “If we don’t clean this up it’s going to get a lot worse. However, after a complete herd negative test, at least you don’t have to deal with Johne’s disease in that herd.”

There are also ethical (and probably legal) questions to consider as well. “I think veterinarians who suspect Johne’s but tell clients not to test may do so out of ignorance of the disease,” adds Collins. “The results of Johne’s testing don’t always bring good news, however testing provides useful information that permits the infection to be managed or eliminated. The standards of practice for veterinarians evolve as science progresses and new diagnostic tools become available. To be unaware of advances in disease diagnosis and new knowledge about the pathogenesis and epidemiology of disease is not an excuse.”

“I don’t think our responsibility is to necessarily encourage a producer to test for Johne’s,” says Roussel. “There are clearly situations where testing is a bad economic choice. A control program that does not include herd testing may be a better choice. I think the veterinarian’s role is to educate the client concerning the disease, its economic impact, its control and eventual ‘eradication.’

“Veterinarians should present the options in an informed and accurate way and help the producer decide what is the best way to deal with this disease — or any disease — in his herd.”

“Livestock producers depend on veterinarians for advice on infectious diseases,” sums Collins. “Given the prevalence of Johne’s disease, its economic impact and the growing concern about the zoonotic potential of this infection, I think it is the responsibility of all veterinarians to help clients decide how and when to confront this problem. It remains the client’s decision, but the veterinarian needs to explain the costs and risks very clearly.”

Editor’s note: For more complete information on Mycobacterium paratuberculosis, read Paratuberculosis, Veterinary Clinics of North America: Food Animal Practice, Volume 12, number 2, July 1996.
Diarrhea: could it be Johne’s?

When you see these pictures of a 7-year-old cow, you might not think she looks too bad, but this is a classic example of deceiving looks. Jason Lombard, DVM, Hartford Animal Clinic, Hartford, Wisc., says his client’s cow had acquired diarrhea of about two weeks duration during the dry period, and he suspected Johne’s disease.

He sent a blood sample to the Wisconsin Animal Health Labs and it was tested with the agar gel immunodiffusion (AGID) test, which came back positive for Mycobacterium paratuberculosis. Lombard notes that he normally requests the IDEXX ELISA, but at the time the lab was temporarily out of that test.

“The owner did ship the cow, unfortunately after she calved,” Lombard says. “She was a home-raised animal in a herd who only buys bulls to breed its heifers. The owner couldn’t remember buying any other animals.

“This one case really stressed to me the fact that Johne’s can be present in an animal for a very long time before it shows signs. I used to think animals would have culled themselves before this age if they had Johne’s. Now I’m trying to convince the client to test the entire herd.”

Lombard says it’s interesting that the herd has not had any other animals that were confirmed Johne’s positive. He says not too long ago there was one animal with diarrhea that was negative on the AGID, and a later ELISA test was also negative.

“This herd has a history of displaced abomasums, and we have concluded that in this case diarrhea was associated with subacute ruminal acidosis, which is present in this herd from time to time, after we talked with the owner more about diarrhea and feeding practices. It’s another differential to think about when presented with a cow with diarrhea.”