Third in a series

Testing for JOHNE'S

Walk through a theoretical scenario typical of discovering Johne's Disease in a beef herd and consider the tests available to detect Johne's.

BY SHAUNA ROSE HERMEL

Editor's note: Rather than quoting the sources, we've put this article together in a different format to make the information easier to understand. Sources of information used in compiling this story include:

- **Bill Rotenberger• practicing veterinarian at the Steele Veterinary Clinic, Steele, N.D., and chair of the U.S. Animal Health Association's (USAHA) Johne's Committee.**
- **Robert Whitlock, University of Pennsylvania School of Veterinary Medicine, New Bolton Center, and co-chair of the National Johne's Working Group (NJWG).**
- **Don Hansen*, Oregon State University Extension veterinarian and chair of the NJWG education subcommittee.**
- **Christine Rossiter, Cornell University Extension veterinarian.**
- **Judy Stabel, chair of the NJWG subcommittee on research status and priorities.**
- **A preview of the handbooks mentioned in the accompanying sidebar.**

***To ensure its accuracy, this article has been reviewed and approved by sources noted with an asterisk.**

Animals infected with M. paratuberculosis will often start to show clinical symptoms of Johne's Disease following an episode of stress, such as calving.

If it were a matter of simply testing your herd for Johne's Disease and culling infected animals (knowing the rest to be disease-free), testing would be straightforward. For infected herds, cleanup would be easy. For herds without symptoms, there would be little risk in testing to establish "Johne's-free" status. Unfortunately it's not that simple, especially for seedstock herds.

Diagnosing infection with *Mycobacterium paratuberculosis,* the bacteria that cause Johne's Disease, is a challenge with the tests currently available (see accompanying sidebar, "The Tests," page 202) especially the subclinical Stage I and Stage II infections. The possibility of a noninfected animal's testing positive (a false positive) makes seedstock producers leary of testing at all. The lack of standard protocols for how veterinarians should handle a positive Johne's test in a seedstock herd has resulted in reactions ranging from "ship the cow" to "quarantine the herd."

Still, as we outlined in earlier articles, this is a disease the beef industry can't afford to let get out of hand. It's already put beef seedstock and commercial cow-calf producers out of business. Experts say Johne's is one disease that appears more prevalent in beef seedstock herds than in commercial beef herds. That may be due in part to using dairy recipients of unknown Johne's status in embryo transfer (ET) programs and more intensive management practices that foster the spread of Johne's Disease (e.g., calving in confined areas).

Ethically, seedstock producers owe it to their customers to provide healthy animals. Legally, the issue of liability for selling Johne's-infected animals has already entered the courtrooms.

Johne's Disease is manageable. It is preventable, and there are protocols for eliminating it from a herd. Some states have structured Johne's monitoring programs in place, and a few even offer monetary assistance for testing to cattlemen trying to eliminate Johne's from their herds. (To see if there is one in your state, contact your state veterinarian.)

The National Johne's Working Group (NJWG), a subcommittee of the U.S. Animal Health Association's (USAHA) Johne's Committee, is currently developing strategies to:

. Educate producers and veterinarians about the disease;

. Recommend standard prevention and control measures;

. Standardize testing procedures and interpretations; and

. Remove, through education, the negative connotations to being a "tested" herd.

By this fall, it's their goal to have in place a structured national voluntary testing program to determine herd status, to verify test-negative herds and to use in programs to eliminate the disease from herds. An outline of that program is currently under review and will be presented to the USAHA membership later this fall.

With that in mind, let's walk through a scenario typical of discovering a Johne's problem in a beef herd and some of the decisions the owner must face in regards to testing. We'll put you in the driver's seat.

Assume you bought a cow-calf pair in 1993. The cow was a 2-year-old with her first calf at side. After calving in March this year, the cow (now 7) came down with a bad case of diarrhea. Certain she hadn't had access to the grain bin, you chalked it up to the lush spring grass at first. But after a few weeks, she still had diarrhea, and she was losing weight.

A Pathfinder, she had earned a little extra attention, and you wanted the calf to do well, so you brought her up close to the house, dewormed her and gave her a little extra grain. She seemed to improve, at least for a while. Then the diarrhea started again. She didn't act sick and she had a good appetite, but the corn seemed to go in one end and out the other, and she was losing weight again.

You might have just shipped other cows for being too thin, but you were hoping to restore her health to get a couple more calves. Buffaloed, you showed her to your vet. He turned a little pale, then said, "We'd better test her for Johne's."

As we discussed in the May issue (see page 118), Johne's Disease can be a hidden time bomb, out of sight until it blows.

The source of infection is usually an animal that was purchased or otherwise brought into the herd. Though the animal appeared healthy when it entered your herd, it may have been harboring the M. *paratuberculosis* organism that causes Johne's Disease.

Cattle are usually infected (though not always) with *M. paratuberculosis* as calves, but they may not show symptoms of disease for 3-5 years after infection, (Some animals in research environments didn't show symptoms for 10 years after initial infection.) By the time they do show symptoms, the infected animals have already had time to contaminate the environment and infect other cattle in the herd.

In what's referred to as the iceberg theory, experts estimate that if one home-raised animal is showing the chronic diarrhea and wasting symptomatic of clinical Johne's, 15-25 other animals in the herd will be infected. Those figures are based primarily on research involving dairy herds. They may be less — 5-15 according to some estimates — in beef herds because of more extensive management styles, which will slow down the spread of Johne's.

Let's go back to our theoretical scenario. You wanted a quick answer, so your veterinarian ran a blood test. The blood test came back positive for Johne's. Tests aren't 100% accurate, and your veterinarian said he could run a more definitive test — a fecal culture — to be sure, but it would take 16 weeks to get the results. He was pretty sure the results of the blood test, when considered with the clinical symptoms, were accurate.

Now you have some decisions to make.

The cow was a purchased animal, not a home-raised one, so the problem may not be quite as widespread as that iceberg theory would suggest. Still, using what we discussed in May, we know the cow could have been shedding millions of organisms at least since the diarrhea started at calving, and probably long before that. We also know that 25-35% of calves born from clinically infected dams (as well as a percentage of calves born to cows not showing symptoms) are born infected, too.

The immediate decisions are easy. You

Workbooks to walk you through

The National Johne's Working Group (NJWG), a subcommittee of the U.S. Animal Health Association's (USAHA) Johne's Committee, has been working to establish uniform standards and guidelines, ranging from prevention and control measures to voluntary herd cleanup programs. In the past, Johne's efforts have been geared to the dairy Industry, but now the NJWG is also addressing concerns of beef herds.

The NJWG, through the USAHA, will soon release two handbooks to help educate beef producers about Johne's Disease:

1. *Johne's Disease: Prevention and Control Measures for Beef Producers* outlines what the disease is, how to prevent it, the tests available and how to interpret results. It also outlines an example of a voluntary testing program to determine a herd's Johne's infection status.

2. *Johne's Disease: Prevention/Control Measures in Beef Herds* is a workbook designed to help veterinarians and producers assess the risk of a particular herd's being infected and the possible prevalence level. It helps walk the producers and veterinarian through selecting a test protocol and management techniques to help prevent, control or eliminate Johne's from the herd according to the herd'sindividual objectives.

The handbooks will be presented for final review and approval at the USAHA annual meeting in October. If approved, they will be released shortly after. To request information on how to receive the handbooks or to be placed on a waiting list, contact Don Hansen at 105 Magruder, College of Veterinary Medicine, Corvallis, OR 97331; phone: (541) 737-6533; e-mail: hansedon@ccmail.orst.edu Chairman of the NJWG education subcommittee, Hansen is also an Extension veterinarian in Oregon.

wean the calf early and ship the cow, pulling a fecal sample before you load her. After all, you're a seedstock producer and if there's a chance the blood test was wrong, you need to know. Though the cow has lost weight and you've lost some income there, she is still saleable if you get her sold before the weight loss is too extreme.

You decide her calf isn't worth taking a chance on, so you'll isolate it for the time being, then put it in the feedlot with the rest of your cull calves after you wean the rest of your herd. Even if infected, the risk is slight that the calf would shed any bacteria to infect other animals at the feedlot or that it would show any clinical symptoms of Johne's before it is harvested. You should get normal feedlot performance and full market value for it when it's sold, so at least that's some good news.

Now the hard part. That cow has been in your herd for eight years, and you don't know when she started shedding the organisms, exposing the rest of the herd to infection. You've kept three daughters, sold one son to a commercial herd and fed out another.

And what about the other cows and calves in your herd?

Evenexhibitingclinicalsymptomsof Johne's Disease(chronicdiarrheaandwasting), animalswillshowanormalappetiteuntil weightlossandmalnutritiontaketheirtoll.

The hardest part of resolving a Johne's problem lies in being able to identify infected animals, especially subclinical cases. Most cattle with subclinical infection with *M. paratuberculosis* can't be diagnosed by a fecal culture. In many, the infection may be detected at postmortem through culture of specific lymph nodes.

Diagnosis of *M. paratuberculosis* infection in live animals is difficult because of the microorganism's slow growth pattern and because of the immune response it elicits. In the initial subclinical stages of infection, *M. paratuberculosis* elicits a cell-mediated response, which basically keeps the infection confined to the intestinal wall. It doesn't produce antibodies in the bloodstream that serology tests could detect. The animal isn't shedding bacteria, so a fecal culture wouldn't indicate an infected animal.

As the infection progresses to clinical disease, that cell-mediated response drops off and a humoral response, which produces antibodies, predominates. The presence of antibodies to ***M.*** *paratuberculosis* makes blood testing possible, but it doesn't protect the host against the disease. Without the cell-

 CONTINUED FROM PAGE 196

mediated response to keep the infection in check, it can spread, and the animal will begin to shed organisms in its feces.

When testing an animal or a herd, it's important to understand a couple of general concepts about tests and test results.

First, they aren't 100% accurate. Four outcomes are possible: true negative (accurate), false negative, true positive (accurate) and false positive. For example, if you test an animal in the preclinical stages of *M*. paratuberculosis infection, before diarrhea develops, the animal will likely test negative — but it's a false negative.

The measures of a test's accuracy are its sensitivity and its specificity. Sensitivity refers to the ability of a test to detect infected animals. The lower the sensitivity, the more false-negatives will occur. Tests with low sensitivity underestimate disease in an infected herd.

Specificity refers to the test's ability to detect noninfected animals. It's the probability or likelihood of a negative test result in animals known to be free of infection. A test with low specificity will produce more false positives than a highly specific test.

The ability of a test to accurately

distinguish an animal's Johne's status will depend on the test, the lab conducting the test and the stage of infection.

Which tests are best to use and in which combination will be unique to your herd's situation. You should thoroughly discuss your options and the ramifications with your herd veterinarian.

CONTINUED ON PAGE 202

The hardest part of resolving a **JOHNE'S** problem lies in being able to identify infected animals, especially subclinical cases.

 CONTINUED FROM PAGE 199

The Tests

Nine tests are currently used to detect Johne's Disease. Four detect the bacterium that causes Johne's Disease, three detect serum antibodies, and two detect ceil-mediated immunity. Six of the tests (denoted by an asterisk) are commonly available for use by veterinary diagnostic laboratories.

Tests for bacteria

- ***Standard bacterial fecal cultures**** require little equipment and are technically simple to perform. Most experts agree fecal culture has been the most accurate diagnostic test to use on live animals. It holds an advantage over serological tests for seedstock producers wanting to limit the chance of false positives.

They do, however, require experienced technologists to conduct the tests and are moderately expensive. It takes up to 16 weeks to get results. That means an animal that's shedding *M. paratuberculosis* could continue to contaminate its environment and infect other animals during the four months it takes to get results back.

Fecal culture sensitivity is considered to be about 30-50% and its specificity to be 99% if done correctly.

Fecal culture is very good at finding cattle shedding *M. paratuberculosis* organisms, but it doesn't identify subclinical cases in which the animal hasn't started to shed any organisms (Stage I and Stage II). Also realize some animals may be intermittent shedders -they may shed the organism for a period, then stop, only to start shedding again. A fecal culture taken when the animal wasn't shedding would not detect infection. Therefore, a negative test result must be interpreted with caution.

- ***Radiometric culture*** is a radioisotope-based detection method adapted from one used to isolate the cause of tuberculosis in humans. Its main advantage is that it can detect low numbers of bacteria and, with results in seven weeks, is faster than standard fecal culture methods. It is, however, more expensive, requires an instrument to read the culture vials and involves handling of radioisotopes.

Radiometric methods can't detect Stage I infections but may detect some animals in late Stage II. Its sensitivity is about 40%; its specificity, 99%. Interpret negative results with caution.

- ***DNA probes*** allow detection of the microbe without having to grow it by culture, so they yield results within three days. Their biggest drawback is cost, which can be twice that of standard fecal cultures.

The probe can't detect animals in Stage I or Stage II infection. Its sensitivity is about 20% and its specificity is nearly 99%.

- ***Histology of tissue**** - microscopic examination of the tissues — requires that specific tissues be collected by a surgical procedure or by necropsy in addition to special preparation before sending to the diagnostic laboratory, which increases its cost.

Sensitivity of the test depends on the stage of the disease and the number and type of specimens examined. It will not detect Stage I and Stage II infection.

Antibody tests

Three serological tests to detect serum antibodies of cattle infected with *M. paratuberculosis* are available in most diagnostic laboratories. The agar gel immunodiffusion (AGID), enzyme-linked immunosorbent assay (ELISA) and complement-fixation test (CFT) are easy to perform but lack sensitivity. They are best used as herd screening tools to identify infected herds. They are reasonably accurate to confirm infection in an animal showing clinical symptoms.

- The AGID* test has a high specificity (>90%) in cattle with clinical signs compatible with Johne's Disease (in late Stage III and Stage IV). Infected cattle without clinical signs are less often positive on AGID. The sensitivity is estimated to be 30% in pre-Stage IV infections.

- The ELISA* has been most widely used for screening herds. Detection of infection by ELISA techniques appears to be dependent upon the disease stage of the animal tested. ELISA sensitivity for clinical cases has been reported to be 85% while the sensitivity is about 15% for subclinical cases. It's also important to note that a new ELISA assay kit has been licensed to replace the one formerly available. Performance of the new kit is still being assessed.

Most experts on Johne's recommend any animal testing positive for Johne's based on ELISA be confirmed infected with Johne's by fecal culture or DNA probe if:

1. There's little other clinical evidence (diarrhea, wasting) to support the diagnosis;
2. The animal is of exceptional genetic value;
3. The pretest Johne's status of the herd was unknown.

- The CFT*, which is required by many countries for export or import, is intermediate in sensitivity and specificity to AGID and ELISA. With many false positives and false negatives, the CFT isn't recommended for routine diagnostic use. Antigens used in the assays in different countries vary in composition depending on the method of preparation.

While in the subclinical stages, infection with* M. paratuberculosis, *the bacteria that cause Johne's Disease, is virtually undetectable.

Tests for cell-mediated response

- Cell-mediated immune responses are considered to be the first and most important response of animals to infection. One way to measure this response is ***skin testing**** - injecting tiny amounts of mycobacterial extracts under the skin, then observing for swelling and redness at the injection site two or three days later. The sensitivity of the Johne's skin test is about 54%; specificity, about 79%.

- The *gamma interferon assay* is a more sophisticated laboratory test for cellular response. Conducted on blood samples, it measures release of a chemical (gamma interferon) from white blood cells. Blood samples must be in the lab within 6-12 hours of collection to assure live white blood cells for testing. Few studies have been published evaluating its diagnostic accuracy. It may detect animals in Stage II or higher.

Laboratory methods vary for conducting fecal cultures and serology tests, which means test results also can vary. A national check test program is in place, coordinated by the National Veterinary Services Laboratories (NVSL), to help standardize laboratory methods and provide laboratories with information on their own performance. NVSL monitors check tests for diagnostic laboratories offering fecal culture, AGID and ELISA for diagnosis of Johne's Disease in cattle. The NJWG recommends using a diagnostic laboratory that has passed NVSL's check test and is accredited for the tests to be used.