Early Detection and Prevention

New EVELISA blood test could be a major step to eliminating Johne's.

Story & photo by ***Ed Haag***

There is a recognized consensus among those scientists, researchers and industry leaders involved in battling Johne's that the shortest road to eliminating the pervasive and economically destructive bacterial infection from our beef and dairy herds is to develop a low-cost, quick-response, high-sensitivity test that identifies Johne's-positive cattle before they can infect their herdmates.

Ken Olson, coordinator for the National Johne's Education Initiative, is encouraged by progress being made to develop new tools to detect the disease.

"There are a variety of tests on the horizon that will enhance our ability to detect Johne's sooner and more accurately," he says, adding that as these tests become available to livestock producers, the industry will be able to shift to more aggressive strategies in dealing with the disease. "The focus will probably change from a control-type program to reduce the incidence of the disease in herds to one of testing and culling the positives, which will more rapidly move toward the elimination of the disease in the herd."

For the cattle industry, the stakes are high. It is difficult to assess Johne's financial impact nationally, but the results of a recent state-wide prevalence study found that 4% of Georgia's beef cattle were Johne's-positive. An accompanying economic analysis determined that the annual cost of Johne's to that state's beef industry was between $2.449 million and $4.898 million annually.

In another study, the U.S. Department of Agriculture (USDA) National Animal Health Monitoring System (NAHMS) tested 10,372 beef cows in 380 herds from 21 states. It estimated, from the results, that 7.9% of the beef herds in the United States were infected with the disease.

As Olson points out, the true economic effect of Johne's on beef herds is often delayed by the glacial progress of the disease. In the infection process, the Johne's bacterium enters and slowly spreads in the small intestine, leading to a thickening in its wall, which, in turn, reduces the intestine's ability to digest nutrients. After a prolonged subclinical period — two to five years — it manifests itself clinically in chronic diarrhea, weight loss, infertility and eventually death.

Because the average cow-calf operator relies on the reproductive longevity of his cows to recover rearing costs, and with the average Johne's-infected animal "going clinical" between 2 and 5 years of age, a cow-calf producer could lose seven to nine years of production off a single infected heifer.

In addition, it has been demonstrated in studies that cattle are capable of shedding live Johne's bacteria in their feces within three months of their initial contact with the disease. Olson notes that because of the lead time between when infected animals start to shed the bacterium in feces and when they demonstrate their first clinical symptoms, it is possible for an untested herd to develop a major population of infected animals before a single cow is visually identified as having Johne's.

Seedstock producers vulnerable

While young calves are more likely to become infected with the Johne's bacteria than adult beef cows, a study published in *Veterinary Microbiology* (Vol. 103, Issues 3-4,

►**Above:** New EVELISA test could help identify Johne's test-positive animals early, reducing the risk of undetected animals infecting others.

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November 2004) confirms that bull calves born in an infected herd can be sources of infection when later used for natural mating or artificial insemination (AI). Subclinically infected bulls release *Mycobacterium paratuberculosis* into semen, consequently infecting the uterine environment of cows.

An earlier study carried out by Merkal et al. (1981) confirms even a small number of *M. paratuberculosis* in the semen of a bull, if not enough to infect, appears to be sufficient to establish hypersensitivity in a recipient cow. In the same study, an inflammatory response to the bacteria was observed in cows inoculated intrauterinely with *M. paratuberculosis*. The inflammation may be responsible for the decreased fertility.

In addition to spreading Johne's through its semen, an infected bull used naturally may shed the organism in his feces, inadvertently exposing calves to the bacteria at a point in time when they are most vulnerable.

Olson sees a growing concern among cattle breeders about the implications of inadvertently introducing Johne's into their herds. He believes that the next generation of tests will encourage more participation on the part of the breeders.

"I talked to a seedstock producer last week who wanted to bring outside genetics into his herd, but the fact that there aren't more herds testing makes him wonder how he will do it without putting his animals at risk," Olson says. "Developing more effective tools will encourage more seedstock producers to test."

Current test limitations

Scott Wells, bovine epidemiologist, University of Minnesota and former chairman of the Johne's committee of the United States Animal Health Association (USAHA), notes that the two most commonly used blood tests for Johne's are both tests for antibodies.

"The problem is that most cattle are infected as calves or as young stock," he says. "It takes at least a couple of years for an infected animal to develop antibodies, and some don't develop them at all."

Wells adds that another test for Johne's involves culturing a fecal sample for *M. paratuberculosis.* Although it costs more than twice as much as the individual antibody tests and takes up to six months to obtain the results, he finds the fecal test particularly useful as a general, cost-effective screen for larger confined herds. Samples taken from heavily traveled areas and lagoons will test positive for Johne's even if only a minute amount of the bacteria is present.

"This doesn't address the issue of individual animals, but it does test the overall herd for the presence of Johne's," Wells says. "It can be a very cost-effective first step."

He adds that using current tests in conjunction with best management practices (BMPs) recommended by the National Johne's Working Group and other organizations dedicated to promoting awareness and control of the disease works for beef producers willing to step forward and avail themselves of one of the Johne's test-negative programs established across the country.

While Bill Shulaw, Ohio State University Extension veterinarian and Johne's committee member, concurs with Wells' assessment of the value of existing testing procedures for controlling and reducing the incidence of Johne's in beef herds, he stresses that with today's technology there is no way to certify that a herd is totally Johne's free.

"The test-negative programs in virtually any state, as well as the national program, do not certify that herds do not have Johne's," Shulaw says. "What the test-negative programs do is to certify the test status and to the extent that the testing program is rigorous gives you some level of confidence about the herd's infection status."

New studies show promise

As Olson stated earlier, it is the limitations associated with existing test methods that ultimately control the effectiveness of the response.

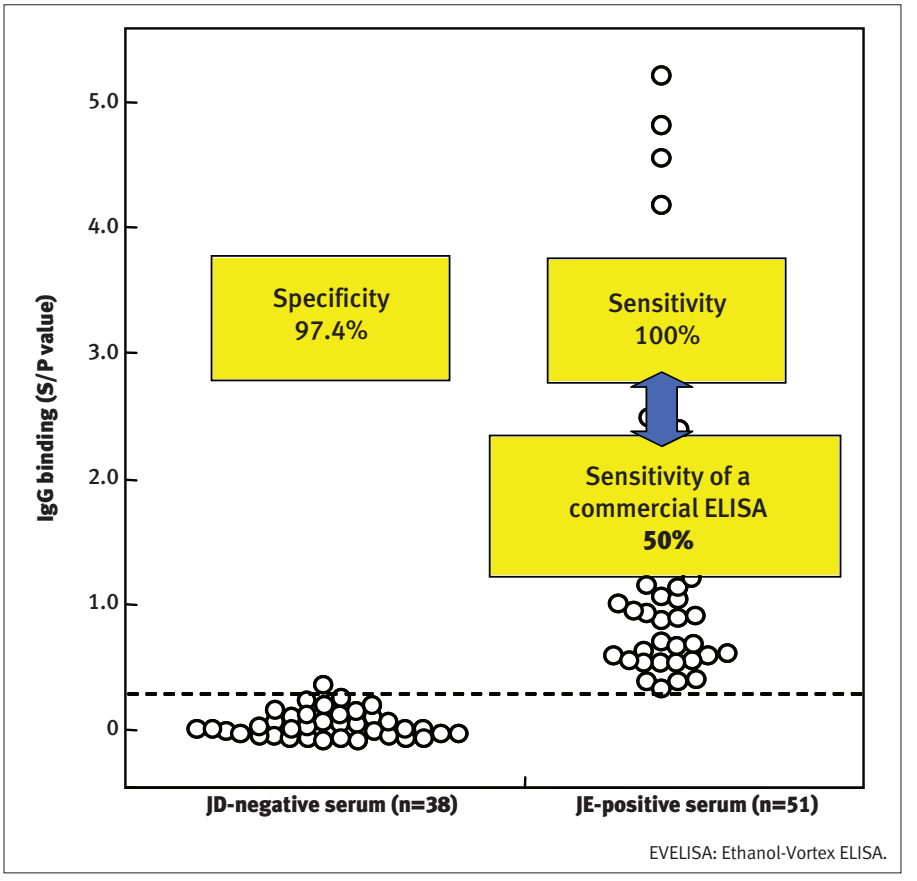
"The current Johne's program is based on the apex that depends on a model of the sensitivity and specificity of the currently available tests," he says. "As we increase both the sensitivity and specificity, we certainly move more rapidly toward control and possible eradication of the disease."

Olson cites brucellosis (Bang's disease) as the example of a disease that once was widespread in this country's beef and dairy herds but through the development of better testing methods has been all but eliminated.

"Other than being in the wildlife population in greater Yellowstone and in the occasional animal imported from Mexico, brucellosis is no longer a significant problem," Olson says.

He believes that one or more of the tests currently being developed in laboratories around the world could serve the same

Fig. 1: Diagnostic specificity and sensitivity of EVELISA



EVELISA: Ethanol-Vortex ELISA.

purpose for Johne's control as new tests did for brucellosis a generation ago.

"There is some very interesting work being done in a number of areas," Olson says, adding that the genetic mapping of the Johne's bacteria has created new opportunities for scientific inquiry. "Researchers are now looking at different identifiers (genetic markers) of the organism as well as looking at the potential difference in the genetic susceptibility of animals."

Olson explains that studying genetic identifiers and traits are not the only lines of inquiry that show promise. Researchers are also making progress developing a vaccine.

From ELISA to EVELISA

One new test that has undergone initial scrutiny and holds the potential of revolutionizing Johne's detection is a modification of an existing commercially available ELISA blood sample test. This new version, dubbed the EVELISA test, was first reported in the American Society for Microbiology's publication, *Clinical and Vaccine Immunology,* August 2006.

Discovered by a research team led by Shigetoshi Eda (Shige), at the Center for Wildlife Health, University of Tennessee, Knoxville, the modification introduces an additional step to the conventional laboratory process that dramatically increases the test's sensitivity.

"The current ELISA test uses the entire lysate (the byproduct of a destroyed bacterial cell)," Eda says. "We just use its surface antigen, which appears to be far more immunogenic than the whole lysate."

He goes on to explain that the surface antigen is dislodged from the source of bacilli by treating it with 80% ethanol followed by a 30-second interval of agitation by vortex.

To evaluate the effectiveness of the new EVELISA assay in comparison with the commercially available ELISA test, a comparative study was launched involving serum samples from 64 fecal-culture-positive cattle categorized as low (<10 colonies; n = 29), middle (10-50 colonies; n = 8), and high (>50 colonies; n = 27) shedders. In this analysis, all of the fecal-culture-positive cattle were included, regardless of the number of colonies formed in the fecal-culture test.

Based on the results of the study, the EVELISA test identified 96.6% of the low shedders and 100% of the middle and high shedders, while the commercial ELISA test detected only 13.7% of the low shedders, 25% of the middle shedders and 96.2% of the high shedders.

Overall, the EVELISA had a sensitivity of 98.4%, detecting all but one fecal-culture-positive animal, whereas the commercial ELISA detected 50% of the fecal-culture positives.

"The difference in sensitivity between the two tests is due to the ability of the new test to detect the early stage of Johne's disease," Eda says, adding that within one year of being intentionally infected with the bacteria, 100% of the infected calves tested positive on the EVELISA assay.

He adds that this ability to detect the disease early — as early as five months after the inoculation of the Johne's bacteria — is particularly significant because if and when the test becomes commercially available, it will, for the first time, offer producers a practical tool to determine, in more cases, if individual animals have Johne's before they can pass the disease on to others in the herd.

"Our study shows the EVELISA was substantially superior to the commercially available ELISA, especially in detecting low-level and midlevel shedders," Eda says. "This leads us to believe the EVELISA may form the basis for a highly sensitive and subspecies-specific test for the diagnosis of Johne's." AJ