

Understanding Johne's Laboratory Tests

The April 2019 Trails Magazine article, "Johne's Disease: What Longhorn Breeders Need To Know", furnished background information leading to this exploration of testing and value-added herds. This month we look at the Johne's tests available, their limitations and recommendations for dealing with this disease in the Texas Longhorn industry. It also introduces the idea of establishing a Johne's free value-added herd.

THE PROBLEM

Most Longhorn breeders have a herd health plan that includes testing for Johne's Disease. The breeder maintains a Johne's free herd and screens all new purchases. One day he purchases a cow at a sale, but before he turns her out in the herd he checks a blood test to protect his Johne's free status. It comes back positive. What should he do? Repeat the test and hope it's negative? Take the cow to the sale barn? Return her to the seller? Quarantine and screen her with more tests later? What if she is pregnant and soon to calve? Is the calf infected or is it salvageable? Well... it depends on multiple factors as you will see.

To complicate things, one requirement before a Longhorn sale is the Bovine TB test within 30 days of the sale. This skin test is regarded as the definitive indicator of infection by the bacterium that causes bovine tuberculosis in cattle - *Mycobacterium bovis* (MB). Unfortunately, *Mycobacterium avium paratuberculosis* (MAP) — the Johne's bacteria — is a close genetic relative to MB. The blood test for MAP can cross react with the TB test giving a false positive. It is a paradox that it is a requirement to test for MB when 99.999% of US herds were not affected from 2003-2017. Even my 65-year-old veterinarian has yet to find a positive TB test despite thousands done since vet school. Alternatively, 10% of U.S. beef herds and 68% of dairy herds contain at least one animal infected with the MAP bacteria. Johne's is 10,000 times more common than bovine tuberculosis in beef cattle, yet it is not mandated to test for it.

JOHNE'S LABORATORY TESTS

(1) BLOOD: The ELISA (Enzyme-Linked Immunosorbent Assay) blood test is designed for screening

large numbers of cattle quickly with results back in a few days. It is relatively inexpensive (\$5.50/animal) and requires 3 cc of blood to test for the MAP antibody. The higher the test result, the more likely the cow is infected. Results are reported as sample to positive ratios (S/P ratio). The typical cut off S/P values are: less than 0.45 is negative; between 0.45 and 0.55 is suspect; greater than 0.55 is positive. There are other blood tests, but the ELISA is the predominant screening test. (2) FECAL: There are two types of fecal tests - the culture and the PCR. A culture takes several weeks and typically costs \$35.00/sample. PCR (Polymerase Chain Reaction) costs about the same and looks for genetic material from MAP instead of the living organism. Most labs provide a PCR result in less than a week.



Concerns have been raised over the possibility of TB tests potentially causing false positives for Johne's due to the genetic closeness of the two disease-causing bacteria.

The sensitivity of the culture and PCR are generally comparable but the breeder gets the answer much faster with PCR.

How accurate are these tests? Not very as it turns out. A good screening test is sensitive with a high probability of detecting the disease and specific in that there is a high probability that those without the disease will screen negative.

The ELISA blood test has many false negative results (the cow has the disease but it is not detected) due to delayed antibody production— especially in young, asymptomatic cows. Michael Collins, DVM, PhD is one of the recognized Johne's experts in the world. He studied over 400 asymptomatic cows with positive MAP fecal cultures and found the commercial ELISA tests were positive in only 28-45% - a low sensitivity. The specificity was much higher — 97-99%. In other words, a positive test was overwhelmingly valid when it occurred. False positive ELISA tests (the cow does not have the disease, but the test says it does) also occur. There are about 140 different species of mycobacteria that are present in the environment which can cause skin or soft tissue infections— abscesses, ulcers, etc. These can cross react with the MAP ELISA test. Examination for these infections will alert you to the potential of a false positive.

The Johne's fecal culture and PCR tests also have limitations with sensitivity in asymptomatic infected cattle. However, there are virtually no false positives like in the blood tests. The gold standard for the indus-

And Establishing The Value Added Herd

try has been the fecal culture, but the results can take several weeks. Studies have revealed the PCR test can detect up to 95 - 100% in heavy shedding herds. For light shedders, it's effectiveness is 40-75% depending on the study. A negative result does not necessarily indicate that the cow is not infected or not shedding. It is the intermittent shedding of the MAP bacteria in feces that creates diagnostic difficulties.

CROSS REACTION BETWEEN BOVINE TB AND JOHNE'S

As mentioned earlier, a common problem in the Longhorn industry is screening new cattle purchases to maintain a Johne's free herd. There is a close biological relationship between MAP (Johne's) and MB (bovine tuberculosis). If an animal is MB tested 30 days before a sale, a cross-reactivity between MAP antibodies and MB antigens may create a false positive.

We decided to test this in our herd. We studied 12 Longhorn heifers 18-22 months of age. Each had a TB skin test on 10/22/18 and MAP ELISA blood tests drawn on 10/22/18 (day 0), 11/26/18 (day 35) and 1/17/19 (day 87) to see if the effect on S/P ratio. The results (see Table 1) revealed that all heifers were negative on the day of the TB skin test. At 35 days post TB test, one sample was suspicious but none turned positive. However, the average S/P ratio had tripled. All 12 S/P ratios returned to normal at 87 days. Susan Moore, PhD at K-State Veterinary Laboratory informed me that the S/P ratios were negative values on 1/17/19 because the negative control was

high. She assured me the results were valid.

There are 2 other studies in the veterinary literature. Aiden examined the impact of a single TB skin test on serum MAP ELISA tests afterwards. Prior to the TB skin test, 8% had positive MAP ELISA. 14 days after the TB test, the positive ELISA jumped to 39% and remained positive for 71 days. In another study by Vargas, 60 animals from a Johne's free herd were given a TB skin test with ELISA blood samples collected at 0, 30, 60 and 90 days. 8% turned positive. This suggests the TB test can create false positive results. This has led to the recommendation that there should be a 90 day waiting period before ELISA testing. Although there is an obvious cross reaction, it is not overwhelming (Aiden 8%-39%; Vargas 0-8%; Gilliland 0-0% but titers tripled) and future follow up is recommended.

IMPLICATIONS FOR THE UNBORN CALF

What about the ELISA positive pregnant cow? A 2017 article from the USDA estimates that up to 25 percent of calves are infected in utero if the cow is in Stage III of the disease. Another study revealed the prevalence of in utero fetal infection in cattle as 9% of fetuses from MAP subclinically infected cows and 39% from clinically affected cows. That incidence rises when calves become infected by exposure to contaminated manure any time in the first year of life. Adaska and Whitlock estimated the overall in utero transmission rate as approximately 2-4.3% in fecal culture positive and ELISA positive cows. That means a genetically superior calf could be weaned at birth, get colostrum, be raised by another disease free cow and have a high chance of being Johne's free.

Table 1

	10/22/18(day 0)	11/26/18 (day 35)	1/17/19 (day 87)
Cow #1	0.029	0.035	-0.09
Cow #2	0.046	0.063	-0.045
Cow #3	0.052	0.071	-0.039
Cow #4	0.113	0.147	-0.055
Cow #5	0.013	0.034	-0.051
Cow #6	0.018	0.017	-0.079
Cow #7	0.04	0.104	-0.082
Cow #8	0.051	0.025	-0.116
Cow #9	0.053	0.347	-0.036
Cow #10	0.062	0.392	-0.033
Cow #11	0.054	0.051	-0.12
Cow #12	0.037	0.459 (SUSPECT)	0.14
AVERAGE	0.047	0.145	-0.054

IMPLICATIONS FOR THE LONGHORN INDUSTRY

There are varying opinions on Johne's because of the cost and imperfection of the tests. There is also an element of denial. The profit margins can be modest in this industry and additional overhead needs to be justified. No Longhorn breeder makes a profit selling \$1500 heifers. It is that infrequent \$50,000 cow or bull sale that pays the overhead. The problem is who would pay \$50,000 for an untested animal that has a 3-5% chance of having asymptomatic Johne's? Not many. We need to try to protect all buyers, and especially new breeders, to get long-term growth in this industry. Each new breeder has to buy from multiple herds to establish his own. These purchased

animals must be Johnes free to the best of the seller's knowledge. As registered breeders, we are not trying to reduce Johnes in an infected commercial herd. We are trying to prevent it 100% in an elite registered Longhorn herd.

There was a previous effort to establish fecal testing for Johnes as a pre-sale requirement for consignors at the 2018 Hudson Valentine Ft. Worth fall sale. The intention was to instill buyer confidence in purchasing a great Longhorn cow verified to be Johnes free. The pre-sale requirement was rescinded due to the confusion with TB tests creating false positives. This is probably a good idea for all sales. If the seller got a blood (\$5.50) and/or fecal test (\$35.00) before any sale, the buyer would have more confidence. This still does not guarantee a Johnes free animal, but it is the best we have available at this time. These laboratory tests are imperfect at best. The ELISA test can miss 50% and the PCR can miss 25%. However, if both are negative multiple times and a regular monitoring program is in place, the buyer should have more confidence in his purchase. Ignoring the problem will only exacerbate it in the future and we should address it as an industry.

ESTABLISHING THE VALUE ADDED HERD

Every Longhorn breeder has a decision to make regarding Johnes and herd health. The following options all have an effect on the market value of your herd.

- (1) **No testing....** If you choose not to test, that virtually guarantees your herd will be infected over time if you are buying and selling cows each year. Serious breeders will lose confidence.
- (2) **Annual/Semi-annual herd testing** –You establish a bovine medical record with multiple negative tests. Hired Hand websites have a column for the medical record – put it there and market it. An ELISA blood test with 50% accuracy performed multiple times over several years greatly enhances the probability of having an MAP free animal. You can combine it with fecal testing on selected cows

- either randomly, for those that have a lower body condition, or for those that are unexpectedly open.

- (3) **New Purchase Quarantine/Testing** –A 90 day quarantine is necessary for ELISA testing to avoid confusion with TB testing. Fecal testing could be implemented after the sale. Both have false negative results but are very specific. Pre-sale testing by the seller makes it easy for the buyer to confidently buy your animal. Be sure to have the auctioneer announce the negative Johnes testing at the sale. It is under-appreciated and frequently unannounced at public auction. This is far easier than a buyer having to quarantine the animal for 90 days before an ELISA and/or FECAL are proven to be Johnes negative.
- (4) **Establish a Johnes free herd and buy only from Johnes free herds** - According to the USDA, the lowest risk possible for maintaining a Johnes free herd is regular blood and fecal testing within your herd and purchasing only from negative tested herds.

CONCLUSIONS

A Johnes free herd has added value. Horn length means nothing if a newly purchased, untested animal is silently infecting your herd. Every Longhorn breeder should have an ongoing monitoring program testing all adult animals over 2 years of age. Annual or semi-annual screening of cattle can be carried out using blood tests combined with fecal tests to clarify the infection status. Although an individual test can miss the infection, multiple negative results over years creates a medical record of wellness and has value. Animals with any positive test results should be treated as a potential source of infection for calves and managed to prevent transmission. False positive and false negative occur with frequency, especially after TB testing. As more is learned about the issue, the Texas Longhorn industry would be well-served by establishing industry standards.

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