Johne's Disease: What have we learned from our Demonstration Herd Project?[1]

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In late 2003, the USDA provided funding for interested states to participate in the National Johne's Disease Demonstration Herd Project. The primary objective of the project is to evaluate the long-term effectiveness and feasibility of various management-related disease-control measures for Johne's disease on dairy and beef cattle operations. Secondarily, the program has provided for educational opportunities for producers and veterinarians highlighting diagnostic testing and management and control strategies. Currently, 17 states with 66 dairy herds and 22 beef herds participate in the program. Ohio enrolled three herds in the summer of 2004, one dairy herd and two beef herds.

In Ohio, the beef cattle herds have been tested by individual animal fecal (manure) culture and blood serum ELISA testing every spring and fall since the fall of 2004. In addition to the individual animal tests, samples of the farm environment have been taken for culture of the causative bacteria; udder skin surfaces have been sampled for culture; and individual animal fecal samples have been pooled in groups of five for culture. Several other "spin off" projects have also been conducted. A large amount of data has accumulated as well as a number of observations concerning management of the disease. So after four years of intensive testing in Ohio, what are some of the things we learned?

Cows shedding *Mycobacterium avium* subspecies *paratuberculosis* (MAP - the cause of Johne's disease) in their manure contaminate the environment with these bacteria which are then ingested by susceptible calves. After an incubation period of several months to several years, these animals may show the classic signs of weight loss and diarrhea and pass large numbers of MAP in their manure. However, most animals shed MAP for months to years before they show any signs of disease. We have known these things for a very long time. Nevertheless, we have only recently begun to appreciate just how many of these bacteria are actually being shed by some cows and how severely the environment can become contaminated. The environmental samples and udder and teat skin samples we have collected show just how severe this can be.

The udder and teat skin samples were collected by rubbing a small section of the skin of the udder at the base and side of the teat for fifteen seconds using a sterile gauze sponge soaked in sterile water. Care was taken to avoid any fresh manure on the skin. The gauze was taken to the lab where any barn dirt was mechanically shaken off, the gauze removed, and the dirt processed for culture much like a fecal sample taken from a cow's rectum. Results from one of the beef herds may serve to illustrate the potential exposure an udder could provide for a calf. During the spring of the first year of the Project, we collected udder scrub samples and fecal samples from all cows in the herd. The herd had calved in a dry lot setting prior to going to pastures, and nearly all the calves had been

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born and were nursing their mothers at the time samples were collected. Of 88 individual animal fecal samples, 7 cows were culture positive, but of the 88 individual udder and teat skin samples, 33 were positive. Furthermore, a few of these skin samples had numbers of MAP in them approximately equal to that of manure samples taken from the rectums of cows that are classed as "heavy" shedders! Remember that these skin samples were taken only one time in a small area around one teat. Imagine the potential exposure a calf nursing that udder several times a day could get. At another sampling session the following year, only two of 117 animals were fecal culture positive, but 4 of 113 udder skin samples were positive, and all of these were on fecal culture-negative cows. Cows shedding MAP into the environment can make the udder of many of their herdmates sources of infection for the calves.

Results from the samples we collected from the environment on these farms were also revealing. At each visit we collected twenty samples, equivalent to about two ounces, of dirt, bedding, or manure slurry from various areas such as free stall alleyways, calving areas, and dry cow pens at the dairy farm, and loafing areas, around round bale feeders, and the calving areas at the beef farms. We strived to get bedding material or dirt from the pens that would mirror what a calf would get exposed to in the pens and lots or on its mother's legs and belly. In general, when the numbers of infected individual cows were very low, the numbers of environmental samples that were positive was also low. However, the presence of many infected cows, or a few so-called "heavy" shedder cows, made it easy to find MAP around round bale feeders, in loafing areas, and in the calving area.

For example on one recent sampling on one of the beef farms, again when all the calves were born and nursing their mothers, a two-year-old heifer was found to be a heavy shedder. There were an additional 7 of 145 animals that were also culture-positive but which were classed as moderate to light shedders. Two of five samples taken from around round bale feeders, and two of five samples taken from a two-acre drylot/loafing area were positive. Because the calving area had been divided into two separate areas for calving and housing the calves and their mothers, and because one of the loafing areas could not be sampled, the remaining ten environmental samples were taken from these two areas. All ten of these samples were culture positive and all ten would have been classed as a "heavy shedder" if they had been taken from an animal! Because the culture-positive two-year-old had access to both these areas, we believe she was responsible for much of the heavy contamination of the entire area. Udder and teat skin samples were not collected from cows at this sampling, but I have no doubt that a high percentage of them taken from cows in this environment would have been culture-positive thus creating great risk of infection for this year's calf crop. Of course the calves were housed in this area too and had exposure to the same environment.

Over the last thirty years, a number of test methods have been developed in an effort to provide producers with a better diagnostic test. The current culture method available at Ohio's Animal Disease Diagnostic Laboratory (ADDL) in Reynoldsburg uses liquid culture media and is perhaps the most sensitive method yet developed. Herd culture results are available in six to seven weeks, and individual positive cultures can be

detected in as little as two weeks. This is a significant improvement over the previously used technology that took up to 12-16 weeks to complete. Culture of manure and tissue is still the "gold standard" for diagnosis of infection with MAP. Blood tests for Johne's disease can be completed in less than a week and are much less expensive than culture. The current method used in most laboratories is referred to as an ELISA, and this test has been markedly improved in the past decade. However current research, including our observations in our demonstration herds, suggests that it is much less sensitive than culture. The ELISA only identifies 70-80% of cows that are heavy shedders, and perhaps only 10-30% of light and moderate shedders compared to manure culture. This test may help a producer determine whether they have Johne's disease in their herd, but testing and culling based on ELISA results may leave animals remaining in the herd to contaminate the environment.

Culturing pools of five individual animals is now a well-accepted technique for detecting an infected herd, and it can be used to estimate the number of infected animals in a herd. In Ohio, veterinarians can now pool the manure samples from individual cows in their offices and submit them to the laboratory. (A recommended procedure is available from the ADDL.) Of course, if one wishes to identify the infected animals in a culture-positive pool of five, the individual animals have to be cultured. However, in herds with a low prevalence of infected animals, or a herd with an unknown disease status, pooling can significantly reduce the cost of testing the herd. The work done with this technique in our demonstration herds suggests that it is much more sensitive than blood testing and reliably allows detection of pools containing heavy and moderate shedding cows and most pools containing one or more light shedders.

The current culture method in use at the ADDL provides results in the form of a number which reflects the number of days of incubation before a positive culture is detected. If they are not detected before, cultures are incubated up to 42 days at which time they are examined by staining and further testing if the stain is positive. Samples from cows which are positive in 25 days of incubation or less are considered "heavy" shedders, and those positive from 26 to 42 days are considered moderate to light shedders. The work we have done with culture of pools of five individual cow samples suggests that there is a close relationship between the days-to-positive of the pooled sample and that of the individual animal in the pool having the shortest days-to-positive. In the previous example in the herd just mentioned, the fecal sample from the two-year-old classed as a heavy shedder was positive at 16 days of incubation. The pool of five cows containing her manure actually had two positive cows, the second at 36 days, but the pooled sample was positive at 18 days of incubation. Another pool also had two culture-positive cows, each at 39 days-to-positive on their individual sample cultures, and the pooled sample was also positive at 39 days of incubation. Pooled sample culture allows the producer and the veterinarian to better characterize the extent of the infection in the herd than with blood testing and to determine if there are individual animals that should receive further testing to identify infected ones. This would also be a more sensitive, and a cost-effective, method of screening newly purchased animals to find infected animals than using blood testing by current ELISA.

Another observation that has emerged from work in the Demonstration Project herds is the need to carefully consider the timing of any diagnostic testing, especially in beef cattle herds. Prior to their enrollment in the project, both of our beef herds had been testing annually for several years using blood samples and fecal culture, however they collected the samples in the fall, and the older method of culture took 12-16 weeks for results to be available. This meant that by the time the results were received, the cows were near calving or had already calved. At that point, if there were fecal culture-positive cows, the calving area was already contaminated, and it was difficult to change the situation by culling or segregation of infected cows. If herds are interested in making meaningful progress toward controlling or eliminating Johne's disease, we would advise using the most sensitive test, culture by individual or pooled sample, and scheduling the sample collection such that results will be available with enough time before calving season begins to cull or segregate infected cows and to remove potential contamination from the calving area before the new calves arrive.

Controlling or eliminating Johne's disease in a herd takes time and commitment. A final observation from the Demonstration Herd Project is that the herd owner must become well informed about the disease and set realistic goals. If this includes making rapid progress, and perhaps eventual elimination, careful consideration regarding retaining home-raised heifer replacements must be given. One of our beef herds had made the decision to try to eradicate Johne's disease from their herd before they were enrolled in the Project in 2004. To their great credit, the owners had recognized the possibility that a replacement heifer could become infected as a calf and not begin shedding for several years. This could destroy a lot of hard work and expense after considerable progress toward that goal had been made by exposing a future calf crop to MAP. Before 2004, they made the decision not to keep their own heifers until they had reason to believe the disease was gone or nearly so. Toward that end, they have purchased some heifers from a herd enrolled in Ohio's Test-Negative Status Program. After removal of two cows following the first sampling in the fall of 2004, and one more after sampling in the spring of 2005, they have had six consecutive, semi-annual, whole-herd tests with all negative culture and blood test results.

In contrast, the other beef herd could not afford to make the decision not to keep some of their own heifers, but they do wish to eventually eliminate Johne's disease from the herd. About 20% of the cows were culture-positive on the first test in the fall of 2004 and 8% the following spring. Subsequently, semi-annual cultures revealed one or two positive cows per sampling. In 2007 all tests were negative. However in the spring of 2008, the apparently healthy, but heavy-MAP-shedding, home-raised heifer cited in the above example of environmental contamination was detected. This is very disappointing but not really surprising. Actually, although it is hard to see any good in this, it is better to have found her as a two-year-old than as a three or four-year-old. This is one of the many frustrating aspects of this insidious disease.

To summarize, some take home messages for beef producer from this Project include:

- Cows shedding MAP into the environment can make the udder of many of their herdmates sources of infection for the calves.

- Beef cattle herd owners need to carefully consider the timing of any diagnostic testing so that results will be available with sufficient time before the calving season begins to cull or segregate infected cows and to remove potential contamination from the calving area before the new calves arrive.
- Pooled sample manure culture can allow the producer and the veterinarian to better characterize the extent of MAP infection in the herd than using blood testing, and they can then determine if there are individual animals that should receive further testing to identify the infected ones.
- The herd owner must become well informed about the disease and set realistic goals in light of their individual situation and current technology.

Direct Federal and state support to producers for control of Johne's disease has dwindled to nearly nothing. However, several million Federal dollars have been allocated for research on the disease over the last five years, and on a worldwide basis, attention on this important disease is increasing in both the livestock and human health sectors. In Ohio, we have been evaluating the usefulness of testing breeding age heifers to find infected animals early in life as well as the potential uses of a more rapid test on manure, the PCR assay. Last April, the National Animal Health Monitoring System released its Dairy 2007 report which showed that at least 68% of US dairy farms are infected. Among other information, the report also states, "These results suggest that at least one-fourth of U.S. dairy operations may have a relatively high percentage of infected cows in their herds." The available information suggests that the situation is not yet so serious for the beef cattle industry. The national Johne's Disease Demonstration Herd Project has given us new insights into the control of this disease, and additional information is expected from analysis of the national database that now exists from this project. We hope beef producers will take advantage of what we know about prevention and control of this disease to reduce spread within and between their herds.

A copy of the NAHMS, Dairy 2007 report on Johne's Disease can be found at:
http://nahms.aphis.usda.gov/dairy/dairy07/Dairy2007_Johnes.pdf

A table illustrating current diagnostic tests available at Ohio's ADDL and their potential uses, advantages, and disadvantages is available on our website at:
http://vet.osu.edu/1985.htm