When the USDA provided funding for states to participate in the National Johne’s Disease Demonstration Herd Project in 2003, William Shulaw, DVM, beef and sheep extension veterinarian, the Ohio State University, jumped at the opportunity. After all, the project 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 could bring forth valuable findings. Shulaw also knew Ohio would have herds wanting to participate in the project. In the end, Ohio enrolled three herds: one dairy herd and two beef herds.

The Ohio beef cattle herds were tested by individual animal fecal (manure) culture and blood serum ELISA testing every spring and fall beginning the fall of 2004. In addition to the individual animal tests, samples of the farm environment were taken for culture of the causative bacteria, udder skin surfaces were sampled for culture and individual animal fecal samples were pooled in groups of five for culture.

Before undertaking the project, Shulaw and his team knew cows shed *Mycobacterium avium* subspecies *paratuberculosis*—MAP, the cause of Johne’s disease—in their manure and contaminate the environment with the bacteria which are then ingested by susceptible calves. They also recognized that most animals shed MAP for months to (Continued on page 2)
years before they show any signs of disease.

“Nevertheless, back in 2004, we had 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,” Shulaw states.

“The environmental samples and udder and teat skin samples we collected showed 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 15 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,” Shulaw states.

During the spring of the first year of the project, udder scrub samples and fecal samples were collected from all cows in the beef herd. The herd had calved in a dry lot setting prior to going to pastures, and nearly all the calves had been born and were nursing their mothers at the time samples were collected.

Of 88 individual animal fecal samples, seven cows were culture positive. 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,” Shulaw states. “Remember, 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 four of 113 udder skin samples were positive, and all of these were on fecal culture-negative cows.

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“Cows shedding MAP into the environment can make the udder of many of their herdmates sources of infection for the calves,” Shulaw points out.

Results from samples collected from the environment on these farms were also revealing.

During each visit the Ohio Demonstration Herd Project team collected 20 samples—equivalent to about two ounces—of dirt, bedding or manure slurry from various areas such as 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,” Shulaw notes.

Shulaw says, in general, when the numbers of infected individual cows were extremely 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 one sampling on one of the beef farms where all the calves were born and nursing their mothers, a two-year-old heifer was found to be a heavy shedder. An additional seven of 145 animals were culture-positive and found to be 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.

Shulaw explains that, 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 10 environmental samples were taken from these two areas.

“All 10 of these samples were culture positive, and all 10 would have been classed as a ‘heavy shedder’ if they had been taken from an animal!” Shulaw states. “Because the culture-positive two-year-old had access to both these areas, we believe she was responsible for

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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.”

Shulaw says the Ohio Demonstration Herd Project showed that controlling or eliminating Johne’s disease in a herd takes time and commitment, and 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.” Shulaw states. “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, the herd owners 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.”

Shulaw says the other beef herd involved in the Ohio Demonstration Herd Project kept their heifers rather than get rid of their heifers, using a different route to eliminate Johne’s disease from their herd. About 20% of this herd’s 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.

While all tests were negative in the fall of 2007, a healthy appearing but heavy-MAP-shedding homeraised heifer was detected in the spring of 2008.

“This is very disappointing but not really surprising,” Shulaw states. “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.”

Shulaw offers these take-home messages for beef producers from the Demonstration Project:

- 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.
What Research Shows

Johne’s disease continues to be a priority for many researchers worldwide, and that is to be expected as Johne’s disease is a worldwide challenge.

The following research studies and results were gleaned from professional journals. While some studies have “dairy” in their study title, the studies also have implications for the beef industry.

Research Study: “Low Rate of Detectable in utero Transmission of Mycobacterium avium subsp. paratuberculosis in a Dairy Herd with a Low Prevalence of Johne’s Disease”
Published in the Journal of Veterinary Diagnostic Investigation, January 2012.
Researchers: J.M. Adaska, R.H. Whitlock

This study focused on in utero transmission of MAP. During the study, researchers cultured tissues from neonatal calves born to cows of known test status for the presence of MAP and found that tissues from a single calf was born to a test-positive cow shedding large numbers of organisms in the feces were positive for MAP.

The detected overall transmission rate was approximately 2% (1/49). The detected transmission rate in cows that were fecal culture positive and serum enzyme-linked immunosorbent assay (ELISA) suspect or positive was approximately 4.3% (1/23).

What does this mean to veterinarians and producers? These research findings agree with previous findings regarding in utero transmission. And, while newborn calves and young animals typically become infected with MAP from ingesting the bacteria on manure-covered teats or via colostrum or milk from infected cows, unborn calves can also become infected in utero if their mothers are infected with MAP.

Research Study: “The Collection of Lymphatic Fluid from the Bovine Udder and its Use for the Detection of Mycobacterium avium subsp. paratuberculosis in the Cow”
Publication: Journal of Veterinary Diagnostic Investigation, January 2012.

This research study evaluated the feasibility of lymph collection from the bovine udder and investigated if the lymphatic fluid might be of diagnostic value in cows infected with MAP.

While collecting lymph fluid from cows, researchers also recorded the level of difficulty associated with collection and the reactions of the cows. They reported that the collection of lymphatic fluid caused no or mild signs of discomfort in 94.6% of the cows. Lymphatic fluid was attained on the first attempt in 51.8% of cows while sample collection was unsuccessful in 12.1%.

MAP was detected in 43.1% of all lymph samples. The bacterium was present in 66.7% of cows with clinical Johne’s disease, in 42.8% of asymptomatic cows with a positive or suspicious ELISA result in blood and in 38.7% of cows with a negative ELISA result in blood.

What does this mean to veterinarians and producers? The study shows that most cows tolerated the procedure well, and the procedure can easily be performed on farm. The isolation of MAP from lymph fluid also suggests that this approach could be used for the early detection of Johne’s disease in cattle.

Research Study: “Fate of Mycobacterium avium subsp. paratuberculosis after Application of Contaminated Dairy Cattle Manure to Agricultural Soils”
Publication: Applied and Environmental Microbiology, March 2011

This research project looked at what happens to MAP when manure slurry is applied to a loamy or sandy soil and whether amount of rainfall impacts the pathogen.

Using a lysimeter—a device for collecting water from the pore spaces of soils and for determining the soluble constituents removed in the drainage, researchers found the greatest proportions of MAP-positive leachates in sandy-soil in the manure-treated group receiving the equivalent of 1,000 mm (39.4 inches) annual rainfall. Under the higher rainfall regimen (78.75 inches per year), MAP was detected more frequently in loamy soil than in sandy soil.

MAP was detected more often in grass clippings than in the soil.

When researchers cultured the soil at different depths, MAP was recovered only from the uppermost levels of the soil.

Factors associated with MAP presence were soil type and soil pH (P < 0.05). For M. paratuberculosis presence in grass clippings, only manure application showed a significant association (P < 0.05).

What does this mean to veterinarians and beef producers? This research indicates that MAP tends to move slowly through soils—but faster through sandy soil. Research findings also show that MAP tends to remain on grass and in the upper layers of pasture soil. Bottom line: Spreading MAP-infected manure slurry on grassland is “a clear infection hazard for grazing livestock and a potential for the contamination of runoff after heavy rains.”