



# Some Johne's cows do more harm than others

We now know there are super-shedders that have an incredible ability to contaminate feed and water. You must find them and remove them.

by Terry Fyock, Robert Whitlock, and Raymond Sweeney

**J**OHNE'S is being recognized in more herds and seems to be more common than previously recognized. The 2007 Dairy NAHMS study reported that at least 68 percent of the dairy herds in the U.S. have Johne's-infected cows. In herds with more than 500 cows, the herd infection rate exceeds 95 percent.

Until recently, fecal test results were reported as either positive or negative for growth of *Mycobacterium paratuberculosis* (MAP). Nearly all cattle with a positive culture were judged to be infected (shedding MAP) and a threat to transmit the disease. A positive fecal test long has been acknowledged as the GOLD standard test for Johne's. Currently, the majority of U.S. labs performing fecal culture for Johne's disease report findings with a shedding classification. The report may include the actual numerical count of colony-forming units (cfu) on the surface of the media for each tube. Or, frequently, the colonies per tube are converted to a classification such as low, moderate, or high shedder.

Standard lab practice across the U.S. has been to report solid media samples with more than 50 colonies as a high shedder. For laboratories using liquid culture, the bacterial concentration in manure sample is recorded as days to positive (DTP). The fewer days required for a tube to show growth, the more mycobacteria present. For example, low shedders require 32 or more days to become positive, moderate shedders require 25 to 31 days, while high shedders require less than 25 days.

## What is a Johne's super-shedder?

Unfortunately, the "low, medium, high" reporting scheme does not take into account the fact that not all high shedders are created equal! Most labs "stop counting" when they get to 50 colonies on a culture tube . . . so a "high" result could mean 50 colonies, or it could mean 5,000 colonies, or more . . . until recently, no one checked any further. Our laboratory has been studying what range of shedding is represented by fecal samples from

cows typically classified as high shedders.

To date, we have retested more than 310 fecal samples from "high" shedders (50 cfu/tube or greater) for this study. These samples were divided into shedding ranges by counting all of the colonies using several serial dilutions of the fecal sample and not stopping at 50 colonies on one tube from an undiluted fecal sample. The table below shows the wide range of shedding among the group of cows classified as high shedders. (Note that 50 colonies per tube corresponds to approximately 2,000 colonies/gram.)

The cows in the last row, with over 1 million CFU/gram, would be classified as Johne's ultra-super-shedders (SS). These cows excrete massive

Cows	%	Range CFU/gram
67	22	2,000 - 9,999
115	37	10,000 - 99,999
95	31	100,000 - 999,999
33	11	1 million up to 4.7 million

numbers of mycobacteria in their manure. They often begin as low shedders but progress over 6 to 24 months to become a super-shedder. This means one ultrasuper-shedder shedding one million cfu MAP per gram of manure results in more mycobacterial contamination than 2,000 moderate shedders or 23,000 low shedders.

Most super shedders appear normal and have normal milk production for extended periods. However, if kept in the herd, they eventually will lose weight and develop chronic intermittent intractable diarrhea.

Super-shedders also are Johne's "super-spreaders." Those cows excrete so many mycobacteria in the environment that just a few drops of manure may infect many other animals if they end up in the feed or water. A 1,400-pound milk cow producing 88 pounds of milk per day will excrete about 100 pounds (45,400 grams) of manure per day or 45.4 billion mycobacteria. One gram of manure from a super shedder may contain 1,000,000 mycobacteria. To help put this number in per-

spective, if we assume 100,000 mycobacteria are required to infect one calf, one day's manure from this super-shedder could infect more than 45,400 newborn calves.

These super-shedders are producing such massive quantities of mycobacteria that normal management plans designed to prevent the spread of Johne's disease easily are overwhelmed. One drop of manure from a super-shedder in a calf milk bucket is enough to infect a newborn calf. Manure on the wheels of a tractor easily could contaminate TMR fed to heifers. Small bits of manure easily can contaminate water troughs, ponds, or other sources of water.

Super-shedders actually can influence the fecal culture results of other uninfected cows. If an uninfected cow eats some feed or drinks water contaminated by a super-shedder, the mycobacteria can "pass through" her digestive tract and show up in her feces as a positive test. This usually occurs at the "light" shedding level. Passive shedders should return to negative when the super-shedder is removed. Super-shedders make it very important to interpret culture or PCR results of "light" or "low" positives carefully.

The majority of super-shedder cows in this study were in good body condition. So, do not think super-shedders are thin cows with diarrhea; they are not. Most super-shedders appear normal and milk normally. Many super-shedders were ELISA (blood test) positive . . . but not all of them. Of course, the ELISA test also can have false positive results, as many ELISA positive cows were culture negative. So we don't recommend culling ELISA positive cows without a fecal test to confirm the test.

## How do you find "super-shedders"?

Manure samples from a super-shedder will be culture positive as early as six weeks with many colonies on each tube which become more apparent after an additional two to four weeks. Diagnostic labs using liquid culture methods report a shorter time to positive, so this could be one way to shorten the time to detect a "super-shedder."

The challenge for Johne's researchers is development of a test to detect super-shedders cost-effectively. That way, these cows can be eliminated from the herd before extensive contamination of the environment.

A rather unique approach for the detection of super-shedders is doing real-time polymerase chain reaction (PCR) testing of pooled environmental manure samples from the herd or a group of cows within the herd. Pooled manure samples are best obtained by your veterinarian who has been trained in these sampling techniques. Basically, four to six individual manure samples are taken from high cow traffic areas and then mixed together as one sample.

Labs running Johne's real-time PCR testing are able to return accurate results within a week of having the samples. Any pooled sample with bacterial counts equivalent to a high shedder suggests there's a super-shedder in the group or herd of cows included in the sample.

Super-shedders could be identified rapidly by doing a real-time-PCR test on a fecal sample from every cow in the herd. However, a more cost-effective approach is to perform real time PCR testing fecal samples from only ELISA positive cows. Alternatively PCR testing of pooled manure samples with follow-up of tests on individual cows from the high-positive pools would reduce costs and the total number of samples tested.

Our experience suggests that virtually every herd infected with Johne's disease will have a super-shedder at some point. Data from our lab indicates that about 10 percent of culture positive animals are super-shedders. So now we have low shedders, moderate and high shedders, and super-shedders which are, in fact, super-spreaders that could take a nasty toll on your herd.

The authors are at the University of Pennsylvania, School of Veterinary Medicine, Kennett Square, Pa.