NATIONAL JOHNE'S EDUCATION INITIATIVE

Johne's Disease – Beef

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Hidden Source of *MAP* Contamination Studied

Researchers have been investigating potential hidden sources of contamination in the farm environment where susceptible animals may be routinely exposed to *Mycobacterium avium* subspecies *paratuberculosis* (*MAP*), the causative agent of the Johne's disease. Recently completed work underwritten by Agricultural Research Service/USDA focused specifically on livestock watering troughs.

"Livestock watering troughs are frequented by all animals on a farm," states researcher Dr. Kimberly Cook, lead scientist on the Bowling Green, Ky., project. "These troughs provide a moist, nutrient-rich environment for bacterial survival, and the trough basin provides a surface for bacterial adhesion, commonly referred to as biofilm formation."

Dr. Cook explains that *MAP* has been shown to have an extremely hydrophic cell wall structure, as do most Mycobacterium species— with this characteristic increasing the propensity for biofilm formation.

The ARS study evaluated the ability of *MAP* to form mixed-community biofilms on the four most commonly used watering trough materials: concrete, plastic, stainless steel and galvanized steel. The study also investigated the ability of *MAP* to persist amid the trough water microbial flora and to become incorporated into an established biofilm on the same trough material.

"High concentrations of *MAP* were detected in biofilms on all trough materials within three days of inoculation into trough water, and *MAP* survived in the biofilms for more than 149 days," Dr. Cook elaborates.

These results suggest that MAP survives well in biofilms present on livestock watering trough materials. To inhibit spread of this organism and exposure of susceptible animals to MAP on infected farms, best management practices aimed at maintaining biofilm-free trough surfaces should be included in any Johne's control plan.

According to this research, trough material composition influenced the survival of *MAP*. The lowest survival was exhibited on stainless steel, followed by plastic, galvanized steel and concrete.

The research team evaluated the effect of chlorine disinfection on survival of *MAP* in the trough biofilms, adding 2ppm chlorine to trough water on a weekly basis.

Dr. Cook notes that chlorine was found to reduce the survival of the organism on stainless steel and galvanized steel trough materials but not on concrete or plastic materials.

"Chlorination may have been effected by higher pH in tanks with concrete trough materials and lower total and free chlorine availability in tanks with plastic trough materials," Dr. Cook states. "These results suggest that the effectiveness of chlorine disinfection depends on trough material construction, pH and chlorine availability.

"Control of pathogens such as *MAP* in livestock drinking water sources may serve as a critical control point for slowing spread of the disease. Optimization of disinfection protocols and elimination of biofilms on trough surfaces should reduce persistence of *MAP* in trough waters."

To inhibit the spread of *MAP* and exposure of susceptible animals to *MAP* on infected farms, best management practices aimed at maintaining biofilm-free trough surfaces should be included in any Johne's disease control plan.

USDA

This e-newsletter is brought to you by the National Johne's Educational Initiative funded by USDA/APHIS/VS and managed by the National Institute for Animal Agriculture. Editor: Teres Lambert. To learn more about Johne's, visit ***www.johnesdisease.org***.

NATIONAL INSTITUTE FOR ANIMAL AGRICULTURE

Survival of *MAP* in the Soil, Environment

Johne's disease-infected animals shedding huge numbers of *Mycobacterium avium* subsp. *paratuberculosis* (*MAP*) in their feces mean that soil and the farm environment can become quite contaminated. And in spite of the bacteria being an obligate parasite, research shows that the bacteria can survive for extended periods in an external environment, facilitating the build-up of soil and pasture contamination levels over time.

In their work addressing the adsorption of *Mycobacterium avium* subsp. *paratuberculosis* to soil particles, researchers Navneet K. Dhand, Jenny-Ann L.M.L. Toribio and Richard J. Whittington, the University of Sydney, New South Wales, Australia, found that "attachment of the bacteria to soil particles could increase their availability to farm animals as well as influence the transportation of *M. avium* subsp. *paratuberculosis* to water sources."

Their research conclusion states: "The results provide indirect evidence that, like many other bacteria, *M. avium* subsp. *paratuberculosis* adsorbs to soil particles. This attachment appears to be dependent on soil pH, with greater adsorption records for soils maintained at acidic pH.

"Further studies are required to substantiate the findings at a range of soil types and pH levels and by direct measurement of viable organisms in the soil, but, if confirmed, these results could explain prior epidemiological observations and have potential repercussions for animal and human health.

"*M. avium* subsp. *paratuberculosis* attached to soil particles might be retained in the upper layers of the soil rather than being leached to the deeper layers, therefore remaining available to grazing animals that normally ingest soil while grazing, thus increasing their likelihood of infection.

"Similarly, the leaching of *M. avium* subsp. *paratuberculosis* from soil to water supplies may be influenced by attachment of *M. avium* subsp. *paratuberculosis* to mobile soil particles."

This full article appeared in the September 2009 issue of *Applied and Environmental Microbiology*, p. 5581-5585 and is available online.

2004 Australian Research

Research funded by Meat and Livestock Australia and NSW Agriculture and published in 2004 focused on the survival and dormancy of *MAP* in the environment.

In this study, researchers Richard J. Whittington, D. Jeff Marshall, Paul J. Nicholls, Ian B. Marsh and Leslie A. Reddacliff found bacteria survival for up to 55 weeks in a dry, fully shaded environment, with much shorter survival times in unshaded locations.

The organism survived for up to 24 weeks on grass that germinated through infected fecal material applied to the soil surface in completed shaded boxes and for up to nine weeks on grass in 70 percent shade.

The research also found that "moisture and application of lime to soil did not affect survival."

Additional Research Findings

The classic reference on the subject of environmental survival of *MAP* traces to a 1944 publication by Lovell et al. This work covers a series of studies using naturally infected bovine feces with the infected fecal matter exposed to a variety of natural conditions such as freezing, drying, sunlight, changes in ambient temperature and rain, with regular attempts to re-isolate *MAP*.

In general the researchers found survival of *MAP* in feces kept outdoors up to 152 to 246 days depending on specific conditions. Although drying of soil appeared to shorten survival, the recommendation came forth that a pasture be considered contaminated by the organism as a potential source of infection for at least one year given the longevity of *MAP*.

Factors that may shorten the estimated survival time of *MAP* in soil are drying, exposure to sunlight, pH above 7.0 and low iron content. Bovine urine is also hostile to *M. paratuberculosis* survival and increasing concentrations of bovine urine (2-10%) caused decreasing survival rates (at pH 6.3 to 6.6).

Survival in Feces Stored in Slurry Pits

Jörgensen published the first comprehensive study of its kind on survival of *MAP* in slurry in Denmark in 1977. In his work Jörgensen used cattle slurry (pH 8.5, dry matter 7%), swine slurry (pH 8.3, dry matter 8.3%) and a mixture of the two (pH8.4, dry matter 7.7%). After spiking each slurry preparation with 3 x 107 *M. paratuberculosis*/ml, the researcher bubbled a mixture of hydrogen and nitrogen gas through the slurry to secure anaerobic conditions and then stored the slurry at 5°C or 15°C.

Jörgensen reported that the number of colonies of *MAP* isolated on modified Löwenstein-Jensen medium dropped drastically between sampling Day 1 and Day 7 but then remained relatively stable until recovery of the organism stopped indicating the limit of survival. At 5°C the survival time was 252 days in all three kinds of slurry. At 15°C the survival time lessened to 182 days in swine slurry, 98 days in cattle slurry and 168 days in mixed slurry.

The second major study on *MAP* in slurry was reported by Olsen, Jörgensen and Nansen in 1985. Their study concerned conditions found during anaerobic digestion of slurry as in bio-gas plants. Slurry was spiked to yield initial counts of 3.3 x 103 to 2.7 x 104 *M. paratuberculosis* /gm slurry and held at mesophilic conditions (moderate temperatures; 35°C or 95°F) or thermophillic conditions (high temperatures; 53-55°C or 127-131°F). At mesophillic conditions *M. paratuberculosis* was re-isolated at 7, 14, and 21 but not 28 days. At thermophillic conditions viable *M. paratuberculosis* could not be detected in as short as 3 hours.

Minnesota Seedstock Producer— Johne's Disease Testing an SOP

Allen Lyon, Lyon River Front Farm, Foley, Minn., has had a Johne's disease prevention and control program in place for close to 10 years. It's a program, he says, that removes the day-to-day concern about Johne's disease—or passing on Johne's disease to other herds.

"When we entered the purebred Limousin seedstock business in 1987, I was aware of the devastation that Johne's disease could do to a herd and knew that I didn't want the bacteria ever infecting my herd," Allen explains. "What a loss of genetics that would be.

"And, as a person selling bulls and females to other cattlemen, I didn't want our genetics to be responsible for infecting any of our customers' herds. Introducing Johne's disease to a herd would be devastating to the customer and, as seedstock producers, we could quickly be out of business."

Lyon River Front Farm is a 100-cow purebred Limousin enterprise that sells bulls and females by private treaty and through consignment auctions. Approximately 90 percent of the offspring are the result of AI, with the remaining 10 percent from an embryo program.

The seedstock herd's original base herd came from purchases from several breeders.

"And that's why, in 2003, we decided to test for Johne's disease," Allen states. "While I had no reason to suspect Johne's disease, I had read about the devastation of the disease and simply wanted to know that we had not unknowingly brought Johne's disease into the herd. It would take just one animal to infect the entire herd and to negate all genetic progress made through the years."

When Lyon River Front Farm initiated its Johne's disease testing program, Dr. Randy McLaughlin, Mille Lacs Veterinarian Clinic of Foley, Minn., pulled fecal samples. Later, when ELISA testing was available, the veterinarian pulled blood, with samples always sent to a USDA-APHIS-VS approved laboratory.

By 2006, the herd had reached the highest level attainable.

During his first five years of testing, Allen says two to three reactors would pop up and require a second round of testing.

"But each animal always came back negative to the second test," he states. "For the last two years, however, we've had no reactors. And that's a nice feeling. You don't have to hold your breath until that second test comes back negative."

Lyon River Front Farm annually tests a minimum of 30 percent of its animals that are three years of age or older.

3 Key Management Strategies

In addition to testing at least 30 percent of adult animals each year, Lyon River Front Farm also quarantines each herd addition for a minimum of 30 days. And, each new addition is purchased from Johne's disease tested herd, then tested for Johne's disease once at the farm and tested yearly thereafter.

Allen Lyon, Lyon River Front Farm, Foley, Minn., is selective on what is retained annually in the herd based on performance and docility—and he's focused on not bringing Johne's disease into the herd or selling it to his customers.

A second strategy is designed to limit exposure of newborn calves to *Mycobacterium avium* subsp. *paratuberculosis*.

"Our cows are wintered in the field, with a couple of fields kept for calving purposes only," Allen states. "Since we have only AI and ET calves, we pretty well know when each cow is going to calve. So we run cows close to calving through the chute and wash their udders.

"This is a precautionary measure because we don't want a calf ingesting fecal matter when it stands up to suckle. Calves are extremely susceptible to the bacteria that cause Johne's disease and, once a calf ingests the bacteria, there is no turning back. Granted, tests show that we have a low-risk herd but you can never be too careful when it comes to Johne's disease."

Allen adds that washing the udders also helps limit the transfer of other bacteria as well, resulting in a healthier calf from Day 1. Plus, he gets a close look at every cow just prior to it calving.

A third strategy practiced at Lyon River Front Farm is that every field where cows have been housed during the winter is plowed down. Manure is moved from topside and is mixed into the earth.

"The more practices in place to limit exposure to the bacteria that cause Johne's disease, the better," Allen summarizes.

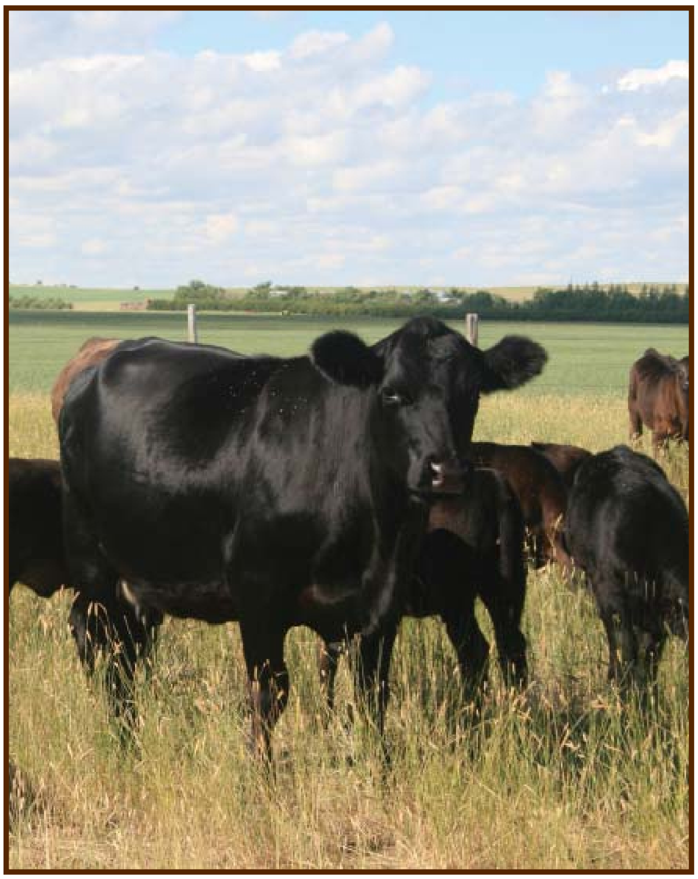
Oh my Gosh, Johne's Disease?

While routinely checking his cows and calves, an Ohio cow-calf producer noticed that a couple of cows were looking a bit thin.

"A few head appeared to be going down in weight quite quickly," he states. "One cow went down pretty near to nothing in what seemed like a short time.

"I'm thinking my herd just might be infected with Johne's disease. And that is quite a shock."

In addition to being losing weight despite a healthy appetite, the cows suspected of having Johne's disease have a couple of other strikes against them in favor of Johne's disease: 1) They are four years of age. 2) They recently underwent a stressful time—calving. 3) They are undergoing a current stressful time—lactating, providing nutrients to some highly demanding offspring. 4) Their manure is watery and lose.



Johne's disease isn't just a dairy disease. It's also a beef disease, with beef producers who have had the disease in their herds urging other producers to take steps to prevent it from entering their herds.

This cattleman is also noticing that the calves at side the suspect cows aren't gaining weight as they should.

Concerned, the Ohio beef producer has contacted his veterinarian and plans to test for Johne's disease. To save time and labor, he plans to test his herd when the cows are gathered and run through the chute to be palpated.

This Ohio producer runs about 90 head, and adds one or two new bulls to his four-bull battery every two years. To date, he hasn't asked his bull suppliers if they have tested for Johne's disease and are a low-risk herd.

"It doesn't matter if my herd has Johne's disease or not. From now on, I'm going to inquire about a herd's Johne's disease status before buying a bull," he interjects. "It's simply to scary to think I might have brought Johne's disease into my herd.

"I can't tell you just how surprised we were to see symptoms of Johne's disease in our herd. It's an awful feeling."

This Ohio cattleman has the right attitude, however.

"If tests show that we have any positive animals or suspect animals, those animals will be removed from the herd," he explains. "I'll do whatever it takes to reduce the incidence of Johne's disease in the herd.

"I once read that Johne's disease is more of a dairy issue than a beef issue. . .that Johne's disease is in only about eight of 100 beef herds in the United States. Well, it's definitely not a good feeling when your herd is among those with Johne's disease. It just makes me wonder if Johne's disease isn't more prevalent in beef herds than we realize."

Editor's Note: The National Johne's Education Initiative has two pieces of information that can help you know more about Johne's disease: *"Johne's Disease Q&A for Bovine Producers"* and *"Beef Producers—Take Proactive Steps to Prevent, Control Johne's Disease."* Both pieces are free and can be in your hands within days when you contact the National Institute for Animal Agriculture. Phone: 719-538-8843. Email: *johnes@animalagriculture.org*

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