



JOHNE'S DISEASE – DAIRY

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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 hydrophobic 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.

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.



Water troughs provide a moist, nutrient-rich environment for bacterial survival, including the bacteria known to cause Johne's disease. —Photo by Shannon Hayes



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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

Jørgensen published the first comprehensive study of its kind on survival of MAP in slurry in Denmark in 1977. In his work Jørgensen used cattle slurry (pH 8.5, dry matter 7%), swine slurry (pH 8.3, dry matter 8.3%) and a mixture of the two (pH 8.4, dry matter 7.7%). After spiking each slurry preparation with 3×10^7 *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.

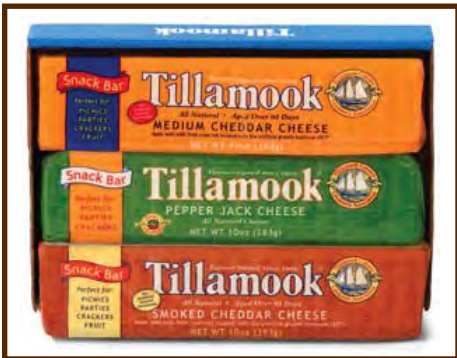
Jørgensen reported that the number of colonies of MAP isolated on modified Löwenstein-Jensen medium dropped drastically between sampling Day 1 and Day 7 but then remained relatively stable until recovery of the organism stopped indicating the limit of survival. At 5°C the survival time was 252 days in 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, Jørgensen 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 3.3×10^3 to 2.7×10^4 *M. paratuberculosis* /gm slurry and held at mesophilic conditions (moderate temperatures; 35°C or 95°F) or thermophilic conditions (high temperatures; 53-55°C or 127-131°F). At mesophilic conditions *M. paratuberculosis* was re-isolated at 7, 14, and 21 but not 28 days. At thermophilic conditions viable *M. paratuberculosis* could not be detected in as short as 3 hours.

Producer Members Benefit from Risk Assessment, Testing

With a strong belief that product starts on the farm, the management staff of Tillamook County Creamery Association of Tillamook, Ore., wants every member aware of and educated about disease, including Johne's disease.

When the focus on Johne's disease was initiated, Tillamook County Creamery Association wasn't concerned that the disease was rampant among its member herds. TCCA simply wanted members to be aware of Johne's disease, its potential cost to producers' bottom lines and how to tackle the disease should the bacteria be found on the dairy.



In the initial years of the program, TCCA requested that all members participate in the state's Johne's Disease Program—and the association even contributed funding to help further USDA funding that was available to producers for testing at the time. All member dairymen had a risk assessment performed on their farm and developed a management plan in partnership with their local veterinarian and frequently the state veterinarian.

Today, TCCA still has an intense interest in Johne's disease, with the state veterinarian more involved. But rather than require producers to be involved in the state program, TCCA has what it calls the "Tillamook Program" in place and the cooperative pays to have an environmental sampling for Johne's disease on every dairy. Samplings go to a National Veterinary Services Laboratory lab, with results returned to each member.

"Tillamook Cheese is a branded

product, and we're sensitive to things that might negatively affect our product," states Mark Wustenberg, DVM, vice president of quality and member services for Tillamook County Creamery Association. "That's why we are proactive when it comes to disease prevention and control. Quality and safety of product starts at the farm.

"If Johne's disease is ever connected to a human health point, then we would be ahead of the game. In addition, none of our producers would be put out of business. During the early years, they became aware of, and implemented, various management strategies to help prevent and control Johne's disease. Annual sampling helps them remain focused on Johne's disease prevention and control and to know their level of risk for Johne's disease. Many dairies have never found a positive culture."

Dr. Wustenberg stresses that TCCA's initial and continued Johne's disease program wasn't—and still is not—"a prescription program but was designed to facilitate a conversation so people could be aware and make decisions." After all, what one doesn't know, one cannot address. In the case of Johne's disease, TCCA's program helped educate producers about Johne's disease. Producers then became aware of management strategies that could be put in place to prevent the spread of disease.

Dr. Bruce Mueller, Oregon's Designated Johne's Coordinator, says he was impressed with the producers' willingness to make management changes when a risk assessment showed areas that could be improved. Two areas where most producers made changes were quickly pulling newborn calves from dams and keeping age groups segregated.

"Both practices help minimize contamination," Dr. Mueller states. "Helping prevent and control Johne's disease is all about manure and minimizing contamination. It's just that simple."

Steve Neahring, a Nehalem, Ore.,

producer who milks 180 head, is among the TCCA members who found significant value from testing his herd for Johne's disease and making several management changes.

"We found out we had more Johne's than we thought we did," Steve states. "Several healthy-appearing, nice-conditioned cows tested positive for Johne's, and that sure surprised me. While it was tough to sell those females, that's what we did when a second round of testing confirmed the first test results.

"We also started giving our calves colostrum from only test-negative cows and switched practices where manure contamination could be an issue."

Dairyman Dave Hogan of Tillamook, Ore., says the key advantage of participating in a Johne's program was testing and identifying cows with Johne's disease. Like Neahring, Hogan eliminated Johne's-positive cows and adjusted various management practices. Today, he says his 2,500-cow dairy has no "blown out sickness."

Dr. Wustenberg points out that TCCA's emphasis on Johne's disease prevention and control has helped producers reap additional benefits. He says that, since Johne's disease is a fecal-born disease, management strategies to help prevent and control Johne's disease have also resulted in lessening the incidence of other diseases such as *Salmonella* and *E. coli*.

**To learn more about
Johne's disease
prevention and control,
please contact your state
Designated Johne's
Coordinator.**

**A list of state DJCs
is available online at
www.johnesdisease.org.**

