of the population was positive for the ELISA test, and 7.03% was positive for either blood- or milk-nested PCR. Apparent prevalence when considering a cow positive, if test-positive in either ELISA or nested PCR tests, for Dairy A, B and C was 23.2%, 15% and 18%, respectively. Maximum possible agreement beyond chance level for all cows, Dairy A, B and C was 17%, 20%, 15% and 16%, respectively. Kappa value (95% CI) in the whole population, Dairy A, B and C was -0.005 (± 0.115), -0.1304 (± 0.071), 0.170 (± 0.277) and -0.087 (± 0.050) respectively, indicating poor agreement of test results in all cases. Negative kappa values indicate that the two sets of results agreed less than would be expected merely by chance. Fisher's Exact Test used to test the alternative hypothesis of positive association between both test outcomes resulted in right-sided p-values of 0.650, 1.00, 0.129 and 1.00 for all animals, Dairy A, Dairy B and Dairy C, respectively. This indicates that in all the cases, there is no evidence to reject the null hypothesis (i.e., there is not a significant association between results of both tests). Complementary sensitivity for ELISA (% of extra cases detected by this test compared to use of PCR alone) was in the range of 116% to 200% and between 36% and 85% for PCR, showing an improvement in the percentage of infected cows detected when both tests were combined. The data presented indicates improvement in sensitivity for detection of MAP when blood ELISA and blood and milk PCR are combined.

### Significance

The compared tests detect different forms and stages of MAP infection because their respective targets (bacteria and antibodies) may not have parallel dynamics. They may identify different populations of infected animals which could be the explanation for low kappa values reported in the present study.

The concept of complementary sensitivity (CS) applied in this study appears to be a useful tool when a "gold standard" is not available in practical terms. CS provides a measure of the efficiency of combining two methods with high specificity to increase sensitivity in MAP detection. In this case, CS for ELISA and PCR indicated in both cases an improvement in percentage of infected cows detected when both tests were combined.

It is concluded that the sensitivity for the detection of MAP is improved when blood ELISA and blood and milk PCR are combined. Further research on the dynamics of MAP in blood and milk could increase the future value of the test combination proposed in this study.



# Environmental Distribution of *Mycobacterium avium paratuberculosis* (MAP) on Michigan Dairy Farms

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### Introduction

Johne's disease (JD) is an important infectious disease of cattle caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). It is estimated that over 50% of US dairy herds are infected with MAP. Environmental contamination with MAP is considered the major reservoir of infection for susceptible cattle. While MAP does not multiply in the environment, it can persist for months in manure, lagoons, manure packs—areas that are found in abundance on most dairy farms—even in below-freezing temperatures. Very little information is published on distribution of MAP on dairy farms. The objectives of this study were to: 1) perform serial environmental culturing on six Michigan dairy herds, enrolled in a JD control program over several years; 2) characterize the distribution of MAP contamination on dairy farms; and 3) determine if and how that distribution changes as herd fecal culture prevalence changes.

## Materials and Methods

The dairy herds sampled in this project are participants in the ongoing Michigan Johne's Disease Control Demonstration Project, and vary in size from 80 to 400 cows milking. Feces from all adult cows were cultured annually for MAP and herd fecal culture prevalence calculated. Additionally, every six months, composite samples of feed, water and flooring were collected and cultured for MAP from each of four animal housing areas on the farm: calf, transition heifer, maternity and lactating cow. Samples were also collected and cultured from the primary manure collection area (lagoon or manure spreader), recycled sand bedding and pasture areas where applicable.

## Results

A total of 547 environmental samples were collected for MAP culture from the six herds from 2003 through 2006. MAP was cultured from 59 (10.8%) environmental samples. The manure collection area was most commonly culture-positive for MAP, with 19 (46.3% manure collection area samples 3.5% all samples (3%), followed by lactating cow floor 16 (29%; 2.6%), maternity floor 7 (16.7; 1.3%), (1%), maternity water with 4 (0.8%), calf floor with 4 (9.1%; .7%), recycled sand bedding 3 (75%; 0.5%) and calf feed with 2 (53%; 4%). Two samples from the heifer floor and one sample each from lactating cows feed, and lactating cow water were positive for MAP. True prev. based on fecal culture of the herds during this time ranged from zero to 48%.

There was a tendency for herds with higher MAP fecal culture prevalence to have more MAP-positive environmental samples. As fecal culture prevalence declined, the number of MAP-positive environmental samples also declined. Also, positive environmental samples tended to go from classification as moderate or heavy shedders to low or very low shedders as herd fecal culture prevalence decreased.

MAP was consistently found (79% of the time) in the manure collection area and/or lactating cow floor when herds had fecal culture prevalence greater than 2%. Once herds reached a prevalence of less than 2%, MAP was never cultured from any area sampled. Conversely, once herds exceeded a fecal culture prevalence of 5%, MAP contamination was found in areas other than the manure collection area and lactating cow floor, most commonly the maternity floor.

## Significance

On the farms in this study, MAP was most commonly cultured from the primary manure collection area and lactating cow floor. These are the areas with the greatest concentration of manure from the greatest number of mature cows, who are at greatest risk of shedding the bacteria. The number of MAP culture-positive environmental samples, and the classification of shedding in those samples, tended to increase as the number of cows shedding MAP in the herd increased. Incidence of environmental contamination with MAP was highest in the primary manure collection area, followed by the lactating cow area and the maternity area. The fact it was not uncommon for MAP to be isolated from the maternity area, particularly the floor, is concerning because calves, the animals most susceptible to MAP infection, are born in these areas. This implies cleanliness and sanitation of the maternity pen must be emphasized when trying to control JD.

Finally, MAP was cultured 79% of the time from the primary manure collection area and/or lactating cow floor in herds with greater than 2% MAP fecal culture prevalence. This suggests targeted environmental sampling could be used to screen dairy herds for JD. Targeted environmental sampling may provide a reliable and cost-effective tool for producers to monitor the progress of their JD control program.