

# Use of Environmental Sampling as a Herd Screening Diagnostic Tool for the Detection of *Mycobacterium avium* Subsp *paratuberculosis* on Minnesota Dairy Farms

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## Introduction

Johne's disease (JD) is a chronic and progressive intestinal disease in ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis* (Map). The usual route of infection is fecal-oral. The disease manifests in adult cows and results in economic losses. Although Map does not propagate in the environment, it survives for long periods in different environmental conditions expected on many dairy farms. Johne's disease control programs have been developed in different countries and in several US states with a goal to test and classify herds of cattle as infected or presumptively non-infected with maximum accuracy and least cost. Generally, these programs utilize recognized laboratory tests such as ELISA or direct microbiological individual fecal culture. However, these tests have several disadvantages, especially when applied in herds with subclinical disease or low prevalence. Since Map is shed into the environment by dairy cattle through fecal contamination and appears to survive well, a better understanding of Map distribution in the environment could lead to improved herd-screening alternatives.

Objectives of the study were: 1) to describe Map distribution and prevalence in the environment on Minnesota dairy farms, and 2) to assess the relationship between culture status of Map in the farm environment and fecal-pooled culture status on dairy farms.

## Materials and Methods

One hundred and eight Minnesota dairy herds were sampled during summer 2002, including 80 herds known to be infected based on previous testing in the Johne's Disease Control Program (JDGP) of the Minnesota Board of Animal Health (MBAH), and 28 herds known to be uninfected based on previous testing in the Voluntary Johne's Disease Herd Status Program

(VJDHSP) of the MBAH. Fecal samples were obtained from up to 100 cows in each herd and were cultured in pools of five cows per pool. Environmental samples were obtained from each farm, with up to two samples collected from various locations including cow alleyways, manure storage, calving barn and sick cow pen. Fecal pools and environmental samples were tested using bacterial culture for Map at the Minnesota Veterinary Diagnostic Laboratory. To determine the best environmental sampling strategy to assess herd infection status, a multivariable logistic regression model was developed using SAS PROC LOGISTIC, where the dependent variable was herd status based on bacterial culture of fecal pools (positive or negative) and the independent variables were environment culture status by location. To estimate prevalence of infection in the herd based on environmental sampling areas identified in the selected logistic regression model, PROC MIXED of SAS with the EMPIRICAL option was used to fit the model for herd fecal pool prevalence. The dependent variable was percent positive fecal pools and the independent variables were maximum colonies per tube (CPT) by environment location sampled (negative, light, moderate and heavy), number of positive samples (0,1,2) from the selected farms areas, herd size (<100, 100-200, >200), and housing type (freestall or tiestall).

## Results

Sixty-four of the 80 JDGP herds (80%) had at least one positive pool; 16 of these herds did not have any positive pools. The farm environment was determined to be contaminated on 61 of the 64 (95%) herds with at least one positive pool, and in one of the 16 JDGP herds with no positive pools. Twenty-six of the 28 VJDHSP herds (93%) had no positive pools; two herds had one positive pool each. One environment sample from these herds was also cultured positive. The farm environment

was determined to be positive in samples from cow alleyways (77% of the herds), manure storage (68%), calving area (21%), sick cow pen (18%), water stream edge and water runoff (6%), and postweaned calves area (3%), but not in preweaned calves or fields near cow area. There was an association between maximum level of colonies per tube from cow alleyways and manure storage and fecal pool prevalence. Herds with both areas cultured negative were estimated to have 0.3-4% fecal pool prevalence. Herds with both areas having a heavy load of bacteria were estimated to have 53-73% fecal pool prevalence.

## Significance

Targeted common and contaminated areas in the farm environment, specifically cow alleyways and manure storage (especially lagoons), suggest a promising alternative strategy for herd screening and JD infection status assessment and for estimating herd fecal prevalence. This strategy has the potential of saving significant economical resources in terms of cost and time.

# Effect of Management Related Disease Control Methods on the Development of Johne's Disease in Dairy Cattle in Texas.

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## Introduction

With the primary objective of evaluating long-term effectiveness and feasibility of management-related disease control methods in large dairies and on beef operations on the development of Johne's disease (JD) in cattle in Texas, USDA-APHIS and Texas A&M University have initiated the Johne's Disease Control Demonstration Project for the Texas dairy and beef industries. The epidemiological and production analysis of one of the dairy demonstration herds (n=3534) is presented, and the effect of JD on the herd's production parameters is evaluated.

## Materials and Methods

A retrospective epidemiological study was conducted from April 2001 until December 2003 on the lactating herd. Serum samples were collected from cows after they had been confirmed pregnant and prior to lactation cessation. The serum samples were tested using an ELISA test (IDEXX Laboratories). Test results >0.25 S/P value were considered positive for JD. Production parameters (305 days milk production, somatic cell counts, total fat, total protein) and reproduction parameters (times bred, days open and times aborted per lactation) were collected. The herd was then stratified and JD-positive cases were compared to negative cases for production and reproduction parameters us-

ing T-test and Mann-Whitney Rank Sum Test using Sigma Stat (SPSS,2001). A colostrum management strategy of using colostrums only from test-negative cases was implemented at the beginning of the study (April, 2001), and results of the serological analysis were incorporated into each cow file and considered during treatment decisions on digestive disorders and when making culling decisions. Cow replacement of home-reared animals with purchased cows was also implemented.

## Results

Laboratory analysis indicated 116 positive cases (8.51%) of a total of 1362 analyzed samples during 2001, 36 positive cases (7.89%) of a total of 456 analyzed samples during 2002 and 83 positive cases (5.61%) of a total of 1477 analyzed samples during 2003.

Prevalence of JD in home-reared cows was 11.73%, and 6.12% and in purchased cows. Cows in lactation one showed no statistically significant differences in any parameter analyzed. Statistically significant differences in 305 days milk (P=0.037), 305 days mature equivalent milk (P=0.006), total fat (P=0.001) and total protein (P=<0.001) were found for cows in lactation two and three. No significant differences in the parameters analyzed occurred in cows in their third or later lactation. The presence of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in fecal material was deter-