

MAP Super-shedders: Another Factor in the Control of Johne's Disease

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Introduction

Traditionally, fecal cultures were either positive or negative for growth of *Mycobacterium paratuberculosis* (MAP). Nearly all culture-positive cattle were judged to be infected, shedding MAP, thus a threat to spread the disease to susceptible cattle and were culled from the herd as quickly as possible. Over time it was recognized that differences in MAP shedding existed among culture-positive cattle. Cattle were classified as low, moderate or high shedders based on the visible colonies of MAP on the surface of solid media, typically Herrold's egg yolk media (HEYM). Rarely did authors provide the estimated MAP colony forming units (cfu) per gram of manure. Additionally, culture methods were not standardized among diagnostic laboratories in the US, which made it difficult to compare results from one laboratory to another. Laboratories rarely used internal quality control fecal samples. As the infection and disease progressed, the intensity of fecal shedding of MAP increased, culminating in clinical disease which was associated with the greatest numbers of MAP present in manure. Since cattle with clinical signs lose weight and have decreased milk production causing them to be unprofitable, they are soon culled from the herd. If not culled from the herd, they would continue to lose weight and become emaciated with muscle weakness, leading to recumbency requiring euthanasia. The fewest colonies reported is one colony on one of four tubes of HEYM, which translates into approximately six cfu/gram of feces, assuming the original sample was two grams; 5 ml was transferred from the fecal water tube to the second decontamination tube with HPC. That centrifugation technique was used and the pellet was re-suspended in 1 ml of antibiotic brew, with 200 ul of the re-suspended pellet inoculated onto each of four tubes of HEYM, all with mycobactin J. Low shedders typically had less than 10 colonies on the four tubes of HEYM. Moderate shedders ranged from 11 to approximately 70 colonies of MAP, while high shedders had more than 70 colonies on any of the four tubes. Calculated on MAP cfu per gram of feces this would be up to 60 cfu for low shedders, 61 to 300 cfu per gram for moderate shedders and more than 300 cfu per gram for heavy shedders.

Over the past five years most laboratories in the US report the number of visible MAP colonies on each

tube of HEYM, but rarely enumerate above 50 to 70 colonies per tube. Accounting for sample preparation, 50 colonies on each of four tubes represents an estimated 1,050 colony forming units of MAP per gram of manure. The objective of this study was to determine the range of MAP cfu/gram of manure in cattle classified as heavy shedders.

Materials and Methods

Fecal samples from cattle classified as high shedders were serially diluted: 1:5, 1:10, 1:50; 1:100, 1:500, 1:1,000, 1:5,000, 1:10,000 and 1:50,000. This initial pilot serial dilution series helped define a closer range to process a larger number of heavy shedders. Fecal samples from more than 200 cattle classified as heavy shedders were cultured with the routine culture methods and serially diluted at 1:100 and 1:1,000. Super-shedders are defined as cattle with fecal samples having more than 10,000 cfu MAP per gram of manure.

Results

The vast majority of heavy shedders would be classified as super-shedders with more than 10,000 cfu MAP per gram of manure. Based on serial dilution of fecal samples, we have demonstrated that some infected cattle not showing clinical signs of Johne's disease (JD) shed more than 1,000,000 cfu of MAP per gram of manure. The typical range of MAP cfu for cattle showing clinical signs of JD is from 50,000 to 250,000 cfu of MAP per gram of manure.

Significance

Based on this commonly accepted numerical assessment of MAP cfu, high shedders represent the greatest threat to spread the disease, but rarely did anyone appreciate that one heavy shedder could excrete adequate MAP in the environment to be equivalent to a high shedder in composite manure samples, or that one heavy shedder could excrete more MAP cfu than 5,000 low shedder cattle. Super-shedders represent the greatest risk to spread Johne's disease among herd mates. Some super-shedders could contaminate the environment with more MAP than 160 heavy shedders, more

than 2,000 moderate shedders and more than 20,000 low shedders. The frequency of super-shedders among culture positive cattle in infected herds is being investigated at this time. Based on this new dimension of super-shedders, a significant proportion of low shedders are likely to represent “pass-through” and not active true infections. As little as 10 ml of manure (1×10^7 cfu of MAP) from a super-shedder could both infect another calf or heifer and result in the manure of the heifer being culture-positive with several colonies per tube. Previous research from this laboratory has shown that “pass-through” can result when cattle consume manure from cattle with clinical Johne’s disease. The challenge to the Johne’s academic community will be to develop diagnostic methods to detect these super-shedders in a cost efficient manner and eliminate them from the herd prior to massive environmental contamination.

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Uterine Bacterial Isolates and Reproductive Performance in Lactating Holstein Cows with Uterine Health Disorders, Large Calves and Fever

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Introduction

Uterine health disorders are common in dairy cows and result in reproductive inefficiency and economic loss in dairy herds. Specific uterine bacteria associated with some uterine health disorders have been described. However, information regarding the relationship between calf birth weight and uterine bacteria is lacking. The aim of this study was to evaluate relationships between uterine bacterial isolates during the first four weeks after calving, uterine health disorders (metritis, retained placenta [RP], clinical and subclinical endometritis), fever and calf birth weight. The impact of uterine health disorders on reproductive performance was also evaluated.

Materials and Methods

One hundred-five Holstein cows were used to investigate the relationship between uterine bacterial isolates, uterine health disorders, fever and calf birth

weight in lactating Holstein cows. Aerobic and anaerobic uterine cultures were performed twice weekly from weeks 1 to 4 postpartum. Cows were examined at 28 days postpartum, for clinical endometritis (purulent cervical discharge on vaginal examination) and at weeks 4 and 8 postpartum for subclinical endometritis (> 15% neutrophils on endometrial cytological exam). Calves were weighed at birth and birth weights were recorded. Rectal temperatures were recorded from days 1 to 10 postpartum, and fever was defined as a rectal temperature $\geq 103^\circ\text{F}$ for \geq two days. The presence and character of uterine discharge (or retained placenta) was recorded daily from days 1 to 10 postpartum. Reproductive performance was measured and related to uterine health disorders. Statistical differences were determined by T-test using Statistix[®] 8 statistical software.

Results

Of 105 cows, 21% developed RP, 22% metritis (fetid discharge), 12% developed clinical endometritis (pres-