Fecal shedding of *Mycobacterium avium* subsp. *paratuberculosis* in calves: Implications for disease control and management

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ABSTRACT

It is widely accepted that most infections caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) occur in utero or in the neonatal animal. The challenge, however, has been to detect the infection in the young animal as this would facilitate more prudent animal management decisions. The objectives of this study are to determine whether: 1) fecal shedding of MAP can be detected in naturally infected calves, 2) there is a relationship between MAP test positive cows (ELISA or fecal) and fecal shedding in their offspring, 3) there is an association between fecal shedding in the calf and their ELISA test status, and 4) pooled fecal sample testing is sensitive enough to detect MAP shedding in calves. This is a two year longitudinal, prospective study using dairy calves of four age groups from seven herds in Lower Michigan with known MAP prevalence.. Fecal, and blood samples, are obtained from ten calves in each of the four age groups at 3 month intervals. The TREK® liquid culture system is used for fecal culture and the Biocor® ELISA for blood. Preliminary results showed (study 40% complete) we were able to detect fecal shedding in calves with a higher proportion of shedding calves originating from test positive dams. Positive ELISA samples from calves showed an association with dam test status but little association with their concomitant fecal status. Also, pool size maybe critical in detection of fecal shedding in calves.

Keywords: calves, dams, shedding, longitudinal study, liquid culture

INTRODUCTION

Johne's disease, *Mycobacterium avium subsp. paratuberculosis* (MAP), is a chronic infectious inflammatory enteric disease of both domestic and non-domestic ruminants. This disease was first described in 1895 in Germany and its etiology was characterized as an acid fast bacillus(Twort,1910). Although MAP is over a century old, it has emerged as a major economic factor in the US dairy industry, primarily over the past three decades. For instance, the cost to the individual US dairy producer is between $22 and $27 per cow, across all herds, with a possible 250 million dollar total annual price tag.(Ott et al.,1999). Although widely variable prevalence figures have been published(Adaska et.al.,2003)(Hirst et.al.,2004), a recent study placed the percent of infected herds in Michigan at 52.4%(Ifearulundu-Johnson et al.,1999) and a national random sample reported that 2.5% of the individual animals tested across the US were fecal positive(USDA-NAHMS,1997).

Calves are often infected before the age of six months (Sweeney,1996) via feces or colostrum and transplacental infection (Kopecky et al.,1967)(Seitz et al.,1989)(Sweeney et al.,1992) also occurs. However, MAP is a slow-growing bacteria and development of clinical signs may take 2-5 years (Harris and Barletta,2001) which presents a challenge to the producer's management of the disease. Attempts to detect naturally infected calves have been unsatisfactory (McDonald et al.,1999)(Ayele et al.,2004) due to low bacterial shedding that is present in young animals. Traditionally, fecal culture has been an ineffective method to detect low bacterial shedders(Kim et al.,2002) and its overall sensitivity may be as low as 33%(Whitlock et al.,2000). Due to reports of increased sensitivity of the recent TREK®ESPII liquid culture system (Stitch etal.,2004) this study is utilizing the liquid culture system to identify fecal shedding in naturally infected dairy calves.

MATERIALS AND METHODS

Study design: This is a longitudinal, prospective, two year study involving calves from seven commercial dairy herds in the lower portion of the State of Michigan

Criteria for herds inclusion: Seven herds were selected for this study due to varied known test prevalence levels (1 – 42%), their involvement in the ongoing USDA Johne's demonstration project, and the invaluable individual cow test data available because of this involvement. With the exception of one 80 cow Jersey herd, the herds are Holstein and herd size ranges from 110 to 511 cows. These herds represent both grazing and confinement management styles.

Selection of calves: Ten heifer calves from each of four age groups: 0-3 months, 4-6 months, 7-14 months, and 15-24 months were selected for fecal culture and ELISA testing during each herd visit. An attempt was made to test all heifer calves from fecal positive dams. We test forty calves from each herd at approximately three month intervals and will repeat this eight times. This will provide approximately 1600 samples at the conclusion of the study.

Sampling: Fecal samples were collected from each calf individually by digital exculpation using individual latex gloves and sterile water for lubrication. At least 6 grams of fecal material were collected as we used 2 grams each for the five and ten calf pooled sample as well as 2 grams for the individual calf culture. 5 cc of blood was collected from the jugular or caudal tail vein for ELISA Paracheck® (Biocor) testing.

Fecal Culture Preparations: Individual fecal samples were identified and refrigerated at 5°C and submitted to lab within 1-2 days. Samples from each age group of 10 calves were divided into 2 pools of 5 as well as a pool containing all 10 calves. Using separate tongue depressors individual samples were transferred to pooled vial and manually mixed for one minute. The pooled samples were submitted at the same time.

Laboratory: All samples were tested at the Diagnostic Center for Population and Animal Health (DCPAH) at Michigan State University which is a USDA certified lab for both liquid (TREK®) and solid (HEYM) media fecal culture for Johne's disease. The Cornell method (Stabel, 1997) was used in preparation of fecal for culture. The samples were placed in the TREK® ESP Culture System II incubator which measured decrease in O2 pressure. This is a semi-quantitative test and positive samples were described as high shedders if it took 7-21 days to turn positive, moderate shedders if 22-28 days to positive, and low shedders if positive at 29- 42 days. If not positive by day 42, they were classified as not shedding. Positive and negative controls were used on each batch of forty samples and the positive control will be consistent across the study, obtained from a serially confirmed low shedding cow. All positives from liquid culture were confirmed with both terminal Acid Fast Disease Staining (AFDS) - Kenyon stain - and IS900 PCR. Real time PCR (Kim et. al.,2004) was used. The blood was centrifuged and the serum tested for MAP antibodies by the Paracheck® (Biocor) ELISA using the manufacturers recommended procedure.

RESULTS

To date 40% of the study is complete and the following are the preliminary results.:

Objective 1- Detection of fecal shedding:

Of the 583 samples cultured 12 were positive (2.1%) with 9 low shedders, 2 moderate shedders, and 1 heavy shedder (Table 1). Of the 4 positive calves tested the 2nd time, 1 was positive and 3 were negative but the pools containing these 2 of these 3 were positive. All but two of the fecal shedding calves were from the first three age groups (≤14 months) and 3 of these were < 6 months of age.

Table 1

Age Distribution of Positive Fecal Shedders and Level (Low, Moderate, Heavy)

	0 – 3 Mos.	4 – 6 Mos.	7 – 14 Mos.	15 – 24 Mos.	Total Fecal Shedders
# Positive Fecal Shedders	1(low)	2(low)	5(low) 1(moderate) 1(heavy)	1(low) 1(moderate)	12

Objective 2 – Relationship between fecal shedding in calf and dam test status (positive dam=positive fecal or ELISA test):

8/11 positive calves represented by the 12 positive samples came from test positive dams (73%) whereas as only 99/583 of total calves tested came from test positive dams (17%).

Objective 3 – Relationship between positive fecal shedding and ELISA results:

Of the 583 blood samples tested, 13 were ELISA positive(2.2%). but only one animal had both a positive fecal and positive ELISA test indicating nearly no relationship between detectable fecal shedding in calves and their ELISA status. However, 8/11 of the sero-positive animals, represented by the 13 positive samples, were from test positive dams (fecal or ELISA).

Objective 4 – Sensitivity of pooling samples:

Of the 8 initial positive individual fecal samples that were in 8 separate pools (10 samples per pool), one pool was positive. We lowered pool size to 5 at that juncture (while also continuing to run pools of 10). With the subsequent 4 positive samples we demonstrated 2 positive pools of 5 while the pools of 10 were negative. We also had three positive pools (2 pools of five and one pool of 10 (containing calves in one of the pools of 5)) that contained no test positive individuals. However, they each contained an animal that had tested fecal positive on a prior test.

DISCUSSION

5 of 7 herds had positive calf samples and the youngest calves testing positive (≤ 6 months of age) came from the two highest prevalence herds (14% and 42% respectively). The fact that some of the fecal positive calves tested negative subsequently leads one to believe that they are low or sporadic shedders or there maybe some pass through of the organism. Follow up sampling may help answer this question. The finding that 11/12 positive fecal tests and the 6 positive pools came from the 339 samples gathered from the four highest prevalence herds leads one to speculate that if fecal culture or pooled fecal culture is used as a tool, it would be most effective in herds with a high prevalence of Johne's. There seems to be a relationship between dam status and fecal shedding as 8/11 positive shedders(73%) came from test positive dams which comprised only 17%(93/583) of the total dams. Considering that the fecal and ELISA positive calves originated almost exclusively from the four herds with the highest test prevalence we also looked at the proportion of test positive dams in this group of four herds and found it to be 57/339(19%) which may not weaken the association between test positive calves and their dam status.

Although nearly the same proportion of fecal samples were positive(12/583) as were ELISA positive(13/583) only one calf was positive for both simultaneously and this suggests that possibly a combination of testing types may be a better way of assessing the infection level in young animals. Quantitative interpretation of ELISA tests may also be a more valid method than simply using positive and negative cutoffs when assessing the ELISA titer(Collins et. al.,2005).

Finally, with only 1/12 of the positive samples turning their pool of ten positive, one may infer that, with low shedding levels in calves, pools of ten are too large (Wells et al.,2002)(Wells et. al.,2003)(van Schaik et al.,2003). We now use 2 pools of five and a pool of ten for each group of ten calves and results seem to have improved. Possibly pooled fecal samples containing five calves in conjunction with environmental testing(Raizman et al., 2004) may be a feasible, inexpensive early predictor of the success of management changes in herds with a high prevalence of MAP. As for accounting for the positive pools containing no positive individuals, it has been shown that, with light shedding, MAP is not spread evenly throughout a fecal sample but rather may exist in pockets.(Kalis et. al., 1999)(Visser,1999).

CONCLUSION

Our preliminary results indicate that it was possible to detect fecal shedding in calves. It is hoped that, by serially testing these calves and testing them later as cows, more questions may be answered as we strive to manage, control, and ultimately eliminate Johne's disease from our dairy cattle industry.

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