Use of a Fecal PCR Assay on Environmental Samples for Detection of Dairy Cattle Herds Infected with Johne's Disease

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Introduction

Johne's disease (JD) is an intestinal disease in ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). MAP is transmitted to susceptible cattle via multiple pathways, including prenatal (in uterus), postnatal (fecal-oral, contaminated colostrum or milk) and later in life through various environmental routes. This bacterium can be detected in biologic samples using culture methods to detect the viable bacteria and by using PCR tests to detect MAP DNA.

The goal of this study was to evaluate the application of fecal PCR assay for diagnosis of *Mycobacterium avium* subspecies *paratuberculosis* in environmental samples and to determine its sensitivity for detection of dairy cattle herds infected with JD.

Materials and Methods

Environmental fecal samples were collected from Minnesota dairy farms known to be infected from previous testing in the Johne's Disease Control Program of the Minnesota Board of Animal Health (MBAH). Herd selection criteria included herds with at least 100 lactating cows, current participation in the Voluntary Minnesota Johne's Disease Control Program from the MBAH and relative proximity (within 150-miles radius) to the University of Minnesota for ease of sampling. All eligible herds were known to be positive based on previous testing and enrollment in the MBAH Johne's Disease Control program (with risk assessment and diagnostic tests performed annually).

Two environmental fecal samples were collected from up to five different locations on each farm: cow alleyways, manure storage (manure push, manure pack or lagoon), calving area, fresh cow area and sick cow area (for a total of six to ten samples total per herd). Environmental samples from Minnesota dairy farms were tested using Taqman PCR assay and fecal culture (FC) for MAP at the Minnesota Veterinary Diagnostic Laboratory. Bacterial culture was performed using the method previously described (Wells *et al*, 2002). Briefly, a sedimentation culture procedure was used with 72 hours of sedimentation prior to inoculation of four tubes containing Herrold's egg yolk medium with Mycobactin J and one without. Molecular detection of MAP was accomplished by PCR amplification of ISMav2 at the MVDL using the technique described by Wells *et al* (to be published). A total of 409 environmental fecal samples were tested from 49 Minnesota dairy herds.

Fecal culture results were defined as light (1-10 colonies per tube), moderate (11-50 CPT), heavy (51-100 CPT) and very heavy bacterial load samples (>100 CPT), and PCR results as positive or negative.

PCR results were compared with solid culture method results at the sample level, at the herd level and by location sampled.

Results

Of 409 environmental samples, 202 (49.4%) were positive by fecal culture (FC). Of those 202 positive samples by FC, 45 (22.3%) were positive also by PCR. At the sample level, the percent agreement was 57% and the kappa value 0.14. At each level, the results were the following: 11% (11/99) of FC light bacterial load samples were positive by PCR; 24% (16/67) of moderate samples; 36% (5/14) of high samples; and 59% (13/22) of very high bacterial load samples were also positive by PCR. There was a significant difference between all the areas overall ($p<0.0001$).

Analysis by location: Within environmental area, highest detection rates were found in samples from fresh cow area (29%), manure storage (27%) and cow alleyways (21%). Lowest detection rates were found in calving area (6.3%) and sick area (13.3%). Within location, the fresh cow area showed the higher rate of high and very high bacterial load samples (27%)

Herd level analysis: Of 49 infected farms at the herd level, 32 were positive by PCR (65.3%) and 44 by FC (90%). From those 44 herds positive by FC, 31 (70.5%) were also positive by PCR.

Comparing both tests' performance, at the sample level, of 113 positive samples by FC, 27 were also positive by PCR (24%). In each level of bacterial load, the results were: 14.5 % (8/55) of FC light bacterial load

samples were positive by PCR, 27.5 % (11/40) of moderate samples, 30 % (3/10) of high samples and 62.5 % (5/8) of very high bacterial load samples.

Analysis of FC and PCR sensitivity at the herd level was made for 2, 4, 6, 8 and up to 10 samples. 34.3 % (12/35) of farms that tested positive by FC were also positive for PCR when two environmental samples (from cow alleyways) were tested. From the 43 herds positive by FC, 27 (62.8%) were also positive by PCR, for six samples (from cow alleyways, manure and fresh area) and 8 samples (cow alleyways, manure, fresh and sick cow area) 30 (69.8%) of the 43 herds positive by FC were also positive by PCR.

From the 44 herds confirmed positive by FC, 48% (21/44) had at least one high or very high bacterial load environmental sample, and PCR was able to detect 71.4 % (15/21) of them.

Significance

To date, this is the first study of application of the PCR test on environmental samples to detect MAP. The study included a large number of well characterized infected herds that allowed us to estimate the sensitivity of the PCR on environmental samples following a specific sampling strategy.

Even though the solid culture method is considered the gold standard for diagnosis of MAP due to its high specificity (100%) and reliability, limitations include low sensitivity (approximately 50% in animals with a patent infection), long growth time and high cost. These limitations show the potential value of a quicker and more practical test for diagnosis, such as PCR.

Since PCR showed a medium sensitivity to detect positive herds, we concluded it would not be a good test to use as a screening method in level 1 herds in the Voluntary Johne's Disease Herd Status Program (VJDHSP). The fecal PCR assay can be effectively used as a pre-screening test for detection of contaminated environmental samples in herds of unknown status because of its lower cost and quicker detection time.

We should evaluate the level of shedding in a herd when considering applying PCR tests. If a high proportion of high shedders are present in a herd, a control program involving PCR methods will result in a rapid improvement, but less effect can be expected in situations in which the level of shedding is low. This test also will allow the option of culling animals shedding high quantities of MAP in the farm environment.

Fecal PCR assay can be used as a quicker test for detection of subclinically infected high to very high fecal shedders, those cattle at highest risk of transmitting infection to susceptible cattle. Our results indicate that the same principle is also applicable for environmental samples since PCR was able to detect those samples with higher bacterial load. In summary, our results demonstrate the applicability of the PCR test on environmental samples as a tool to screen the status of unknown herds.