

Evaluation of Diagnostic Tests for Detection of Paratuberculosis in Young Cattle

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Background

Johne's disease is an insidious chronic enteric infection of ruminants caused by *Mycobacterium avium* ssp. *paratuberculosis* (MAP). MAP is taken up by phagocytic cells in the ileum and gradually spreads to regional lymph nodes and other body organs in the later stages of the disease.

One author describes four stages of Johne's disease (Smith, 2002). The first stage is the "silent infection" stage. Most animals are infected as young calves via fecal-oral transmission. The organism proliferates slowly in the ileal mucosa and then slowly spreads to regional lymph nodes. Animals in stage I are rarely detected even with the most sensitive laboratory tests, including fecal culture. The silent phase of infection usually lasts at least 2 years and may last as long as 10 years. Stage II is the "inapparent carrier adults" stage. These animals do not show weight loss or diarrhea but may have an altered immune response. They may also have an increased gamma interferon response by T-cells that are sensitized to specific mitogens or increased antibody response to MAP. Some, Stage II animals, may test positive on fecal culture, shedding organisms in their manure and contaminating the environment. They may serve as a source of infection for other animals on the farm. The third stage is "clinical disease", characterized by weight loss and chronic or intermittent diarrhea. The fourth stage is "advanced clinical disease", characterized by profuse diarrhea and intermandibular edema. Death usually follows soon after Stage IV begins due to dehydration and cachexia.

Justification and Objective

The diagnostic problem that is evident is that detection of MAP by fecal culture is usually ineffective in heifers younger than two years of age. The objective of this study is to detect MAP infection in calves less than two years of age in order to avoid the costs of raising a heifer that ultimately may be culled due to MAP infection. In addition, identification of early clinically silent infections may help to prevent the insidious spread of the disease within young stock groups. Our approach to this problem was to evaluate the use of diagnostic tests for MAP infection based on the detection of cell mediated immunity (CMI) in young cattle (2-8 months old) for identification of heifers infected with MAP and to determine whether there is any association between the infection status of the dam and their respective heifers.

Materials and Methods

Three hundred eight dams and their heifers were selected from herd A with 11% seroprevalence by IDEXX enzyme-linked immunosorbent assay (ELISA) for MAP

infection. Infection in this herd has been qualified by fecal culture and presence of clinical signs in culled cattle. Dams and heifers were also selected from a low seroprevalence herd B (2%) that does not observe clinical signs of Johne's disease in culled cattle. Because an adequate gold standard test for MAP infection status is not available, and the currently available tests have a sensitivity of less than 50%, it is very difficult to identify and determine a "disease free" status of herds. Thus, herd B was included in the study as the best option available for a negative control herd. Selection of the dam-calf pairs (n = 308) was performed on the basis of the dam's serologic status. ELISA, intradermal skin test of with MAP purified protein derivative (PPD) in randomized cervical or caudal fold sites, and Gamma-interferon (IFN) tests were performed in the heifers at 2, 4, 6 and 8 months of age. In addition, fecal culture and PCR were run on samples from each dam at enrollment and in the heifers when they reached 8 months of age.

Results

Preliminary results on 308 dams and heifers enrolled indicate that skin test reactions in calves are more numerous when testing is performed between 4 and 6 months of age and that the majority of the skin test reactions correspond to the cervical injection site rather than the caudal fold site. The ELISA result in calves remains negative despite the repeated intradermal injection of MAP PPD. There was a significant loss of heifers born from ELISA positive dams due to perinatal death or premature culling because of repetitive pneumonia or delayed growth. Skin test reactions in calves did not seem to be associated with the dam's ELISA status. PCR tests performed in feces collected from 8-month-old heifers demonstrate that some calves are shedding MAP at this age. No association between the heifer's IFN test and the serologic and or shedding status of their dams has been observed.

Conclusions

The overall goal of this study was to evaluate the use of diagnostic tests for MAP infection based on the detection of CMI in young cattle (2-8 months old) for identification of heifers infected with MAP and to determine whether there is any association between the infection status of the dam and their respective heifers. We will not be able to evaluate the diagnostic tests until the infection status of calves is confirmed with fecal culture, fecal PCR or necropsy which may require waiting until heifers are greater than 2 years of age. However, positive PCR results from fecal samples of 8-month-old calves are promising in that we may be able to confirm infection status earlier than expected.

References

Whitlock, R. Johne's Disease. In, Smith B, ed. Large Animal Internal Medicine. Mosby Inc., St. Louis, 2002, pp 799-782.

