Comparison of the γ-Interferon ELISA and the Skin Test for the Detection of Sub-clinical Johne's Disease in Cattle

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Introduction

The diagnosis of Johne's disease in cattle can be difficult. Cattle are often infected for years before they begin shedding *Mycobacterium avium* subsp. *paratuberculosis* in their feces or mount a detectable humoral immune response. Current diagnostic tests, serology and fecal culture, are effective diagnostic tests to detect animals in advanced stages of disease, but fail to reliably detect infection in young replacement animals.

However, it is thought that the majority of animals elicit a detectable cell mediated immune (CMI) response before fecal shedding occurs, and a detectable humoral immune response after fecal shedding. Therefore, it is important to evaluate CMI diagnostic tests as potential tools to use in the detection of subclinical infection, especially in replacement populations. There are two common methods for evaluating the CMI response to *M. paratuberculosis*, skin testing and the γ-interferon (IFN-γ) test.

In the past, skin testing has not been viewed favorably in the literature. This may be in part due to the use of a gold standard (either fecal/tissue culture) to determine Johne's-positive status of individual animals. If the CMI response occurs before lesions are detected or organisms are at high enough concentrations to culture, then the skin test would be viewed as lacking specificity. In addition, no studies are published that carefully evaluate the skin test in known negative populations.

Although the IFN-γ has worked well in research settings, field studies must be done to evaluate the usefulness of this test in production settings. Experience and research with the skin test and IFN-γ ELISA for the detection of *Mycobacterium bovis* has shown there is high agreement between the two tests. Therefore, our objectives in this study were to evaluate the agreement between the IFN-γ and skin test for the detection of Johne's disease, and to see if these tests detect the disease status of young animal populations.

Materials and Methods

Known negative and positive beef herds were identified. On day one, 0.1 ml of Johnin was injected intradermally in a shaved area of the neck. Both a serum sample and heparinized whole blood sample was obtained as well as ~50g of fecal material. On day two the heparinized whole blood was set up for the IFN-γ test. One ml each of whole blood was cultured with pokeweed mitogen (PWM), johnin PPD, avium PPD and one well was left as a non-stimulated control. The blood was then incubated for 18 hours and plasma was harvested and used to measure γ-IFN production by an ELISA (Bovigam, Biocor, Omaha, NE 68134). Seventy-two hours post injection, the skin test site was read and measured.

Results and Conclusions

Preliminary results indicate that in one infected beef herd, 14% (64/452) of all cattle six months and older were positive on skin testing, and 10% of animals (9/90) between 7 and 9 months of age were positive. In this herd every other animal was IFN-γ tested. The initial run of IFN-γ tests were all negative. Herd sampling was performed in December with an ambient temperature of 33° F. During sampling, blood was transferred every 20 minutes from chute-side to a heated vehicle. Upon completion of the sampling, tubes were transferred to the laboratory by van (2.25 hours). The next morning, the assay was set up for overnight culture. The cold shock appeared to only affect the antigen specific IFN-γ responses as cells responded normally to PWM, the positive control, with an average absorbance reading of 1.3. Then, one month later, the herd was revisited and 3/35 samples were γ-IFN positive. The average PWM response across all 35 samples was 1.8. It appears that PWM may not be an acceptable positive control for the IFN-γ test. Further results will be discussed.