

Johne's Disease

Natural Exposure of Purchased Heifers in a Johne's Positive Herd

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Introduction

Johne's disease (JD), caused by *Mycobacterium avium* sub species *paratuberculosis* (MAP), is an infectious enteric disease causing chronic diarrhea, emaciation, and eventual death. However, cattle with subclinical disease may appear normal except some may have evidence of diarrhea. Control strategies include vaccination, diagnostic testing and management.⁶ The limitations and difficulties of the available diagnostic tests for JD present a challenge to producers and veterinarians trying to develop herd management protocols and herd entrance requirements to decrease the prevalence or prevent entry of JD in a herd. Diagnostic tests that identify MAP, such as fecal culture and polymerase chain reaction (PCR), fail to detect infected cattle prior to shedding. Tests that measure the humoral immune response or antibody response, such as ELISA, lack sensitivity to accurately identify MAP-infected cattle early in the progression of disease prior to shedding of MAP. In one study the ELISA detected only one of four cows shedding MAP⁴. Tests that measure a cell-mediated immune response, such as the intradermal skin test or gamma-interferon assay, have been used to identify cattle in the early stages of infection that have been exposed to MAP^{2,7}. These cattle have a high risk for shedding infectious organisms into the environment and exposing other cattle and for developing clinical disease resulting in losses due to reduced production. Identifying and removing MAP-infected cattle in the early stages of disease, prior to shedding of the organism, will allow producers to prevent entry or spread of the causative agent of JD within a herd.

Materials and Methods

Johne's Positive herd: Numerous cattle had been culled from the herd in the 1990s because of clinical signs associated with JD. An annual cull rate due to JD was estimated at 25%, typically in cows 3-5 years of age and

in peak milk production. The original owner was concerned about dispersing a herd with infected cattle; therefore, his local veterinarian collected and submitted blood from the entire milking herd twice at a six month interval to test for JD using a commercial ELISA. Results of ELISA revealed that five of 49 (10%) cows were positive. Intradermal skin tests were conducted using Johnin PPD (NVSL Lot #9801) prepared from the culture filtrate of the Neotype Strain of MAP ATCC 19698⁵. The skin test was conducted in the mid-cervical region by injecting 0.1 ml of PPD. The increase in dermal thickness (mm) was determined by measuring the skin thickness at the injection site before injection of the PPD and at 72 hours. Blood and fecal specimens were collected at six month intervals from each of the cows in the herd for laboratory examinations.

Project heifers: Ten two-year-old Holstein heifers were purchased from a herd having a five year history with no clinical evidence of JD (diarrhea, weight loss, poor production). Blood ELISA, in combination with PCR and/or fecal culture, failed to identify MAP infection in the project heifers. The ten heifers were added to the Johne's-positive herd in the fall of 2000. Fecal culture, blood ELISA and the intradermal skin test using MAP PPD (NVSL Lot # 9801) were conducted at six month intervals for three years. The IDEXX ELISA was conducted in the Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA according to the protocol outlined by the manufacturer. Mycobacteriologic examinations were conducted by procedures described in: "Laboratory Methods in Veterinary Mycobacteriology".³ In the fall of 2002, one project heifer positive on fecal culture was removed from the herd, and in the fall of 2003 the nine remaining project heifers were euthanized and tissues collected for laboratory examinations.

Results

The results of diagnostic tests on the Johne's positive herd are shown at the onset of the study; nine of 79

cows tested on ELISA were positive, and 21 of 79 cows were positive on skin test (increase of skin thickness of 3 mm or greater). Mycobacteriologic examinations on fecal specimens revealed that nine of 84 cows were positive at 18 months. None of the ten project heifers displayed any clinical signs of JD while in the John's positive herd. Fecal culture and ELISA were negative for each of nine heifers at the time intervals tested. Positive skin test responses were observed in two heifers at six months post-exposure; no positive responses were observed on follow-up skin tests. MAP was identified in a fecal sample (PCR) from one of the ten heifers (Ruth) at 12 months following addition to the John's positive herd. Positive skin tests responses were observed at 18 and 24 months post-exposure. ELISA reactions were observed at 12, 18 and 24 months. MAP was isolated from fecal specimens collected at 18 and 24 months post-exposure. The animal was removed from the herd at 25 months post-exposure. MAP was isolated on culture of tissues (duodenum and ileum) collected at necropsy.

Significance

It is important that the project heifers entered the John's positive herd as negative, non-infected two-year old heifers and remained productive for three years while John's disease was a serious problem, resulting in the culling of 25% of the cows. This leads one to conclude that if cattle are purchased from a closed herd that has no history of JD and tested negative on skin test using MAP PPD, the likelihood of the cattle remaining in the herd and staying productive is very high. The intradermal skin test has been found to have a specificity of 97%, meaning that of 100 truly negative cattle tested, 97 would be test negative and only three would be false positives. This is useful when purchasing cattle. If a group of cattle is tested and all are negative, there is little risk that a positive animal will be purchased. However, if a positive test result is found, that animal has

likely been exposed to MAP. Therefore, the entire group of cattle should not be purchased. If a producer is interested in decreasing JD within a herd, testing home-raised replacements prior to breeding can be useful to limit the spread of MAP. Selecting breeding heifers that are skin-test negative and culling skin-test positive heifers may reduce the number of infected cattle entering the milking herd. In conclusion, to prevent monetary and production losses from early culling, it is important to identify a source herd(s) for purchasing cattle for herd additions that have not had a history of JD for the past five years. In addition, it is important to emphasize testing of cattle of unknown origin with a test that measures the cell-mediated immune response, such as the intradermal skin test or gamma-interferon assay, to identify cattle in the early stages of infection following exposure to MAP. This will provide assurance that herd additions can be introduced with confidence, and the losses due to John's disease will be of minimal importance.

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