The Significance of a Negative Map ELISA Test for Mycobacterium Avium Subspecies Paratuberculosis

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ABSTRACT

The significance of a negative USDA certified Map ELISA test was assessed in three separate study designs.

- Part I: The sera of seven out of nine necropsied cows with documented Johne's disease and whose feces was positive for mycobacterium DNA tested negative using the ParaChek® Map ELISA test system (Prionic, Switerland).
- Part II: Of the sera from 42 cows whose fecal specimens had been characterized by the Trek® Diagnostic System as having a significant amount of Map, 39 specimens tested negative in the ParaChek® test. When serological testing was extended for 14 additional months, 20 of the 21 animals remained sero-negative.
- Part III: Comparative serological analysis done on the same serum samples from a dairy herd demonstrated that 42% of the cows test certified as having been Map-free had anti-Map antibodies.

INTRODUCTION

A positive USDA certified Map ParaChek® ELISA test identifies an animal infected by Mycoberterium avium subspecies paratuberculosis with a high probability of developing disease. The threshold for a positive test had apparently been set at a level best correlated with the presence of disease (Johne's disease) or the potential for progression to it. Antigen selection had been predicated upon specificity for identifying Mycobacterium avium subspecfies paratuberculosis. What the ParaChek® test is not is a comprehensive assessment for the presence or absence of Map specific antibodies. Neither Prionic nor the United States Department of Agriculture (USDA) has stated in print the significance of a negative Map ELISA test.

The demonstration of Map ELISA seronegativity has come to be interpreted in an absolute sense, and not as a qualified statement. The ambiguity as to the significance of a negative Map ELISA test has fostered the erroneous concept that if an animal's Map ELISA test is negative, it is Map-free

Demonstration that viable Map can be introduced into the human food supply through milk and milk products has forced

an alteration in the Map paradigm.[1-5] This fact, coupled with the acceptance of Map as a human zoonotic pathogen and circumstantial evidence specifically linking Map to human gastrointestinal disease, has given urgency to ascertaining the significance of a negative Map ELISA test.[6-10]

The purpose of this paper is to present three divergent sets of observations that speak to the significance of a negative Map ELISA test.

PART I: TESTING OF SERA FROM COWS WITH NECROPSY CONFIRMED JOHNE'S DISEASE

MATERIALS AND METHODS

Study Population

The study population was derived from the bovine necropsy files of the University of Florida College of Veterinary Medicine. Cows that the met gross and microscopic diagnosis of Johne's disease and had both corresponding fecal and serum samples available were selected. Confirmation of a mycobacterium etiology was achieved using polymerase chain reaction (PCR) primers. After removal of PCR inhibitor and DNA extraction, the samples were probed with two pairs of primer: IS1-IS2 nested primers IS3 -IS4.

Both pairs of primers are based upon the IS1311 insertion sequence. Primers IS1-IS2 (CGA TTT ATC AGG CAC TCA TCG/CAA ATA GGC CTC CAJ CAC CA) recognize a 242 base pair sequence of Map IS1311 and primers IS3-IS4 (ATG AAC GGA GCG CAT CAC /CGA CCG AAG CTT GGG AAT) overlap and span a 104 base pair region within the insertion sequence. The IS1-IS2 primers recognize a 242 base pair sequence of Map IS1311 and IS3-IS4 primers overlap and span a 104 base pair region within the insertion sequence. Positive and negative controls were used in each confirmation test.

Serological Testing: Testing of Sera from Cows with Necropsy Confirmed Johne's Disease

After comparative testing of various Map ELISA tests, the State of Florida Veterinary Diagnostic Laboratory had previously selected the ParaChek® (Prionic, Switerland), Prionic's Map ELISA test as its test of choice. The study serum samples were forwarded directly to the State of Florida Veterinary Diagnostic Laboratory. After being analyzed, the sera were then redirected to Veterinary Diagnostic Laboratory of the University of Florida College of Veterinary Medicine. Both laboratories had been certified by USDA to perform Map ELISA testing.

RESULTS

Using the ParaChek® Map ELISA test, the State of Florida Veterinary Diagnostic Laboratory identified one serum as being positive and one being suspicious for Map Laboratory. Using a modification of the Richardson Map ELISA test, the Veterinary Diagnostic Laboratory of the University of Florida College of Veterinary Medicine identified six sera as having Map antibodies.

PART II: CORRELATION BETWEEN HEAVY FECAL SHEDDING COW AND MAP ELISA TEST RESULTS

Study Population

The fecal samples were obtained from two dairy herds that participated in USDA's Florida Johne's Disease Dairy Herd Demonstration Project.

The fecal samples collected onsite at the dairies were sent via Federal Express next day shipment in coolers with ice packs to the Animal Disease Diagnostic Laboratory, School of Veterinary Medicine at Purdue University, and to the Veterinary Diagnostic Laboratory of the Department of Infectious Diseases at the University of Florida College of Veterinary Medicine. The number of fecal samples analyzed in the study was determined by the number of samples concomitantly made available to the Diagnostic Laboratory of the Department of Infectious Diseases, University of Florida College of Veterinary Medicine on a related study.

Corresponding serum samples were shipped directly to the State of Florida Vet-

Table 1. *Serial ParaChek® Map ELISA serological results of 21 cows identified by the Trek® Diagnostic System as being heavy or moderate Map fecal shedders*

Cow ID Number	ParaChek® Result	
	12/2006	2/2008
Serial serological observations – heavy shedders		
550	-	-
906	-	-
2216	-	**culled**
2854	0.93	-
3079	-	-
3162	no data	**- (culled)**
3308	-	-
3335	-	-
3537	-	-
3697	-	-
Serial serological observations – moderate shedders		
994	-	**6.12**
2372	-	-
2693	-	-
2833	-	-
3057	no data	-
3066	-	-
3160	-	-
3332	-	-
3310	-	-
3592	-	-
3748	-	-

+ = positive test result; - = negative test result

erinary Diagnostic Laboratory.

Fecal Culture

The fecal samples were assessed for the presence or absence of Map by the Trek® Diagnostic System. The tests were conducted in accordance with the manufacturer's instructions and the laboratories standing protocols. The Animal Disease Diagnostic Laboratory, School of Veterinary Medicine at Purdue University is annually certified by USDA for the performance in identifying Map in fecal samples.

Serum Map ELISA Test

The serum Map ELISA testing was done at the State of Florida Veterinary Diagnostic Laboratory at Live Oak, Florida, in accordance with that laboratory's established protocols. The State of Florida Veterinary Diagnostic Laboratory' Map ELISA test is annually certified by The United States Department of Agriculture.

Data Computation

The test results from the veterinary diagnostic laboratories were sent as developed, directly to the USDA Office in Gainesville, Florida. There the data was compiled and forwarded to Infectious Diseases Incorporated for analysis.

RESULTS

Of the 327 fecal specimens analyzed, 22 animals were identified as heavy shedders and 21 animals as moderate shedders based upon assessment of their fecal culture sample.

The State of Florida Veterinary Laboratory reported results from 43 available sera. In the heavy shedder group of cows, two were ParaChek-positive and another had a suspicious titer. Sera from all 21 culture identified "moderate shedders" tested negative in the ParaChek® Map ELISA tests in December of 2006. In total, 39 of 42 animals with either heavy or moderate Map fecal shedding had tested negative in the ParaChek® Map test.

In February of 2008, 18 of the original 43 cows were retested. One cow now tested positive. The 16 cows who had tested negative 14 months previously were still

ParaChek® negative. The cow whose sera had had a high suspicious ParaChek® level in 2006 tested ParaChek® negative in 2008

PART III: RE-ANALYSIS OF A ParaChek® MAP CHARACTERIZED HERDS

Study Population

Dairies #1and #2 were situated within one mile of each other. Based on serial evaluations and aggressive culling of sero-positive animals, Dairy #1 was considered as being Johne's disease-free. Dairy #2 was known to have had a number of Johne's diseased animals.

Sera from two dairies located in South Florida were shipped directly to the State of Florida Diagnostic Veterinary Laboratory for testing. These sera were then re-routed to the Veterinary Diagnostic Laboratory of the University of Florida College of Veterinary Medicine where they were retested using an antigen enhanced Richardson Map ELISA test.

Results

Of the 23 sera from Dairy #1, none tested positive in the Map ParaChek® ELISA test. Of 43 sera from Dairy #2, two tested positive and 10 tested suspicious.

When the sera were re-tested at UF-CVM's Veterinary Diagnostic Laboratory using a modification of the Richardson Map ELISA test,11) the sera from 12 of the 23 cows from Dairy #1 were identified as having anti-Map antibodies (60%). From Dairy #2, 18 sera demonstrated the presence of Map antibodies (42%).

DISCUSSION

ParaChek®'s lack of significant correspondence with either necropsy confirmed case of Johne's disease or animals identified by culture as being moderate or heavy Map fecal shedders can only be partially accounted for by a high threshold for test positivity. Darcel and Logen-Handsame had long postulated that the failure of the certified Map ELISA tests to identify all clinically ill Map animals had been due to a lack of representation of the entire range of immunodominant test antigens.[12] That the Richardson Map ELISA tests identified but six of the nine cases of Johne's disease that had been confirmed by IS1311 DNA fecal analysis, is consistent with the postulate that not all mycobacteria capable of inducing Johne's disease are detectable by tests predicated on the IS900 insertion sequence. Frothingham and then Turenne et al. have contended that the current array of veterinary pathogenic mycobacteria evolved from Mycobacterium avium (Maa).[13, 14] Based on restriction fragment length polymorphism analysis, Coffin using IS900 polymerase chain reaction in et al have demonstrated that some Map isolates are more Maa-like than Map.[15] Using IS900 polymerase chain reaction, other groups have identified mycobacteria distinct from Map.[16-18]

In this study, the Parachek® initially identified only 2 out of 42 Map shedding animals. When serological analysis was extended another 14 months, only one additional infected animal subsequently tested positive. The inability of a USDA certified Map ELISA test to identify infected animals is well documented in the literature by Cocito et al showed that Map was more likely to be identified in feces when the commercial Map ELISA tests were into their projected positive zone.[19] Collins et al demonstrated that the two prevailing Map ELISA tests used in the United States identified less than 29% of the fecal culture positive cows.[20] Sweeney and co-workers suggested that the commercial Map ELISA tests might have a sensitivity rate lower than 13.5%.[21] Sockett et al reported the sensitivity of commercial Map ELISA tests to be 8.9% to 32.1% for low shedders, but improved to 47.1% to 62.9% for more extensive fecal shedders.[22]

Quality of merchandise is primarily addressed through the animal's health certificate. The Code of Federal Regulations (CFR) was intended to restrict the interstate movement of Map infected animals except to recognized slaughter establishments. Approving an elevated threshold for a positive Map serological test, USDA prevented pertinent CFR regulations from effectively

addressing the quality of merchandise issue. By not stipulating on an animal's certificate of health its Map status in a manner comparable to M. bovis, animals with subclinical infection have been transported across interstate and national boundaries. The net result is not only the introduction of infected animals into uninfected herds, but an overall increased prevalence of Map infection in the nation's herds. Between 2002 and 2007, the number of U.S. dairy herds containing infected animals has more than doubled (23). Fifty-four percent of infected/diseased animals detected by the Japanese Animal Quarantine Service have come from the United States.[24]

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