Research Summaries 4

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Analysis of the Association between ELISA and Nested PCR on Blood and Milk for *Mycobacterium avium subsp. paratuberculosis* Detection in Holstein Cows

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Introduction

Paratuberculosis is a chronic, infectious disease of ruminants characterized by progressive weight loss and profuse diarrhea. It is caused by the acid-fast bacillus *Mycobacterium avium* subspecies *paratuberculosis* (MAP), has a worldwide distribution and is categorized by the OIE as a List B disease that has serious economic impact or is a public health concern. Disease diagnosis is hampered by a lack of sensitive tests. Available methods fail to identify all infected animals, and many produce substantial numbers of false positives and false negatives. This is of particular importance, as they relate to detection of the organism in subclinically infected animals. Several commercial, enzyme-linked immunosorbent assay (ELISA) tests are available, but it is generally accepted that their sensitivity in detecting infected animals is only about 50%. In fact, because of its low sensitivity, the ELISA test is rarely positive in animals under two years of age. Polymerase chain reaction (PCR) tests based on the insertion element IS900 have been the most widely used for MAP identification. However, detection of the etiologic agent is limited by frequency and number of organisms present in the fluid or tissue. A combination of serologic tests, such as ELISA, and agent detection through nested PCR could be a useful strategy to improve the sensitivity of paratuberculosis diagnosis, especially when detectable target levels for each test follow different temporal patterns.

The objective of this study was to compare the performance of ELISA testing of sera, and nested PCR in milk and blood, for diagnosis of paratuberculosis in Holstein cows.

Materials and Methods

Blood and milk samples were collected from 256 Holstein cows in three dairies near Gainesville, Florida. The ELISA originally developed by WD Richards (Allied Laboratories, Inc, Ames, IA) was performed with crude, soluble protoplasmic antigen (Allied Monitor Missouri). Test sera were pre-absorbed over night with *Mycobacterium phlei.* ELISA results were calculated from wavelength readings (OD at 405 nm) of triplicates and recorded as negative (<1.5 OD), suspicious (1.5 to 1.9 OD) and positive (>2.0 OD). For PCR analysis, milk and blood samples were probed with primers P90, P91 for IS900, which specifically recognizes a 413 bp sequence of MAP followed by a second set of primers J1, J2 overlapping and spanning a 333 base pair region within the insertion sequence.

Overall and herd-group ELISA and blood/milk PCR results were analyzed to establish association between both tests. Maximum possible agreement beyond chance level and kappa coefficient were used as a measure of agreement between the two tests. Fisher's Exact Test was used to determine whether there was a non-random association between results of both tests. Complementary sensitivity (CS) was estimated as the ratio of positive results in only one method to the total number of positive results in the other method, accounting for the additional detection efficacy of one method over the other.

Result

A total of 17.9% of animals tested were positive for ELISA or/and nested PCR (parallel testing). Some 11.7%

of the population was positive for the ELISA test, and 7.03% was positive for either blood- or milk-nested PCR. Apparent prevalence when considering a cow positive, if test-positive in either ELISA or nested PCR tests, for Dairy A, B and C was 23.2%, 15% and 18%, respectively. Maximum possible agreement beyond chance level for all cows, Dairy A, B and C was 17%, 20%, 15% and 16%, respectively. Kappa value (95% CI) in the whole population, Dairy A, B and C was -0.005 (± 0.115), -0.1304 (± 0.071), 0.170 (± 0.277) and -0.087 (± 0.050) respectively, indicating poor agreement of test results in all cases. Negative kappa values indicate that the two sets of results agreed less than would be expected merely by chance. Fisher's Exact Test used to test the alternative hypothesis of positive association between both test outcomes resulted in right-sided p-values of 0.650, 1.00, 0.129 and 1.00 for all animals, Dairy A, Dairy B and Dairy C, respectively. This indicates that in all the cases, there is no evidence to reject the null hypothesis (i.e., there is not a significant association between results of both tests). Complementary sensitivity for ELISA (% of extra cases detected by this test compared to use of PCR alone) was in the range of 116% to 200% and between 36% and 85% for PCR, showing an improvement in the percentage of infected cows detected when both tests were combined. The data presented indicates improvement in sensitivity for detection of MAP when blood ELISA and blood and milk PCR are combined.

Significance

The compared tests detect different forms and stages of MAP infection because their respective targets (bacteria and antibodies) may not have parallel dynamics. They may identify different populations of infected animals which could be the explanation for low kappa values reported in the present study.

The concept of complementary sensitivity (CS) applied in this study appears to be a useful tool when a "gold standard" is not available in practical terms. CS provides a measure of the efficiency of combining two methods with high specificity to increase sensitivity in MAP detection. In this case, CS for ELISA and PCR indicated in both cases an improvement in percentage of infected cows detected when both tests were combined.

It is concluded that the sensitivity for the detection of MAP is improved when blood ELISA and blood and milk PCR are combined. Further research on the dynamics of MAP in blood and milk could increase the future value of the test combination proposed in this study.

Environmental Distribution of *Mycobacterium avium paratuberculosis* (MAP) on Michigan Dairy Farms

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Introduction

Johne's disease (JD) is an important infectious disease of cattle caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). It is estimated that over 50% of US dairy herds are infected with MAP. Environmental contamination with MAP is considered the major reservoir of infection for susceptible cattle. While MAP does not multiply in the environment, it can persist for months in manure, lagoons, manure packs—areas that are found in abundance on most dairy farms—even in below-freezing temperatures. Very little information is published on distribution of MAP on dairy farms. The objectives of this study were to: 1) perform serial environmental culturing on six Michigan dairy herds, enrolled in a JD control program over several years; 2) characterize the distribution of MAP contamination on dairy farms; and 3) determine if and how that distribution changes as herd fecal culture prevalence changes.