New Rapid Diagnostic Test for Johne's Disease in Cattle

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Summary

A new rapid test for diagnosis of Johne's disease in cattle is described. This unique format Tip-Test™ utilizes the same principles as the Enzyme-linked Immunosorbent Assay (ELISA) test with similar sensitivity and specificity, but can be done in the practitioner's office with the results available in 25 minutes. Two formats of the test are available: a single Tip-Test™ using a tuberculin syringe with disposable supplies provided with the kit, and a 12-sample multi-tip pipetter. The test is designed to detect animals in the later stages of infection and to provide quick laboratory confirmation of clinical cases of Johne's disease.

Introduction

Chronic diarrhea and weight loss with a normal appetite remain the classic clinical signs of Johne's Disease, or paratuberculosis, in adult cattle. However, several other diseases including renal amyloidosis, abdominal neoplasia, caudal venal caval thrombosis and heart failure all may cause chronic diarrhea in adult cattle to clinically resemble Johne's Disease. Thus, laboratory evaluation may be necessary to make a more definitive diagnosis of clinical Johne's Disease. Since most cattle infected with *Mycobacterium paratuberculosis* do not show clinical signs or otherwise manifest evidence of infection,[10] diagnostic tests become mandatory to detect subclinical disease. However, earlier stages of infection have a reduced bacterial load with less antibody produced, making laboratory diagnosis more challenging for both the practitioner and the diagnostician.

One of the earliest reported serological diagnostic tests for Johne's disease was the complement fixation (CF) test, which is still required for exportation and importation of cattle among different countries.[2] With a relatively low sensitivity (10.9% to 38.4%) and specificity compared to newer serologic tests, the CF test is rarely used for clinical diagnosis.[6,7] The agar gel immunodiffusion test (AGID) was developed later as a relatively quick test to detect animals showing clinical signs.[4,5] The specificity and sensitivity of the AGID test for animals with clinical signs compatible with Johne's disease are very high (99% and 96.9%.[4] The ELISA tests were developed as more sensitive tests to detect infected animals earlier in the disease process.[11] Other antigen preparations have been evaluated in a variety of test formats to detect antibodies or sensitized cells produced as part of the host response to infection with *M. paratuberculosis.*[1]

At this time, both the AGID and the ELISA tests have high specificity (>99%) and sensitivity (>85%) to confirm infection in animals with clinical signs, and 33% for subclinically infected cattle.[5] However, no existing diagnostic test has adequate sensitivity and specificity to detect all infected cattle with subclinical infection.[10] The new rapid diagnostic test described here not only detects cattle with clinical signs of Johne's Disease, but also detects some animals in the later subclinical phases of the disease, making it superior to the AGID test and similar in performance to the commercial ELISA test. Since the test does not require sophisticated laboratory instrumentation, such as an ELISA reader and washer, it can easily be done in the veterinarian's office.

Agar Gel Immunodiffusion Test

The AGID, or Rapid Johne's Test (RJT™) (available commercially from ImmuCell Portland, ME), has significant diagnostic value if the tested animal has weight loss and/or diarrhea, with a sensitivity of 96.9% and a specificity of 94%.[4] A positive test correlates well with clinical signs when the weight loss and/or diarrhea are due to Johne's disease. Lack of sensitivity, or failure to detect animals that are fecal-culture positive but not showing clinical signs, is the major drawback with the AGID test. The reported sensitivity of the AGID test to detect paratuberculosis-infected cattle ranges from 18.9%[6] in subclinicalyl infected cattle to 96.9% in cattle with clinical signs.[4] The test should be reserved for the individual cow with clinical signs compatible with Johne's disease. If the AGID test is positive (laboratory time 48-72 hrs), then the cow has a greater than 98% probability of being infected with the Johne's organism.

Materials and Methods

TIP-TEST™

A unique format, individual sample, practice-lab-suitable test has been developed for Johne's Disease in

cattle. The test, based on the ELISA principle, is more sensitive than the AGID test, with a sensitivity resembling the commercial ELISA test (45%).[8] The test can be completed in 20 minutes after a blood sample is obtained. Whole blood with an anticoagulant such as EDTA is added to a well within a sealed, disposable tray. Alternatively, either plasma or serum are both suitable samples for the Tip-test™. By passing the sample through a series of solutions contained within the tray provided with the test kit, then through a disposable pipette tip containing polyester test elements attached to a tuberculin syringe, the results are visualized by assessing color development of the elements contained within the tip. The test is simple to perform, quick, and can be done in the practitioner's office. This test is further supplemented by a multichannel version of the Tip-test that also can be done in the practitioner's office using serum or plasma and a multichannel pipette. The multichannel test allows up to 10 samples at a time, with 25 minutes to final results. The Tip-test format has been applied to the rapid diagnosis of *Escherichia coli* 0157 in raw meat.[3]

Tip-Test design

The tray format consists of a filter tip, wash buffer, prediluted *M. phlei* in the sample diluent to absorb cross-reacting antibody, conjugate and substrate with a syringe-to-tip adapter. The detector tip is an immunoassay formatted in a micropipette tip. The pipette tip is filled with a large number of cellulose fibers packed together in 3 distinct segments. The top segment is the positive test control indicator, the middle segment is the negative control indicator and the bottom segment the test sample indicator.[9] The fibrous cellulose capture element is designed to optimize surface area to sample volume ratio, thus enhancing test sensitivity. Antigens, relatively specific for *M. paratuberculosis*, are covalently bound to the test filaments. The target antibody, if present in the sample, will bind to the antigens attached to test filaments in the bottom segment resulting in a purple color which is compared to the top, or positive control, segment and the middle segment, the negative control segment. Sample testing can be done with a single tip or up to 12 tips simultaneously using a 12-channel pipetter.

The fibrous capture element in the porous micropipette tip presents a much larger surface area (100 to 1000-fold greater) to the sample, compared to receptors coated onto a solid support, such as microtiter plates.

Preliminary Results and Discussion

In a group of 42 adult dairy cattle with clinical signs of Johne's disease, each of which was found culture-positive for *M. paratuberculosis,* 40 were positive with the Tip-test for a sensitivity of 95.2% for cattle with clinical signs of Johne's disease. Only 19 of those 42 sera were AGID-positive. In a group of 28 adult dairy cattle without evidence of clinical signs of Johne's disease, but that were fecal-culture positive and tissue-culture positive for *M. paratuberculosis,* 16 (57.1%) were positive on the Tip-test. Colony counts for the fecal samples ranged from 2,1,0,0 colonies per tube to "too numerous to count" for each of the 4 tubes of these subclinically infected cattle. To assess specificity, the Tip-test was run on 30 sera from animals that always were fecal-culture negative, and all the tissues examined at slaughter were culture-negative for *M. paratuberculosis*. Four sera were classified positive, for a specificity of 86.7% or a false-positive rate of 13.3%. Larger numbers of serum samples will be run in the future to more critically assess both sensitivity and specificity.

This preliminary evaluation of the Tip-test would suggest the test will detect nearly all animals that have clinical signs of Johne's Disease and most animals shedding higher numbers of organisms in their fecal samples. This is similar sensitivity to the commercially available ELISA test.[8] Since the test employs most of the elements of the ELISA test, very few false-positive tests are reported.

Conclusions

A major advantage of the new Tip-test is the short time necessary to perform the test, less than 30 minutes. It is possible to do the test at the office or small laboratory by an employee of the practice. Thus, results would be available the same day the sample is obtained. As each Tip-test includes both positive and negative controls, additional laboratory control samples are unnecessary to run simultaneously. The authors believe this new diagnostic test for Johne's Disease offers the advantages of being quick and easy to run, with sensitivity and specificity data similar to the commercially available ELISA test, but available within minutes of sample acquisition. The test is designed specifically to detect animals with clinical signs of Johne's Disease and animals in the later stages of infection (high shedders); not herd-replacement heifers, for example, that may be infected, but with little circulating antibody and unlikely to be detectable by fecal culture.

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References

1. Collins MT. Diagnosis of Paratuberculosis. The Veterinary Clinics of North America, Food Animal Practice, pp 357-371, July, 1996.
2. De Lisle GW, Sequin P, Samagh BS, Corner AH, Duncan JR: Bovine paratuberculosis I. A herd study using complement fixation and intradermal tests. Can J Comp Med 44:177-182, 1980.
3. Firstenberg-Eden R, Sullivan NM. A new rapid method for the detection of E coli 0157 in raw meat and processed meat. Proc 82 Annual IAMFES Mtg, July, 1995.
4. Sherman DM, Markham RJF, Bates F. Agar gel immunodiffusion test for diagnosis of clinical paratuberculosis in cattle. J Am Vet Med Assoc 185:179-182, 1984.
5. Sherman DM, Bray B, Gay JM, Bates F. Evaluation of the agar gel immunodiffusion test for diagnosis of subclinical paratuberculosis in cattle. Am J Vet Res 50:525-530, 1989.
6. Sherman DM, Gay JM, Bouley DS, Nelson GH: Comparison of the complement-fixation and agar gel immunodiffusion tests for diagnosis of subclinical paratuberculosis in cattle. Am J Vet Res 51:461-465, 1990.
7. Sockett DC, Conrad TA, Thomas CB, Collins MT. Evaluation of four serological tests for bovine paratuberculosis. J Clinical Micro 30:1134-1139, 1992.
8. Sweeney RW, Whitlock RH, Buckley CL, et al. Evaluation of a commercial enzyme-linked immunosorbent assay for the diagnosis of paratuberculosis in dairy cattle. J Vet Diagn Invest 7:488-493, 1995.
9. Wainwright N, Boyd SH. Aligned fiber diagnostic chromatography with positive and negative controls. US Patent office # 5,876,918, March 2, 1999.
10. Whitlock RH, Buergelt C. Preclinical and clinical Manifestations of paratuberculosis (including pathology). The Veterinary Clinics of North America, Food Animal Practice, pp 345-356, July, 1996.
11. Yokomizo Y, Merkal RS, Lyle PAS. Enzyme-linked immunosorbent assay for detection of bovine immunoglobulin G_1 antibody to a protoplasmic antigen of *Mycobacterium paratuberculosis* Am J Vet Res 44:2205-2207, 1983.