Evaluation of two rapid assays for detecting *Cryptosporidium parvum* in calf feces

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Objective—To evaluate 2 rapid, patient-side assays for detection of *Cryptosporidium parvum* in feces from neonatal calves with diarrhea.

Design—Diagnostic test evaluation.

Sample Population—Fecal samples from 96 neonatal (1 to 30 days old) calves with diarrhea.

Procedure—Results of the rapid assays were compared with results of microscopic examination of fecal smears that had been stained with diamant fuchsin stain.

Results—One of the rapid assays correctly identified 56 of 62 (90%) fecal samples positive for *C parvum* oocysts and 33 of 34 (97%) fecal samples negative for oocysts. The other assay correctly identified 53 of 62 (85%) fecal samples positive for oocysts and 33 of 34 (97%) fecal samples negative for oocysts.

Conclusions and Clinical Relevance—Results suggest that these 2 rapid assays are accurate when used to detect *C parvum* in fecal samples from neonatal calves with diarrhea. (*J Am Vet Med Assoc* 2004;225:1090–1092)

RUMINANTS

Cryptosporidium parvum is a protozoan parasite that has been identified as a cause of diarrhea in neonatal calves.[1] The infective form of *C parvum* is a 4- to 6-µm-diameter, colorless, transparent oocyst shed in large numbers in the feces of infected animals.[2] Environmental contamination facilitates a fecal-oral mode of transmission. Generally, the highest prevalence of infection is seen in calves between 1 and 3 weeks old.[3] Infection results in profuse, watery diarrhea lasting up to 2 weeks.[2] Cryptosporidiosis is not responsive to most antimicrobials, and while experimental treatments appear promising,[4] treatment is currently limited to supportive therapy. Oocysts are extremely resistant to many disinfectants[5] and can survive in the environment after UV light irradiation.[6] Intense rearing environments such as those used on dairy farms are ideal for the spread of *C parvum*.

Conventional diagnostic methods for detection of *C parvum* include direct microscopic examination of fecal smears after staining with acid-fast or diamant fuchsin stain, fecal flotation, antigen-detection ELISAs, direct immunofluorescence assays, and **polymerase chain reaction (PCR)** assays.[7] All of these methods require laboratory equipment and therefore result in a delay between the time of examination of affected animals and the diagnosis of cryptosporidiosis. A rapid, accurate diagnostic test that could be used at the time of animal examination would facilitate timely and appropriate intervention strategies. If cryptosporidiosis is diagnosed immediately, control measures to prevent the spread of infection to other animals and to prevent zoonotic transmission could be instituted immediately.

Two rapid, patient-side assays[a,b] have been developed to detect *C parvum* in human feces.[8-10] Both are based on detection of *Cryptosporidium*-specific antigen, can be completed in 15 to 20 minutes, require minimal supplies, and have been shown to be accurate in the diagnosis of cryptosporidiosis in people. The purpose of the study reported here was to determine whether these 2 assays can be used to detect *C parvum* in the feces of neonatal calves with diarrhea.

Materials and Methods

Fecal specimens—Fecal samples from 96 calves between 1 and 30 days old that had diarrhea were used in the study. Sixty samples were collected from calves on 7 farms within 75 miles of East Lansing, Mich. The remaining 36 samples had been submitted to the Michigan State University Diagnostic Center for Population and Animal Health. Twenty-one samples had been stored at –20°C prior to testing. However, *Cryptosporidium* oocysts have been shown to remain viable and permeable to vital stains after freezing.[11,12] The remaining 75 samples were refrigerated at 4°C until tested.

Assay procedure—All fecal samples were tested with both rapid assays. For the first assay (assay A[a]), fecal samples were diluted 1:4 with distilled water, and 2 drops of sample treatment buffer were added to each diluted specimen. A micropipette provided in the kit was used to transfer approximately 60 µL of the diluted specimen to the specimen dilution tube. Two drops of biotinylated rabbit anti-*Giardia* antibody in diluent buffer and 2 drops of colloidal dye-labeled monoclonal antibodies to *Giardia* spp and *Cryptosporidium* spp in diluent buffer were added to the specimen dilution tube, and contents were mixed by manually swirling the tube. The contents of the specimen dilution tube were then transferred to the sample well of the test device. After 10 minutes of incubation at room temperature, results were read. Results were considered positive if black lines were seen at the control and *Cryptosporidium* positions and were considered negative if a black line was seen only at the control position. Results were considered invalid if a black line was not seen at the control position.[13]

For the other assay (assay B[b]), a cotton-tipped applicator was immersed in each fecal sample until fully saturated. The

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applicator was then transferred to a specimen dilution vial containing 2 mL of specimen dilution buffer. The applicator was swirled in and pressed against the sides of the specimen dilution vial to release as much of the fecal material as possible before being discarded. The specimen dilution vial was capped with a filter tip provided with the assay kit. Four drops of the diluted specimen were placed in the center of the reaction device membrane. After 2 minutes, 10 drops of wash buffer were added to the reaction device, followed by 4 drops of rabbit anti-*Cryptosporidium* antigen labeled with biotin in rabbit serum. After 2 minutes, 10 drops of wash buffer were added to the reaction device, followed by 4 drops of horseradish peroxidase-streptavidin. After 2 minutes, 10 drops of wash buffer were added, followed by 4 drops of chromogen color substrate. After 3 minutes, 4 drops of buffered stop solution were added, and test results were read within 10 minutes. Results were considered positive if a blue control dot and a second blue dot were seen and were considered negative if only a blue control dot was seen. Results were considered invalid if a blue control dot was not seen.[14]

Microscopic examination of fecal smears—Microscopic examination of fecal smears stained with diamant fuchsin for *C parvum* oocysts[15] was used as the gold standard method for determining whether fecal samples did or did not contain *C parvum*. Briefly, approximately 2 µL of each fecal sample was mixed with 2 µL of diamant fuchsin stain. The mixture was smeared on a clear glass slide and examined microscopically at 1,000× magnification. Samples were considered positive for *C parvum* if refractile, 4- to 6-µm-diameter, oocyst-like structures were seen and negative for *C parvum* if such structures were not seen.

Fecal samples for which results of the 3 tests (ie, the 2 rapid assays and microscopic examination of a fecal smear) were discordant were tested by use of a PCR assay. Briefly, DNA extracted from the fecal sample was amplified with primers that define a 194-base pair fragment of *C parvum* DNA, as described.[16,17]

Data analysis—Sensitivity, specificity, and κ values for the 2 rapid assays were calculated with standard software.[c] Results of microscopic examination of fecal smears were used as the gold standard. κ Values represent the degree of agreement between results of 2 independent tests.

Results

Microscopic examination of fecal smears stained with diamant fuchsin stain indicated that 62 of the 96 fecal samples contained *C parvum* oocysts and 34 did not. Assay A correctly identified 56 of the 62 fecal samples positive for *C parvum* oocysts (sensitivity, 90%; 95% confidence interval, 83% to 98%) and 33 of the 34 fecal samples negative for oocysts (specificity, 97%; 95% confidence interval, 91% to 100%; $\kappa = 0.846$). Assay B correctly identified 53 of the 62 fecal samples positive for oocysts (sensitivity, 85%; 95% confidence interval, 77% to 94%) and 32 of the 34 samples negative for oocysts (specificity, 94%; 95% confidence interval, 86% to 100%; $\kappa = 0.761$).

For 83 of the 96 fecal samples, results of all 3 tests (ie, the 2 rapid assays and microscopic examination of a fecal smear) were in agreement. For the remaining 13 samples, results of the 3 tests were discordant and these samples were tested for *C parvum* DNA with a PCR assay. For 2 of the 13 samples, results of microscopic examination were positive but results of both rapid assays and the PCR assay were negative. For 9 samples, results of microscopic examination and the PCR assay were positive but results of 1 or both rapid assays were negative. For 1 sample, results of the PCR assay and 1 of the rapid assays were positive but results of the other assay and microscopic examination were negative. Finally, for the remaining sample, results of microscopic examination and the PCR assay were negative but results of both rapid assays were positive.

Discussion

Results of the present study suggest that both of these rapid assays[a,b] were sensitive and specific when used to detect *C parvum* in fecal samples from neonatal calves with diarrhea. Sensitivities of the 2 assays in the present study were 86% and 90%. In contrast, reported sensitivities for these 2 assays when used to detect *C parvum* in human fecal samples range from 99% to 100%.[8-10] It is possible that factors present in bovine feces that are not present in human feces may have reduced the effectiveness of reagents used in both assays, resulting in lower sensitivity. If so, then optimizing assay reagents and procedures for use with bovine feces might improve the performance of both assays. Alternatively, it is possible that sensitivity of the rapid assays depends on the concentration of *C parvum* oocysts in fecal samples and that for various anatomic, physiologic, and pathophysiologic reasons, humans shed higher concentrations of *C parvum* oocysts in their feces than do cattle. Finally, the lower sensitivities observed in the present study may be attributable in part to a lack of specificity of the gold standard test. For 2 samples in the present study, for instance, results of microscopic examination of fecal smears were positive but results of both rapid assays and the PCR assay were negative. Reexamination of the fecal smears for these 2 samples suggested that oocysts considered to be *C parvum* were morphologically more similar to *Cryptosporidium muris*.[18] Although of less clinical importance, *C muris* oocysts can be found in bovine fecal samples[19] and can be mistaken for *C parvum* oocysts during examination of stained fecal smears. A disadvantage of the 2 rapid assays used in the present study is that they are unable to detect *Cryptosporidium* organisms other than *C parvum*.

For both rapid assays in the present study, specificity was > 94%. There were only 2 samples for which results of microscopic examination were negative, but results of 1 or both rapid assays were positive. For one of these samples, results of the PCR assay were negative, and for the other, results of the PCR assay were positive. False-positive results for these 2 samples could have been a result of cross-reacting antigens[8] or other factors present in the samples.[10]

Subjectively, assay A was easier to use than assay B in the present study in that it required fewer steps. Also, results were available in approximately 15 minutes, rather than the approximately 20 minutes required to obtained results with assay B. In addition, assay A has the added advantage of simultaneously being able to detect *Giardia lamblia*, another potential cause of diarrhea in calves.[20,21] However, whether assay A was an accurate method of detecting *Giardia lamblia* in calf feces was not evaluated in the present study.

The availability of a rapid diagnostic assay for *C parvum* should help facilitate diagnosis, allowing for

timely implementation of treatment and control procedures, such as separation of affected animals and disinfection of contaminated facilities. Results of the present study suggest that both rapid assays could be used to detect *C parvum* in feces from neonatal calves with diarrhea.

[a]ColorPAC *Giardia/Cryptosporidium* rapid assay, Becton, Dickinson & Co, Sparks, Md.
[b]ProSpecT *Cryptosporidium* rapid assay, Alexon-Trend, Ramsey, Minn.
[c]WinEpiscope version 2.0, Department of Animal Sciences, Wageningen Agricultural University, The Netherlands.

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