Detection of *Cryptosporidium parvum* Using the Kinyoun Acid-Fast Stain

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Recent evidence from our laboratory indicates that the Kinyoun acid-fast stain is a reliable, low cost, and simple method performed in the practice setting to detect *Cryptosporidium parvum* in equine feces. This paper explains how this test is performed. Author’s address: Dept. of Large Animal Medicine and Surgery, Texas A&M University, College Station, TX 77843-4475. © 1997 AAEP.

1. Introduction
As infection with *Cryptosporidium parvum* becomes increasingly recognized as a cause of outbreaks of foal diarrhea, a rapid method of oocyst detection in equine feces that can be done easily in the practice setting is desirable. Protozoal identification techniques that have been described include the evaluation of stained fecal smears or preparations of floated feces, using both light and phase-contrast microscopy. Immunologic methods include immunofluorescent assays (IFA’s) and enzyme-linked immunosorbent assay techniques. Commercial veterinary diagnostic laboratories most commonly use acid-fast staining or IFA methods.

Poor sensitivity and specificity have been associated with the acid-fast staining of fecal specimens in human studies, and these data have been extrapolated to equine reports. Because IFA procedures have been reported to be ten times more sensitive than acid-fast techniques, many veterinarians routinely submit fecal samples for evaluation to commercial laboratories for IFA testing.

The submission of fecal samples to commercial laboratories can be labor intensive and result in either delayed results or even a failure to submit samples for evaluation. A reliable, low cost, and simple method that can be done in the practice setting is necessary to enable the practitioner to arrive quickly at a diagnosis and prevent potential complications. Recent evidence from our laboratory indicates that the Kinyoun Acid-Fast Stain will perform as well or better than an IFA in this setting.

2. Materials and Methods
A sample of diarrheic feces collected from the affected foal can be directly smeared on a slide and air dried for staining. If the fecal smear is not prepared within 24 h of collection, it should be diluted in a 10% buffered formalin solution in a parvum 1:3 ratio (stool:formalin) prior to smearing it on a slide. Fecal smears should be thinly smeared on the slide for easiest evaluation, and thicker samples can be diluted slightly in physiological saline. The slide does not have to be heat fixed prior to staining. The manufacturer’s instructions outline the staining procedure well and include a three-step process of staining, decolorizing, and counterstaining. The entire procedure does not require more than 10 min per slide.

An evaluation of the stained fecal smear should be
done at 400× and 1000× magnification. Cryptosporidium parvum oocysts stain as pale to bright pink spheres against a dark green or purple background. The oocysts are 4–6 µm in diameter. They are roughly the size of a red blood cell. To be clinically significant, oocysts should be readily identifiable on the slide and many high dry fields should have more than one oocyst at a time.

3. Results
In a recent study using fecal samples inoculated with known concentrations of C. parvum oocysts, the acid-fast stain performed at least as well as the IFA conducted at a commercial laboratory. In this study, feces were collected from three clinically healthy adult horses and inoculated with preserved C. parvum oocysts in varying concentrations. An evaluation of the samples was done by using the Kinyoun Acid-Fast Stain, and the IFA procedure was performed at a commercial veterinary diagnostic laboratory. In each group of inoculated samples there were three negative control samples included. The acid-fast stain detected oocysts in all the preparations that were immunofluorescent positive. Consequently, the acid-fast stain performed as well as the IFA in detecting clinically significant numbers of oocysts in the fecal material.

On at least one occasion, foals examined in the clinic and found to have C. parvum oocysts in acid-fast stained preparations and on postmortem evaluation were found negative on immunofluorescent evaluation conducted at the commercial veterinary laboratory. This suggests that the acid-fast stain may be more sensitive than the immunofluorescent procedure.

4. Discussion
The acid-fast stain has not been universally recommended for the detection of C. parvum oocysts in fecal samples largely because of previous data that suggested a low specificity and sensitivity. In our study, however, the Kinyoun Acid-Fast Stain performed at least as well as the immunofluorescent procedure done at a commercial veterinary laboratory. In some clinical cases, the acid-fast staining method was found to be more sensitive than the IFA.

The acid-fast stain does not require special preparation of the fecal smear (i.e., flotation or heat fixing) and can easily and cheaply be conducted in the clinical setting. Because recent evidence suggests that cryptosporidiosis has been previously underrecognized in foals, it is important for equine practitioners to consider this disease in the young foal (younger than 4 weeks) with diarrhea. Given the potential for C. parvum to cause outbreaks of diarrhea in foal populations and the potential for zoonotic transmission, it is important to diagnose this disease efficiently so that proper control measures can be taken quickly. Submitting samples to a diagnostic laboratory can be potentially more costly, time consuming, and less sensitive than conducting the acid-fast stain in the practice setting.

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