A Comparison of Dichromate Solution Floatation and Fecal Smears for Diagnosis of Cryptosporidiosis in Calves

P.J. WILLSON AND S.D. ACRES

Veterinary Infectious Disease Organization, 124 Veterinary Road, Saskatoon, Saskatchewan, Canada S7N 0W0

SUMMARY

Fecal samples from eleven calves experimentally infected with cryptosporidia were examined by two methods to evaluate their sensitivity and ease of use as diagnostic techniques. Comparison of a dichromate solution floatation and fecal smear techniques indicated that the former method was more sensitive. Oocysts were detected in feces earlier and for a longer period following exposure, and were easier to visualize using the dichromate solution floatation procedure. In addition, the dichromate solution floatation technique eliminated *Candida albicans* which can sometimes be confused with cryptosporidial oocysts in fecal smears. Another advantage was that oocysts could readily be detected in fecal samples stored as long as 120 days in dichromate solution. Using the dichromate solution floatation technique, it was shown that shedding of cryptosporidial oocysts in feces occurred from two to 20 days after oral challenge but was not always accompanied by diarrhea. Infection with cryptosporidia was confirmed in two calves at necropsy. Calves which were not sacrificed for portmortem examination recovered without treatment. The dichromate solution floatation technique is simple, rapid, inexpensive and should facilitate detection of cryptosporidia by diagnostic laboratories and some veterinary practitioners.

RÉSUMÉ

Comparaison entre une solution de flottation à base de dichromate et des impressions de matières fécales, pour le diagnostic de la cryptosporidiose du veau

Cette expérience consistait à examiner des échantillons de matières fécales de 11 veaux, infectés expérimentalement avec des cryptosporidies, à l'aide de deux méthodes dont on voulait évaluer la sensibilité et la simplicité, comme techniques de diagnostic. La comparaison entre une solution de flottation à base de dichromate et des impressions de matières fécales révéla une plus grande sensibilité, de la part de la première méthode. Elle permit en effet de détecter des oocystes dans les fèces, plus tôt et pendant plus longtemps après l'infection; ils étaient aussi plus faciles à visualiser qu'avec l'autre méthode. La technique de flottation permit en plus d'éliminer *Candida albicans,* champignon qu'on peut parfois confondre avec des cryptosporidies, dans les impressions de matières fécales. Un autre avantage consistait dans le fait qu'on pouvait facilement détecter des oocystes dans les échantillons mélangés à la solution de dichromate depuis aussi longtemps que 120 jours. L'emploi de cette technique de flottation révéla aussi que l'élimination d'oocystes de cryptosporidies se produisit de deux à 20 jours après l'infection buccale expérimentale, sans s'accompagner infailliblement de diarrhée. La nécropsie permit de confirmer la cryptosporidiose, chez deux des veaux expérimentaux. Ceux qu'on ne sacrifia pas pour en effectuer la nécropsie, guérirent sans traitement. La technique de flottation dans une solution à base de dichromate représente par conséquent une méthode simple, rapide et peu coûteuse qui devrait faciliter la détection de cryptosporidies, tant dans les laboratoires de diagnostic que dans les cabinets des praticiens.

INTRODUCTION

Cryptosporidiosis is an infection of the microvillus epithelium caused by organisms of the genus *Cryptosporidium,* which are protozoan parasites of the subclass *Coccidiasina,* suborder *Eimeriorina,* and hence are related to coccidian parasites of domestic animals. Cryptosporidia have been reported in mammals, including humans, reptiles and birds (7) and recent reports suggest that there may only be one species of *Cryptosporidium* which is capable of infecting many different host species (9,16).

Diagnosis of bovine cryptosporidiosis has been most frequently made by observing cryptosporidial oocysts in fecal smears stained by the Giemsa method (9,13,17), or the Grocott-Gomeri methanamine silver method (4), or in histological sections taken at necropsy (8,10,12,14,15). These methods present several difficulties when used for routine diagnosis. Oocysts have poor affinity for Giemsa's stain and tend to fade into the background of protein and other debris present in fecal smears, hence they may be missed especially when present in low numbers. In addition, cryptosporidial oocysts can be confused with yeast in Giemsa stained smears because of their similar size and color (4). The silver stain differentiates cryptosporidial oocysts from yeasts, which stain intensely black; however the oocysts are poorly stained by this method and characteristic features are difficult to visualize (4). In addition, oocysts cannot be detected in fecal samples which have been stored frozen making large scale epizootiologic studies difficult. Diagnosis from histological sections is time consum-

Published with permission of the Director of VIDO as journal series number 18.

TABLE I
CALVES EXPERIMENTALLY CHALLENGED WITH CRYPTOSPORIDIUM

Calf Number	Colostral Status	Source of Feces for Inoculum	Age at Inoculation (Days)
80-56	Deprived	1615	2
81-2	Deprived	80-56	4
81-6	Deprived	81-2	4
81-7	Deprived	81-6	12
81-8	Deprived	81-6	11
81-23	Fed	81-7	1.5
81-27	Fed	81-23	24
81-31	Fed	81-23	1
81-32	Fed	81-23	5
81-39	Deprived	81-23 and 81-27	16
81-40	Fed	81-23 and 81-27	15

ing, expensive, requires specialized equipment, and cannot be done on live calves.

Therefore, a rapid, simple, and sensitive antemortem diagnostic procedure for cryptosporidial infection in calves would provide practicing veterinarians and diagnostic laboratories with a useful tool when investigating outbreaks of calf diarrhea and would facilitate more detailed studies on the epizootiology of cryptosporidiosis. This report describes a dichromate solution floatation (DSF) technique for diagnosis of cryptosporidial infections from fecal specimens which is adaptable to both clinical veterinary practice, diagnostic and research situations. Although floatation methods have been described previously (3,8), this report compares the sensitivity of a DSF technique combined with phase contrast microscopy with the Giemsa stained fecal smear technique.

MATERIALS AND METHODS

Animals — Calves were obtained from dairies in the Saskatoon area or were born to Hereford cross cows housed at the Veterinary Infectious Disease Organization (VIDO) research facility. Some calves were colostrum fed, and others were deprived of colostrum and fed 2% ultra-high-temperature (UHT) pasteurized milk.[1] They were challenge fed cryptosporidia once as described below at the ages indicated in Table I.

Fecal Samples — Fecal samples collected from experimentally infected calves were mixed with 2.5% potassium dichromate solution (one part feces to two parts potassium dichromate solution) which was used to store and preserve the oocysts, and for diagnosis as described below. Samples were stored at room temperature for as long as 61 days between calf passages. Also, 27 fecal samples were obtained from 18 calves raised at a local dairy.

Experimental Transmission — A sample of diarrheic feces containing cryptosporidial oocysts preserved in 2.5% potassium dichromate solution was obtained from Dr. Bruce Anderson, University of Idaho, Caldwell Veterinary Teaching Center, Caldwell, Idaho. His sample 1615 was used to inoculate calf 80-56 used in the present study.

To prepare the inoculum for each calf, 18 to 25 mL of fecal dichromate solution (6 to 8 mL of calf feces) was dialyzed against distilled water for 24 h to remove the potassium dichromate. The inoculum was added to 0.5 to 1 L of UHT pasteurized milk and fed to the calf by nipple bottle. Fecal samples containing oocysts were serially passaged through six calves as shown in Table I.

Tissue Specimens — Seven days after experimental inoculation, two calves (nos. 81-7 and 81-23) which were shedding large numbers of cryptosporidial oocysts were anesthetized with sodium pentobarbital and intestinal samples were taken at 11 equally spaced locations between the pylorus and the cecum. The first section was taken 50 cm distal to the pylorus and the last 50 cm proximal to the ileocecal junction. An additional sample was taken from the middle of the spiral colon. These samples were preserved in 10% neutral buffered formalin, sectioned and stained by routine histological methods. Samples for transmission electron microscopic examination taken from the same locations in the small intestine were originally fixed in 10% neutral buffered formalin, postfixed in 2% osmium tetroxide, dehydrated in ethanol, embedded in EPON-812, sectioned at 1 μm and stained with toluidine blue. Specific areas of the 1 μm sections containing the parasite were cut with an ultramicrotome, mounted on grids, stained with uranyl acetate and lead citrate and examined under an electron microscope[2] (5).

Detection of Cryptosporidial Oocysts — Fecal samples were examined for the presence of cryptosporidial oocysts by two methods. a) DSF technique — three mL of fecal-dichromate solution mixture was placed in a screw cap centrifuge tube and mixed with 5 mL of saturated sucrose solution (1.26 g/mL) prepared by dissolving 825 g of sucrose in 500 mL of distilled water. Five mL of distilled water was added to the tube so that the total volume was 13 mL with a final density of 1.10 g/mL or greater. Tubes were inverted ten times to mix the contents and were then centrifuged at 800x g for ten minutes (setting of 5 on IEC clinical centrifuge). After centrifuging, the meniscus was removed by placing three loopfuls of liquid on a glass slide using a wire inoculating loop (4 mm diameter), covered with a cover glass and examined for the presence of cryptosporidial oocysts. Examination was facilitated by scanning the perimeter of the cover glass using 400X magnification and phase contrast illumination at a focal plane just beneath the cover glass. b) Fecal smears — a thin smear of the fecal material was made on a glass slide by dipping a cotton tipped swab in the fecal sample and rolling it lightly on the glass slide. The slides were air dried, methanol fixed, and stained with an Ames Hema-tek using the Wright-Giemsa H-pack stain.[3]

[1]2% UHT partly skimmed milk, Palm Dairies, Calgary, Alberta.
[2]Hitachi, Model HS-8 electron microscope, Tokyo, Japan.
[3]Ames Hema-tek, Ames, Iowa, A.J.P. Scientific, Clifton, New Jersey.

TABLE II
GRADING SYSTEM USED TO SCORE FECAL SAMPLES

Fecal Score	Dichromate Solution Floatation	Fecal Smear
Tr	1 - 2/slide[a]	1 - 2/slide[a]
1	3 - 10/slide[a]	3 - 10/slide[a]
2	2 - 6/field[b]	2 - 4/30 fields[c]
3	7 - 12/field[b]	5 - 10/30 fields[c]
4	> 13/field[b]	> 11/30 fields[c]

[a]Oocysts per preparation.
[b]Oocysts per field (400X under phase contract illumination).
[c]Oocysts per 30 fields (1000X under bright field illumination).

Stained smears were observed at 1,000X magnification for cryptosporidial oocysts using bright field illumination.

Both floatation mounts and fecal smears were examined and graded for the presence of cryptosporidial oocysts as described in Table II.

Appearance of Yeast — Yeast found in calf feces can be confused with cryptosporidial oocysts in stained smears (4), therefore experiments were done to determine if the DSF technique would eliminate this problem. Five slides were prepared using feces from calf 81-39 as follows: a) fecal smear prepared and stained with the Wright-Giemsa technique, b) DSF preparation, c) a slide identical to the second one except that one drop of a heavy suspension of *Candida albicans,* grown on Sabauraud's dextrose agar, was added just before the cover glass was applied, d) a Giemsa stained fecal smear made from a mixture of three parts feces and one part heavy suspension of *C. albicans* and e) a DSF preparation from the same mixture of feces and yeast as used in d. Replicate slides were made from each of the smears (slides a and d) and each series was stained using Wright-Giemsa, methenamine silver or Kinyoun acid-fast techniques.

Rotavirus Diagnosis — The presence of rotavirus in feces was determined using an enzyme-linked immunosorbent assay (ELISA).

Enteropathogenic Escherichia coli — Selected diarrheic fecal samples were examined for enteropathogenic *E. coli* (EEC) by streaking the sample on MacConkey's agar and incubating at 37°C for 24 h. Pink colonies were picked for additional biochemical tests using commercial media, and those which did not utilize citrate or produce urease, but which did produce indole from tryptophan, and did produce an acid butt and slant with gas but no hydrogen sulfide on TSI agar were identified as *E. coli.* These isolates were examined for expression of K99 antigen and production of heat stable enterotoxin. The production of K99 antigen was assayed using a slide agglutination method (1), and heat stable enterotoxin was assayed by a suckling mouse test (6).

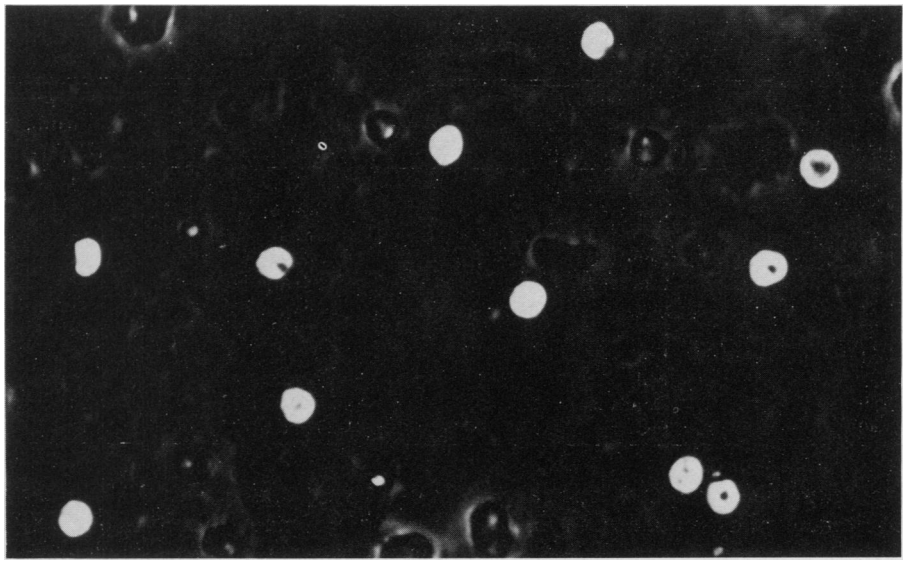

FIGURE 1. Cryptosporidial oocysts in dichromate solution floatation preparation of feces from calf 81-39 under phase contrast illumination (X400). Note: Bright refractile bodies some of which have a central black dot.

RESULTS

When observed at the margin of the cover glass using phase contrast illumination of the DSF preparation, the cryptosporidial oocysts were bright refractile bodies, circular in profile, having a distinct border, and internal structures which sometimes appeared as an eccentric black dot (Figures 1-3). The mean density of oocysts was 1.08 g/mL which was less than that of the floatation solution and, therefore, they floated to the underside of the cover glass. In contrast, yeast organisms which have a higher mean density, were not concentrated in the

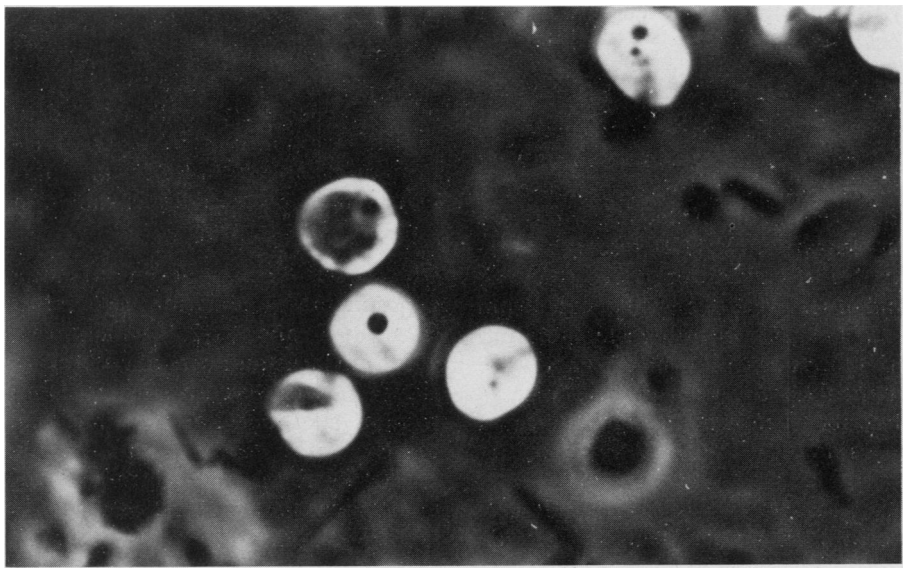

FIGURE 2. Same as Figure 1 (X1000). Note presence of eccentrically located black dot within the oocysts.

meniscus and were not observed in the preparations even after a heavy suspension of yeast was added to the fecal sample as in slide e prepared from calf 81-39 (Figure 3). When the yeast were mixed with oocysts directly on the slide and observed at 1000X magnification with phase contrast illumination, the yeast had an oval shape, were not refractile and lacked the black dot (Figure 4).

In contrast to the above, the oocysts observed in Giemsa stained fecal smears were pale blue, often vacuolated, and occasionally exhibited dark reddish bodies around the interior periphery. When scanning the slide they were most readily recognized as circular areas devoid of background debris and bacteria (Figures 5 and 6). Yeast have been observed in fecal smears made from naturally and experimentally infected calves (4) and, like the oocysts, appeared as pale blue to violet vacuolated bodies (Figure 6). The morphology of the oocysts observed on the silver stained slide was distorted and there was little affinity for the stain, consequently the oocysts were difficult to recognize. Differentiation from yeast was easy however, since the *C. albicans* stained intensely black.

The acid-fast stain produced a slide on which the oocysts were easy to recognize as pink or red circles on a blue background. The yeast, debris and most bacteria were blue. The only other pink objects observed were occasional pollen grains or other plant material.

Using the DSF technique cryptosporidial oocysts were detected sooner, in larger numbers and over a longer period of time than with the smear technique (Table III). The data from five calves which is presented in Table III is representative of the 11 experimentally infected calves. Bacterial enteropathogens were not detected in the diarrheic samples from any of these calves. Shedding of oocysts did not correlate well with the presence of diarrhea and younger calves concurrently infected with rotavirus shed oocysts for a longer period than the older calves not concurrently shedding rotavirus. Oocysts were shed for a mean period of eight days following experimental infection. Both colostrum fed and colostrum deprived

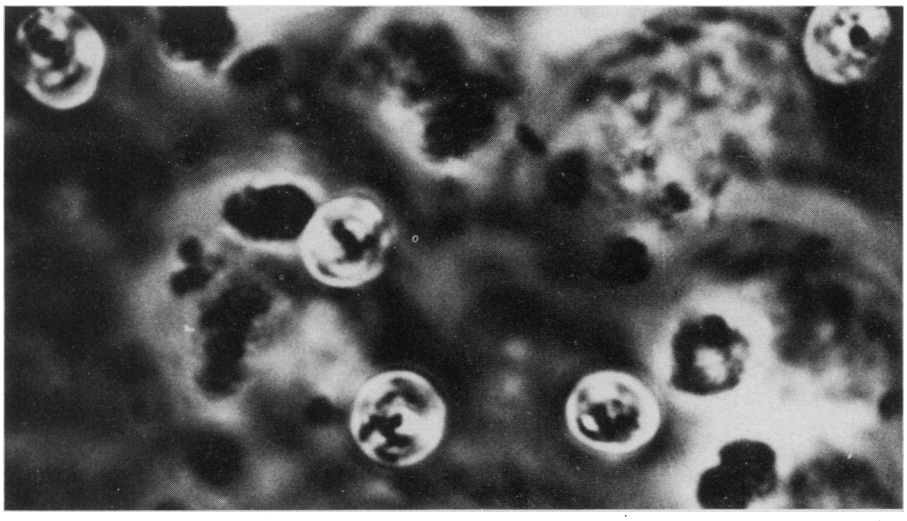

FIGURE 3. Yeast (*C. albicans*) were added to feces of calf 81-39 which contained cryptosporidial oocysts and the dichromate solution floatation procedure was performed and observed under phase contrast illumination (X1000). Several cryptosporidial oocysts are present and seen as bright refractile bodies with internal structure. Yeast were eliminated by the procedure and are not seen in the slide.

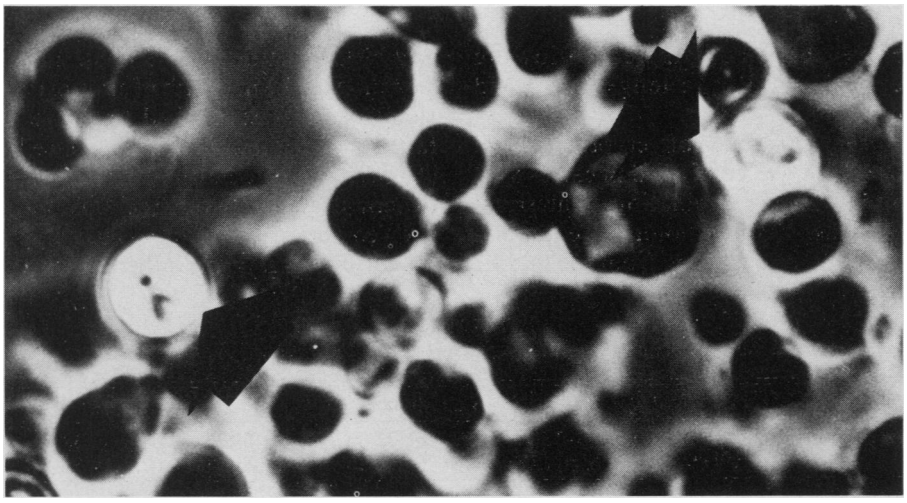

FIGURE 4. Yeast (*C. albicans*) were added to a DSF preparation from feces of calf 81-39. Wet mount under phase contrast illumination (X1000). Yeast appear as dark, nonrefractile bodies. Two cryptosporidial oocysts are seen (arrows).

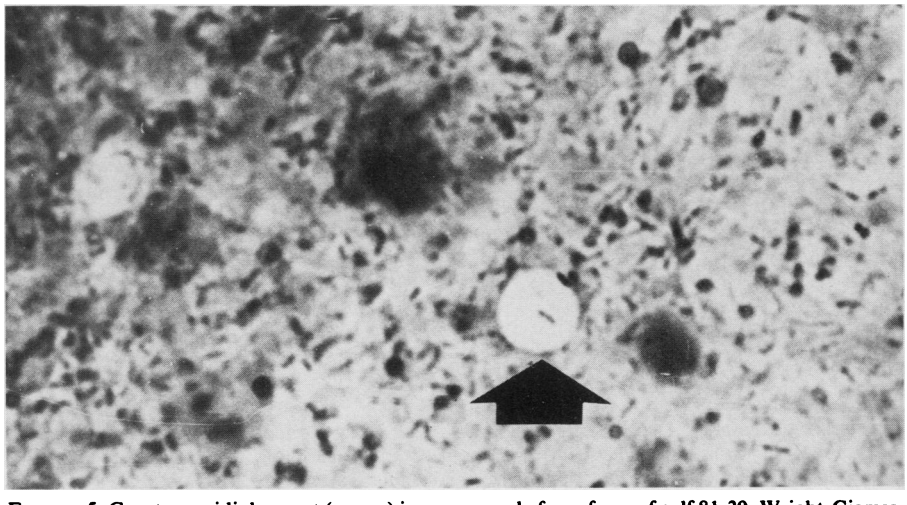

FIGURE 5. Cryptosporidial oocyst (arrow) in smear made from feces of calf 81-39. Wright-Giemsa satin (X1000). Note the pale circular area devoid of most bacteria and debris.

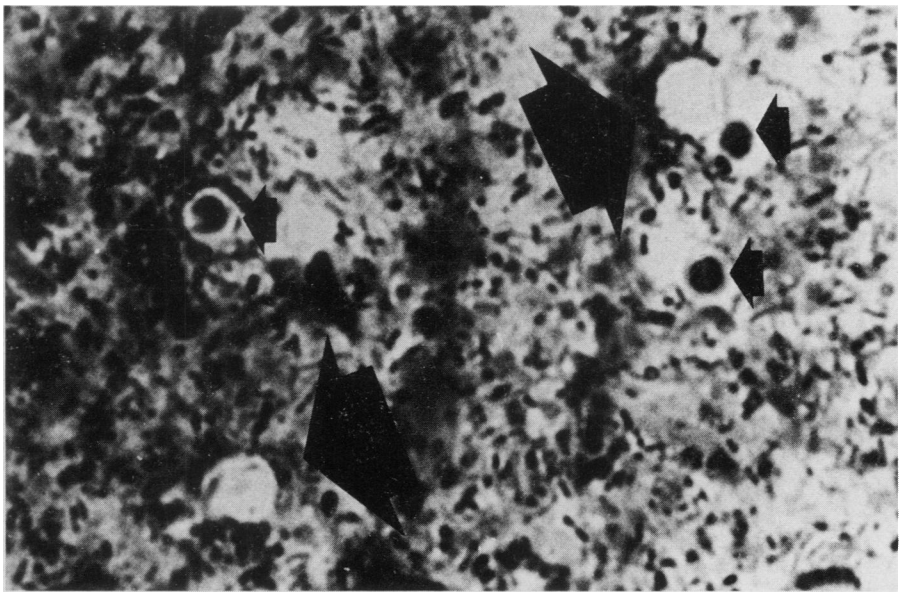

FIGURE 6. Direct smear prepared from feces of calf 81-39 to which *Candida albicans* were added. Wright-Giemsa stain (X1000). Two cryptosporidial oocysts (large arrows) and three yeast (smaller arrows) are present.

calves became infected following experimental challenge and shed oocysts.

The presence of cryptosporidial infection was confirmed in calves 81-7 and 81-23 by examination of intestinal sections. Histopathological examination of sections taken from the middle and distal portions of the small intestine showed the presence of cryptosporidial organisms attached to the surface of the intestinal epithelial cells (Figure 7). Electron microscopic examination of tissue sections also showed the presence of typical cryptosporidial organisms adherent to the villus surfaces (Figure 8).

Using the dichromate solution floatation technique cryptosporidial oocysts were detected in one fecal sample from a naturally infected calf housed at a local dairy which was passing feces of normal consistency. Oocysts were not observed in the Giemsa stained smear made from the same fecal sample. The one positive calf was part of a population of nine calves so the prevalence rate of cryptosporidiosis was 11%. During the next 12 months 27 samples from 18 calves in the same herd were examined, but there were no additional positive samples.

TABLE III
COMPARISON OF TECHNIQUES FOR DETECTION OF CRYPTOSPORIDIOSIS

	Calf Number															
	81-6				81-7				81-23				81-27				81-39			
	F[a]	S[b]	R[c]	D[d]	F	S	R	D	F	S	R	D	F	S	R	D	F	S	R	D
Day PI																				
0	-	-	Tr	-	..	-	-	-	-	-	-	-	-	-	-	-	-	-	Tr	-
1	-	-	+	-	-	-	-	-	-	-	-	-	..	..	-	-	-	-	+	-
2	-	-	+	-	1	-	-	-	-	-	-	-	..	..	-	-	-	-	-	-
3	..	..	..	-	1	Tr	-	+	Tr	Tr	-	-	..	..	-	-	-	-	-	-
4	..	..	Tr	-	4	3	-	+	1	Tr	-	+	..	..	-	-	-	-	-	-
5	..	Tr	-	-	4	1	-	+	2	1	-	-	..	..	-	-	-	-	-	-
6	..	..	..	-	4	3	-	+	4	2	-	-	..	..	-	-	-	-	-	-
7	4	1	..	-	3	4	-	-	4	3	-	-	..	..	..	-	-	-	-	-
8	4	Tr	..	-	Sacrificed				Sacrificed				1	-	-	-	-	-	-	-
9	3	1	-	-									2	-	-	-	-	-	-	-
10	2	-	..	-									1	-	-	-	-	-	-	-
11	2	1	..	-									1	-	-	-	-	-	-	-
12	1	-	..	-									1	-	-	-	-	-	-	-
13	1	Tr	..	-									1	-	-	-	-	-	-	-
14	..	..	..	-									1	-	-	-	Tr	-	-	-
15	-	-	..	-									Tr	-	-	-	1	Tr	-	-
16																	2	2	-	-
17																	3	4	Tr	+
18																	3	1	-	+
19																	2	1	-	+
20																	Tr	-	-	-

[a]Detection of cryptosporidial oocysts by dichromate solution floatation.
[b]Detection of cryptosporidial oocysts by Giemsa stained fecal smear.
[c]Rotavirus present in feces.
[d]Diarrhea.

DISCUSSION

In this study we compared a DSF technique and fecal smears stained with Wright-Giemsa technique for routine diagnosis of cryptosporidiosis in calves. Using the DSF technique we were able to detect oocyst shedding from experimentally infected calves earlier and over longer intervals than with the smear method, and from a naturally infected calf when oocysts were not observed in the fecal smear. Oocysts were readily observed along the margin of the coverglass even when few oocysts were present in the fecal sample because they were concentrated in the meniscus during the DSF procedure. Besides being more sensitive than the smear technique, the floatation technique was more specific because it removed yeast which can be confused with oocysts in Giemsa stained fecal smears. Another advantage of the dichromate solution technique is that fecal specimens stored at room temperature for up to 120 days retained morphological characteristics and were readily identified. Fecal samples which had been stored frozen cannot be examined for cryptosporidia since this destroys the oocysts. Processing of nondiarrheic fecal samples is also facilitated because the dichromate solution suspends the oocysts and allows them to rise in the medium, hence the floatation process concentrates the oocysts from approximately 1 g of feces which is much more than is present in a smear.

When phase contrast illumination is used and the preparation is examined at 400X magnification, a large area can be examined in a short time ena-

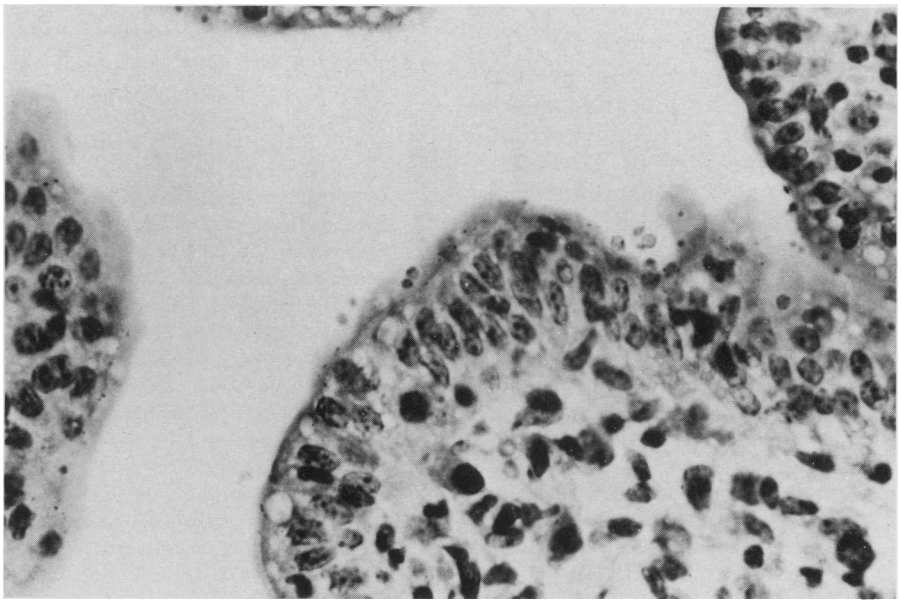

FIGURE 7. Tissue section of ileum of calf 81-7 on day 7 postinoculation with cryptosporidia along microvillus border. H&E. X1000.

bling a rapid and accurate diagnosis to be made. Positive preparations can be readily recognized because there is a very significant contrast between the bright, refractile oocysts and darker floatation medium and debris when the preparation is observed along the margin of the cover glass. Oocysts which are observed in the center of the preparation have less contrast from the background than those which are observed at the margin of the coverglass. The oocysts are visible under normal bright field illumination but they are most difficult to detect since there is no enhancement of contrast between the oocysts and the medium. A Giemsa stained smear contains less fecal material for examination and takes longer to scan because 1000X magnification must be used to differentiate the oocysts from debris. It is likely that the DSF technique is adaptable for diagnosis of cryptosporidiosis in other species.

If phase contrast illumination is not available, the meniscus from the DSF tube can be suspended in a centrifuge tube containing normal saline, the mixture centrifuged, and the sediment spread on a slide. The slide can be stained with the acid-fast technique and observed at 1000X magnification under bright field illumination. This combines the benefits of concentration by DSF and the high contrast of pink oocysts against a blue background.

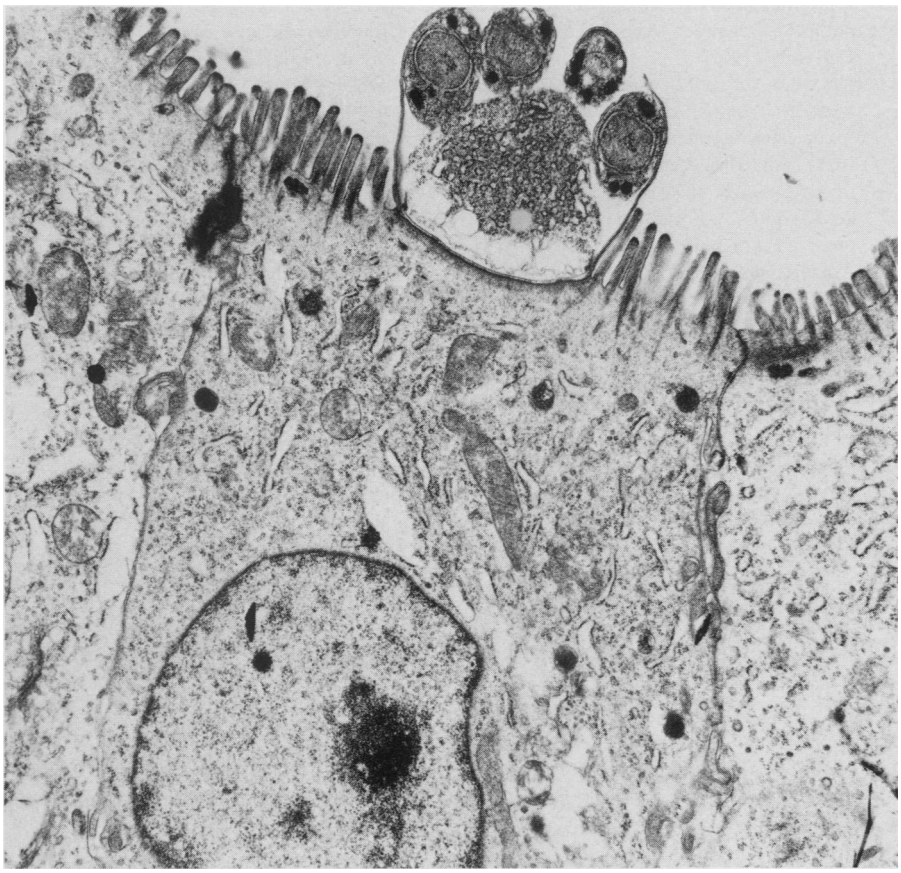

FIGURE 8. Tissue section of ileum of calf 81-7 on day 7 postinoculation with cryptosporidium showing schizont with developing merozoites embedded in microvillus border of enterocytes. X9750.

Scours in neonatal calves has long been recognized as a source of significant economic loss to cattle producers. A variety of etiological agents and management practices which contribute to disease have been described (2,10). Recently cryptosporidia have been implicated as another cause contributing to the calf scours complex (3,9,13). However, most of the calves experimentally challenged during this study did not develop diarrhea, and those which had a transient diarrhea recovered without treatment. In addition, the calf raised on a local dairy which was shedding cryptosporidial oocysts was not diarrheic. Histopathological lesions are observed in infected calves, however and the potential of this parasite to produce significant disease in calves in the absence of other contributing enteropathogens must be investigated further.

Other investigators have consistently reproduced diarrhea by orally challenging calves with cryptosporidia (9,16), however cryptosporidia have been occasionally detected from nondiarrheic calves by histological examination (10), microscopic examination of ileal smears (11), and DSF of feces in the present study. The clinical significance is unclear because most experimentally challenged calves recover

spontaneously, while in field cases severe diarrhea and death may result. The natural disease is often complicated by concurrent infection with other enteropathogens such as enteropathogenic *E. coli,* rotavirus and coronavirus (10,11,13,14,15); however, there are reports in which no other enteropathogen was detected (8,10,11,17). A summary of the current reports indicates that most clincial cases occur in calves one to three weeks old, although older calves and cows may shed cryptosporidial oocysts, and that the disease is more likely to be fatal if there are concurrent viral infections. Sulfonamides and antibiotics do not appear to be effective treatments in some cases (8,14,15), but other cases showed marked clinical improvement after sulfonamide and/or antibiotic treatment (3,10,11, 17). Calves not infected with other agents may recover spontaneously; however the herd may remain infected for several months (13). The prevalence of cryptosporidiois is unknown but one report suggests that it may occur in as high as 33% of diarrheic dairy calves in Quebec (10). Utilization of the DSF technique should enable future studies to determine the prevalence of cryptosporidiosis, the role of cryptosporidia in pathogenesis of neonatal calf scours and the potential utility of treatment. A survey to establish the prevalence of cryptosporidiosis in calves in western Canada is underway.

ACKNOWLEDGMENTS

The authors wish to thank Dr. J.E.C. Bellamy, Western College of Veterinary Medicine for preparing and examining tissue sections. Technical assistance provided by G. Falk, S. Feschuk and K. Dukeshire is also gratefully acknowledged.

REFERENCES

1. ACRES, S.D., A.J. FORMAN and R.A. KAPITANY. An antigen extinction profile in pregnant cows using a K99-containing whole cell bacterin to induce passive protection against enterotoxigenic colibacillosis of calves. Am. J. vet. Res. 43: 569-575. 1982.
2. ACRES, S.D., J.R. SAUNDERS and O.M. RADOSTITS. Acute undifferentiated neonatal diarrhea of beef calves: the prevalence of enterotoxigenic *E. coli,* Reo-like (Rota) virus and other enteropathogens in cow-calf herds. Can. vet. J. 18: 113-121. 1977.
3. ANDERSON, B.C. Patterns of shedding of cryptosporidial oocysts in Idaho calves. J. Am. vet. med. Ass. 178: 982-984. 1981.
4. ANGUS, K.W., I. CAMPBELL, E.W. GRAY and D. SHERWOOD. Staining of faecal yeasts and cryptosporidium oocysts. Vet. Rec. 108: 173. 1981.
5. BELLAMY, J.E.C. and S.D. ACRES. Enterotoxigenic colibacillosis in colostrum-fed calves: pathologic changes. Am. J. vet. Res. 40: 1391-1397. 1979.
6. DEAN, A.G., Y.C. CHING, R.G. WILLIAMS and L.B. HARDEN. Test for *Escherichia coli* enterotoxin using infant mice: Application in a study of diarrhea in children in Honolulu. J. infect. Dis. 125: 407-411. 1972.
7. KENNEDY, G.A., G.L. KREITNER and A.C. STRAFUSS. Cryptosporidiosis in three pigs. J. Am. vet. med. Ass. 170: 348-350. 1977.
8. MEUTEN, D.J., H.J. VAN KRUININGEN and D.H. LEIN. Cryptosporidiosis in a calf. J. Am. vet. med. Ass. 165: 914-917. 1974.
9. MOON, H.W. and W.J. BEMRICK. Fecal transmission of calf cryptosporidia between calves and pigs. Vet. Path. 18: 248-255. 1981.
10. MORIN, M., S. LARIVIERE, R. LALLIER, M. BEGIN, R. ROY and R. ETHIER. Neonatal calf diarrhea: pathology and microbiology of spontaneous cases in dairy herds and incidence of the enteropathogens implicated as etiological agents. Proc. VIDO Int. Symposium on Neonatal Diarrhea. 2: 347-369. 1978.
11. NAGY, B., A. ANTAL and J. LAKNER. Significance of intestinal cryptosporidiosis in calf diarrhea. Proc. Int. Symposium of Vet. Lab. Diagnosticians 2. (3): 431-434. 1980.
12. PEARSON, G.R. and E.F. LOGAN. Demonstration of cryptosporidia in the small intestine of a calf by light, transmission electron and scanning electron microscope. Vet. Rec. 103: 212-213. 1978.
13. POHLENZ, J., H.W. MOON, H.F. CHEVILLE and W.J. BEMRICK. Cryptosporidiosis as a probable factor in neonatal diarrhea of calves. J. Am. vet. med. Ass. 172: 452-457. 1978.
14. POWELL, H.S., M.A. HOLSCHER, J.E. HEATH and F.F. BEASLEY. Bovine cryptosporidiosis (a case report). Vet. Med. small Anim. Clin. 71: 205-207. 1976.
15. SNODGRASS, D.R., K.W. ANGUS, E.W. GRAY and W.A. KEIR. Cryptosporidia associated with rotavirus and an *Escherichia coli* in an outbreak of calf scours. Vet. Rec. 106: 458-460. 1980.
16. TZIPORI, S., K.W. ANGUS, I. CAMPBELL and E.W. GRAY. *Cryptosporidium:* evidence for a single-species genus. Infection & Immunity 30: 884-886. 1980.
17. TZIPORI, S., I. CAMPBELL, D. SHERWOOD and D.R. SNODGRASS. An outbreak of calf diarrhea attributed to cryptosporidial infection. Vet. Rec. 107: 579-580. 1980.

ABSTRACT

Safety and efficacy of zeranol. R.G. Brown, (International Minerals & Chemical Corporation, Terre Haute, Indiana).

When selecting an anabolic implant it is important to use one which is safe and nontoxic to cattle and to the consumer who eats the meat. Zeranol is a nonsteroid anabolic implant which has been tested for toxicity in nine different species of animals. It has been tested in dogs for seven years at a daily dose which is 12,000 times the daily dose absorbed by a 200-kg steer and ten years in monkeys at 25,000 times the daily steer dose. At interim sacrifices and at the end of the tests there were no signs of toxicity such as carcinogenicity. In university trials, when suckling calves are implanted properly, there were 10 extra kg of beef produced by one 36-mg implant and 24 kg when reimplanted. In grazing cattle 12 extra kg of beef was produced by 36 mg and in growing-finishing cattle 20 extra kg with one reimplant and 29 kg with two reimplants. The use of zeranol and Rumensin produces an additive effect in weight gain and feed efficiency. An additive effect is also produced in calves given zeranol and an anthelmintic. When zeranol implanted calves are exposed to heat stress, the signs of heat stress toxicity are decreased.

Scientific Program of the Canadian Veterinary Medical Association Convention, Winnipeg 1981.