ISSN 0008-5286

THE CANADIAN VETERINARY JOURNAL

LA REVUE VÉTÉRINAIRE CANADIENNE

Volume 23 December-décembre No. 12

Bovine Cryptosporidiosis: Clinical and Pathological Findings in Forty-two Scouring Neonatal Calves

S. E. SANFORD AND G. K. A. JOSEPHSON

Veterinary Services Laboratory, Ontario Ministry of Agriculture and Food, Huron Park, Ontario N0M 1Y0

SUMMARY

Cryptosporidia organisms were identified in 42 of 161 (26%) neonatal, diarrheic calves, over a 32 month period commencing July 1979. Forty of the 161 calves were submitted alive and cryptosporidiosis was diagnosed in 63% (25 of 40) of them. The cryptosporidia infected calves were usually one to two weeks old and came from 26 herds where the typical history was profuse, watery diarrhea in nearly all neonatal calves. The diarrhea usually started around one week of age, was unresponsive to all conventional antidiarrhea therapies, lasted for two or more weeks and was usually fatal. Twenty-nine (69%) of the cryptosporidia infected calves were submitted between December and February. These calves were often hutch reared.

Histopatholoical examination revealed large numbers of the coccidial parasite *Cryptosporidium* sp embedded in the microvilli of jejunal and ileal absorptive enterocytes of all affected calves. The organisms were identified as trophozoites and schizonts (asexual stages) and macrogametes (female sexual stages) with the electron microscope. Microgametes (male sexual stages) were not identified. Occasionally a merozoite (asexual stage) was also seen apparently burrowing into or about to be enveloped by a host microvillus. Observation of the organisms was much easier when diarrheic calves were submitted alive. Enterotoxigenic *Escherichia coli* were often cultured from intestines of dead calves and occasionally from calves submitted alive. Coronavirus particles were seen in one calf. In the last year of this study, oocysts were identified in fecal smears stained with May-Grünwald-Giemsa stain and fecal samples using a dichromate solution flotation technique.

RÉSUMÉ

La cryptosporidiose bovine: observations cliniques et pathologiques, chez quarante-deux veaux atteints de diarrhée néo-natale

Cette étude portait sur 161 veaux qui souffraient de diarrhée néonatale et elle a permis, au cours d'une période de 32 mois qui débuta en juillet 1979, d'identifier des cryptosporidies chez 42 de ces veaux, i.e. 26% du groupe. Les auteurs en reçurent 40 qui étaient encore vivants et ils diagnostiquèrent la cryptosporidiose chez 25, i.e. 63% d'entre eux. Les veaux atteints de cette maladie étaient ordinairement âgés d'une à deux semaines et ils provenaient de 26 troupeaux dont les propriétaires se plaignaient d'une diarrhée profuse et liquide, chez la plupart de leurs veaux nouveau-nés. La diarrhée débutait ordinairement une semaine après la naissance, résistait à tous les traitements antidiarrhéiques conventionnels, durait environ deux semaines ou plus et s'avérait généralement fatale. Vingt-neuf, i.e. 69% des veaux atteints de cryptosporidiose furent soumis au laboratoire entre décembre et février. Il s'agissait, la plupart du temps, de veaux gardés dans des cages.

L'examen microscopique de la muqueuse intestinale permit de démontrer une multitude de coccidies du genre *Cryptosporidium*, imbriquées dans la bordure en brosse des entérocytes de tous les veaux parasités. La microscopie électronique permit par ailleurs de reconnaître des formes asexuées et sexuées du parasite, à savoir des trophozoïtes et des schizontes, ainsi que des macrogamètes. On ne réussit toutefois pas à démontrer la présence de formes sexuées mâles, i.e. des microgamètes. De temps en temps, il s'avéra possible de voir un mérozoïte, une autre forme asexuée du parasite, en train de s'enfouir dans une microvillosité intestinale ou sur le point d'être enveloppé par elle. La démonstration des cryptosporidies se révéla beaucoup plus facile, dans l'intestin des veaux soumis vivants. Un examen bactériologique de l'intestin se solda par l'isolement de colibacilles entérotoxinogènes chez plusieurs veaux reçus morts, mais chez seulement quelques-uns de ceux qui arrivèrent vivants au laboratoire. Un de ces veaux arborait aussi des particules de coronavirus. Durant les derniers 12 mois de cette étude, des impressions de matières fécales, colorées selon la méthode de May-Grunwald-Giemsa, et des échantillons de matières fécales, soumis à la flotation dans une solution de dichromate, recelaient des oocystes.

INTRODUCTION

In recent years, *Cryptosporidium* sp has been identified as a sole diarrhea-causing agent in calves by several investigators in different countries (1,2,3,4). It has also been associated with diarrhea in lambs (5,6) and goat kids (7). Its pathogenicity for pigs is less convincing (8).

A single-species genus has been proposed for *Cryptosporidium* based on the demonstration that oocysts from diarrheic calves were infective to and capable of inducing diarrhea in seven different animal species (9). This lack of host specificity is even more disconcerting because of the apparent zoonotic potential of *Cryptosporidium* (10).

The number of reports of outbreaks of diarrhea associated with enteric cryptosporidiosis in various species has multiplied rapidly (3,4,5,7,11). Despite this, there is a conspicuous absence of recommendations for treatment and control once cryptosporidiosis has been diagnosed. A recent study has revealed that several different types of antiprotozoal drugs were ineffective in stopping the shedding of oocysts in scouring calves experimentally infected with cryptosporidia (12).

The clinical and pathological findings in 42 calves submitted to our diagnostic laboratory from 26 farms over a period of 32 months and identified as being infected with enteric cryptosporidia are reported.

MATERIALS AND METHODS

In the 32 month period commencing July 1979, 161 neonatal, diarrheic, calves were submitted to the Huron Park Veterinary Services Laboratory for routine diagnostic investigation. Calves originated from farms where several other calves had died despite vigorous electrolyte, broad spectrum antibiotic and other antidiarrheal treatments over a period of weeks or even months. Forty of these calves were submitted alive and usually within 72 hours of the commencement of their diarrhea. Many of these had not received treatment. The calves were killed with an overdose of barbiturate. Complete necropsies were performed on all calves. Tissue samples for histopathology were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 6 μm and stained with hematoxylin and eosin (H & E).

Formalin-fixed tissues from four calves submitted alive were prepared for electron microscopy by postfixing in 1% osmium tetroxide, dehydrated in acetone and embedded in Epon. Semi-thin (1 μm) sections were stained with methylene blue and examined by light microscopy. Ultrathin sections of selected areas were stained with lead citrate and uranyl acetate and examined in a Hitachi transmission electron microscope, model no. HS9 (TEM).

Portions of intestine, spleen, occasionally liver, kidney, gall bladder, mesenteric lymph node and lung were streaked on 5% bovine blood agar plates and incubated at 37°C for 24 hours. Enterotoxigenic strains of *Escherichia coli* isolated were identified using the slide agglutination test.

Direct electron microscopic examinations were performed on the ileal and colonic contents from 22 calves submitted alive after negative staining with 3% phosphotungstic acid (13). Intestines from these calves were also ground and inoculated into embryonic bovine spleen culture.

In the last year of this study, fecal smears from selected calves were stained with May-Grünwald Giemsa's stain and examined for oocysts. Fecal samples were examined by phase contrast microscopy using the dichromate solution flotation (DSF) technique (14).

RESULTS

Forty-two of the 161 (26%) scouring, neonatal, calves were found to be infected with enteric cryptosporidia. These 42 calves came from 26 farms. Twenty-five of the 40 calves (63%) submitted alive were infected. Forty-one of the calves with cryptosporidiosis were Holstein-Freisian and one was a Hereford. Calves with cryptosporidiosis ranged between seven and 30 days of age, but were usually seven to 14 days old. They were dehydrated and had a protracted, yellow, watery diarrhea with mucus.

During the 32 months of this study, 29 (69%) of the cryptosporidia-infected calves were submitted between December and February (Figure 1). During these months, calves were frequently reported to have been reared in hutches.

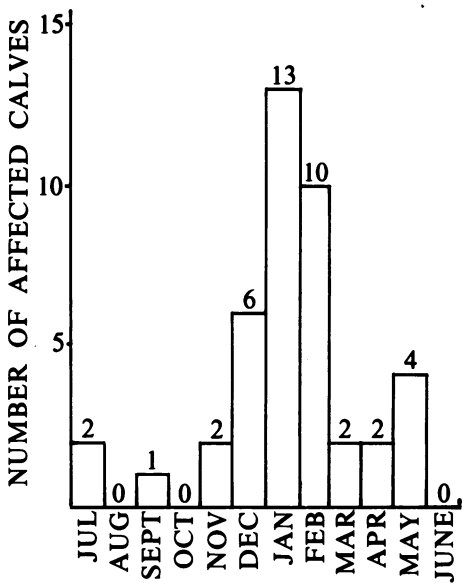

FIGURE 1. Monthly prevalence of cryptosporidiosis seen in 42 affected calves submitted to the Huron Park Laboratory.

HISTOPATHOLOGICAL FINDINGS

The ileal and jejunal villi exhibited mild to moderate atrophy with occasional bridging and fusion of adjacent villi. The lamina propria was invaded by large numbers of mononuclear cells and some neutrophils. Occasionally, crypts were dilated and contained necrotic debris and neutrophils. Large numbers of cryptosporidia organisms were embedded in the microvillous border of the jejunal and ileal absorptive enterocytes (Figures 2 and 3). In cases with even larger numbers of parasites, the organisms were also seen in the microvillous border of the duodenal, cecal and colonic enterocytes. Organisms were best identified in calves submitted alive.

ELECTRON MICROSCOPICAL FINDINGS

Numerous cryptosporidia at various stages of development were seen with the TEM (Figures 4, 5 and 6). Trophozoites, schizonts and macrogametes were seen embedded in microvilli and attached to enterocytes (Figures 4, 5 and 6). They were similar to those described by others (2,5,6,14). Microgametes were not identified. Organisms usually displaced or replaced microvilli. Microvilli and

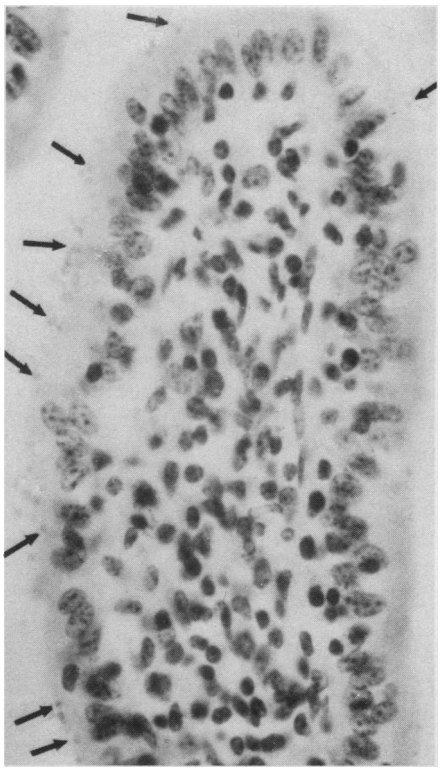

FIGURE 2. *Cryptosporidia* organisms (arrows) embedded in the brush border of a jejunal villus of a three week old calf. Moderate numbers of mononuclear cells have invaded the lamina propria. H & E. X125.

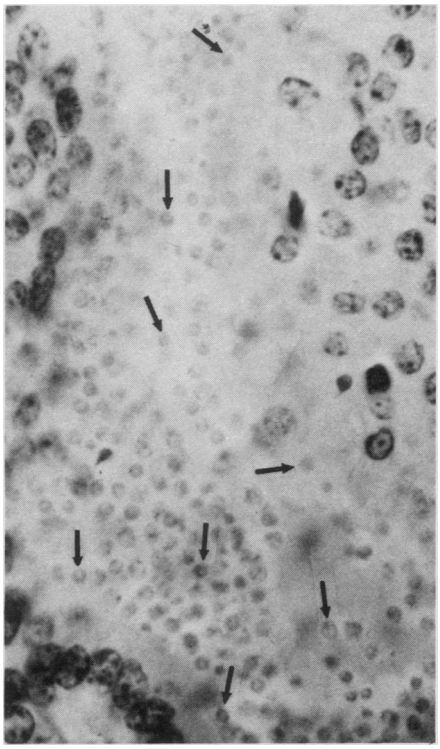

FIGURE 3. A high power photomicrograph of a large number of cryptosporidia organisms (arrows) attached to, and free between, epithelial cells of adjacent villi. H & E. X650.

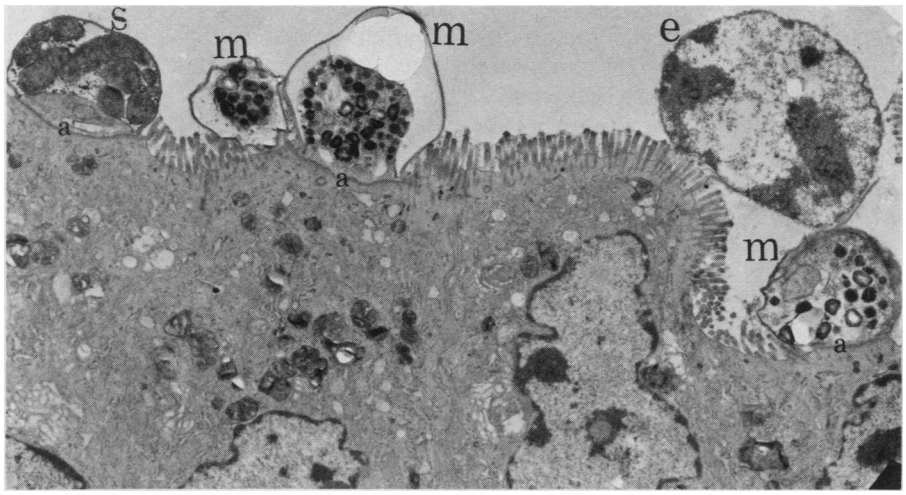

FIGURE 4. A transmission electron micrograph of several cryptosporidia organisms attached to enterocyte cell surfaces. Microvilli and rootlets are absent or distorted in areas of parasite attachment. a = electron dense attachment zone; e = effete epithelial cell; m = macrogamete; s = schizont. N.B. The two electron lucent vacuoles within the central macrogamete are artefacts. TEM. X5080.

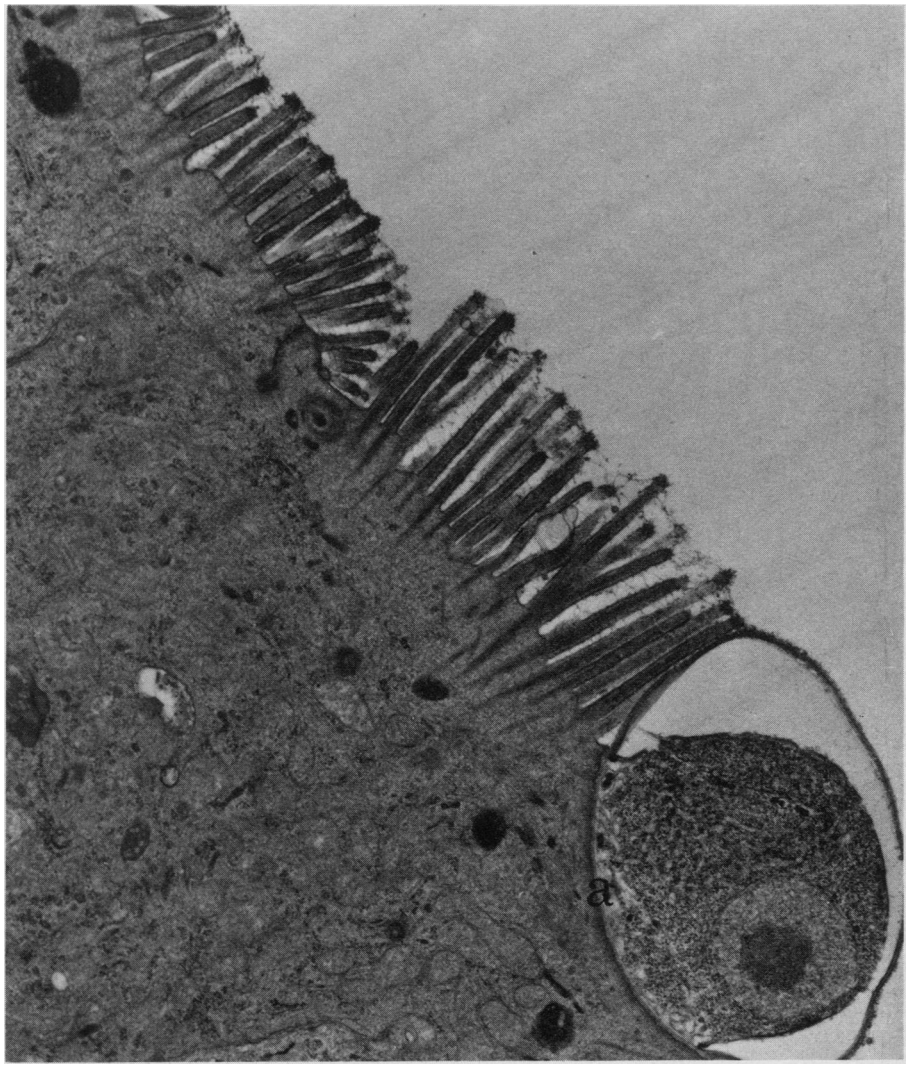

FIGURE 5. Ileal enterocytes with trophozoite (below) and merozoite (above). Merozoite is apparently embedding into or about to be enveloped by a microvillus. a = electron dense attachment zone. TEM. X14 465.

rootlets were absent from sites of parasite attachment (Figures 4 and 5), but were present adjacent to the organisms (Figures 4 and 5). Parasitized enterocytes generally had more apical cytoplasmic lysosomes than nonparasitized cells. Occasionally, effete, epithelial cells and various stages of cryptosporidia were seen in the lumen. Each attached organism was covered by a parasitophorous membrane which consisted of unit membranes of host origin (Figures 4 and 5). Occasionally a merozoite was seen burrowing into a microvillus or apparently about to be enveloped by it (Figures 5 and 6). Organisms were attached to the epithelial cell surface at a specialized, electron dense attachment zone (Figures 4 and 5).

MICROBIOLOGICAL FINDINGS

Large numbers of enterotoxienic *E. coli* were cultured from the intestines of 17 calves (11 submitted dead, six submitted alive). Large numbers of *E. coli* were also cultured from the spleen, kidney and other internal organs of four calves submitted dead and one calf submitted alive. *Pseudomonas* sp was cultured in large numbers from the intestines of six calves (four submitted dead and two alive). *Corynebacterium pyogenes* was isolated from the lung of three calves.

Corona virus particles were identified in intestinal contents of one calf. No viruses were isolated on tissue culture.

Large numbers of cryptosporidia oocysts, 3 to 5 μm in diameter, were seen on fecal smears stained with May-Grünwald Giemsa's stain. Oocysts were round and frequently contained three to seven red granules and a central vacuole (Figure 7). Large numbers of yeast organisms were often present also. These organisms were oval, stained a uniform mauve and had no granules. Using phase contrast illumination with the DSF preparation, cryptosporidia oocysts were seen as bright, silvery, circular bodies.

DISCUSSION

The histological and ultrastructural characteristics of the intestinal parasite identified in these diarrheic calves are consistent with *Cryptosporidium* sp described by several others (2,5,6,15). Enterotoxigenic *E. coli*,

FIGURE 6. Higher magnification of merozoite from figure 5. Note the nucleated portion of the merozoite has already been enveloped by the microvillus. TEM. X65 010.

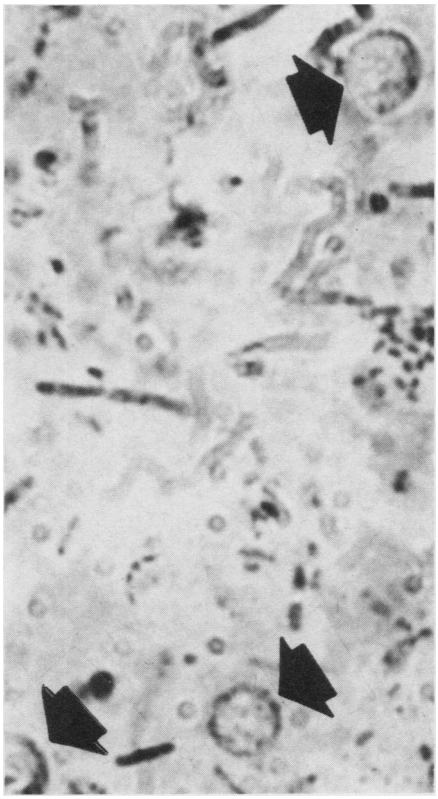

FIGURE 7. Three cryptosporidia oocysts (arrows) among large numbers of bacteria in a fecal smear. The red granules are just visible in the lower right oocyst. Giemsa X1250.

rota and/or coronaviruses have frequently been identified in calves with cryptosporidiosis (1,11). There are several reports in which *Cryptosporidium* sp are the only enteric organisms identified in some or all diarrheic calves (1,2,3,4). Other enteric pathogens were identified in less than 50% of our calves. Enterotoxigenic *E. coli* was concomitantly cultured from 17 calves. Since most of these 17 calves had been submitted dead, there is the distinct possibility of postmortem overgrowth by *E. coli*. A coronavirus was identified in only one calf. The isolation of *Pseudomonas* sp from the intestines of six calves and the identification of large numbers of yeast along with oocysts in some fecal smears were interpreted as opportunistic overgrowth by these organisms after prolonged antibiotic therapy had altered the enteric microflora.

Moderate villous atrophy associated with bovine cryptosporidiosis has been reported (1,4,5). Microvilli were altered or absent where organisms were attached to enterocytes. Both changes combined to reduce the total absorptive surface area of the intestine and could lead to the malabsorptive diarrhea as seen with many viral infections (16).

Controversy still exists over whether the parasite, attached to the luminal border of the enterocyte, is covered by host or parasite membranes (2,8,15). In our study a merozoite was seen apparently burrowing into or about to be enveloped by a microvillus (Figures 5 and 6). We feel that although this juxtaposition of parasite and microvillus may have been a fortuitous association, it is very tempting evidence that the parasitophorous envelope is of host microvillus origin. One may then speculate that the parasite eventually penetrates the microvillus *via* enzymatic breakdown at the point of contact, forms its basal attachment and feeder organelle zones, and the microvillus is reunited above it.

The concentration of our cases to the late fall and winter months may have been an aberration of the submission pattern to the diagnostic laboratory. The concomitant history of calves reared in hutches makes us wonder if the confinement necessary in winter, combined with a lack of sanitation and strict management practices may have contributed to the build up of oocysts in these hutches and allowed continuous reinfection to successive generations of calves. Although hutches were thoroughly cleaned between calves, they frequently, because of climatic conditions, were not moved to a new, clean location.

To date, information on treatment and control of enteric cryptosporidiosis has been spread largely by personal communications among colleagues. Oral dosing of all calves in chronically infected herds with 30 mL of sulphamethazine[1] daily for the first ten to 14 days of life has been used empirically as a preventative measure with apparent success. A few practitioners have had similar success recommending amprolium[2] or decoquinate[3] as alternative prophylactic treatments. Small numbers of oocysts were identified in fecals from medicated calves, and renewed outbreaks of diarrhea occurred when there was any lapse in prophylaxis. Furthermore, once a calf has started scouring, it is our experience that none of the above drugs has effected a cure.

ACKNOWLEDGMENTS

We thank the many practitioners and producers who have submitted these cases. We also thank M. Halfpenny, electron microscopy section, for excellent technical assistance, and Drs. A. Gagnon, D.W. Key and J. Lynch for assistance with virology.

REFERENCES

1. MORIN M, LARIVIÈRE S, LALLIER R. Pathological and microbiological observations made on spontaneous cases of acute neonatal calf diarrhea. Can J Comp Med 1976; 40: 228-240.
2. PHOLENZ J, BEMRICK WJ, MOON HW, CHEVILLE NF. Bovine cryptosporidiosis: A transmission and scanning electron microcope study of some stages in the life cycle and of the host-parasite relationship. Vet Pathol 1978; 15: 417-427.
3. TZIPORI S, CAMPBELL I, SHERWOOD D, SNODGRASS DR. An outbreak of calf diarrhea attributed to cryptosporidial infection. Vet Rec 1980; 107: 579-580.
4. JERRETT IV, SNODGRASS DR. Cryptosporidia associated with outbreaks of neonatal calf diarrhoea. Aust Vet J 1981; 57: 434-435.
5. ANGUS KW, TZIPORI S, GRAY EW. Intestinal lesions in specific-pathogen-free lambs associated with a cryptosporidium from calves with diarrhea. Vet Pathol 1982; 19: 67-78.
6. BARKER IK, CARBONELL PL. *Cryptosporidium agni* sp. n. from lambs, and *Cryptosporidium bovis* sp. n. from a calf, with observations on the oocysts. Z Parasitenkd 1974; 44: 289-298.
7. MASON RW, HARTLEY WJ, TILT L. Intestinal cryptosporidiosis in a kid goat. Aust Vet J 1981; 57: 386-388.
8. KENNEDY GA, KREITNER GL, STRAFUSS AC. Cryptosporidiosis in three pigs. J Am Vet Med Assoc 1977; 170: 348-350.
9. TZIPORI S, ANGUS KW, CAMPBELL I, GRAY EW. *Cryptosporidium:* Evidence for a single-species genus. Infect Immun 1980; 30: 884-886.
10. ANDERSON BC, DONNDELINGER T, WILKINS RM, SMITH J. Cryptosporidiosis in a veterinary student. J Am Vet Med Assoc 1982; 180: 408-409.
11. SNODGRASS DR, ANGUS KW, GRAY EW, KEIR WA. Cryptosporidia associated with rotavirus and an *Escherichia coli* in an outbreak of calf scour. Vet Rec 1980; 106: 458-459.
12. MOON WH, WOODE GN, AHRENS FA. Attempted chemoprophylaxis of cryptosporidiosis in calves. Vet Rec 1982; 110: 181.
13. FLEWETT TH. Electron microscopy in the diagnosis of infectious diarrhea. J Am Vet Med Assoc 1978; 173: 538-543.
14. WILLSON PJ, ACRES SD. A comparison of dichromate solution floatation and fecal smears for diagnosis of cryptosporidiosis in calves. Can Vet J 1982; 23: 240-246.
15. INMAN LR, TAKEUCHI A. Spontaneous cryptosporidiosis in an adult female rabbit. Vet Pathol 1979; 16: 89-95.
16. MOON HW. Mechanisms in the pathogenesis of diarrhea: a review. J Am Vet Med Assoc 1978; 172: 443-448.

[1]Sulfamethazine 12.5%, P.V.U. Inc., Victoriaville, Québec.
[2]Amprol premix, Merck Sharp and Dohme of Canada Ltd., Pointe Claire, Québec.
[3]Decox, May and Baker Canada, Ltd., Toronto, Ontario.