

## Detection of *Cryptosporidium parvum* Oocysts in Bovine Feces by Monoclonal Antibody Capture Enzyme-Linked Immunosorbent Assay

KRZYSZTOF Z. ANUSZ,<sup>1†</sup> PATRICIA H. MASON,<sup>1</sup> MICHAEL W. RIGGS,<sup>2</sup> AND LANCE E. PERRYMAN<sup>1\*</sup>

Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, Washington 99164-7040,<sup>1</sup>  
and Department of Comparative and Experimental Pathology, University of Florida, Gainesville, Florida 32610<sup>2</sup>

Received 10 July 1990/Accepted 24 September 1990

A monoclonal antibody enzyme-linked immunosorbent assay (ELISA) was developed to detect *Cryptosporidium parvum* oocysts in bovine feces. Fecal oocysts were concentrated by centrifugation through Formalin-ethyl acetate solution and captured with monoclonal antibody 18.280.2 reactive with *C. parvum* oocysts. Captured oocysts were detected with goat anti-oocyst serum, following the addition of a peroxidase conjugate of rabbit anti-goat immunoglobulin and *O*-phenylenediamine substrate. The assay was specific for *Cryptosporidium* sp. oocysts and did not detect oocysts of *Eimeria auburnensis*, *Eimeria bovis*, *Eimeria ellipsoidal*, or *Eimeria zuernii*. Assay sensitivity allowed detection of  $3 \times 10^5$  oocysts per ml of feces, compared with  $1 \times 10^6$  oocysts per ml detected by examination of acid-fast-stained fecal smears and  $1 \times 10^3$  oocysts per ml detected by indirect immunofluorescence. The capture ELISA was suitable for diagnostic analysis of bovine fecal samples and for assessment of oocyst shedding in experimentally infected calves. This assay may also prove useful for diagnostic assessment of humans in which cryptosporidiosis is suspected.

Cryptosporidiosis, caused by the sporozoan *Cryptosporidium parvum*, has emerged as an important disease of humans and calves (3, 11-13, 15, 20, 25). Infection of immunocompetent humans results in self-limiting diarrhea and most often occurs in travelers, infants in day-care settings, and individuals working with cattle or other livestock. In contrast, immunodeficient patients with cryptosporidiosis experience severe diarrhea which may be life-threatening (11-13, 15, 25). Persistence of infection, in conjunction with absence of reliable chemotherapy, accounts for the severity of disease in immunodeficient patients (11-13, 15) and has stimulated investigation of passive immunotherapy as a means of treating cryptosporidial infections (9, 14, 34, 45).

Cryptosporidiosis in cattle was first reported in 1971 (32). It is now recognized as a common infection, observed primarily in young calves from birth to 30 days of age (3, 15, 20, 23, 31). *C. parvum* is one of the major causes of neonatal diarrhea in calves and thus contributes to significant economic loss to the beef and dairy cattle industries (23). *C. parvum* oocysts, which are widely distributed in the environment of many cattle operations, are ingested by the newborn calf. Following an incubation period of 72 to 96 hours, diarrhea is observed for 2 to 10 days, during which time oocysts are demonstrable in feces. Oocyst shedding continues for a variable number of days following cessation of diarrhea.

Diagnosis of cryptosporidiosis is based on detection of fecal oocysts. Detection methods include concentration (4, 7, 8, 18, 22, 24, 27, 33, 39, 46, 47, 49), staining of fecal smears (5-8, 10, 18, 19, 21, 30, 35, 36, 42, 43), latex agglutination immunoassay (37), and demonstration by immunofluorescence using monoclonal or polyclonal antibodies reactive with *C. parvum* oocysts (5, 17, 26, 40, 41, 44). Here we report the development of a monoclonal antibody (MAb)-

based capture enzyme-linked immunosorbent assay (ELISA) for the detection of *C. parvum* oocysts in bovine feces.

### MATERIALS AND METHODS

**Production of polyclonal and monoclonal antibodies to *C. parvum* oocysts.** Oocysts were isolated from bovine feces by a combination of sieving, ether extraction, and sodium hypochlorite digestion as previously described (39). An adult female goat was immunized, intramuscularly, three times at biweekly intervals with 2 ml containing  $10^8$  oocysts incorporated in monophosphoryl lipid A-trehalose dimycolate adjuvant (Ribi Immunochemical Research, Inc., Hamilton, Mont.). The titer of serum from this goat was 1:20,000 when evaluated on isolated oocysts in an indirect immunofluorescence assay (39). Preimmunization goat serum was used as a control in all ELISA results included in this report. The control serum did not react with *C. parvum* oocysts by immunofluorescence when tested at a dilution of 1:50.

MAbs were produced as previously described (38). Ascites fluids containing oocyst-reactive MAb were produced by intraperitoneal injection of  $10^7$  cloned hybridoma cells into BALB/c mice previously conditioned by intraperitoneal injection of pristane. Immunoglobulin content of ascites was determined by radial immunodiffusion (TAGO, Burlingame, Calif.). Pooled ascites fluids were clarified and delipidated as previously described (29). Immunoglobulin M (IgM) was precipitated with 10% polyethylene glycol 6000, and the precipitate was dissolved in 0.02 M phosphate-buffered saline (PBS) (pH 7.4) containing 0.5 N NaCl. IgM concentration was determined by single radial immunodiffusion. The reactivity of the MAb against *C. parvum* oocysts and isolated sporozoites was assessed by Western blots (immunoblots) as previously described (38). Since oocysts contain four sporozoites,  $1.25 \times 10^9$  solubilized oocytes per ml and  $5 \times 10^9$  isolated and solubilized sporozoites per ml were compared to provide equivalent sporozoite quantities.

**Preparation of plates for ELISA.** Flat-bottom Immulon I

\* Corresponding author.

† Permanent address: Department of Infectious Diseases, Veterinary Faculty, Agricultural Academy, 03-849 Warsaw, Poland.

plates (Dynatech Laboratories, Inc., Alexandria, Va.) were coated with 5  $\mu\text{g}$  of MAb per ml in 0.05 M sodium carbonate-bicarbonate buffer (pH 9.6) by adding 50  $\mu\text{l}$  per well and incubating them overnight at 4°C. Wells were washed five times with 200  $\mu\text{l}$  of blocking buffer (PBS containing 2% bovine serum albumin [PBS-2% BSA]) per ml at room temperature and 2 min per wash. The plates were then incubated for 2 h at 4°C with 200  $\mu\text{l}$  of blocking buffer per well. Plates were used on the day of preparation or stored at -20°C for up to 7 weeks before use. Immediately before use, the plates were blocked a second time with 100  $\mu\text{l}$  of PBS-10% BSA per well for 30 min at 37°C.

**Preparation of fecal samples for analysis.** Three sources of bovine feces were analyzed in this study. The first consisted of normal feces collected from 1-day-old calves prior to infection with *C. parvum*. These feces were diluted (1 g in 10 ml of PBS) and mixed well to obtain material with the consistency of diarrhetic feces. Known numbers of isolated *C. parvum* oocysts were added to samples of this preparation. The second source of feces was from cattle naturally or experimentally infected with *Cryptosporidium* spp. or with *Eimeria* spp. Feces containing *C. parvum* oocysts were obtained from calves in New York, Florida, and Washington. Feces containing *C. parvum* and *Cryptosporidium muris*-like oocysts (1) were obtained from cattle in Idaho. The third source of feces was from three calves experimentally infected at 3 to 6 days of age with  $10^8$  *C. parvum* oocysts. Daily fecal specimens were collected from each calf prior to and for 13 days after oral exposure to oocysts. These samples were used to evaluate the kinetics of fecal oocyst shedding following experimental infection.

Specimens were processed as follows (48). Ten milliliters of well-mixed feces was sieved through several layers of gauze into a 15-ml conical polypropylene tube (Sarstedt, Princeton, N.J.) and centrifuged at  $500 \times g$  for 2 min. All supernatant was discarded, as well as the pellet down to the 0.75-ml mark of the tube. Nine milliliters of neutral buffered 10% Formalin was added to the tube, followed by 4 ml of ethyl acetate (Baker Chemical Co., Sanford, Maine). The tube was tightly capped and shaken vigorously for 30 s to completely suspend the pellet. The tube was centrifuged at  $450 \times g$  for 2 min. All supernatant was discarded, and the pellet was suspended in 0.5 ml of PBS-2% BSA.

**Performance of ELISA.** Samples (50  $\mu\text{l}$ ) of concentrated fecal pellet were added to appropriate wells and incubated for 30 min at 37°C. Plates were washed five times at 2 min per wash with PBS-2% BSA. Samples (50  $\mu\text{l}$ ) of immune or nonimmune goat serum (1:1,000) were added to appropriate wells. Following 30 min of incubation at 37°C, the plates were washed five times at 2 min per wash with PBS-2% BSA. Peroxidase conjugate of rabbit anti-goat IgG (50  $\mu\text{l}$ ; 1:500; Organon Teknika Corp., West Chester, Pa.) was added to wells and incubated for 30 min. The plates were then washed five times at 2 min per wash with PBS containing 0.05% Tween 20. *O*-Phenylenediamine (50  $\mu\text{l}$ ) (Sigma Chemical Co., St. Louis, Mo.) was added to the wells and incubated in the dark for 15 min at room temperature. Finally, 50  $\mu\text{l}$  of 1 N HCl was added to each well. Reactions were then scored visually, and optical densities at 492 nm ( $\text{OD}_{492}$ ) were measured with an automated plate reader (Titertek Multiskan MCC/340 MKII; Flow Laboratories, McLean, Va.).

**Comparative sensitivities of *C. parvum* oocyst detection assays.** Three assays were compared for their ability to detect *C. parvum* oocysts in feces from calves. The assays were performed on the same day, utilizing identical fecal

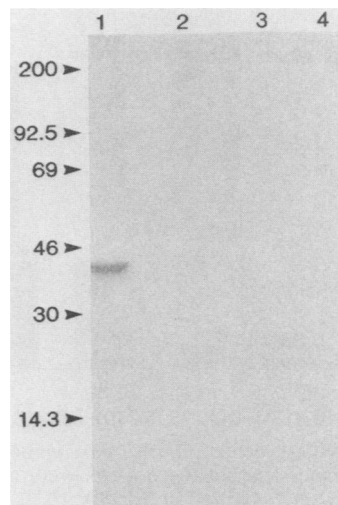


FIG. 1. Western blot analysis of *C. parvum* oocysts (lanes 1 and 3) and isolated *C. parvum* sporozoites (lanes 2 and 4). Lanes 1 and 2 were probed with MAb 18.280.2. Lanes 3 and 4 were probed with an IgM isotype control MAb. The positions of molecular weight standards are indicated on the left.

samples containing known numbers of *C. parvum* oocysts ranging from  $10^1$  to  $10^8$  oocysts per ml, in half-log increments. The MAb capture ELISA was performed as described above. The indirect immunofluorescence assay was performed with a commercial kit (Merifluor Cryptosporidium Kit; Meridian Diagnostics, Cincinnati, Ohio). Nonconcentrated fecal samples were applied to slides provided in the kit. The slides were then processed and examined according to the manufacturer's instructions. The third assay consisted of light microscopic analysis of fecal smears stained with a modified Kinyoun acid-fast stain as previously described (18).

## RESULTS

**Characteristics of MAb 18.280.2.** This IgM MAb binds diffusely to the exterior surface of *C. parvum* oocysts, but not sporozoites, when analyzed by indirect immunofluorescence following procedures we previously described (38). It binds to oocyst antigen migrating with an apparent molecular mass of 40 kDa when analyzed by Western blots (Fig. 1, lane 1). No reaction was observed when the antibody was tested with isolated sporozoites in numbers equivalent to those contained within oocysts (Fig. 1, lane 2).

**Assay configuration.** Checkerboard titrations of MAb and goat anti-oocyst antibody were performed to optimize the assay. Best results were obtained with IgM MAb 18.280.2 at a coating concentration of 5  $\mu\text{g}/\text{ml}$  and a goat serum dilution of 1:1,000. Background was minimized by using polyethylene glycol-precipitated ascites as opposed to untreated ascites containing the MAb and by blocking the coated plates a second time just prior to use. Intact oocysts are captured and retained throughout the incubation and washing steps of the assay. The oocysts are visible in the wells of ELISA plates when examined microscopically.

**Sensitivity and specificity determinations.** Figure 2 shows the relationship of fecal oocyst numbers to  $\text{OD}_{492}$  observed in the ELISA procedure. The oocysts formed a virtual monolayer when feces contained  $\geq 10^7$  oocysts per ml. The  $\text{OD}_{492}$  observed with control goat serum probably reflect absorbance of light by oocysts, as no color was visible by

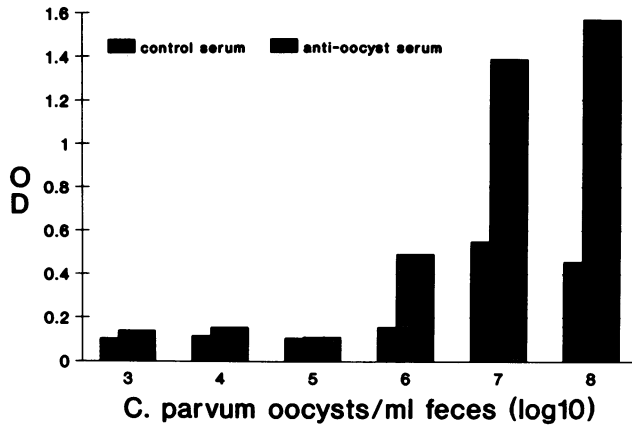


FIG. 2. Sensitivity of detection of *C. parvum* oocysts in bovine feces. Known numbers of isolated oocysts were added to noninfected bovine feces and analyzed by ELISA.

direct observation. Positive results were readily visualized by direct observation when oocyst numbers were  $3 \times 10^5$ /ml of feces or greater.

The comparative sensitivities of three assays for detecting fecal oocysts were determined. ELISA results are analyzable immediately on completion of the assay. The test was more sensitive in detecting oocysts ( $3 \times 10^5$ /ml of feces) than was microscopic examination of acid-fast-stained fecal smears ( $1 \times 10^6$ /ml of feces) when performed by a trained technician examining slides at  $\times 400$  magnification. The indirect immunofluorescence assay was more sensitive ( $10^3$  oocysts per ml of feces) than ELISA, when smears were diligently examined at  $\times 400$  magnification by a trained technician.

Different geographical isolates of *C. parvum* oocysts were also evaluated (Table 1). Oocysts were detected by ELISA in feces from calves infected with Florida, New York, and Washington isolates of *C. parvum*. Specificity was assessed by evaluating feces from calves infected with *Eimeria bovis*, *E. zuernii*, *E. ellipsoidalis*, and *E. auburnensis*. The results presented in Table 1 show the assay is specific for *Cryptosporidium* sp. oocysts. Similar studies were performed on feces from adult cattle containing *C. parvum* oocysts and oocysts with the morphologic characteristics of *C. muris*. Positive reactions were obtained, as would be expected from the presence of *C. parvum* oocysts in the samples. However,

TABLE 1. Specificity of ELISA for detection of *Cryptosporidium parvum* oocysts

Sample	OD <sub>492</sub> <sup>a</sup>
<i>C. parvum</i> <sup>b</sup>	
Washington	0.677
Florida	0.855
New York	1.176
<i>Eimeria</i> spp. <sup>c</sup>	0.052
<i>Eimeria</i> spp. <sup>d</sup>	0.070
Feces without oocysts	0.051

<sup>a</sup> OD<sub>492</sub> of oocysts plus immune goat serum minus OD<sub>492</sub> of oocysts plus control goat serum.

<sup>b</sup> Feces from naturally or experimentally infected calves in the identified states. Variations among OD<sub>492</sub> values reflect differing numbers of oocysts in fecal specimens.

<sup>c</sup> Bovine feces containing oocysts of *E. auburnensis*, *E. bovis*, *E. ellipsoidalis*, and *E. zuernii*.

<sup>d</sup> Bovine feces containing oocysts of *E. bovis*, *E. ellipsoidalis*, and *E. zuernii*.

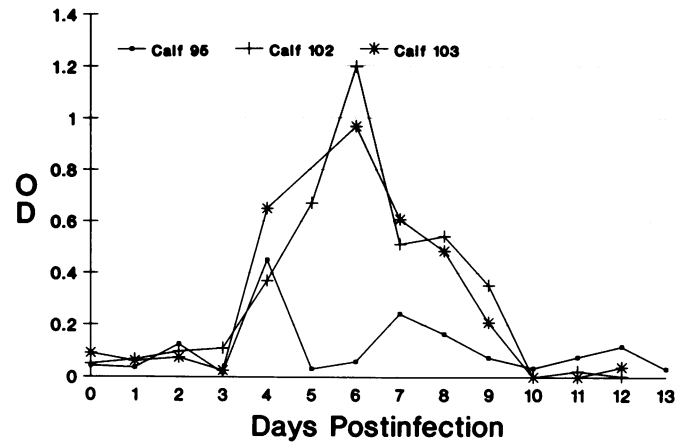


FIG. 3. Kinetics of fecal shedding of *C. parvum* oocysts by calves experimentally infected with  $10^8$  *C. parvum* oocysts on day 0.

oocysts with the characteristics of *C. muris* (1) were also captured on the ELISA plates. Furthermore, MAb 18.280.2 and the immune goat serum employed in the ELISA both reacted with *C. muris*-like oocysts when analyzed by indirect immunofluorescence. Therefore, the ELISA reported here does not distinguish between *C. parvum* oocysts and those produced in adult cattle with abomasal infections with *Cryptosporidium* spp.

**Assessment of oocyst shedding in calves experimentally infected with *C. parvum*.** Figure 3 shows results of fecal oocyst shedding in three calves infected on day 0 with  $10^8$  *C. parvum* oocysts. Calf 95 never demonstrated diarrhea following infection, and only small numbers of oocysts were detected on days 4 and 7 postinfection. Calf 102 exhibited diarrhea on days 4 to 6 and excreted detectable numbers of oocysts on days 4 to 9 postinfection. Calf 103 excreted detectable numbers of oocysts and had diarrhea on days 4 to 9 following exposure to oocysts. All samples from a single calf were analyzed at one time, but the assays for each calf were performed on separate days. An oocyst antigen standard was included with each assay. The mean OD<sub>492</sub> for the standard was 0.641, and the coefficient of variability for the three test days was 3.28%.

## DISCUSSION

The results presented in this report describe a new assay for detecting *C. parvum* oocysts in bovine feces. The assay depends on a MAb to capture oocysts from fecal concentrates. The MAb employed reacts with an oocyst antigen with an apparent molecular mass of 40 kDa and has sufficient affinity to bind and retain oocysts throughout the incubation and wash steps required to perform the ELISA.

The assay is rapid and simple to perform. A two-step centrifugation procedure applied to fecal specimens prior to assay removes fecal debris, concentrates oocysts, and reduces background reactivity. Ten specimens can be processed in approximately 30 min. The ELISA incubation time is 30 min, allowing a complete assay to be performed in 4 h, once the fecal concentrates have been prepared. Results are readily interpretable by direct visual examination of the plate, and a permanent record of results is easily obtained by measuring OD<sub>492</sub>. This eliminates the subjectivity of analysis which may occur when specimens are individually examined and scored following staining procedures or immunofluorescence protocols.

The MAb capture ELISA described in this report is suitable for diagnostic analysis of *C. parvum* infection in calves. Coefficient-of-variability calculations demonstrated the reproducibility of the assay. Sensitivity is sufficient to detect oocysts in animals with clinical signs of disease ( $10^5$  to  $10^7$  oocysts per ml of feces [28]), as well as in calves which have recovered from diarrhea but continue to shed oocysts for several days. The assay detects *C. parvum* oocysts from geographically dispersed areas of the United States, suggesting that the antigen defined by the capture MAb and the antigen(s) recognized by the goat anti-oocyst serum are conserved. Specificity is acceptable since the assay did not capture or detect *Eimeria* sp. oocysts, another cause of diarrhea in calves (16).

We found the test did not distinguish *C. parvum* from *C. muris*-like oocysts. The latter organism has been demonstrated to infect the abomasum of adult cattle (1) but is not associated with diarrhea caused by intestinal infection with *C. parvum* in young calves. Since *C. parvum* is not considered an important cause of diarrhea in adult cattle, it is unlikely that the reported assay would be employed in the diagnostic evaluation of mature cattle. Therefore, inability to distinguish between *C. parvum* and *C. muris* should not limit the usefulness of this MAb capture ELISA for detection of *Cryptosporidium* oocysts.

*Cryptosporidium parvum* is known to infect several mammalian species including humans, and bovine sources of *C. parvum* have been reported to cause disease in laboratory personnel, veterinary students, and livestock workers (2, 11–13, 15, 25). We have used the ELISA to detect *C. parvum* oocysts in diarrheic feces from calves, llamas, and young horses with severe combined immunodeficiency. The assay may also prove useful for diagnostic assessment of humans in which cryptosporidiosis is suspected.

#### ACKNOWLEDGMENTS

We are grateful to B. Anderson, Caldwell Veterinary Teaching Center, University of Idaho, Caldwell, and W. Foreyt, Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, for providing specimens containing *C. muris*-like oocysts. We thank M. Frongillo, Department of Microbiology, Immunology, and Parasitology, New York State College of Veterinary Medicine, Cornell University, Ithaca, N.Y., for providing *C. parvum* oocysts from calves in New York, and D. Reduker, Department of Pathology, Colorado State University, Fort Collins, for supplying specimens containing *E. bovis*. We acknowledge the excellent technical assistance provided by Roberta Konzek and John Ahmann.

This work was supported by Public Health Service grant AI 25731 from the National Institutes of Health, grant 86-CRSR-2-2861 from the U.S. Department of Agriculture, Immunology training grant AI 07025 from the National Institutes of Health, grant 10A-3073-0845 from the Washington State Agricultural Research Center, and funds from the Washington Dairy Products Commission.

#### LITERATURE CITED

- Anderson, B. C. 1987. Abomasal cryptosporidiosis in cattle. *Vet. Pathol.* **24**:235–238.
- Anderson, B. C., T. Donndelinger, R. M. Wilkins, and J. Smith. 1982. Cryptosporidiosis in a veterinary student. *J. Am. Vet. Med. Assoc.* **180**:408–409.
- Anderson, B. C., and R. F. Hall. 1982. Cryptosporidial infection in Idaho dairy calves. *J. Am. Vet. Med. Assoc.* **181**:484–485.
- Arrowood, M. J., and C. R. Sterling. 1987. Isolation of *Cryptosporidium* oocysts and sporozoites using discontinuous sucrose and isopycnic Percoll gradients. *J. Parasitol.* **73**:314–319.
- Arrowood, M. J., and C. R. Sterling. 1989. Comparison of conventional staining methods and monoclonal antibody-based methods for *Cryptosporidium* oocyst detection. *J. Clin. Microbiol.* **27**:1490–1495.
- Asahi, H., M. Kumada, K. Kato, and T. Koyama. 1988. A simple staining method for cryptosporidian oocysts and sporozoites. *Jpn. J. Med. Sci. Biol.* **41**:117–121.
- Baron, E. J., C. Schenone, and B. Tanenbaum. 1989. Comparison of three methods for detection of *Cryptosporidium* oocysts in a low-prevalence population. *J. Clin. Microbiol.* **27**:223–224.
- Baxby, D., and N. Blundell. 1983. Sensitive, rapid, simple methods for detecting *Cryptosporidium* in faeces. *Lancet* **ii**:1149.
- Bjorneby, J. M., M. W. Riggs, and L. E. Perryman. 1990. *Cryptosporidium parvum* merozoites share neutralization-sensitive epitopes with sporozoites. *J. Immunol.* **145**:298–304.
- Bronsdon, M. A. 1984. Rapid dimethyl sulfoxide-modified acid-fast stain of *Cryptosporidium* oocysts in stool specimens. *J. Clin. Microbiol.* **19**:952–953.
- Current, W. L. 1983. Human cryptosporidiosis. *N. Engl. J. Med.* **309**:1326–1327.
- Current, W. L. 1988. The biology of *Cryptosporidium*. *ASM News* **54**:605–611.
- Current, W. L., N. C. Reese, J. V. Ernst, W. S. Bailey, M. B. Heyman, and W. M. Weinstein. 1983. Human cryptosporidiosis in immunocompetent and immunodeficient persons. Studies of an outbreak and experimental transmission. *N. Engl. J. Med.* **308**:1252–1257.
- Fayer, R., L. E. Perryman, and M. W. Riggs. 1989. Hyperimmune bovine colostrum neutralizes *Cryptosporidium* sporozoites and protects mice against oocyst challenge. *J. Parasitol.* **75**:151–153.
- Fayer, R., and B. L. P. Ungar. 1986. *Cryptosporidium* spp. and cryptosporidiosis. *Microbiol. Rev.* **50**:458–483.
- Fitzgerald, P. R. 1962. Coccidia in Hereford calves on summer and winter ranges and in feedlots in Utah. *J. Parasitol.* **48**:347–351.
- Garcia, L. S., T. C. Brewer, and D. A. Bruckner. 1987. Fluorescence detection of *Cryptosporidium* oocysts in human fecal specimens by using monoclonal antibodies. *J. Clin. Microbiol.* **25**:119–121.
- Garcia, L. S., D. A. Bruckner, T. C. Brewer, and R. Y. Shimizu. 1983. Techniques for the recovery and identification of *Cryptosporidium* oocysts from stool specimens. *J. Clin. Microbiol.* **18**:185–190.
- Garza, D., R. L. Hopfer, C. Eichelberger, S. Eisenbach, and V. Fainstein. 1984. Fecal staining methods for screening *Cryptosporidium* oocysts. *J. Med. Technol.* **1**:560–563.
- Heine, J., J. F. L. Pohlenz, H. W. Moon, and G. N. Woode. 1984. Enteric lesions and diarrhea in gnotobiotic calves monoinfected with *Cryptosporidium* species. *J. Infect. Dis.* **150**:768–775.
- Henricksen, S. A., and J. F. L. Pohlenz. 1981. Staining of cryptosporidia by a modified Ziehl-Neelsen technique. *Acta Vet. Scand.* **22**:594–596.
- Heyman, M. B., L. K. Shigekuni, and A. J. Ammann. 1986. Separation of *Cryptosporidium* oocysts from fecal debris by density gradient centrifugation and glass bead columns. *J. Clin. Microbiol.* **23**:789–791.
- House, J. A. 1978. Economic impact of rotavirus and other neonatal disease agents of animals. *J. Am. Vet. Med. Assoc.* **173**:573–576.
- Kilani, R. T., and L. Sekla. 1987. Purification of *Cryptosporidium* oocysts and sporozoites by cesium chloride and Percoll gradients. *Am. J. Trop. Med. Hyg.* **36**:505–508.
- Ma, P. 1986. Cryptosporidium—biology and diagnosis. *Adv. Exp. Med. Biol.* **202**:135–152.
- McLauchlin, J., D. P. Casemore, T. G. Harrison, P. J. Gerson, D. Samuel, and A. G. Taylor. 1987. Identification of *Cryptosporidium* oocysts by monoclonal antibody. *Lancet* **i**:51.
- McNabb, S. J. N., D. M. Hensel, D. F. Welch, H. Heijbel, G. L. McKee, and G. R. Istre. 1985. Comparison of sedimentation and flotation techniques for identification of *Cryptosporidium* sp. oocysts in a large outbreak of human diarrhea. *J. Clin. Microbiol.* **22**:587–589.
- Moon, H. W., and D. B. Woodmansee. 1986. Cryptosporidiosis.

- J. Am. Vet. Med. Assoc. **189**:643-646.
29. Neoh, S. H., C. Gordon, A. Potter, and H. Zola. 1986. The purification of mouse monoclonal antibodies from ascitic fluid. *J. Immunol. Methods* **91**:231-235.
  30. Ongerth, J. E., and H. H. Stibbs. 1987. Identification of *Cryptosporidium* oocysts in river water. *Appl. Environ. Microbiol.* **53**:672-676.
  31. Ongerth, J. E., and H. H. Stibbs. 1989. Prevalence of *Cryptosporidium* infection in dairy calves in western Washington. *Am. J. Vet. Res.* **50**:1069-1070.
  32. Panciera, R. J., R. W. Thomassen, and F. M. Garner. 1971. Cryptosporidial infection in a calf. *Vet. Pathol.* **8**:479-484.
  33. Perry, J. L., J. S. Mathews, and G. R. Miller. 1990. Parasite detection efficiencies of five stool concentration systems. *J. Clin. Microbiol.* **28**:1094-1097.
  34. Perryman, L. E., M. W. Riggs, P. H. Mason, and R. Fayer. 1990. Kinetics of *Cryptosporidium parvum* sporozoite neutralization by monoclonal antibodies, immune bovine serum, and immune bovine colostrum. *Infect. Immun.* **58**:257-259.
  35. Pohjola, S. 1984. Negative staining method with nigrosin for the detection of cryptosporidial oocysts: a comparative study. *Res. Vet. Sci.* **36**:217-219.
  36. Pohjola, S., L. Jokipii, and A. M. M. Jokipii. 1984. Dimethylsulphoxide-Ziehl-Neelsen staining technique for detection of cryptosporidial oocysts. *Vet. Rec.* **115**:442-443.
  37. Pohjola, S., E. Neuvonen, A. Niskanen, and A. Rantama. 1986. Rapid immunoassay for detection of *Cryptosporidium* oocysts. *Acta Vet. Scand.* **27**:71-79.
  38. Riggs, M. W., T. C. McGuire, P. H. Mason, and L. E. Perryman. 1989. Neutralization-sensitive epitopes are exposed on the surface of infectious *Cryptosporidium parvum* sporozoites. *J. Immunol.* **143**:1340-1345.
  39. Riggs, M. W., and L. E. Perryman. 1987. Infectivity and neutralization of *Cryptosporidium parvum* sporozoites. *Infect. Immun.* **55**:2081-2087.
  40. Rose, J. B., L. K. Landeen, K. R. Riley, and G. P. Gerba. 1989. Evaluation of immunofluorescence techniques for detection of *Cryptosporidium* oocysts and *Giardia* cysts from environmental samples. *Appl. Environ. Microbiol.* **55**:3189-3196.
  41. Rusnak, J., T. L. Hadfield, M. M. Rhodes, and J. K. Gaines. 1989. Detection of *Cryptosporidium* oocysts in human fecal specimens by an indirect immunofluorescence assay with monoclonal antibodies. *J. Clin. Microbiol.* **27**:1135-1136.
  42. Scott, C. D. 1988. Screening faecal smears for *Cryptosporidium* and *Isospora belli* using a modified Ziehl-Neelsen stain. *Aust. J. Med. Lab. Sci.* **9**:80-82.
  43. Smith, H. V., A. McDiarmid, A. L. Smith, A. R. Hinson, and R. A. Gilmour. 1989. An analysis of staining methods for the detection of *Cryptosporidium* spp. oocysts in water-related samples. *Parasitology* **99**:323-327.
  44. Stibbs, H. H., and J. E. Ongerth. 1986. Immunofluorescence detection of *Cryptosporidium* oocysts in fecal smears. *J. Clin. Microbiol.* **24**:517-521.
  45. Ungar, B. L. P., D. J. Ward, R. Fayer, and C. A. Quinn. 1990. Cessation of *Cryptosporidium*-associated diarrhea in an AIDS patient after treatment with hyperimmune bovine colostrum. *Gastroenterology* **98**:486-488.
  46. Waldman, E. S., S. Tzipori, and J. R. L. Forsyth. 1986. Separation of *Cryptosporidium* species oocysts from feces by using a Percoll discontinuous density gradient. *J. Clin. Microbiol.* **23**:199-200.
  47. Willson, P. J., and S. D. Acres. 1982. A comparison of dichromate solution flotation and fecal smears for diagnosis of cryptosporidiosis in calves. *Can. Vet. J.* **23**:240-246.
  48. Young, K. H., S. L. Bullock, D. M. Melvin, and C. L. Spruill. 1979. Ethyl acetate as a substitute for diethyl ether in the Formalin-ether sedimentation technique. *J. Clin. Microbiol.* **10**:852-853.
  49. Zierdt, W. S. 1984. Concentration and identification of *Cryptosporidium* sp. by use of a parasite concentrator. *J. Clin. Microbiol.* **20**:860-861.