Prophylactic use of decoquinate for infections with *Cryptosporidium parvum* in experimentally challenged neonatal calves

Dale A. Moore, DVM, PhD, DACVPM; E. Robert Atwill, DVM, PhD; John H. Kirk, DVM, MPVM; Dipa Brahmbhatt, VMD; Laura Herrera Alonso, DVM; Lingling Hou, DVM, MS; Matthew D. Singer; Tracy D. Miller

Objective—To evaluate the effect of daily oral administration of decoquinate to neonatal calves experimentally challenged with various numbers of *Cryptosporidium parvum* oocysts.

Design—Clinical trial.

Animals—75 calves.

Procedure—Calves were purchased from a commercial dairy during a 5-week period. Calves were housed in individual hutches and fed milk replacer with or without decoquinate (2 mg/kg [0.9 mg/lb per day]). Calves were randomly assigned to treatment and 1 of 5 challenge groups (0, 50, 100, 1000, or 10,000 *C parvum* oocysts in 60 mL of saline [0.9% NaCl] solution administered PO on the day after arrival). Calves were maintained in the study for as long as 28 days. Calves were clinically assessed for diarrhea and dehydration. Fecal samples were submitted for oocyst enumeration 3 times each week.

Results—Treatment did not affect number of days to first watery feces (diarrhea), number of days to first oocyst shedding, or duration of diarrhea or oocyst shedding. Duration of oocyst shedding was significantly associated with challenge dose of oocysts administered to calves and number of days to first oocyst shedding. Duration of diarrhea and number of days to first oocyst shedding were significantly associated with week of arrival and number of days to first watery diarrhea.

Conclusions and Clinical Relevance—Daily treatment with decoquinate at the dosage used in this study did not affect oocyst shedding or clinical signs associated with cryptosporidiosis. However, there was an indication that if the number of oocysts calves received could be reduced, then the duration of oocyst shedding and, hence, environmental loading of *C parvum* oocysts could be reduced. (*J Am Vet Med Assoc* 2003;223:839–845)

Cryptosporidiosis is a leading cause of diarrhea in preweaned calves and represents an important public health risk.[1,2] *Cryptosporidium parvum* infection typically occurs between the first and third week of age and is often self-limiting.[3-5] The organism may be observed in young calves as the only etiologic agent associated with diarrhea and death caused by dehydration and malnutrition.[6] Cryptosporidiosis may result in death of neonatal calves if there is a failure to provide supportive treatment or if the calf is also infected with other disease agents.

Although many therapeutic and prophylactic agents have been tested in vivo for efficacy against *C parvum*,[7] only paromomycin[8] and halofuginone[9,10] were found to be effective in calves. These 2 treatments are not licensed for use in food animals in the United States.[11] Results of 1 study[12] performed with Holstein calves and decoquinate and 1 study[13] with mice and lasalocid indicated that coccidiostats may reduce oocyst shedding and clinical signs of cryptosporidiosis. Many veterinarians have experimented with feeding decoquinate in milk or milk replacer to neonatal calves at rates higher than the labeled dose for coccidiosis control for the first 4 weeks of life. Dairy producers have the impression that the use of decoquinate reduces clinical signs, and perhaps oocyst shedding, from cryptosporidiosis. If an intervention could reduce *C parvum* oocyst shedding, it could potentially reduce the risk of transmission to other calves, thereby reducing morbidity and mortality rates, treatment costs, and the environmental loading rate of oocysts produced per calf.

Decoquinate is a coccidiostatic drug and is effective at controlling or reducing the severity of coccidiosis in cattle after weaning.[14] In preweaned calves, administration of decoquinate results in reduced coccidial oocyst shedding and increased weight gains, compared with untreated calves.[15] The recommended dosage for control of coccidiosis is 0.5 mg/kg (0.23 mg/lb per day). Experimentally, decoquinate was found to have little activity against *C parvum* in vitro or in vivo in suckling mice.[16] One study[12] with a small number of experimentally challenged calves suggested that treatment might be effective if decoquinate was fed at 2.5 mg/kg (1.14 mg/lb per day). Another study[17] investigated the efficacy of decoquinate (2.5 mg/kg per day for 21 days) in neonatal goat kids experimentally infected with 10^6 oocysts derived from infected calves. Diarrhea was not observed in the medicated group but was observed in the nonmedicated, challenged kids.

From the Veterinary Medicine Teaching and Research Center, School of Veterinary Medicine, University of California, Davis, CA 95616 (Moore, Atwill, Kirk, Brahmbhatt, Herrera Alonso, Hou); the Department of Dairy Science, California Polytechnic University, San Luis Obispo, CA 93407 (Singer); and the Department of Animal Science, California State University, Chico, CA 95929 (Miller).

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Address correspondence to Dr. Moore.

Medicated kids also gained more weight and had a lower peak of oocyst shedding (approx 1.2×10^6 oocysts/g of feces) 6 days after administration of decoquinate, compared with nonmedicated kids (1.8×10^6 oocysts/g of feces). Every kid in the study shed oocysts; however, treated kids shed for 11.6 days, compared with 14.1 days for the nonmedicated controls.

The reported experimental studies each used a single challenge dose; however, the dose was different among studies. It is possible that different oocyst challenge doses could affect the efficacy of the treatment. The purpose of the study reported here was to evaluate the extent to which feeding decoquinate alters the oocyst shedding pattern (onset, duration, and intensity of shedding), the clinical course of cryptosporidiosis, or body weight gain in neonatal calves experimentally challenged with *C parvum* oocysts.

Materials and Methods

Calves—The study was conducted at the Veterinary Medicine Teaching and Research Center, University of California, Tulare, Calif. The study was approved by the institutional animal care and use committee. To identify clinical disease problems at the farm of origin, reports from all submissions to the local animal diagnostic laboratory were reviewed for 2 months before and 2 months after initiation of the study. No cryptosporidiosis was identified from 3 calves submitted to the local diagnostic laboratory during that time period.

Prior to initiation of the study, 5 calves were purchased from the source dairy farm, fed milk replacer, housed where study calves would be housed, and maintained for 17 days. Fecal samples from these calves were collected every other day for enumeration of *C parvum* oocysts to determine if study calves may be already infected at the time of arrival or if the study housing area provided a source of infection. No *C parvum* oocysts were detected in any of the fecal samples for the 17 days. Seventy-five Jersey bull calves, obtained at 1 to 24 hours of age, were purchased from 1 dairy in central California from June 26 to July 26, 2001. Calves were to be given colostrum at the dairy (2 L at birth and another 2 L if calves remained at the dairy at 12 hours of age). Calves were held in a pen outside the calving area before being transported to the study site. All calves were weighed and given a colostrum substitute[a] on arrival at the study site. Calves were housed in individual calf hutches that were separated by at least 1 meter on ground that had not had livestock on it for > 5 years. Hutches had slatted sides, had a roof over the rear half of the hutch, and were bedded with rice hulls. Milk replacer was fed to calves twice daily by bottle (10% of body weight/d). Calves that refused to suckle were fed through an esophageal feeder. Water in buckets was available to calves at all times. Calf-starter grain was provided to all calves within the first week of life and continued throughout the 4-week study period.

Calf caregivers were not masked to treatment or challenge; they mixed decoquinate into the treated calves' milk replacer and knew which calves were challenged. An individual bottle was used for each calf throughout the study. Caregivers separated bottles used by nonchallenged calves from those of challenged calves. Bottles and esophageal feeders were cleaned with soap and water and rinsed with ammonia and water after each feeding. All nonchallenged calves were fed before challenged calves. Separate disposable gloves were worn between handling of calves, and disposable coveralls and plastic boots were used if caregivers were required to enter hutches to treat calves or take fecal samples. Separate esophageal feeders were used for challenged and nonchallenged calves.

Experimental challenge and treatment—Calves were randomly assigned to 1 of 10 challenge-treatment groups. Random numbers were generated by use of computer software.[b] Decoquinate[c] was fed to calves in milk replacer twice daily at a rate of 2 mg/kg (0.9 mg/lb) from the day after arrival for a maximum of 28 days. The control group received only milk replacer. Within each treatment group, calves were challenged only once orally with a suspension of 0 (group E) or 50, 100, 1,000, or 10,000 (groups A, B, C, and D, respectively) viable *C parvum* oocysts through a 60-mL catheter-tipped syringe on the day of arrival with the evening feeding.

Oocysts used for challenge were isolated from naturally infected dairy calves from 1 commercial dairy (not the source herd). Fecal samples were screened for high concentrations of oocysts by use of an acid-fast procedure.[3] By use of a modification of an isolation technique,[18] fecal samples containing *C parvum* were passed sequentially through size 40, 100, 200, and 270 mesh sieves and then poured into 50-mL centrifuge tubes. Tubes were centrifuged at $1,000 \times g$ for 10 minutes, the supernatant was aspirated, and the sediment was resuspended in sterile deionized water. The oocyst suspension was layered onto the top of a double sucrose layer (specific gravity, 1.103 and 1.064 g/mL, respectively) in 50-mL centrifuge tubes and centrifuged at $1,500 \times g$ for 30 minutes. Top and middle layers from each tube were pooled into a 15-mL centrifuge tube and centrifuged at $1,000 \times g$ for 10 minutes. The final pellet was washed twice in sterile deionized water with 0.2% (v/v) Tween 20[d] and resuspended in an antimicrobial solution (100 mL of sterile deionized water with 0.25 mg of amphotericin B, 6 mg of penicillin G, and 10 mg of streptomycin sulfate) so that the final working solution of oocysts was approximately 10^3 oocysts/mL, as determined by use of a phase-contrast hemocytometer and 8 separate counts. Oocysts were stored for no more that 14 days at 4°C before use, during which time their viability was monitored. Only oocyst solutions with ≥ 90% viability, as determined by complete or partial excystation,[19] were used for oral inoculation after dilution to the appropriate total challenge dose.

Clinical assessments—Calves were assessed for fecal consistency (score of 1, 2, or 3 for formed, semiformed, or watery feces, respectively), attitude, appetite, and hydration status (determined by skin tent response). Primary observers (DB, JK, DM) assessed clinical criteria for calves on Mondays, Wednesdays, and Fridays for 4 weeks after challenge. Additional clinical assessments were made by clinicians when requested by calf caregivers. Clinical assessments were made without knowledge of treatment or challenge group assignment. Treatment records were kept for electrolyte therapy (PO), antimicrobials, and anti-inflammatory drugs when administered to calves. Body weights were obtained with a scale that was accurate to the nearest pound on days 1 and 28 and converted to kilograms.

A blood sample was obtained from each calf the day after arrival to assess passive immunity status. Serum **total protein (TP)** concentrations were determined by use of a refractometer. Packed cell volume was evaluated from blood collected in EDTA collection tubes. To minimize misclassification of failure of passive transfer caused by assessing TP in a potentially dehydrated calf, calculations of dehydration on

the basis of PCV and calculations of an adjusted TP on the basis of dehydration were made by use of models.[20] An adjusted TP concentration of ≤ 5.0 g/dL was classified as failure of passive transfer.

All calves with fecal scores of 2 or 3 were provided electrolytes[e] in water orally by use of bottles (0.75 to 1.0 L) twice daily in addition to milk replacer. Calves that refused to eat were fed milk replacer and electrolytes through an esophageal feeder twice daily. For animal welfare reasons, calves with signs of depression and that did not respond to electrolyte administration (unable to stand in 1 day) were euthanized with a solution of pentobarbital sodium and phenytoin sodium[f] (390 mg/4.54 kg and 50 mg/4.54 kg, respectively, administered at 1 mL/10 lb, IV). Eight calves that died or were euthanized during the study were submitted to a laboratory[g] for postmortem examination.

Sample collection, oocyst detection, and enumeration— Fecal samples were collected per rectum on Monday, Wednesday, and Friday of each week and assessed for oocyst shedding by use of quantitative immunofluorescent microscopy.[21] Percentage recovery was estimated by comparing these fecal samples to oocyst-spiked fecal samples to estimate the intensity of shedding (oocysts/g of feces). Flies (*Musca domestica)* were captured during the study, and tissues were submitted for histologic examination (H & E stain) for identification of *C parvum* oocysts. Oocysts found on flies were not enumerated.

Statistical analyses—Data were entered into a computerized spreadsheet program[b] and analyzed by use of a statistical software package.[h] Risk factors potentially associated with death (challenge dose and treatment) were analyzed by use of χ^2 statistics. Study variables included the number of days to first oocyst shedding, number of days to peak oocyst shedding, total number of days shedding oocysts, number of days required to reach a fecal score of 3 (watery feces), number of oocysts at peak oocyst shedding, log of the number of oocysts at peak oocyst shedding, number of days to removal (death, euthanasia, or end of the study), number of days required to reach a fecal score of 1 (formed feces) from a fecal score of 3 (watery feces), and **average daily gain (ADG)**. Treatment group, challenge dose, failure of passive transfer, oocyst shedding parameters, clinical parameters, and week of arrival were used as potential covariates in general linear models[22] by use of backwards elimination to assess the effects of treatment and dose and associations with clinical and oocyst shedding outcomes. Variables were removed from the model if the *P* value from the partial *F* test was > 0.10. Only calves with complete data were used in the analyses.

Results

Seventy-five calves were enrolled in the study at a rate of 3 to 5 calves/d, 4 d/wk. Forty-three calves completed the study, and 32 (42%) died or were euthanatized before 28 days of age **(Table 1)**. There was no significant difference in mortality rate between treated and nontreated calves (relative risk, 0.67; 95% **confidence interval [CI]**, 0.39 to 1.14; *P* = 0.13). There was no significant association between mortality rate and dose of oocysts (*P* = 0.88). There were no significant differences in the proportion of calves that were euthanized among treatment groups. Of 32 calves that died, 3 that died before day 3 died from atresia coli (n = 1), respiratory dysfunction (1), and unknown causes (1). All subsequent evaluations and analyses were performed on the remaining calves. Of 29 calves that died after day 3, 8 were submitted for necropsy. *Salmonella* sp, *C parvum*, rotavirus, and coronavirus were recovered from these calves. All calves that died arrived in weeks 3 and 4 of the study, were 6 to 14 days old, died from July 10 to 27, and received oocyst doses ranging from 0 to 1,000; 3 calves had received treatment. All calves had cryptosporidiosis, 5 calves had *Salmonella* spp isolated from the intestines, 4 had abomasitis, and 4 had coronavirus infections. These calves were selected from 2 cohorts (arrival weeks 3 and 4 of the study) on the basis of convenience during a period with a high number of deaths, independent of treatment or challenge group. Histologic examinations of tissue specimens from 15 flies captured during the study revealed *Cryptosporidium* spp oocysts on the legs and mouthparts.

There was a significant (*P* = 0.02) association between week of arrival and mortality rates in all groups. Two of 9 calves died during the first week of arrival, and 4 of 19, 7 of 17, 9 of 17, and 10 of 13 calves died during weeks 2 through 5 of arrival, respectively. Mean ± SD adjusted TP concentration for all calves was 6.2 ± 1.5 g/dL. Twelve calves were reclassified regarding passive immunity transfer status on the basis of adjusted TP concentrations because of dehydration that was calculated from PCV. Seventeen (24%) calves were classified as having failure of passive transfer of immunity. There was no significant association between failure of passive transfer and mortality rate for all calves (*P* = 0.30).

Mean ± SD values for continuous variables measured in the study were determined **(Table 2)**. All calves that survived the first week of the study, including those not experimentally challenged, became infected and shed oocysts. Thirty-five calves that completed the study and had before and after body weight measurements gained a mean of 0.47 kg/d (1.03 lb/d). Average daily

Table 1—Mortality rates in neonatal calves fed milk replacer with (Decoq) and without (Con) decoquinate (2 mg/kg [0.9 mg/lb] per day) for 28 days after oral administration of single challenge doses containing different numbers of *Cryptosporidium parvum* oocysts

Challenge dose (oocysts/g)	Live		Dead		Euthanatized		Mortality rate (proportion)	
	Decoq	Con	Decoq	Con	Decoq	Con	Decoq	Con
50	6	4	2	1	1	3	0.33	0.50
100	2	3	3	3	0	2	0.60	0.63
1,000	4	4	1	2	3	2	0.50	0.50
10,000	6	5	1	2	1	1	0.25	0.38
0	7	2	0	1	1	2	0.13	0.60
Total	**25**	**18**	**7**	**9**	**6**	**10**		

Table 2—Observations from neonatal calves fed milk replacer with (treatment) and without (control) decoquinate (2 mg/kg per day) for 28 days after oral administration of single challenge dose containing different numbers of *C parvum* oocysts

	Control			Treatment		
Measurement	**No. of calves**	**Mean**	**SD**	**No. of calves**	**Mean**	**SD**
Adjusted total protein (g/dL)	37	6.2	1.4	37	6.3	1.5
Days to removal	37	19.4	8.7	37	20.2	8.7
Days to first oocyst shedding	37	7.4	1.2	34	7.5	2.2
Days to watery feces	25	5.6	4.2	35	5.1	3.8
Number of days shedding	22	14.7	3.4	25	15.0	3.6
Days to formed feces[a]	21	10.2	6.4	25	13.6	5.4
Log of peak oocyst shedding[b]	25	7.5	0.3	20	7.6	0.3
Average daily gain (kg)	17	0.6	0.34	18	0.4	0.3

[a]No. of days after detection of watery feces until formed feces were observed. [b]Log_{10} of the highest value of oocysts per gram of feces detected per calf.

gain was not significantly associated with treatment group, challenge dose, or any other variable ($P > 0.10$).

For calves that survived beyond the first week of the study, clinical signs of watery feces preceded detection of oocysts by a mean of almost 2 days ($P = 0.001$). The number of days from the first sign of watery feces to finding formed feces was 2 days less than the total number of days a calf shed detectable oocysts ($P = 0.02$). Median daily fecal scores did not differ by treatment group (**Fig 1**) or challenge group (**Fig 2**) with time. However, those calves that acquired infection through the environment (unchallenged Group E) occasionally had lower median peak fecal scores, compared with experimentally challenged calves, although the difference was not significant ($P = 0.10$).

Peak oocyst shedding and the log of peak oocyst shedding were evaluated for association with treatment, challenge dose, and the number of days to first oocyst shedding, controlling for week of arrival. No significant associations were found. In addition, there were no significant differences between treatment groups or among challenge groups with regards to the number of days to peak oocyst shedding ($P = 0.10$).

From multivariate analyses, the number of days a calf shed *C parvum* oocysts was not significantly associated with treatment, week of arrival, or failure of passive transfer but was significantly associated with challenge dose and the number of days to first oocyst shedding (**Table 3**). As challenge dose increased, the number of days shedding oocysts increased. The sooner calves began shedding oocysts, the higher the total number of days of shedding. These 2 variables explained 21% of the variation in the total number of days a calf shed oocysts.

The total number of days of diarrhea (time from when a calf first had watery feces to the day the calf had its first formed feces) was not significantly associated with treatment, failure of passive transfer, or dose but was significantly associated with the week of arrival and the total number of days after challenge that a calf started with watery diarrhea. As the number of days until diarrhea began increased, the total number of days of diarrhea decreased by 1 day.

The number of days after challenge until the first oocyst was shed was not significantly associated with treatment group, failure of passive transfer, or challenge dose. Days to first shedding were associated with week of arrival and the number of days until watery diarrhea first appeared after challenge. As the days to first appearance of watery diarrhea increased (controlling for week of arrival), the days to first oocyst shedding increased.

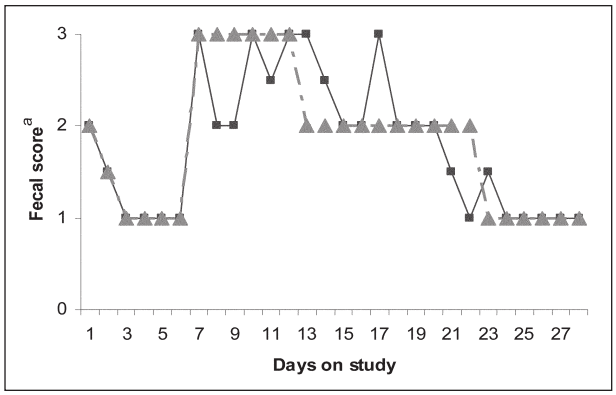

Figure 1—Median fecal scores for neonatal calves fed milk replacer with (treatment; triangles) and without (control; squares) decoquinate (2 mg/kg [0.9 mg/lb] per day) for 28 days after oral administration of single challenge doses containing different numbers of *Cryptosporidium parvum* oocysts. [a]1 = Formed feces. 2 = Semiformed feces. 3 = Watery feces.

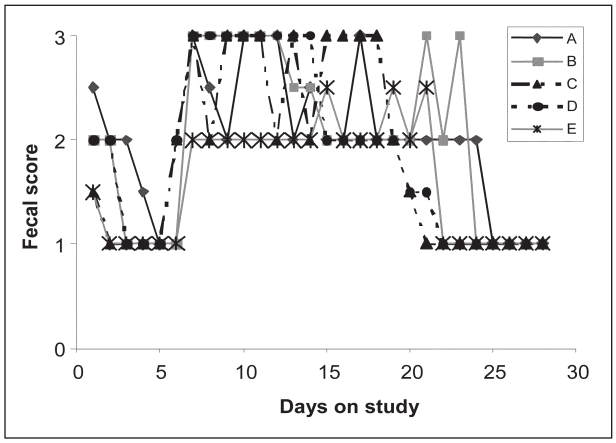

Figure 2—Median fecal scores for neonatal calves fed milk replacer with and without deconquinate (2 mg/kg per day) for 28 days after oral administration of single oral administration of challenge doses containing different numbers of *Cryptosporidium parvum* oocysts. Group A = 50 oocysts (n = 8), B = 100 (8), C = 1,000 (8), D = 10,000 (8), and E = 0 (5). *See* Figure 1 for remainder of key.

Table 3—Three general linear models for the outcomes of total number of days of oocyst shedding (model 1), number of days of diarrhea (model 2), and the days to the first oocyst detection in feces (model 3) for neonatal calves fed milk replacer with and without decoquinate (2 mg/kg per day) for 28 days after oral administration of single challenge doses containing different numbers of *C parvum* oocysts

Model/Outcome	*df*	Variable	Estimate	*P* value	R^2
Model 1	46		NA	0.005	0.21
Days oocysts shed		Oocyst dose at challenge	0.000111	0.068	
		Days to first oocyst shed after challenge	–0.69	0.005	
Model 2	45		NA	0.0001	0.41
Days of diarrhea		Week of arrival	NA	0.04	
		Days to watery diarrhea	–0.77	0.02	
Model 3	68		NA	0.003	0.25
Days to first oocyst shed after challenge		Week of arrival	NE	0.027	
		Days to watery diarrhea	0.17	0.004	

df = Degrees of freedom. NA = Not applicable. NE = Not estimable.

Discussion

To our knowledge, this is the second reported study of the effects of feeding decoquinate on the course and shedding patterns of cryptosporidiosis in neonatal calves. In the study reported here, we did not find an association between results of daily treatment of neonatal calves with decoquinate in milk replacer and any beneficial changes in the clinical or laboratory findings. Our study results are similar to results obtained from mice given decoquinate at dosages of 2.0 and 5.9 mg/kg (2.7 mg/lb) per day after challenge[16]; no reduction in oocyst shedding among treatment groups was found. Compared with another study[12] in Holstein calves, calves in our study had greater mean days to first shedding, greater mean number of days of shedding, and greater total number of days of diarrhea after challenge. The daily dose of decoquinate we used was different than the dose used in that study and was chosen on the basis of what was generally being used in California. Although there was an indication of treatment effect in that study, the investigators used a different decoquinate formulation. In our study, we used a newer formulation of decoquinate[c] presently used by producers and designed for ease of mixing in milk or milk replacer.

Decoquinate is labeled for use in calves with coccidiosis caused by *Eimeria bovis* and *E zuernii* and administered at 0.5 mg/kg (0.23 mg/lb) per day, PO, for at least 28 days during periods of likely exposure to coccidia. Any use other than the approved label guidelines is considered extralabel by the AMDUCA[23] and is permitted under the supervision of a veterinarian with a valid veterinarian-client-patient relationship for drugs used for treatment purposes, dosage-form drugs, and drugs administered in water but not feed, and the drug must be accompanied by instructions for the specific extralabel use.

Although this study was conducted with an awareness of biosecurity to reduce calf-to-calf transmission, we used field conditions representative of commercial calf-raising operations. Despite attention to biosecurity measures intended to prevent cross-contamination, unchallenged calves nonetheless became infected. Results from this and another study[4] indicate that small numbers of oocysts (< 50) can result in infected calves. In that study, 100% of calves became infected without direct contact with other calves. The potential source of infection for nonchallenged calves could be bottles or nipples washed in the same sink used for washing equipment contaminated with *C parvum* (although they were washed separately in our study) or by flies. Several flies were captured during our study, and oocysts from *Cryptosporidium* spp were detected on their outer surfaces. Flies have been reported to be mechanical transports for *C parvum* oocysts.[24-26] Although sound management and careful attention to hygiene during the handling of infected calves are recommended,[11] *C parvum* may require so few oocysts to infect individual calves that these recommendations would be futile. When an outbreak of cryptosporidiosis is ongoing, it appears difficult to deter transmission of oocysts from calf to calf.

An important finding in our study was the association between challenge dose and duration of shedding. Higher challenge doses were associated with a longer duration of shedding. In addition, the sooner a calf began to shed oocysts after challenge, the longer the duration of shedding. The importance of these findings is that higher doses of oocysts (eg, oocyst exposure from the environment) can increase the duration of shedding, total amount of oocysts shed, and, hence, the ever-increasing load of oocysts in the calf's environment.

The infectious dose used in this study was small, and 2-day-old calves became infected with challenge doses as low as 50 oocysts. In mice given oocysts from a human isolate, 22% became infected with as few as 100 oocysts, and the **dose at which 50% became infected (ID_{50})** was 100 to 500 oocysts.[27] As few as 23 oocysts from a bovine isolate infected 2 of 25 mice with an ID_{50} of 79 oocysts.[28] The ID_{50} for humans, determined by use of a bovine isolate, was 132 oocysts, and 1 of 5 humans became infected with only 30 oocysts.[29]

Severity of clinical signs and intensity of shedding appear to vary from study to study. Less than half of infected and shedding individuals had enteric signs in experimental infection in humans.[29] However, individuals with diarrhea had higher total shedding.[30] In 6-day-old goat kids inoculated with 6×10^6 oocysts from a bovine isolate, all became lethargic and had decreased appetite 72 hours after inoculation.[31] Nonchallenged kids kept in a separate pen developed clinical signs 8 days after challenged kids were inoculated. For challenged and control groups, diarrhea preceded shedding by 2 days, which is similar to our findings. In a study[17] of goat kids given a bovine iso-

late and either no treatment or decoquinate (2.5 mg/kg [1.2 mg/lb] per day) for 21 days, treated kids shed fewer oocysts had fewer days of soft feces and oocyst shedding. Neither treated nor control kids had severe clinical signs. Calves challenged with *C parvum* oocysts in 1 study[32] had diarrhea on the third day after inoculation, and the diarrhea lasted for 4 to 16 days and varied in severity among the calves. In our study, diarrhea preceded shedding and lasted approximately 12 days; shedding began 7 days after challenge and lasted for approximately 15 days, and signs were severe enough to result in death or require euthanasia. Thus, there appear to be differences in shedding patterns and clinical signs among different experimental challenge studies.

The focus of our study was on the association between oocyst shedding and diarrhea. However, mortality rate was high in our study and could be attributable to management, source, breed, and sex of calves. Necropsy was not a routine part of the study design because of cost; however, data from 8 calves that died or were euthanatized were included. Certain calves had *Salmonella* sp and enteric viral infections in addition to *C parvum*. Because certain calves died soon after arrival, a likely source of other agents was the farm of origin. Euthanatized calves were included in mortality rates. We opted to euthanatize calves if they did not respond to standard treatment for diarrhea rather than administer prolonged treatment. Infections with other organisms could potentially confound certain clinical manifestations; however, the patterns of shedding and fecal scores were consistent, indicating that the clinical signs observed could be attributable to *C parvum* infection. In addition, concurrent or mixed infections in neonatal calves are common in the field,[6] making this study similar to a field trial except for the experimental challenges with *C parvum*.

Results of clinical trials with decoquinate for *C parvum* infections are inconsistent. If there is no effective treatment or prophylactic method available for controlling *C parvum* infections and young animals can be infected with small numbers of oocysts, effective prevention strategies will be difficult to find. Considering these 2 factors, the most promising preventive measure may be an orally administered vaccine that was found to be successful in an experimental trial[33] but not yet successful in the field.[3] An integrated approach that would include management measures to reduce fly populations, transmission, and environmental loading rates remains the alternative available to dairy producers and veterinarians to minimize consequences of *C parvum* infections in neonatal calves.

[a]Colostrx, Schering Plough Animal Health Corp, Kenilworth, NJ.
[b]Excel, Microsoft Corp, Bellevue, Wash.
[c]Deccox-M, Alpharma Animal Health Pty Ltd, Alpharma Inc, Fort Lee, NJ.
[d]Fisher Scientific, Santa Clara, Calif.
[e]Biolyte, Pharmacia Animal Health, Kalamazoo, Mich.
[f]Beuthanasia-D Special, Schering-Plough Animal Health Corp, Union, NJ.
[g]California Animal Health and Food Safety Laboratory, Tulare, Calif.
[h]PROC GLM, version 8, SAS Institute Inc, Cary, NC.

References

1. Anderson BC. Cryptosporidiosis in bovine and human health. *J Dairy Sci* 1998;81:3036–3041.
2. Nydam DV, Wade SE, Schaaf SL, et al. Number of *Cryptosporidium parvum* oocysts or *Giardia* spp cysts shed by dairy calves after natural infection. *Am J Vet Res* 2001;62:1612–1615.
3. Harp JA, Jardon P, Atwill ER, et al. Field testing of prophylactic measures against *Cryptosporidium parvum* infection in calves in a California dairy herd. *Am J Vet Res* 1996;57:1586–1588.
4. O'Handley RM, Cockwill C, McAllister TA, et al. Duration of naturally acquired giardiosis and cryptosporidiosis in dairy calves and their association with diarrhea. *J Am Vet Med Assoc* 1999;214: 391–396.
5. Sischo WM, Atwill ER, Lanyon LE, et al. Cryptosporidia on dairy farms and the role these farms may have in contaminating surface water supplies in the northeastern United States. *Prev Vet Med* 2000;43:253–267.
6. Moore DA, Zeman DH. Cryptosporidiosis in neonatal calves: 277 cases (1986–1987). *J Am Vet Med Assoc* 1991;198: 1969–1971.
7. Blagburn BL, Soave R. Prophylaxis and chemotherapy: human and animal. In: Fayer R, ed. *Cryptosporidium and cryptosporidiosis*. Boca Raton, Fla: CRC Press Inc, 1997;111–128.
8. Fayer R, Ellis W. Paromomycin is effective as prophylaxis for cryptosporidiosis in dairy calves. *J Parasitol* 1993;79:771–774.
9. Naciri M, Mancassola R, Yvore P. The effect of halofuginone lactate on experimental *Cryptosporidium parvum* infections in calves. *Vet Parasitol* 1993;45:199–207.
10. Villacorta I, Peeters JE, Vanopdenbosch E, et al. Efficacy of halofuginone lactate against *Cryptosporidium parvum* in calves. *Antimicrob Agents Chemother* 1991;35:283–287.
11. Harp JA, Goff JP. Strategies for the control of *Cryptosporidium parvum* infection in calves. *J Dairy Sci* 1998;81: 289–294.
12. Redman DR, Fox JE. The effect of varying levels of Deccox on experimental *Cryptosporidia* infections in Holstein bull calves, in *Proceedings*. 26th Annu Conv Am Assoc Bov Pract 1993;157–159.
13. Castro-Hermida JA, Freire-Santos F, Oteiza Lopez AM, et al. In vitro and in vivo efficacy of lasalocid for treatment of experimental cryptosporidiosis. *Vet Parasitol* 2000;90:265–270.
14. Hoblet KH, Charles TP, Howard RR. Evaluation of lasalocid and decoquinate against coccidiosis resulting from natural exposure in weaned dairy calves. *Am J Vet Res* 1989;50:1060–1063.
15. Heinrichs AJ, Bush GJ. Evaluation of decoquinate or lasalocid against coccidiosis from natural exposure in neonatal dairy calves. *J Dairy Sci* 1991;74:3223–3227.
16. Lindsay DS, Woods KM, Upton SJ, et al. Activity of decoquinate against *Cryptosporidium parvum* in cell cultures and neonatal mice. *Vet Parasitol* 2000;89:307–311.
17. Mancassola R, Richard A, Naciri M. Evaluation of decoquinate to treat experimental cryptosporidiosis in kids. *Vet Parasitol* 1997;69:31–37.
18. Arrowood MJ, Sterling CR. Isolation of *Cryptosporidium parvum* and sporozoites using discontinuous sucrose and isopycnic Percoll gradients. *J Parasitol* 1987;73:314–319.
19. Robertson L, Campbell AT, Smith HV. In vitro excystation of *Cryptosporidium parvum*. *J Parasitol* 1993;106:13–19.
20. Constable PD, Walker PG, Morin DE, et al. Clinical and laboratory assessment of hydration status of neonatal calves with diarrhea. *J Am Vet Med Assoc* 1998;212:991–996.
21. Pereira M, Atwill ER, Jones T. Comparison of sensitivity of immunofluorescent microscopy to that of a combination of immunofluorescent microscopy and immunomagnetic separation for detection of *Cryptosporidium parvum* oocysts in adult bovine feces. *Appl Environ Microbiol* 1999;65:3236–3239.
22. *SAS/STAT User's guide: version 6 edition*. Cary, NC: SAS Institute Inc, 1994;891–996.
23. FDA. AMDUCA. 2002. Available at: www.avma.org/scientact/amduca/amduca1.asp. Accessed on May 13, 2003.
24. Graczyk TK, Cranfield MR, Fayer R, et al. House flies (*Musca domestica*) as transport hosts of *Cryptosporidium parvum*. *Am J Trop Med Hyg* 1999;61:500–504.
25. Graczyk TK, Fayer R, Cranfield MR, et al. Filth flies are

transport hosts of *Cryptosporidium parvum*. *Emerg Infect Dis* 1999;5: 726–727.

26. Graczyk TK, Fayer R, Knight R, et al. Mechanical transport and transmission of *Cryptosporidium parvum* oocysts by wild filth flies. *Am J Trop Med Hyg* 2000;63:178–183.

27. Ernest JA, Blagburn BL, Lindsay DS, et al. Infection dynamics of *Cryptosporidium parvum* (Apicomplexa: Cryptosporiidae) in neonatal mice (*Mus musculus*). *J Parasitol* 198;72:796–798.

28. Finch GR, Daniels CW, Black EK, et al. Dose response of *Cryptosporidium parvum* in outbred neonatal CD-1 mice. *Appl Environ Microbiol* 1993;59:3661–3665.

29. DuPont HL, Chappel CL, Sterling CR, et al. The infectivity of *Cryptosporidium parvum* in healthy volunteers. *N Engl J Med* 1995; 332:855–859.

30. Chappel CL, Okhuysen PC, Sterling CR, et al. *Cryptosporidium parvum*: intensity of infection and oocyst excretion patterns in healthy volunteers. *J Infect Dis* 1996;173:232–236.

31. Koudela B, Jiri V. Experimental cryptosporidiosis in kids. *Vet Parasitol* 1997;71:273–281.

32. Fayer R, Gasbarre L, Pasquali P, et al. *Cryptosporidium parvum* infection in bovine neonates: dynamic clinical, parasitic and immunologic patterns. *Int J Parasitol* 1998;28:49–56.

33. Harp JA, Goff JP. Protection of calves with a vaccine against *Cryptosporidium parvum*. *J Parasitol* 1995;81:54–57.