Farm Factors Associated with Reducing *Cryptosporidium* Loading in Storm Runoff from Dairies

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A systems approach was used to evaluate environmental loading of *Cryptosporidium* oocysts on five coastal dairies in California. One aspect of the study was to determine *Cryptosporidium* oocyst concentrations and loads for 350 storm runoff samples from dairy high use areas collected over two storm seasons. Selected farm factors and beneficial management practices (BMPs) associated with reducing the *Cryptosporidium* load in storm runoff were assessed. Using immunomagnetic separation (IMS) with direct fluorescent antibody (DFA) analysis, *Cryptosporidium* oocysts were detected on four of the five farms and in 21% of storm runoff samples overall. Oocysts were detected in 59% of runoff samples collected near cattle less than 2 mo old, while 10% of runoff samples collected near cattle over 6 mo old were positive. Factors associated with environmental loading of *Cryptosporidium* oocysts included cattle age class, 24 h precipitation, and cumulative seasonal precipitation, but not percent slope, lot acreage, cattle stocking number, or cattle density. Vegetated buffer strips and straw mulch application significantly reduced the protozoal concentrations and loads in storm runoff, while cattle exclusion and removal of manure did not. The study findings suggest that BMPs such as vegetated buffer strips and straw mulch application, especially when placed near calf areas, will reduce environmental loading of fecal protozoa and improve stormwater quality. These findings are assisting working dairies in their efforts to improve farm and ecosystem health along the California coast.

Water quality has become a complex and important issue as human demands on coastal ecosystems continue to increase (Daszak et al., 2001). Humans and their animals produce significant amounts of fecal byproducts that must be managed to maximize water quality and minimize public health risks in these coastal ecosystems (Fayer et al., 2004). The Tomales Bay watershed, along the northern California coast, supports livestock, aquaculture, and recreation industries that are currently working together to improve water quality and ecosystem health. Because exposure to even low doses of *Cryptosporidium* oocysts from contaminated water and food sources is a public health concern (Okhuysen et al., 1999), a better understanding of the environmental ecology and control of these parasites in coastal watersheds is key to designing preventive measures.

A systems approach by Lewis et al. (2005) evaluated stormwater quality on various dairy farm loading units near Tomales Bay, and the study findings suggest that the high concentrations of fecal microbes mobilized in overland runoff during storm events may be more cost-effectively controlled by implementing beneficial management practices (BMPs) targeting point sources such as dairy dry lots and other high use areas, as compared to nonpoint source areas such as pastures. Vegetated buffers are BMPs that can be used on farms to mitigate contaminants in storm runoff, and in recent controlled studies evaluating their capacity to reduce the load of *Cryptosporidium* oocysts in overland runoff, a 1 to 3 $\log_{10}$ reduction/m buffer was found (Atwill et al., 2002, 2006; Davies et al., 2004; Tate et al., 2004; Trask et al., 2004). This suggests that buffers, when matched appropriately to local environmental conditions, can significantly improve stormwater microbial quality when placed between fecal sources and downstream waterways.

The current study extends our previous evaluations (Atwill et al., 2002, 2006; Tate et al., 2004) of vegetated buffers for reduction of protozoal loading in storm runoff to a working farm setting. Five dairy farms near Tomales Bay, California, participated in the 2-yr study. The goals were to (i) evaluate factors associated with detection of *Cryptosporidium* oocysts in runoff from dairy

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Table 1. Precipitation, storm runoff, and management conditions of dairy lot study sites, 2002–2004, Tomales Bay, California.

Lot characteristic	Mean	Median	Min.	Max.
Precipitation and discharge:				
24 hour precip. (mm.)	23	20	10	83
Cumulative precip. (mm.)	388	481	37	713
Slope (°)	8.7	4	1	26
Instantaneous flow (m^3/sec.)	0.009	0.002	0.00004	0.2
Storm runoff (hectare-mm.)	13.0	8.1	0.0002	72.8
Management:				
Size (hectare)	1.8	0.4	0.04	32
Stock number	51	30	5	390
Animal conc. (#/hectare)	84	47	1	309
Ground cover (%)†	40	30	0	99
Buffer length (m.)‡	30	21	6	152

† Ground cover consisted of vegetation and/or straw mulch application.

‡ Conditions described are for the 17 lots where beneficial management practices were studied.

high use areas, and (ii) evaluate the efficacy of BMPs to reduce the *Cryptosporidium* load in storm runoff from treated dry lots compared to adjacent control dry lot sites. In addition to vegetated buffers, BMPs evaluated included straw mulch application, cattle exclusion, and scraping of lots to remove manure. We hypothesized that *Cryptosporidium* would be detected most often in storm runoff collected near calf areas, and that BMPs such as vegetated buffer strips would significantly decrease the protozoal load in storm runoff from dairy high use areas.

Materials and Methods

Study Sites

Five dairy farms were selected based on voluntary participation and location within the Tomales Bay watershed. The Mediterranean climate has cool wet winters and dry hot summers, with annual cumulative precipitation ranging from 600 to 1300 mm (Fischer et al., 1996). The farms were situated on hilly pasture terrain above the bay, with an average slope of 9° (range 1–26°) and an average of 104 animals per hectare (range 5–309). A variety of farm sites were evaluated but sampling efforts focused mainly on locations that received extensive cattle use (e.g., corrals, lots) because of the high rate of fecal coliforms discharged from these areas during the 1999 to 2001 water years (Lewis et al., 2005).

Thirty five cattle lots on the five dairies were enrolled in the study. Typically positioned next to barns, these units ranged from several hectares to less than a hectare in size (Table 1). The lots are used to provide daily exercise to milking cows, who do not move far from the barn or milking facilities. In general, these areas hold high densities of dairy cattle during the summer, are scraped of manure in the fall, and are not used in the winter. All lots were characterized by area, slope, animal density, and ground cover factors. Real-time precipitation data for the 24 h and 5 d preceding sample collection, and for cumulative seasonal precipitation estimates was collected from Point Reyes and Tomasini Point, CA (http://cdec.water.ca.gov/snow_rain.html [verified 19 May 2008]).

Beneficial Management Practice Implementation on Farms

A variety of BMPs were evaluated that were either already in practice by one or more dairy farms, or newly installed for this project. These included modifying the density of cattle or complete exclusion of cattle from the lot during the duration of the rainfall season, and using a tractor to scrape and remove the accumulated layer of manure from the lot before the onset of winter rains.

Building on these preexisting practices, vegetative surface treatments were implemented in October before the onset of winter rains on 13 of the dairy lots. Vegetative surface treatments involved seeding the lot with 112 kg/hectare of annual barley (*Hordeum vulgare*) and 28 kg/hectare rye grasses (*Lolium multiflorum*) that would provide cover and soil stabilization during later winter storms. On the same day, 5.4 metric tons/hectare straw were applied to the lots to provide soil surface cover during early winter storms (Lennox et al., 2007). Percent straw cover was expected to decrease from over 60 to 20% within 2 mo of application, with corresponding increases in cover by the seeded grasses.

Lastly, existing or newly implemented practices below 17 lots were evaluated to improve the quality of storm runoff after it left the lot. In this case runoff was channeled from the lot through one of three BMPs: vegetative buffer strips (14 lots); grassed waterways (2 lots); or impoundment (1 lot), as a means to reduce the load of waterborne fecal protozoa.

Stormwater Collection and Analysis

Throughout the 2002 to 2003 and 2003 to 2004 water years, surface runoff grab samples were collected during each storm to represent both early and late flushing events at each study site. Samples were collected from small channels which collect and drain stormwater runoff from each lot. Instantaneous runoff rate at the time of each sample collection was determined at each sample site by the area velocity method (Mosley and McKercher, 1993). Water samples were collected and transported chilled in 500 mL plastic bottles within 72 h of collection from the farm study sites to the University of California, Davis for laboratory analysis. Turbidity in nephelometric turbidity units (ntu) was determined using a turbidimeter (Orbeco Analytical Systems, Farmingdale, NY). To each 500 mL water sample, 125 μL 0.2% Tween 80 and 25 mL 50 mmol L^{-1} morphilinopropane sulfonic acid (MOPS) sodium extraction solution (Fisher Scientific, Pittsburg, PA) were added. Samples were hand mixed and decanted to two 250 mL spin bottles. Bottles were agitated for 5 min on a hand-wrist shaker at speed 7 and then centrifuged at 1100 × *g* for 10 min. Supernatant was aspirated down to a 1:1 pellet/liquid ratio.

For *Cryptosporidium* quantitation, pellets less than 50 μL were analyzed by direct fluorescent antibody (DFA) assay without immunomagnetic separation (IMS) concentration, by drying 10 μL loop aliquots onto three-well Merifluor (Meridian Bioscience Inc., Cincinnati, OH) slides for direct DFA analysis. For larger pellets, up to 0.5 mL of the pellet was processed by *Cryptosporidium* IMS (Dynal Biotech, Oslo, Norway) and then DFA (Meridian Bioscience) analysis according to the manufacturer's instructions. All slides were read by the same microscopist.

After parasite quantification, selected slides were scraped with a razor blade and washed with sterile phosphate buffered saline (PBS) into a microcentrifuge tube for subsequent DNA extraction, polymerase chain reaction (PCR) amplification, and DNA sequence analysis (Miller et al., 2005). For each dairy, five oocyst-positive runoff samples were processed for molecular determination of *Cryptosporidium* genotypes. *Cryptosporidium andersoni* oocysts were readily distinguished by their typical 5- × 7-μm morphology compared to the smaller 5-μm diameter spherical *C. parvum*-like oocysts, and were thereby excluded from all *C. parvum* calculations in this cohort of dairy farms.

Two conventional 18S small subunit ribosomal DNA PCR protocols (Morgan et al., 1997, Xiao et al., 1999) were used to genotype *Cryptosporidium* spp. detected in stormwater samples. For DNA extraction, a 50-μL pellet was mixed with 180 μL QIAGEN ATL buffer and suspended in liquid nitrogen for 4 min, then boiling water for 4 min. Using the QIAGEN DNA Mini Kit tissue protocol (QIAGEN Inc., Valencia, CA), each sample was digested, bound to a QIAamp column, washed, and the DNA eluted with 50 μL of 95°C PCR water. The PCR and DNA sequence analysis methods targeted 300 and 850 bp 18S regions.

Stormwater Spiking Trials

The stormwater spiking experiments were designed to simulate a range of field conditions and contained a known number of oocysts so that recovery efficiency of the water processing protocols could be evaluated. *Cryptosporidium parvum* oocysts were obtained from calf fecal samples submitted to the Veterinary Medical Teaching Hospital in Davis, California. Calf feces were sieved through #40, 100, and 200 mesh strainers with 1X PBS and purified by sucrose flotation methods (Arrowood and Sterling, 1987). Oocyst concentrations were determined from the mean of eight hemacytometer counts and confirmed with DFA enumeration. Oocyst suspensions were kept at 4°C and used within 1 mo of collection.

Soil and cattle manure from the participating farms were pre-tested and then used to adjust stormwater turbidity to 1, 100, or 500 ntu. Oocyst doses of 0, 10, 50, or 500 oocysts per 500 mL water sample were mixed with each water sample and refrigerated overnight before processing. Four replicates were created for each oocyst dose and turbidity combination, and were processed identically to the field samples.

Statistical Analyses

Percent recovery values for oocyst DFA and IMS-DFA procedures were estimated by fitting a negative binomial regression model to the observed oocyst count data over the range of turbidity values, with the number of spiked oocysts as the offset variable (Hardin and Hilbe, 2001; Atwill et al., 2003). In addition to the percent recovery estimates, the assay sensitivity S(c), defined as the probability of detecting at least one oocyst per sample, was calculated as shown in Eq. [1], where $e^{\beta x}i$ is the percent recovery of the assay, α is an ancillary parameter for modeling dispersion, c_i is the number of oocysts spiked per 500 mL stormwater sample, and W_i is the proportion of sample tested in the assay (Atwill et al., 2003).

$$S(c_i) = 1- [1/(1 + \alpha c_i W_i e^{\beta x}i)]^{1/\alpha} \qquad [1]$$

In the field study, water samples were first classified as *Cryptosporidium* positive or negative for use in prevalence calculations. The percent recovery estimates were used to adjust the oocyst count data from water samples to estimate the true concentration of parasites. Adjusted parasite concentrations were then used to calculate instantaneous parasite load by multiplying the stormwater concentration by the instantaneous flow for each sample, as seen in Eq. [2]:

$$\text{Load (oocysts/sec)} = \text{Oocyst concentration (oocysts/L)} \times \text{Runoff flow (L/sec)} \qquad [2]$$

Negative binomial regression was used to identify farm factors and BMPs associated with detection of *Cryptosporidium* oocysts in storm runoff. The stormwater outcome variables were oocyst concentration and instantaneous parasite load, with study site functioning as a group effect to adjust for repeated sampling at the same sites over time. Categorical predictor variables included month of sampling (November-March), cattle age class (dry and milking adults, calving adults, 3–6 mo old calves, 1–2 mo old calves), and dry lot BMP status (control site, vegetated buffer, mulch straw application, cattle exclusion, scraping of manure). Continuous predictor variables included instantaneous flow, 24 h precipitation, cumulative annual precipitation, percent slope, acreage, cattle stocking number, and cattle density. Forward stepping multivariate regression models were created and used to estimate the effect of changing predictor variables on protozoal stormwater concentration and load. Statistical significance was defined as $P < 0.10$. The McFadden likelihood-ratio index was used to estimate the percent variance explained by the model, calculated as R^2 = 1-{(log likelihood of the full model/log likelihood of the intercept-only model)} (Hardin and Hilbe, 2001).

Results

Between November 2002 and March 2004, a total of 350 stormwater samples were processed from high use areas on the five dairy farms. Overall, *Cryptosporidium* oocysts were detected in 21% of storm runoff samples. The highest prevalence was in runoff collected near calves 0 to 2 mo of age, with 59% of stormwater samples testing positive for *Cryptosporidium* oocysts. In comparison, *Cryptosporidium* oocysts were detected in 38% of runoff discharging from sites housing 3- to 6-mo-old calves and in 10% of runoff discharging from locations with dry, milking, or calving adult cattle.

Cryptosporidium parvum-like and *C. andersoni*-like oocysts were detected in runoff from four of the five dairy farms. Amplification and sequence analysis of up to five randomly selected DFA-positive samples per farm confirmed that both *C. parvum* and *C. andersoni* were present but not other genotypes reported in cattle such as *C. bovis* or cervine genotypes. Results herein focus on 5 μm oocysts resembling *C. parvum*, with *C. andersoni* excluded from the loading calculations and analysis.

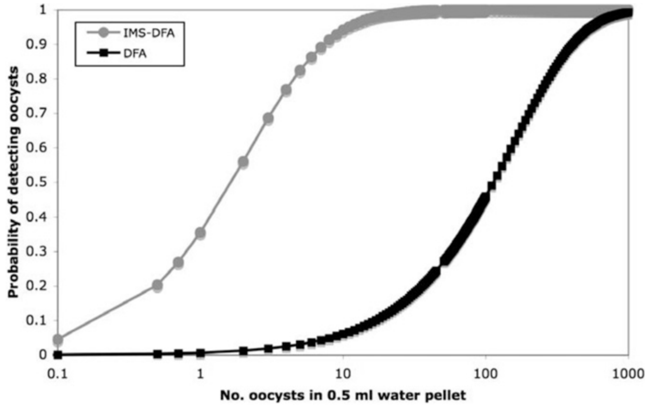

Fig. 1. Sensitivity of direct fluorescent antibody (DFA) testing and immunomagnetic separation (IMS) with DFA testing for detection of *Cryptosporidium* oocysts spiked into stormwater samples.

In the percent recovery experiments, no negative controls tested positive as part of the 48 stormwater samples spiked with 0, 10, 50, or 500 oocysts that were used to estimate the recovery efficiencies of the *Cryptosporidium* detection methods over a range of turbidities. Detection by DFA alone resulted in 21% recovery of spiked oocysts, whereas IMS concentration before DFA detection improved recovery to 47% overall, as determined by negative binomial regression modeling of the experimental data. The percent recovery values were used to adjust the raw oocyst count data from field samples so that the true parasite concentrations in the storm runoff samples could be estimated for the statistical analyses.

Figure 1 illustrates the assay sensitivity curves for *Cryptosporidium* oocysts detected in stormwater samples by DFA and IMS-DFA. The IMS-DFA analytic sensitivity improved by 1 to 2 $\log_{10}$ units, shown by the IMS-DFA curve left shifted from the DFA curve. When IMS concentration was performed before DFA detection, the 50% probability detection threshold (DT_{50}) improved from approximately 100 to 2 oocysts per 500 mL stormwater sample, and the DT_{90} improved from 400 to 10 oocysts.

Cryptosporidium oocysts were detected in stormwater runoff throughout the wet season sampling periods, with the highest concentrations and loads exceeding 10,000 oocysts/L or sec in December, February, and March. Mean concentrations and loads were around 11 oocysts/L and 50 oocysts/sec in dairy runoff during November and January, respectively. In December the mean concentration and load was 130 oocysts/L and 245 oocysts/sec, in February they were 372 oocysts/L and 294 oocysts/sec, and in March they were 1150 oocysts/L and 780 oocysts/sec, respectively. Figure 2 shows box plots of positive stormwater samples by month for *Cryptosporidium* concentrations (oocysts/L) and loads (oocysts/sec). The mean overland flow peaked at 18 L/sec in the spring, concurrent with the highest levels of *Cryptosporidium* detected during the study.

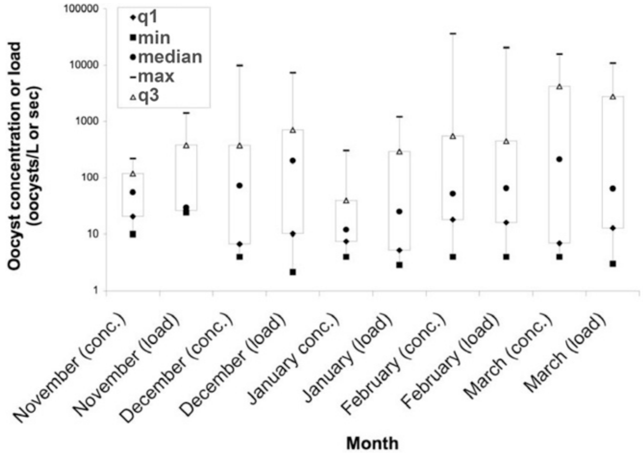

Fig. 2. Monthly concentration and load distributions for *Cryptosporidium*-positive samples collected from dairy lots during runoff conditions, 2002–2004, Tomales Bay, California.

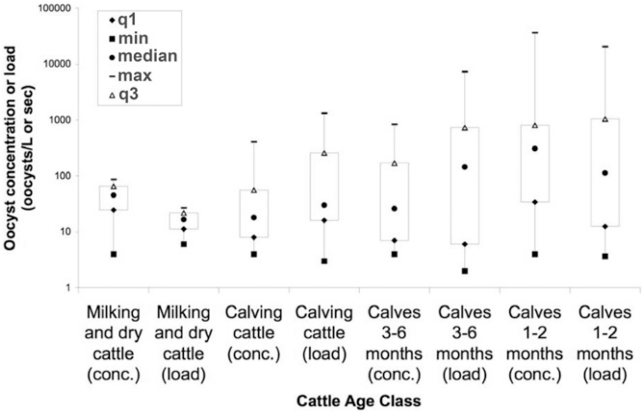

Fig. 3. Concentration and load distributions for *Cryptosporidium*-positive samples collected from dairy lots during runoff conditions stratified by cattle age class, 2002–2004, Tomales Bay, California.

The highest mean parasite concentration (2000 oocysts/L) and load (1400 oocysts/sec) was detected in runoff discharging from sites housing calves under 2 mo of age, compared to other cattle age classes. Stormwater discharging from sites housing calves 3 to 6 mo of age had a mean concentration of 50 oocysts/L and mean instantaneous load of 320 oocysts/sec. In contrast to these higher concentrations and loads from calf-rearing locations, oocyst concentrations and loads in runoff from sites housing dry, milking, or calving cows were less than 10% of these values, and in some cases less than 0.1%. For example, runoff from sites housing milking or dry cows had a mean concentration and load of about 1 oocyst/L or sec, and runoff collected near calving cows had a mean values of 6 oocysts/L and 20 oocysts/sec, respectively. Figure 3 shows box plot distributions of data from *Cryptosporidium*-positive samples, with the maximum values seen to exceed 10,000 oocysts/L and sec in runoff collected near calves under 2 mo old.

Table 2 shows that cattle age class, 24 h precipitation, and cumulative seasonal precipitation were significantly associated with oocyst concentration in storm runoff, as were the BMPs of covering dairy cattle high-use areas with straw mulch and passing runoff through a vegetated buffer strip (McFadden $R^2 = 0.10$). Given that these predictor variables were in the model, the other variables such as percent slope, lot acreage, cattle stocking number, cattle density, cattle exclusion, and manure removal were not significantly associated with oocyst concentration. By exponentiating model coefficients, the relative change in stormwater oocyst concentra-

Table 2. Negative binomial regression model for the effect of cattle age, beneficial management practices, and precipitation on the concentration of *Cryptosporidium* (oocysts/L) discharging from dairy lots during runoff conditions, 2002–2004, Tomales Bay, California.

Factor	Coefficient‡	95% CI‡	*P*-value
Type of dairy cattle			
Cow: milking or dry†	0.0	–	–
Cow: calving	0.20	(−0.91, 1.31)	0.72
Calf: 2.1 to 6.0 mo of age	2.90	(1.09, 4.71)	0.002
Calf: 0.1 to 2.0 mo of age	7.65	(6.19, 9.10)	< 0.001
Percent ground covered with straw (%)	−0.032	(−0.055,−0.009)	0.007
Length of vegetated buffer (m)	−0.028	(−0.043,−0.012)	0.001
24-hour precipitation (mm)	0.18	(0.058, 0.29)	0.003
24-hour precipitation^2 (mm)^2	−0.005	(−0.007,−0.002)	< 0.001
Cumulative precipitation (mm)	−0.005	(−0.009,−0.002)	0.003
Constant or intercept term for the model	1.76	(−0.42, 4.0)	0.11

† Referent condition for the categorical variable, type of dairy cattle.

‡ Adjusted for potential lack of independence due to repeated sampling of lots across storms.

tion can be determined. For example, using milking or dry adult cattle as the referent condition, oocyst concentration in stormwater collected near calves under 2 mo old was increased by a factor of 2100 ($e^{7.65}$), and increased by a factor of 18 for calves 3 to 6 mo of age ($e^{2.90}$). For continuous predictor variables, each 10% increase in straw mulch application to dairy high-use areas resulted in the oocyst concentration decreasing by a factor of 0.73 ($e^{-0.032 \times 10}$), and each additional meter of vegetated buffer established below cattle high-use areas resulted in the stormwater oocyst concentration decreasing by a factor of 0.97 ($e^{-.028 \times 1.0}$). Figures 4a and 4b show how the stormwater oocyst concentrations changed in relation to the BMP factors for each cattle age class. Figures 4c and 4d show how the preceding day (24 h) and cumulative seasonal precipitation affected stormwater oocyst concentrations for each cattle age class. Concentrations were found to increase when precipitation in the 24 h preceding stormwater collection was less than 20 mm, and to decrease at greater rain volumes. For cumulative seasonal precipitation, each additional 10 mm of rain decreased the oocyst concentration by a factor of.95 ($e^{-.005 \times 10}$).

Similar to the oocyst concentration model, Table 3 shows that cattle age class and 24 h precipitation were significantly associated with stormwater oocyst load, as were the BMPs of covering a dairy cattle high-use area with straw mulch and having runoff pass through a vegetated buffer strip (McFadden $R^2 = 0.08$). Using oocyst load in runoff discharging from sites housing milking and dry cows as the referent condition, oocyst load in runoff from sites housing calves under 2 mo old was increased by a factor of 728 ($e^{6.59}$), runoff from sites housing calves 3 to 6 mo old was increased by a factor of 302 ($e^{5.71}$), and runoff from sites housing periparturient cows was increased by a factor of 8 ($e^{2.13}$). In Fig. 5a, each additional 10% of straw mulch coverage placed on dairy cattle high-use areas was associated with a reduction in stormwater oocyst load by a factor of 0.76 ($e^{-0.028 \times 10}$). In Fig. 5b, each meter increase in vegetative buffer length was associated with a reduction in the stormwater oocyst load by a factor of 0.98 ($e^{-0.018}$). In Fig. 5c, each additional 10 mm of rain that fell in the 24 h preceding stormwater sampling was associated with a reduction in the stormwater oocyst load by a factor of 0.58 ($e^{-0.054 \times 10}$).

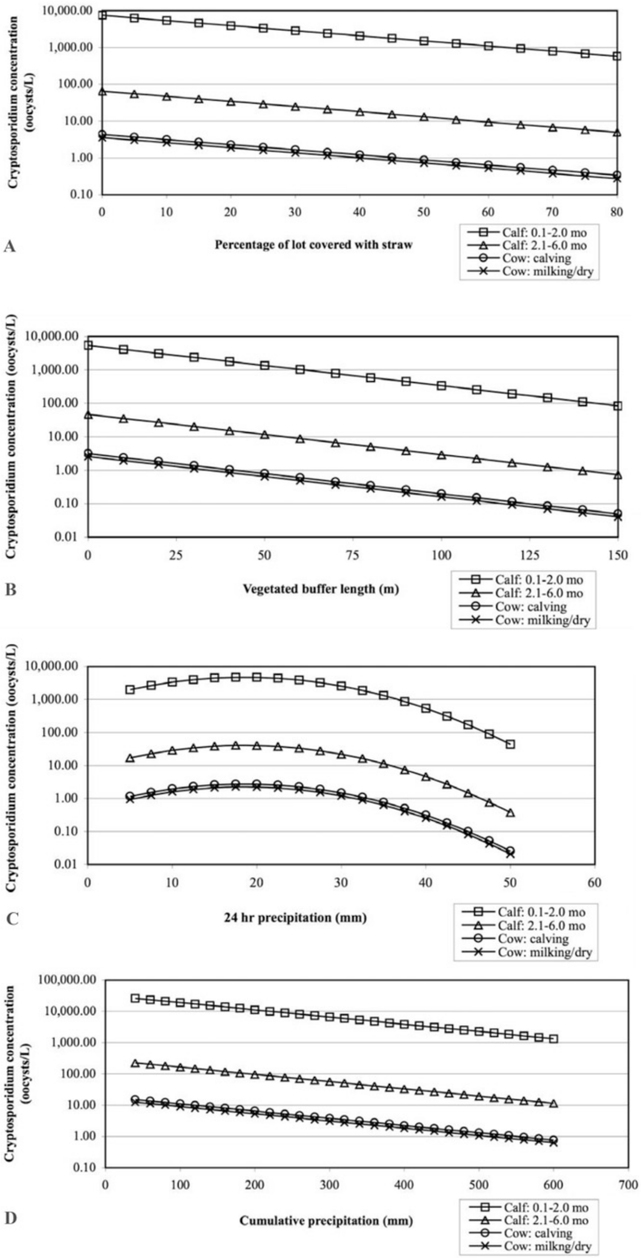

Fig. 4. Predicted *Cryptosporidium* oocyst concentrations (oocysts/L) in storm runoff from dairy lots for cattle of various age classes as a function of (a) percentage of each lot covered in straw, (b) length of vegetated buffer, and (c) 24 h precipitation, and (d) cumulative precipitation.

Discussion

This multi-year epidemiologic study evaluated the efficacy of on-farm BMPs to reduce protozoal loading in storm runoff on working dairies, along with the distribution and magnitude of *Cryptosporidium* loading from dairy high use areas. Both *C. parvum* and *C. andersoni* were detected on these coastal dairies, consistent with studies from other parts of the world that have genotyped *Cryptosporidium* in fecal and farm runoff samples (Olson et al., 1997; Peng et al., 2003). BMPs such as vegetated buffer strips have been shown to reduce

Table 3. Negative binomial regression model for the effect of cattle age, beneficial management practices, and precipitation on the instantaneous load of *Cryptosporidium* (oocysts/sec) discharging from dairy lots during runoff conditions, 2002–2004, Tomales Bay, California.

Factor	Coefficient‡	95% CI‡	*P*-value
Type of dairy cattle			
Cow: milking or dry†	0.0	–	–
Cow: calving	2.13	(0.78, 3.48)	0.002
Calf: 2.1 to 6.0 mo of age	5.71	(4.25, 7.17)	< 0.001
Calf: 0.1 to 2.0 mo of age	6.59	(5.32, 7.85)	< 0.001
Percent ground covered with straw (%)	−0.028	(−0.058, 0.001)	0.06
Length of vegetated buffer (m)	−0.018	(−0.031,−0.005)	0.007
24-hour precipitation (mm)	−0.054	(−0.10,−0.004)	0.04
Constant or intercept term for the model	2.01	(0.43, 3.59)	0.01

† Referent condition for the categorical variable, type of dairy cattle.

‡ Adjusted for potential lack of independence due to repeated sampling of lots across storms.

not only levels of protozoal parasites as in this study, but also the level of bacteria, nutrients, particulates, and pesticides in runoff (Stout et al., 2005; Syversen 2005; Blanco-Canqui et al., 2006; Tate et al., 2000, 2006), supporting their role in improving overall water quality and ecosystem health.

Vegetated buffers and straw mulch BMPs demonstrated their potential to significantly reduce the load of *Cryptosporidium* oocysts in storm runoff on working dairy farms. Both BMPs function in part as barriers to impede overland flow and provide surfaces to trap oocysts, thereby removing them from runoff, as well as slowing down the runoff to allow more time for infiltration and sedimentation of microorganisms (Atwill et al., 2002; Tyrrel and Quinton 2003; Blanco-Canqui et al., 2006). Moreover, application of straw mulch is a proven method for reducing soil erosion created by rain drop impact and soil particle entrainment on bare soil (USDA-NRCS 2004). Given the very slow settling velocity of oocysts, it is thought that one mechanism of removal is by sedimentation when attached to particulate matter (Medema et al., 1998; Searcy et al., 2006), though the Kaucner et al. (2005) study suggests that oocysts may often remain as single entities in overland runoff instead of attaching and settling out. Consistent with our findings, the Davies et al. (2004) and Trask et al. (2004) studies found reduced oocyst concentrations in runoff from vegetated soil plots compared with paired devegetated plots. Additional management approaches such as applying anionic polyacrylamide (PAM) to increase flocculation and soil infiltration were not evaluated in our study but may also prove useful for improving stormwater quality (Sojka et al., 2005).

The Atwill et al. (2002, 2006) and Tate et al. (2004) *Cryptosporidium* studies found a 1 to 3 $\log_{10}$ reduction per meter in controlled soil box experiments or field-plot studies, whereas in the current study oocyst concentrations and instantaneous loads were reduced by a factor of 0.97 and 0.98 respectively, which corresponds to a 0.01 $\log_{10}$ reduction per meter. The decreased magnitude of protozoal retention per meter of buffer length in the current study is likely due to a combination of factors. For example, in the present study only a single point in the pathogen breakthrough curve was measured, preventing a calculation of the total number oocysts retained during the storm event due to the presence of a vegetated buffer strip. If total runoff or runoff duration was shortened by the presence of a buffer, our calculation of oocyst retention via calculating concentration or instantaneous load would not reflect this reduction in total storm load reductions. In the work reported by Atwill et al. (2002), the entire runoff event was captured so that a complete mass balance could be calculated (entire pathogen load above and below the buffer was measured in surface runoff). In Atwill et al. (2006), the efficacy of a meter of vegetated buffer to retain *C. parvum* was based on capturing the entire surface runoff generated by each storm event for the entire storm season for two separate years, thereby allowing the calculation of buffer retention on an annualized basis. The calculations reported by Tate et al. (2004) were based on repeated subsampling across the duration of a set of runoff events, somewhat similar to the work reported in Atwill et al. (2002).

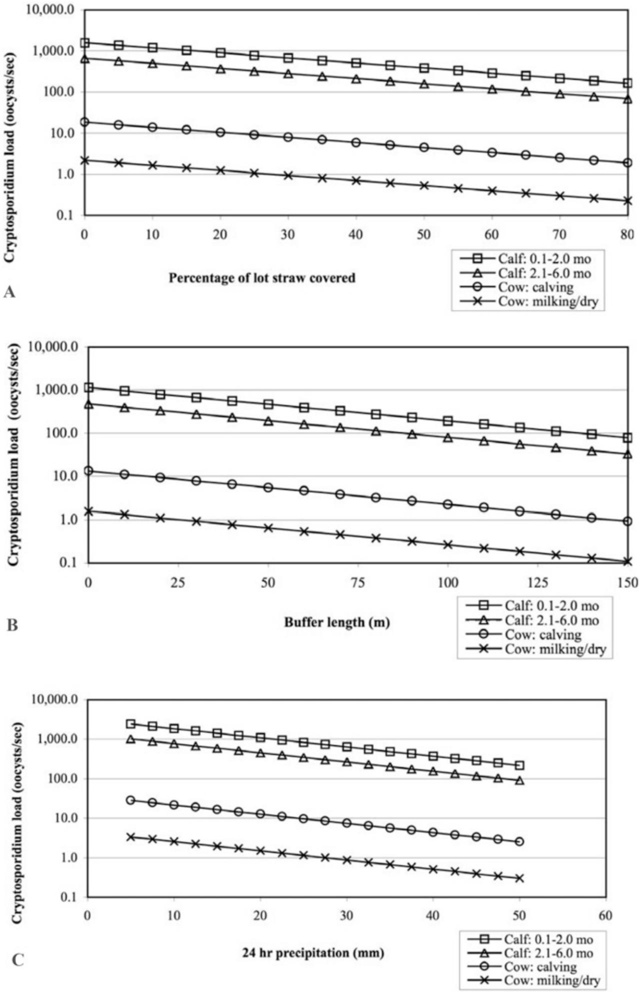

Fig. 5. Predicted *Cryptosporidium* oocyst loads (oocysts/sec) in storm runoff from dairy lots for cattle of various age classes as a function of (a) percentage of each lot covered in straw, (b) length of vegetated buffer, and (c) 24 h precipitation.

Furthermore, differences between these studies with respect to soil type, buffer composition and its morphology, hydrologic behavior of runoff, and storm dynamics likely influence buffer efficacy calculations. The vegetated buffers in this study were typically grassy channels that resulted in channelized flow, in contrast to the hill-slope conditions (Atwill et al., 2006) or soil-box buffers (Atwill et al., 2002; Tate et al., 2004) that created more sheet-like flow conditions. This reduction in buffer retention due to channelized flow would be consistent with the finding of Tate et al. (2004) and Atwill et al. (2006) where they found that as rate of precipitation or runoff volume increased, the efficacy of buffer strips to retain *C. parvum* oocysts decreased. Careful site selection for installation of buffers and ensuring that percent cover is maintained may increase buffer efficacy on dairy farms to a greater extent than our models currently predict.

Cattle age class was significantly associated with *Cryptosporidium* loading in storm runoff. Consistent with other dairy fecal studies (Olson et al., 1997; Huetink et al., 2001), we found the highest prevalence and oocyst concentrations in runoff collected from sites housing calves less than 2 mo of age. Given that oocyst concentrations and loads in storm runoff collected from sites housing young calves were 2100 and 728 times greater than runoff collected from sites housing adult cows, respectively, designing BMPs to filter or reduce runoff from calf areas may be one of the best strategies for improving on-farm water quality. The BMPs could involve installing vegetative buffers to reduce runoff from calf areas, or removing calf feces before it can become entrained in runoff events, either with sanitation measures or indoor calf housing. It is also possible that confounding factors are causing us to underestimate the potential for oocyst loading from adult cattle, such as covered calving pens with straw bedding leading to decreased runoff flow and oocyst loading even though the calving adults could be shedding significant numbers of oocysts.

Seasonal precipitation factors were also significantly associated with *Cryptosporidium* discharging in storm runoff from high use areas. Increasing volumes of rain in the watershed during the 24 h preceding sample collection (e.g., late in storm events), and increasing cumulative precipitation (e.g., late in the storm season) were associated with decreased storm runoff concentrations (Fig. 4c, 4d). These findings represent general trends in oocyst loading as related to precipitation events, and are useful when considering seasonal patterns and the associated farm management practices. They are consistent with the idea that early in a storm event and early in the storm season is when accumulated fecal matter will be mobilized in overland runoff, and are consistent with the Sischo et al. (2000) study that found a decreased probability of detecting oocysts in farm-associated streams as 5-d cumulative precipitation increased. Our results are also consistent with the Bodley-Tickell et al. (2002) study in which samples taken early in the storm season were more likely to contain oocysts than samples taken during other times.

While *Cryptosporidium* oocysts were detected on most dairies (4/5), they were detected in a minority of the total stormwater samples tested (21%). These findings are similar to the Sischo et al. (2000) study that detected *Cryptosporidium* on 10 of 11 dairy farms in the northeastern United States, but in only 9% of the farm-associated stream samples. The findings are in contrast to a Japanese study which detected *Cryptosporidium* spp. in 88% of dairy associated river samples (Tsushima et al., 2001), and a study in the United Kingdom that found *Cryptosporidium* in 79% of surface water samples collected near a farming site (Bodley-Tickell et al., 2002). Differences in detection methods do not sufficiently explain the broad range of study findings. Taken together, these studies demonstrate that even with widespread endemic cryptosporidiosis in dairy operations, the environmental loading of these parasites may vary across farm units, depending on both biotic animal factors and abiotic environmental factors.

Although the variables in Tables 2 and 3 were significantly associated with the occurrence of *Cryptosporidium* oocysts in runoff from dairy lots, collectively these variables did not explain the majority of the variance associated with *Cryptosporidium* oocysts in dairy runoff. The calculated McFadden similarity index for maximum likelihood models emulates the R^2 values typical of least squares theory (Hardin and Hilbe, 2001). The goal of this study was to develop a model that estimates the association between the various independent variables and the outcome variable of *Cryptosporidium* oocysts in dairy runoff, not to completely explain the occurrence of oocysts in dairy runoff.

The water spiking trials were critical to accurately assess the water processing recovery efficiency, to adjust the raw oocyst counts in the field samples and estimate the true parasite concentration in each sample. The overall percent recovery of 21% by DFA alone, and 47% when IMS concentration preceded DFA analysis, is similar to what has been found in other studies (Pereira et al., 1999; Atwill et al., 2003). The Pereira et al. (1999) study reported a mean percent recovery of 28 to 34% for DFA when 1000 or more oocysts were spiked into a fecal sample and analyzed by DFA alone. The same study found an improved percent recovery of up to 48% when 100 or more oocysts were spiked into a fecal sample and concentrated with IMS before DFA analysis. Similarly, the Atwill et al. (2003) study reported an IMS-DFA recovery of 32 to 40% when 10 to 500 oocysts were spiked into a fecal sample. The Atwill et al. (2002) study reported higher recovery values, 63 to 81% oocyst recovery by DFA, in runoff samples from vegetated buffer soil box experiments.

Conclusions

This study of BMP efficacy and farm factors associated with protozoal loading in storm runoff has taken the next steps toward understanding the environmental ecology and control of *Cryptosporidium* on coastal California dairies. Factors associated with environmental loading of *Cryptosporidium* oocysts included cattle age class, 24 h precipitation, and cumulative seasonal precipitation, but not percent slope, lot acreage, cattle stocking number, or cattle density. Vegetated buffer strips and straw mulch application significantly reduced the protozoal concentrations and loads in storm runoff, while cattle exclusion and removal of manure did not. The study findings suggest that BMPs such as vegetated buffer strips and straw mulch application, especially when placed near calf areas, will reduce environmental loading of fecal protozoa and improve stormwater quality. Using a systems approach to evaluate the flow and control of pathogens from specific farm loading units such as dry lots, pastures, roads, and waste management systems will help farmers and regulatory agencies prioritize their management efforts in the future (Lewis et al., 2005;

Oborn et al., 2005). The BMPs evaluated in this study represent practical on-farm solutions to improve water quality and decrease pathogen loading in storm runoff.

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References

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.

Atwill, E.R., L. Hou, B.M. Karle, T. Harter, K.W. Tate, and R.A. Dahlgren. 2002. Transport of *Cryptosporidium parvum* oocysts through vegetated buffer strips and estimated filtration efficiency. Appl. Environ. Microbiol. 68:5517–5527.

Atwill, E.R., B. Hoar, M.D.G. Pereira, K.W. Tate, F. Rulofson, and R. Nader. 2003. Improved quantitative estimates of low environmental loading and sporadic periparturient shedding of *Cryptosporidium parvum* in beef cattle. Appl. Environ. Microbiol. 89:4604–4610.

Atwill, E.R., K.W. Tate, M.D.G. Pereira, J. Bartolome, and G. Nader. 2006. Efficacy of natural grassland buffers for removal of *Cryptosporidium parvum* in rangeland runoff. J. Food Prot. 69:177–184.

Blanco-Canqui, H., C.J. Gantzer, and S.H. Anderson. 2006. Performance of grass barriers and filter strips under interrill and concentrated flow. J. Environ. Qual. 35:1969–1974.

Bodley-Tickell, A.T., S.E. Kitchen, and A.P. Sturdee. 2002. Occurrence of *Cryptosporidium* in agricultural surface waters during an annual farming cycle in lowland UK. Water Res. 36:1880–1886.

Daszak, P., A.A. Cunningham, and A.D. Hyatt. 2001. Anthropogenic environmental change and the emergence of infectious diseases in wildlife. Acta Trop. 78:103–116.

Davies, C.M., C.M. Ferguson, C. Kaucner, M. Krough, N. Altavilla, D.A. Deere, and N.J. Ashbolt. 2004. Dispersion and transport of *Cryptosporidium* oocysts from fecal pats under simulated rainfall events. Appl. Environ. Microbiol. 70:1151–1159.

Fayer, R., J.P. Dubey, and D.S. Lindsay. 2004. Zoonotic protozoa: From land to sea. Trends Parasitol. 20:531–536.

Fischer, D.T., S.V. Smith, and R.R. Churchill. 1996. Simulation of a century of runoff across the Tomales Bay watershed, Marin County, California. J. Hydrol. 186:253–273.

Hardin, J., and J. Hilbe. 2001. Generalized linear models and extension. p. 141–158. Stata Press, College Station, TX.

Huetink, R.E.C., J.W.G. van der Giessen, J. Noordhuizen, and H.W. Ploeger. 2001. Epidemiology of *Cryptosporidium* spp. and *Giardia duodenalis* on a dairy farm. Vet. Parasitol. 102:53–67.

Kaucner, C., C.M. Davies, C.M. Ferguson, and N.J. Ashbolt. 2005. Evidence for the existence of *Cryptosporidium* oocysts as single entities in surface runoff. Water Sci. Technol. 52:199–204.

Lennox, M.S., D.J. Lewis, J. Gustafson, K.W. Tate, and E.R. Atwill. 2007. Water quality treatment for livestock feeding and exercise areas on California coastal dairy farms and ranches. Univ. of California Div. of Agriculture and Natural Resources. Publ. 8210. 9 p.

Lewis, D.J., E.R. Atwill, M.S. Lennox, L. Hou, B. Karle, and K.W. Tate. 2005. Linking on-farm dairy management practices to storm-flow fecal coliform loading for California coastal watersheds. J. Environ. Monitor. Assess. 107:407–425.

Medema, G.J., F.M. Schets, P.F.M. Teunis, and A.H. Havelaar. 1998. Sedimentation of free and attached *Cryptosporidium* oocysts and *Giardia* cysts in water. Appl. Environ. Microbiol. 64:4460–4466.

Miller, W.A., E.R. Atwill, I.A. Gardner, M.A. Miller, H.M. Fritz, R.P. Hedrick, A.C. Melli, N.M. Barnes, and P.A. Conrad. 2005. Clams (*Corbicula fluminea*) as bioindicators of fecal contamination with *Cryptosporidium* and *Giardia* spp. *In* freshwater ecosystems in California. Int. J. Parasitol. 35:673–684.

Morgan, U.M., C.C. Constantine, D.A. Forbes, and R.C.A. Thompson. 1997. Differentiation between human and animal isolates of *Cryptosporidium parvum* using rDNA sequencing and direct PCR analysis. J. Parasitol. 83:825–830.

Mosley, M.P., and A.I. McKercher. 1993. Streamflow. p. 8.1–8.39. *In* D.R. Maidment (ed.) Handbook of hydrology. McGraw-Hill, New York.

Oborn, I., A. Modin-Edman, H. Bengtsson, G.M. Gustafson, E. Salomon, S.I. Nilsson, J. Holmqvist, S. Jonsson, and H. Sverdrup. 2005. A systems approach to assess farm-scale nutrient and trace element dynamics: A case study at the Ojebyn dairy farm. Ambio 34:301–310.

Okhuysen, P.C., C.L. Chappell, J.H. Crabb, C.R. Sterling, and H.L. DuPont. 1999. Virulence of three distinct *Cryptosporidium parvum* isolates for healthy adults. J. Infect. Dis. 180:1275–1281.

Olson, M.E., N.J. Guselle, R.M. O'Handley, M.L. Swift, T.A. McAllister, M.D. Jelinski, and D.W. Morck. 1997. *Giardia* and *Cryptosporidium* in dairy calves in British Columbia. Can. Vet. J. 38:703–706.

Peng, M.M., M.L. Wilson, R.E. Holland, S.R. Meshsnick, A.A. Lal, and L. Xiao. 2003. Genetic diversity of *Cryptosporidium* spp. in cattle in Michigan: Implications for understanding transmission dynamics. Parasitol. Res. 90:175–180.

Pereira, M.D.G., E.R. Atwill, and T. Jones. 1999. 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. 65:3226–3239.

Searcy, K.E., A.I. Packman, E.R. Atwill, and T. Harter. 2006. Deposition of *Cryptosporidium* oocysts in streambeds. Appl. Environ. Microbiol. 72:1810–1816.

Sischo, W.M., E.R. Atwill, L.E. Lanyon, and J. George. 2000. *Cryptosporidia* on dairy farms and the role these farms may have in contaminating surface water supplies in the northeastern United States. Prev. Vet. Med. 43:253–267.

Sojka, R.E., J.A. Entry, W.J. Orts, D.W. Moishita, C.W. Ross, and D.J. Horne. 2005. Synthetic and bio-polymer use for runoff water quality management in irrigated agriculture. Water Sci. Technol. 51:107–115.

Stout, W.L., Y.A. Pachepsky, D.R. Shelton, A.M. Sadeghi, L.S. Saporito, and A.N. Sharpley. 2005. Runoff transport of faecal coliforms and phophorus released from manure in grass buffer conditions. Lett. Appl. Microbiol. 41:230–234.

Syversen, N. 2005. Cold-climate vegetative buffer zones as pesticide-filters for surface runoff. Water Sci. Technol. 51:63–71.

Tate, K.W., E.R. Atwill, J.W. Bartolome, and G. Nader. 2006. Significant *Escherichia coli* attenuation by vegetative buffers on annual grasslands. J. Environ. Qual. 35:795–805.

Tate, K.W., G.A. Nader, D.J. Lewis, E.R. Atwill, and J.M. Connor. 2000. Evaluation of buffers to improve the quality of runoff from irrigated pastures. J. Soil Water Conserv. 55:473–478.

Tate, K.W., M.D.G. Pereira, and E.R. Atwill. 2004. Efficacy of vegetated buffer strips for retaining *Cryptosporidium parvum*. J. Environ. Qual. 33:2243–2251.

Trask, J.R., P.K. Kalita, M.S. Kuhlenschmidt, R.D. Smith, and T.L. Funk. 2004. Overland and near-surface transport of *Cryptosporidium parvum* from vegetated and nonvegetated surfaces. J. Environ. Qual. 33:984–993.

Tsushima, Y., P. Karanis, T. Kamada, H. Nagasawa, X. Xuan, I. Igarashi, K. Fujisaki, E. Takahashi, and T. Mikami. 2001. Detection of *Cryptosporidium parvum* in environmental water in Hokkaido, Japan. J. Vet. Med. Sci. 63:233–236.

Tyrrel, S.F., and J.N. Quinton. 2003. Overland flow transport of pathogens from agricultural land receiving faecal wastes. J. Appl. Microbiol. 94:87S–93S.

USDA-NRCS. 2004. Standards and specifications 342A, USDA-NRCS field office technical guide. Available at http://www.nrcs.usda.gov/search.asp?site=NRCS&client=usda&output=xml&newparam=&restrict=NRCS_NHQ&q=standards+and+specifications+342A&Go.x=0&Go.y=0&Go=Search (verified 19 May 2008). USDA-NRCS, Washington, DC.

Xiao, L., U.M. Morgan, J. Limor, A. Escalante, M. Arrowood, W. Shulaw, R.C.A. Thompson, R. Fayer, and A.A. Lal. 1999. Genetic diversity within *Cryptosporidium parvum* and related *Cryptosporidium* species. Appl. Environ. Microbiol. 65:3386–3391.