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Science of the Total Environment 321 (2004) 47–58
Science of the Total Environment
An International Journal for Scientific Research into the Environment and its Relationship with Humankind
www.elsevier.com/locate/scitotenv

Cryptosporidium parvum oocyst inactivation in field soil and its relation to soil characteristics: analyses using the geographic information systems

Satomi Kato[a,*], Michael Jenkins[b], Elizabeth Fogarty[a], Dwight Bowman[a]

[a]*Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA*
[b]*USDA-ARS, Watkinsville, GA 30677, USA*

Received 29 April 2003; accepted 13 August 2003

Abstract

The need exists to understand the environmental parameters that affect inactivation of *Cryptosporidium parvum* oocysts in soil under field conditions. The inactivation of *C. parvum* oocysts placed in the natural environment was studied at a dairy farm in western New York State, USA. Seventy sampling points were arranged in a grid with points 150 m apart using the Geographic Information System. The sampling points were distributed among three distinct areas: woodland, corn field and pasture. Purified oocysts were inoculated into chambers filled with soil from each sampling point, and buried in the surface of each respective sampling point. To compare *C. parvum* oocyst survival with another organism known to survive environmental stresses, *Ascaris suum* eggs were also placed in soil contained in chambers and buried at the same sampling points as the oocysts. As controls oocysts and eggs in distilled water were also placed at each sampling point. Oocyst and egg viability, soil pH and percent gravimetric water content were measured at all sampling points at 0, 60 and 120 day sampling periods. Soil organic content was determined for each sampling point. At 120 days after placement, mean viability of *C. parvum* oocysts was 10% although at a few sampling points, 30% of oocysts were still potentially infective; whereas 90% of *A. suum* eggs were viable at all sampling points. Statistically significant differences were not observed among the three different sampling areas, and no statistically significant predictors were found by regression analysis. Results exemplified the heterogeneity of soil parameters and oocyst viability across a landscape; such results make predictive models for *C. parvum* inactivation problematical. The long-term survival of *C. parvum* oocysts in soil under field conditions, as this study demonstrated, emphasizes their potential as a risk to contaminate surface waters.

Keywords: *Cryptosporidium parvum* oocysts; Geographic information system (GIS); *Ascaris suum* eggs; ArcView; Dye permeability assay; Inactivation rates; Soil moisture content; Soil organic content; Soil pH

*Corresponding author. *Present address*: Department of Family Medicine and Community Health, Tufts University School of Medicine, Boston, MA 02111, USA. Tel.: +1-617-636-3452; fax: +1-617-636-4017.
E-mail address: Satomi.Kato@tufts.edu (S. Kato).

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doi:10.1016/j.scitotenv.2003.08.027

1. Introduction

Since the first waterborne outbreak of *Cryptosporidium parvum* was reported in 1985 in Texas, USA, at least 19 additional outbreaks have been identified around the world, and more than 427 000 people have been infected by this parasite (Smith and Rose, 1998). Dairy farms are known to serve as a potential source for the distribution of *C. parvum* oocysts because one infected calf alone can release billions of oocysts into the environment (Walker and Stedinger, 1999). Although studies have shown that both dairy cows and wildlife contribute to the load of *Cryptosporidium* oocysts in surface waters of watersheds (Jellison et al., 2002), Hansen and Ongerth (1991) reported that oocyst concentrations in surface waters impacted by dairy farms were an order of magnitude higher than surface waters impacted by wildlife alone.

No effective means has been developed to measure the potential effects of watershed environments on *C. parvum* oocyst contamination. Barwick (1998, unpublished dissertation, Cornell University, Ithaca, New York, USA) examined the prevalence of *C. parvum* oocysts in the soil of dairy farms in Southeastern New York State, USA. Out of 782 soil samples from 37 farms, the prevalence of *Cryptosporidium* was 17%. The study, however, did not examine if the type of soil on a farm site would affect the inactivation of oocysts in these areas. Walker and Stedinger (1999) generated a decay and transport model for oocysts, but soil characteristics were also not included within their modeling scheme. Jenkins et al. (2002) performed a laboratory pot study to determine if soil type (silty clay loam, silt loam and loamy sand), temperature (4 °C, 20 °C and 30 °C), and soil water potential (−0.033, −0.5 and −1.5 MPa) affected oocyst survival. Their results indicated that the range of soil water potentials that they tested had no significant effect on oocyst inactivation, however, survival was significantly greater in the silt loam compared to the other two soil types, and temperature appeared to have the greatest effect on inactivation, with greatest survival associated with 4 °C. Under constant laboratory conditions oocyst survival was significantly greater in all three soil types than in distilled water (Jenkins et al., 2002). Also, under laboratory conditions, the oocyst inactivation exposed to repeated freeze–thaw events was faster in soil than in water (Kato et al., 2002).

Because the experiments reported by Jenkins et al. (2002) and Kato et al. (2002) were under laboratory conditions, field experiments are needed to test further the hypothesis that soil properties such as soil type (texture), soil water content, pH and soil organic carbon under winter conditions affect oocyst inactivation. To test this hypothesis: (1) a field experiment was established on a dairy farm in New York State, USA that compared the inactivation kinetics of *C. parvum* with *Ascaris suum* (an helminthic parasite used to test disinfection processes for biosolids); and (2) Geographic information system (GIS) technique of spatial statistical analysis and multiple regression analysis were used to identify possible soil factors affecting oocyst inactivation.

2. Materials and methods

2.1. C. parvum oocysts

C. parvum oocysts were obtained from naturally infected, 7–14-day-old calves in Tompkins County, New York, USA. A sucrose/Percoll™ (Pharmacia, Uppsala, Sweden) flotation method was used to extract oocysts from calf feces (Jenkins et al., 1997). After extraction, oocysts were stored at 4 °C in water with antibiotics (100 U of penicillin G sodium ml^{-1}, 100 μg of streptomycin sulfate ml^{-1} and 0.25 μg of amphotericin B ml of $suspension^{-1}$) and were used within 1 month of collection. The viability of the purified oocysts was determined by the dye permeability assay (described below). At the time of inoculation, 99% of the oocysts were viable.

2.2. A. suum eggs

Ascaris suum eggs were harvested from the feces of naturally infected pigs. The eggs were purified by washing and sieved by a No. 400 US Standard mesh sieve (38 μm diameter opening). The extracted eggs were used within 3 to 4 weeks

of collection and purification. They were stored at 4 °C in water with 0.5% formalin. The viability of eggs was determined as described below; eggs were 98% viable at the time of inoculation.

2.3. Sentinel chambers

The sentinel chamber method developed by Jenkins et al. (1999) was used to monitor *C. parvum* oocyst and *A. suum* egg viability in the soils. The chambers into which *C. parvum* oocysts were inoculated consisted of an acrylic cylinder with dimensions of 9.5 mm inside diameter by 25 mm length with seated washers holding in place 10 μm-pore-size nylon mesh at each end. Chambers into which *A. suum* eggs were inoculated consisted of polycarbonate cylinders with dimensions of 13 mm inside diameter by 25 mm length with No. 400 US standard stainless steel mesh fastened to each end to form a seal. Each chamber was designed to contain a representative soil sample, to equilibrate with the solute and moisture regime of the external environment, and to be easily retrievable and processed for analysis. Soil from each sampling site in the field (described below) was collected, air-dried, sieved through a No. 10 US standard mesh sieve (2 mm diameter opening), and approximately 0.7 g was placed into the chambers. The chambers were then placed into a foam rack and partially submerged in a pan of distilled water until all the soil in each of the chambers had wicked up water to near the water holding capacity. Oocysts, 10^7 in 40 μl of distilled water, and eggs, 10^4 in 40 μl of distilled water, were then inoculated into the soil filled chambers. The open end of the chambers was then sealed.

2.4. Field and experimental design

The field sampling site was established on the Teaching and Research Center (Department of Animal Science, Cornell University, New York, USA) at Harford, New York, USA. The field site was composed of two soil series, a Holly (a silty clay loam) and a Langford (a channery silt loam) (Maxon, 1917; Howe, 1924). The longitude and latitude of 70 sampling points, each 150 m apart were recorded using a global positioning system (GPS) unit. The field sampling sites were divided in three categories: corn field (33 sampling points), pasture (16 sampling points), and non-field (wooded) areas (21 sampling points) (Fig. 1). These categories were made to identify field effects on the viability of oocysts. From each sampling point, soil samples were collected, air-dried and water content and pH were measured. A small quantity (0.7 g) of each sieved and dried soil sample was put into chambers that were inoculated with oocysts and eggs as described above. Inoculated chambers were then buried in the surface 3–4 cm at the same sampling points from which the soil samples were initially taken. Control oocysts, 10^6 oocysts in distilled water in microcentrifuge tubes, and control *A. suum* eggs, 10^4 eggs in distilled water in microcentrifuge tubes were also buried at the same sampling sites.

The chambers and microcentrifuge tubes were placed in the field in November 2000. Chambers were sampled at 0, 60 and 120 days after placement, and oocyst and egg viability was determined. Soil samples at each sampling point were also collected at the same sampling times for analysis. Temperature probes were buried at four sampling points to record temperature changes.

2.5. Oocyst extraction from soil

To extract oocysts from the chambers, soil was removed from the chambers and put into a 15 ml polypropylene centrifuge tube containing 7 ml of a 0.5% 7× detergent (Linbro 7×, Thomas Scientific, Swedesboro, New Jersey, USA) in phosphate buffered saline (PBS). A small amount of Zirconia/silica beads (0.5 mm, BioSpec Products, Inc., Bartlesville, Oklahoma, USA) was added, and the soil, beads and detergent were mixed for 20 s using a vortex mixer. The 7 ml mixture was then underlaid with the 7 ml of cold sugar solution (sp gr. 1.18). After the samples were centrifuged at $1800 \times g$ for 20 min at 4 °C, approximately 3 ml of the interface was gently removed and transferred to a second tube. Distilled water, 12 ml, was added to the sample; the diluted sample was mixed thoroughly and centrifuged again at $1800 \times g$ for 20 min at 4 °C. The supernatant was aspirated, leaving approximately 1 ml in which the pellet

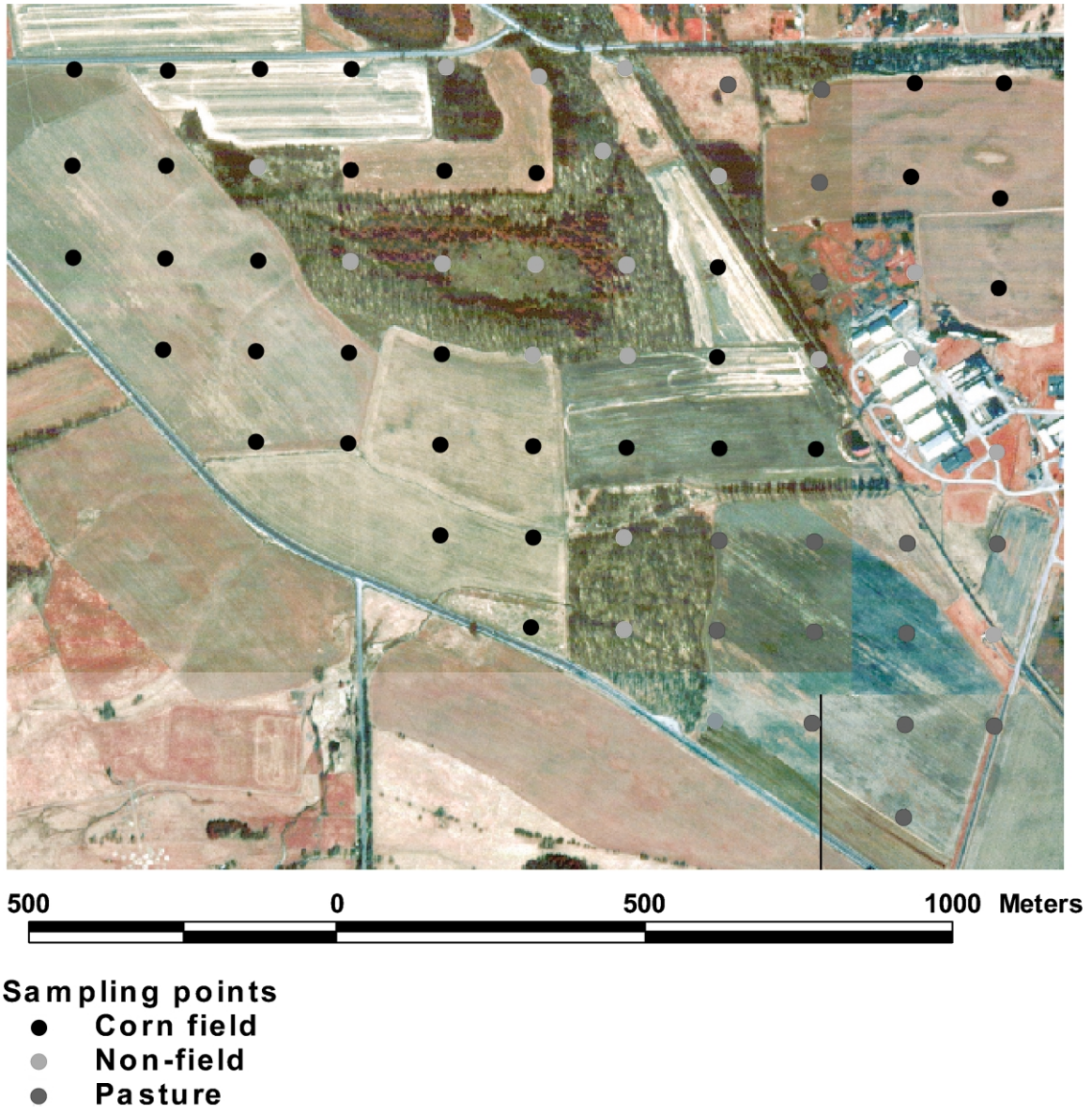

Fig. 1. Digital aerial photograph of the field sampling site at the Teaching and Research Center of Cornell University, Harford, New York, USA. Seventy sampling points were established 150 m apart, using a GPS unit. The sampling sites were distributed among three distinct areas as indicated on the photograph: corn field, woodland and pasture.

was resuspended, transferred to a microcentrifuge tube, and sedimented at 11 000×*g* for 5 min at 4 °C. The supernatant was aspirated and the pellet was resuspended in 100 μl PBS in preparation of the dye permeability assay.

2.6. Egg extraction from soil

The material in the chambers was washed into 15 ml polypropylene conical centrifuge tubes using distilled water. The tubes were filled with distilled

water and centrifuged at 1800×*g* for 1 min at 4 °C and the supernatant was discarded. Aqueous magnesium sulfate (sp gr. 1.20), 4 ml, was added to the pellet, and the pellet was fully suspended using applicator sticks and a vortex mixer. The tube was then filled to 15 ml with the magnesium sulfate solution, and the suspended material was centrifuged again at 1000×*g* for 3 min at 4 °C. The suspension at the top of the tube was then poured over a No. 400 US standard mesh sieve, and the material on the sieve was collected and transferred to T25 flasks with vented tops.

2.7. Dye permeability assay for oocyst viability

A dye permeability assay using propidium iodide (PI) (Sigma, St. Louis, Missouri, USA) was performed to assess oocyst viability as described by Jenkins et al. (1997). Stock solutions of PI (1 mg in 0.1 M phosphate-buffered saline [PBS] ml^{-1}) were prepared. The dye was added to aliquots of recovered oocyst suspensions at the ratio of 1:10 (vol/vol), mixed gently, and incubated for 2 h at 37 °C in the dark. After the oocysts were labeled with the fluorescent dye PI, they were stained with a monoclonal antibody (Hydrofluor, Strategic Diagnostics, Inc., Newark, Delaware, USA) specific for the oocyst wall of *Cryptosporidium* as described by Jenkins et al. (1997). Oocysts that did not take up PI, PI negative (PI^-) and displayed outlines of sporozoites were considered viable (Anguish and Ghiorse, 1997; Jenkins et al., 1997); oocyst that took up PI (and fluoresced red), PI positive (PI^+) were considered non-viable.

2.8. Microscopy

Samples were examined by epifluorescence and differential interference contrast (DIC) microscopy (Eclipse, E600, Nikon, Tokyo, Japan). Excitation bands of 546/10 and 450–490 nm were used. At least 100 oocysts in each sample were examined.

2.9. A. suum egg viability determination

The viability of *A. suum* eggs was determined by culture in T25 flasks in water with 0.5% formalin. The eggs were examined to verify the number out of 100 that had successfully completed embryonation (Reimers et al., 1982).

2.10. Soil pH

Soil samples were taken from each sampling point at 0, 60 and 120 days after placement to measure the pH of the soil using a standard method (Thomas, 1996).

2.11. Water percentage in soil by weight

Soil samples were taken from each sampling point at 0, 60 and 120 days after placement to measure the percentage of water content by weight using a modification of a standard method (Clesceri et al., 1998). Soil samples, approximately 40 g, were measured and dried at 56 °C for overnight, and the weight of dried soil samples was measured. Percentages of water content by dried soil weight were calculated.

2.12. Soil organic content

The soil organic content of each sampling site was measured using a standard method (Clesceri et al., 1998).

2.13. Statistical analysis

Inactivation: Analysis of variance for mean oocyst viability in soil and water was performed using a general linear model (GLM) (Minitab 13, Minitab Inc., State College, Pennsylvania, USA). The viability was compared using the Bonferroni multiple comparison test at $P=0.05$.

Inactivation prediction model: A prediction model of oocyst inactivation was generated using Minitab 13. A step-wise regression analysis was used to generate the prediction model for oocyst inactivation. Alpha-levels for entry to and removal from a model are 0.15. The regression model is:

$$Y=\beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_3+\ldots+\beta_7X_7+\varepsilon \quad (1)$$

where Y is the response (the percentage of viable oocysts), X_1 is sampling time, X_2 is oocyst placement (in water or soil), X_3 is field areas (corn field, non-field (wooded) and pasture areas), X_4 is the percentage of viable *A. suum* eggs, X_5 is soil pH, X_6 is water percentage in soil by weight and X_7 is soil organic content. Sampling time (X_1) and oocyst placement (X_2) are the primary factors, and the other variables are potential confounding factors. The β_0, β_1, β_2, β_3, and β_7 are the regression coefficients, and ε is an error term with a normal distribution and a mean of 0 and standard deviation σ.

Inactivation rate: The inactivation rates of oocysts and days required to achieve 99% inactivation in the field were determined using a first order kinetic equation (Kato et al., 2002):

$$Y_t = Y_0 \times e^{-kt} \quad (2)$$

where Y_t is the percentage of viable oocysts at time t, Y_0 represents the initial percentage of viable oocysts, k is the coefficient of inactivation and t is the time (days). Based on Eq. (2), the coefficient of inactivation (k) was determined by regressing $\ln(Y_0/Y_t)$ against time (t). Confidence intervals (95%) were determined using the Student t-value at the appropriate degrees of freedom at an α-level (two-sided) of 0.025. The number of days required for the oocysts to reach 99% inactivation was determined using the equation:

$$t = \ln(Y_0) \times k^{-1} \quad (3)$$

where t is the number of days required to achieve a 99% inactivation, Y_0 is the initial percentage of viable oocysts and k is the coefficient of inactivation calculated in Eq. (2).

2.14. Graphical presentation and GIS analysis

ArcView GIS Spatial Analyst (ESRI, Redlands, California, USA) was used to produce a surface map (a surface grid theme) of oocyst inactivation and other environmental factors in the field soil over the sampling period. The inactivation of *C. parvum* oocysts at each sampling point was analyzed by the ArcView Spatial Analyst with the inverse distance weighted (IDW) technique and was graphically represented (a grid theme). Other soil factors and *A. suum* egg viability were also graphically presented with the ArcView Spatial Analyst using the IDW technique.

Table 1
Mean percent viability of *Cryptosporidium parvum* oocysts and *Ascaris suum* eggs in field soil

Sampling day	Placement	Mean% ± S.D.
C. parvum		
60	Soil	39.4 ± 9.2 (n = 66)
	Water	45.8 ± 11.3 (n = 66)
120	Soil	10.2 ± 8.1 (n = 63)
	Water	11.2 ± 8.8 (n = 64)
A. suum		
60	Soil	95.6 ± 2.6 (n = 66)
	Water	95.8 ± 1.5 (n = 66)
120	Soil	96.0 ± 2.1 (n = 63)
	Water	94.7 ± 4.1 (n = 63)

3. Results

3.1. C. parvum oocysts placed in the field soil

Mean oocysts viability 60 days after placement was 39.4% in soil and 45.8% in water (Table 1) indicating no significant difference whether oocysts were in soil or water. After 120 days, oocyst viability was reduced to 10.2% in soil and 11.2% in water. The range of percent viability of oocysts in chambers containing soil and in microcentrifuge tubes containing distilled water at 60 and 120 days after placement in the field was from 20% to 60% and from less than 5% to 30%, respectively (Figs. 2 and 3). The distribution of these ranges of viability is represented in Figs. 2 and 3. At four sampling points, between 25 and 30% of oocysts were still viable in soil 120 days after placement (Fig. 2). Between 25 and 30% of oocysts in microcentrifuge tubes with water were also viable at four sampling points 120 days after placement, but at the different sampling points (Fig. 3). Oocysts in soil and water were inactivated faster in the central part of the sampling site that included areas across the corn field and wooded non-field (Figs. 2 and 3).

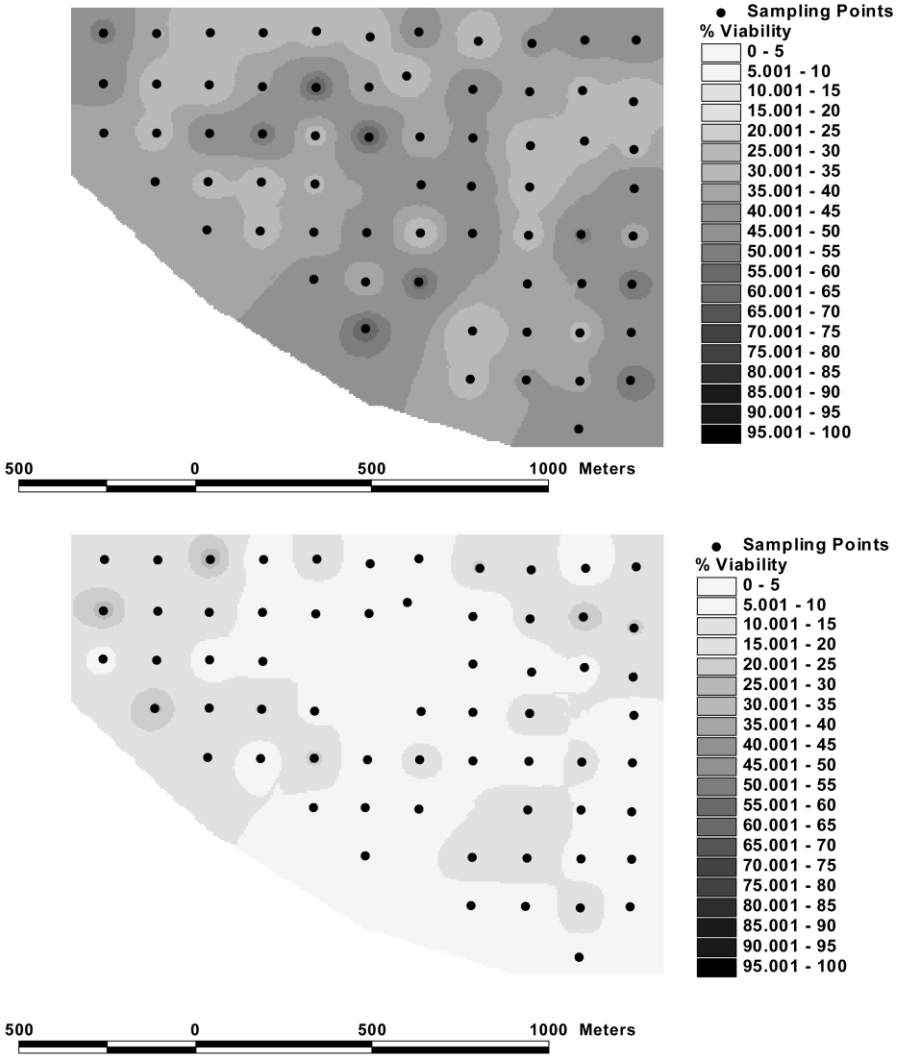

Fig. 2. Surface maps of the percentage viability of *Cryptosporidium parvum* oocysts. (a) 60 days after placement and (b) 120 days after placement at each sampling point in chambers containing soil from each sampling point.

3.2. A. suum eggs placed in the field

Across all sampling points, more than 90% of *A. suum* eggs were viable 60 days after placement in soil and water (data not shown); mean viability was 96% in both soil and water. Little decrease in viability occurred 120 days after placement. Mean viability in soil was 96 and 95% in water. No statistical differences were observed between the viability of eggs placed in soil and water. No significant correlation was observed between the inactivation of *C. parvum* oocysts and *A. suum* eggs placed in soil and water. No apparent relationship was observed between oocyst viability and soil texture.

3.3. Soil temperature

The ground temperature at the sampling site was recorded using temperature probes placed at

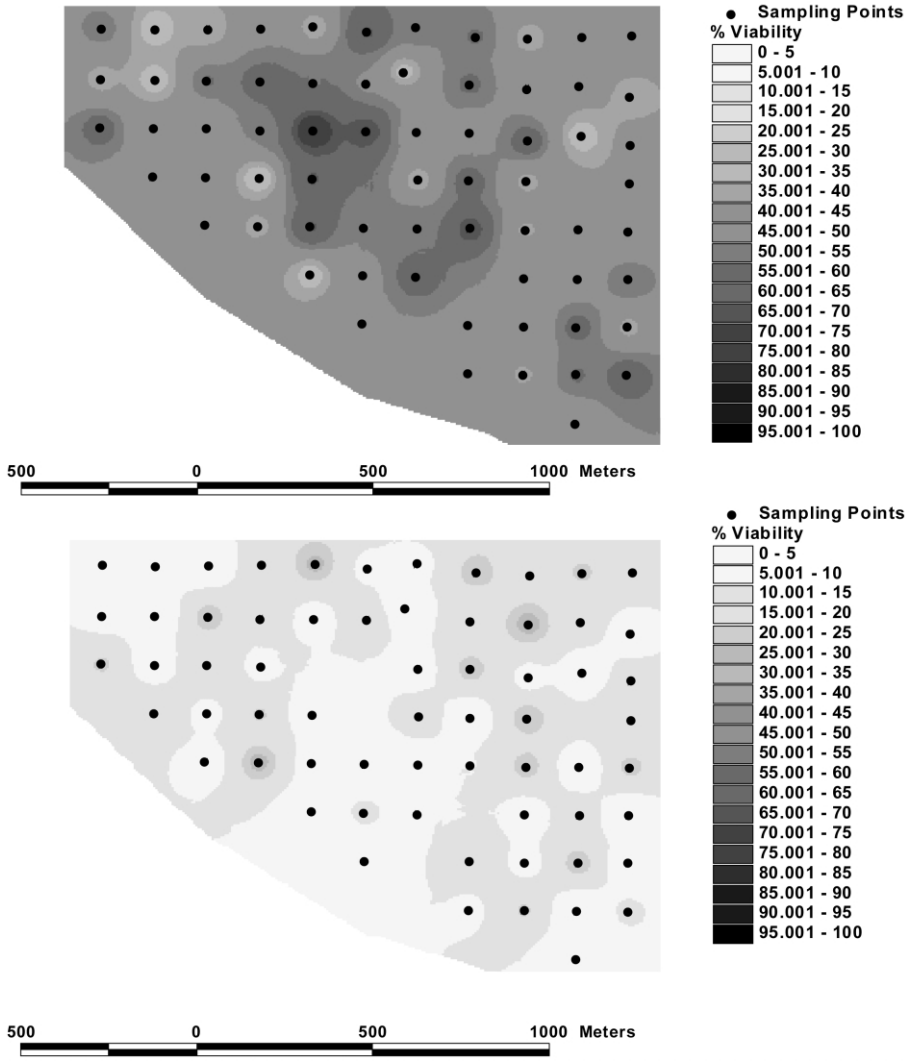

Fig. 3. Surface maps of the percentage viability of *Cryptosporidium parvum* oocysts. (a) 60 and (b) 120 days after placement at each sampling point in microcentrifuge tubes containing distilled water.

four sampling points. One of the four probes failed and did not function during the trial. There were no differences observed among the other three probes buried in the field. Data recorded by one representative probe (Fig. 4) indicated that the soil temperatures remained between 0 and 1 °C over most of the 120 day study period with short periods of temperatures below 0 °C and some apparent freeze–thawing.

3.4. Statistical analysis

Using regression analysis, a significant relationship appeared to exist between the sampling time and the percentage of viable *C. parvum* oocysts ($P = 0.0$) but not *A. suum* eggs. Inactivation of *A. suum* eggs was negligible compared to *C. parvum* oocysts. Oocyst viability decreased on the average by 0.54% for each day after placement in the field.

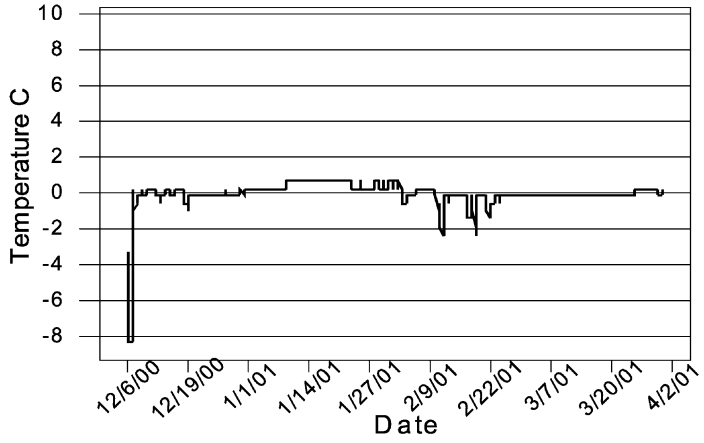

Fig. 4. Representative average daily surface soil temperatures over the duration of the study.

Using a step-wise regression analysis, no statistically significant independent variables were observed relative to the percentage of viable *C. parvum* oocysts among the percentage of viable *A. suum* egg or the measured soil factors: field areas, soil pH (although the range was from 4 to 7), percentage of water by weight and soil organic content.

Inactivation rates of oocysts and days required to achieve 99% inactivation were calculated (Table 2). Inactivation rates for oocysts placed in soil and water were not significantly different: 0.028 in soil and 0.030 in water. The days required to achieve 99% inactivation of oocysts (Table 2) were 163 days and 160 days for the oocysts placed in soil and water, respectively. There were no statistical differences observed among the viability of oocysts in the three field areas (Table 3).

4. Discussion

4.1. Oocyst and egg inactivation

C. parvum oocysts were more susceptible to environmental stresses than were *A. suum* eggs. *A. suum* eggs placed in soil and water in field soil were resistant to environmental stresses, and 95% of eggs were still viable 120 days after placement. *A. suum* eggs were known to be very resistant to various treatment processes (Reimers et al., 1982; Larsen and Roepstorff, 1999). At both 60 and 120 days, the viability of oocysts in the chambers filled with soil was not significantly different from the viability of oocysts in water. These data agree with one of two field studies incorporating chambers that Jenkins et al. (1999) reported. They observed no difference in the inactivation of oocysts in

Table 2
Rates and days to reach 99% inactivation of *Cryptosporidium parvum* oocysts in field soil

Oocyst placement	Inactivation rate (95% CI[a]) day^{-1}	Days to reach 99% inactivation
Soil	0.028 (0.023–0.033)	163
Water	0.030 (0.023–0.034)	160

[a] Confidence interval.

Table 3
Rates and days to reach 99% inactivation of *Cryptosporidium parvum* oocysts in the different field areas: corn field, woodland and pasture

Oocyst placement	Area	*n*	Inactivation rate (95% CI[a]) day^{-1}	Days to reach 99% inactivation
Soil	Corn field	33	0.028 (0.021–0.035)	164
	Woodland	21	0.031 (0.018–0.043)	150
	Pasture	16	0.028 (0.016–0.039)	166
Water	Corn field	33	0.026 (0.020–0.033)	174
	Woodland	21	0.035 (0.022–0.047)	133
	Pasture	16	0.025 (0.017–0.034)	183

[a] Confidence interval.

chambers filled with soil and oocysts in water filled microcentrifuge tubes over 45 days with soil temperatures between 0 and 5 °C. In their other field study, and contrary to the results of this present study, they reported observing a greater decline in viability of oocysts in soil than in water over 75 days in which the soil temperature was below 0 °C and in which seven freeze–thaw events occurred. In this study, no significant difference in rates of inactivation existed between oocysts in soil filled chambers and water filled microcentrifuge tubes and are, thus, contrary to a pot study Jenkins et al. (2002) reported. Kato et al. (2002) reported the inactivation rates of oocysts exposed to repeated freeze–thaw events under laboratory settings and the inactivation rates of oocysts were faster in soil than in water. Jenkins et al. (2002) observed greater inactivation rates of oocysts in water than in soil. The inactivation rates in the soil filled chambers in this study were an order of magnitude greater than those of Jenkins et al. (2002) reported for their pot study, and indicates the differences between a field study under varying environmental conditions and the constant conditions of a laboratory study.

4.2. Regression analysis

The lack of significant correlation between oocyst inactivation and soil water content also reflects observation reported by Jenkins et al. (2002), in which they showed that soil water potential between −0.033 MPa (a soil at or near full water holding capacity) and −1.5 MPa (a dry soil associated with permanent wilt) had no effect on oocyst inactivation. No correlation between soil pH and oocyst inactivation further underscores oocyst resistance to pH levels between 3 and 11 as previously observed (Jenkins et al., 1998, 1999, 2002). The two soil series, Holly (a silty clay loam), and Langford (a channery silt loam) did not make a difference in oocysts inactivation under the field conditions of this study. Jenkins et al. (2002) observed a significant difference in oocyst inactivation between a silty clay loam and a silt loam. These two contrasting observations regarding the effects of soil texture and other parameters associated with a particular soil series reflect either the difference between a field and laboratory study, or indicate that some differences between soil types cannot be generalized.

Although no statistical differences were observed in oocyst inactivation rates in the three field areas (non-field (wooded), corn field and pasture areas), the inactivation of oocysts placed in soil at wooded areas was higher than those in the other two areas. The sampling points high in soil organic content, percentage of water by weight and low in pH were located in the woods. At those sampling points, most of the oocysts were inactivated (0%–5% viability). Although the biotic aspect of soil was not a parameter, it may play a role in oocyst inactivation. In particular, protozoan predation may inactivate oocysts. Fayer et al. (2000) reported that under laboratory conditions six genera of rotifers ingested *C. parvum* oocysts. In their study, they could not determine if ingestion lead to digestion or inactivation of the oocysts. They observed that some rotifers excreted boluses

containing several oocysts. Since these rotifers are found in damp soils (Fayer et al., 2000) as in the wooded areas of the field site, they and other protozoa known to ingest *C. parvum* oocysts (Stott et al., 2001) may affect the oocyst inactivation rates. Under the conditions of this field study, however, the low soil temperatures would preclude insignificant predatory activity.

Because no significant correlation was observed between the viability of oocysts and the measured soil factors and *A. suum* eggs, no prediction model could be made to estimate the inactivation of oocysts in field soil. Results indicate the difficulty in predicting oocyst inactivation by the measured soil factors and by comparison to *A. suum* inactivation in the natural environment. The presence of a few sampling points that retained between 25 and 30% potentially infective oocysts 120 days after placement indicates the heterogeneity across a landscape, and the potential presence of high concentrations of viable *C. parvum* oocysts that could be transported to surface waters.

This study could be useful to extension programs and the farmers they serve, especially in or near watershed environments, in which the potential threat of applying animal wastes containing viable *C. parvum* oocysts in present as Walker and Stedinger (1999) emphasized. Large scale studies, including more frequent sampling would appear to be required to establish an oocyst inactivation prediction model for watershed environments. In this study, sampling was prohibited on Day 90, the middle of the study period because of a heavy snow accumulation on the field. Also, samples had to be removed from the field in the spring because of plowing.

4.3. Conclusions

Under the field conditions of this study inactivation of *C. parvum* oocysts was significantly greater than *A. suum* eggs, which showed negligible inactivation over the study's duration. The high level of *A. suum* egg survival in the landscape underscores the necessity of disinfecting sewage sludge before its application to agricultural fields. Although no statistical correlation was observed between the viability of oocysts and the measured soil factors, and no effective predictive model on the inactivation of *C. parvum* oocysts could, therefore, be developed, this study, nevertheless, has established the groundwork for such a predictive model. The results indicated that a significant fraction of *C. parvum* oocysts were inactivated over the 120 days in field soils. The fact that as much as 5–30% of oocysts remained viable and potentially infective after their deposition indicates, however, that they continued to persist and if they had been naturally deposited they could be transported by runoff from rain events to surface waters. Establishing appropriate methods of manure and biosolids management is, therefore, important for protecting watershed environments.

Acknowledgments

This material is based upon work supported by the Cooperative State Research, Education and Extension Service, US Department of Agriculture, under Agreement No. 98-35102-6503.

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