Transport of manure-borne *Cryptosporidium parvum* oocysts through saturated and unsaturated soil columns

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A b s t r a c t. Release of manure-borne *Cryptosporidium parvum* oocysts is the most probable cause of ground and surface water contamination with this pathogen. Our objective was to assess the effect of water saturation on transport of manure-borne oocyst through soil cores. Manure seeded with oocysts was applied on the surface of 10 cm columns filled with either sandy loam or clay loam soils. An eight-hour rainfall simulation provided saturated flow in one set of columns whereas the other set had suction *ca.* 5 kPa applied at the bottom. The convective-dispersion model with exponential release boundary condition, instantaneous adsorption and first-order kinetic removal of oocysts was used to simulate the transport. Transport parameters were found by fitting the van Genuchten analytical solution to the oocyst profile distributions. Oocysts stayed mostly within the top 8 cm of soil columns. Cumulative oocyst contents in leachates from unsaturated columns were less than 0.1% whereas the saturated columns allowed breakthrough of 0.4 and 1.3% in sandy loam and clay loam soil cores, respectively. The model mimicked the profile distributions of oocysts very well, but failed to simulate breakthrough of small amounts of oocysts. Values of retardation coefficient were less than unity in saturated columns and greater than unity in unsaturated columns. Values of the soil partition coefficient K_d derived from the retardation coefficient values were much less than values reported earlier from batch experiments with the same soils. The removal rates were much higher in saturated than in unsaturated columns. Soil water saturation substantially affected the manure-borne oocyst transport.

K e y w o r d s: *Cryptosporidium parvum*, soil water, transport

INTRODUCTION

Cryptosporidium parvum, the causal agent of crypto-sporidiosis, is a widespread protozoan parasite afflicting numerous mammalian species, including humans. Several outbreaks of cryptosporidiosis have occurred in the past decade, the most severe in Milwaukee, WI, where over 400 000 people were infected (Mackenzie *et al*., 1994). *C. parvum* is a particularly serious health threat to immuno-deficient individuals *eg* AIDS, cancer patients because there are no effective treatments for the disease.

An important mode of transmission to humans is believed to be via contaminated drinking water and/or recreational waters. Although wildlife (Atwill *et al*., 1997; Graczyk *et al*., 1998) and sewage outflows (States *et al*., 1997) have been implicated in watershed contamination, dairy/beef animals are considered to be a major source of contamination because of their numbers and distribution, incidence of infection, and extent of oocyst excretion. Based on a survey of 7 369 calves from 1 103 dairy farms (located in 28 states), Garber *et al*. (1994) concluded that virtually all herds with >100 cows are infected with *C. parvum*.

Water contamination can occur via surface transport of oocysts from land-applied manures or fecal excretion, or vertical transport via preferential flow to groundwater *eg* karst groundwater. Previous laboratory studies have documented the potential for oocyst runoff or leaching. Atwill *et al*. (2002) investigated the impact of vegetated buffer strips on overland oocyst transport. Mawdsley *et al*. (1996a, 1996b) investigated oocyst fate and transport through intact soil cores and soil blocks. Although absolute numbers of oocysts in leachate or runoff were substantial, percent recoveries were relatively low. Darnault *et al*. (2004) found that in saturated conditions from 14 to 86% of

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applied oocysts could pass through soil columns with well defined macropores.

Several researchers have modeled vertical oocyst transport through porous media. Brush *et al*. (1999) and Harter *et al*. (2000) used the classic convective dispersion equation in conjunction with sorption-desorption processes to simulate oocysts transport in relatively homogeneous porous media, such as sand and alluvial sediments. Their data suggest that oocysts are relatively mobile, and that oocyst transport can be modeled using these equations. These and other similar studies were conducted with purified oocysts. Oocyst contamination is more likely to occur from land-application of manure, or fecal deposition on pastures, of adult animals. Recent research by Kuczynska *et al*. (2004) indicates that manures affect attachment to soil particles in a complex manner. Diluted manure initially enhanced oocyst attachment to soil particles; however, it also appeared to facilitate subsequent detachment from soil particles.

The objective of this study was to assess the effect of water saturation on transport of manure-borne oocyst through soil cores.

MATERIALS AND METHODS

The characteristics of the sandy loam and clay loam soils used in this study are described in Table 1. Soils were air dried and sieved (< 5 mm), but not crushed. The soil columns were 11 cm high and 11.5 cm in diameter. The columns were placed inside of PVC Buchner funnels (7 cm high and 12.5 cm in diameter). A layer of coarse sand (1 cm depth) was placed at the bottom of the column. The remaining 10 cm was packed with air-dried sieved soil in 100 g increments to provide a homogenous soil matrix: bulk density was *ca*. 1.38 g cm^{-3}. Percent porosity was *ca*. 50% according to the equation: % porosity = (1 - ρ_b/ρ_d) x 100; where: ρ_b – bulk density and ρ_d – particle density assumed to be 2.65 g cm^{-3}. After packing, each column was slowly saturated from the bottom of the funnel and then allowed to drain overnight. An outer cylinder (*ca*. 12.5 cm high) was placed snugly inside the PVC funnel. A wooden cylinder with a diameter slightly less than the inner cylinder was placed on the top of the soil column to hold it intact. As the inner cylinder was slowly lifted, the emerging gap between column wall and the outer cylinder was immediately filled

with Raketite cement (*ca*. 3:1 cement : water) to prevent wall flow (Isensee and Sadeghi, 1992). The rainfall simulator used in these studies has been previously described (Isensee and Sadeghi, 1992; Sigua, *et al*., 1993). It consists of a turn-table capable of supporting 12 soil cores attached to a va-cuum pump, and providing variable rainfall intensities.

Unless otherwise specified, all the experiments were conducted with bovine manure from the Animal Research Facility, Beltsville Agricultural Research Center. Manure was initially oocyst-free; 50 g of manure was seeded with 7.1 x 10^6 oocysts and applied to the surface of soil cores just prior to the initiation of rainfall. Cores were covered with straw to prevent splashing and soil crusting. Rainfall was applied at *ca*. 1.6 cm h^{-1} for 8 h. Two cores per soil were prepared for each experiment: for one set a light suction (~5 kPa) was applied during rainfall to simulate field drainage conditions; for the other set, no suction was applied. Leachate was collected and volume measured at one hour intervals.

At the end of the experiment, (i) the manure on the surface of soil cores was carefully removed; and (ii) cores were sliced into five 2 cm sections, mixed, and a 25 g sub-sample removed for analysis. Soil samples were refrigerated overnight and oocysts were extracted on the following day.

Leachate and soil samples were processed using the NaCl flotation method as previously described (Kuczynska and Shelton, 1999). Three 10 μl aliquots (of the final 100 μl suspension) were pipetted onto slide wells (5 mm diameter), dried on a slide warmer, and stained using a commercial immunofluorescence antibody (IFA) kit (Merifluor; Meridian Diagnostic, Inc., Cincinnati, OH, USA). Samples were examined by epifluorescence microscopy at 200X magnification (Olympus). Numbers of oocysts per 25 g soil sample were obtained by multiplying the average number of oocysts in three wells by 10. Percent recoveries from leachate were *ca*. 5% as determined by spiking blank leachate samples with 10^4 purified oocysts and processing as previously described.

TRANSPORT MODELING

The oocyst transport was simulated assuming steady-state water flow and neglecting variations in water content

T a bl e 1. Selected soil properties

Soil type	Grain size distribution (%)			C_{org} (g kg^{-1})	pH
	Sand	Silt	Clay		
Sandy loam	78	18	4	1.1	6.8
Clay loam	40	48	12	3.0	5.8

Soil texture with the hydrometer method, C_{org} with chromic acid oxidation (Walkley and Black), pH in 1:1 suspension.

and pore structure along the soil profile. The model was chosen in the form:

$$\frac{\partial}{\partial t}(\theta_a c + \rho s) = \frac{\partial}{\partial x}\left(\theta_a D \frac{\partial c}{\partial x} - J_w c\right) - \mu_l \theta_a c - \rho \mu_s s. \quad (1)$$

Here c is the volume-averaged oocyst concentration in the soil solution, cells cm^{-3}, θ_a is the porosity available for oocyst transport in soil (m^3 m^{-3}), ρ is soil bulk density (g cm^{-3}), s is the adsorbed amount of oocyst (cells g^{-1}), D is the dispersion coefficient (cm^2 h^{-1}), J_w is the volumetric water flux density (cm h^{-1}), μ_l and μ_s (h^{-1}) are first-order removal coefficients to simulate removal of oocyst from solution and solid phase due to deposition and entrainment (Harter *et al.*, 2000; Kouznetsov *et al.*, 2004).

Oocyst adsorption by the solid phase is described with a linear isotherm (Harter *et al.*, 2000) as:

$$s = K_d c, \quad (2)$$

where: K_d is a distribution constant (cm^{-3} g^{-1}). Using Eq. (2), one rewrites Eq. (1) as:

$$R \frac{\partial c}{\partial t} = \frac{\theta a}{\theta} D \frac{\partial^2 c}{\partial x^2} - v \frac{\partial c}{\partial x} \mu c, \quad (3)$$

where: $v = J_w/\theta$ (cm h^{-1}) is the average pore-water velocity, θ (cm^3 cm^{-3}) is the volumetric soil water content, R_a is the retardation factor given by:

$$R = \frac{\theta a}{\theta} + \frac{\rho K_d}{\theta}, \quad (4)$$

and μ is the combined first-order removal rate coefficient:

$$\mu = \mu_l \frac{\theta_a}{\theta} + \frac{\rho K_d \mu_s}{\theta}. \quad (5)$$

This coefficient is proportional to the colloid filtration coefficient λ (Harter *et al.*, 2000), $\mu = v\lambda$, v is the average pore-water velocity.

The third-type boundary condition was used for the oocyst flux on the surface:

$$\left(J_w c - \theta_a D \frac{\partial c}{\partial x}\right)_{x=0} = J_w c_m(t). \quad (6)$$

The concentration of oocyst released from manure c_m (cells cm^{-3}) was assumed to decrease exponentially with time:

$$c_m(t) = c_0 \exp(-\Lambda t), \quad (7)$$

where: c_0 is the initial concentration (cells cm^{-3}) and Λ (h^{-1}) is the oocyst release rate constant. The exponential decrease model has been selected after inspection of data published

by Bradford and Schijven (1992) who studied the release of manure particulates and *Cryptosporidium* oocysts from manure under simulated rainfall. Figure 1 shows their data in the semilogarithmic scale. Given the observed scatter, the log-linear model:

$$\log(c_m) = A - \Lambda t, \quad (8)$$

seems to be appropriate to simulate the dependencies in Fig. 1. The Eq. (8) is equivalent to Eq. (7) after the conversion of common logarithms to natural logarithms. We note that the boundary condition (7) was successfully used to simulate the release of manure particulate and manure-borne bacteria in the work of Shelton *et al.* (2003).

Using dimensionless variables: $C = c/c_0$, $Z = x/L$, $T = vt/L$, $P = vL\theta/(D_a\theta_a)$, $\mu^E = \mu L/v$, and $\lambda^E = \Lambda L/v$, Toride *et al.* (1999) presented the solution of the boundary problem Eqs (3)-(7) for the infinite domain with zero initial concentration for arbitrary X and T values:
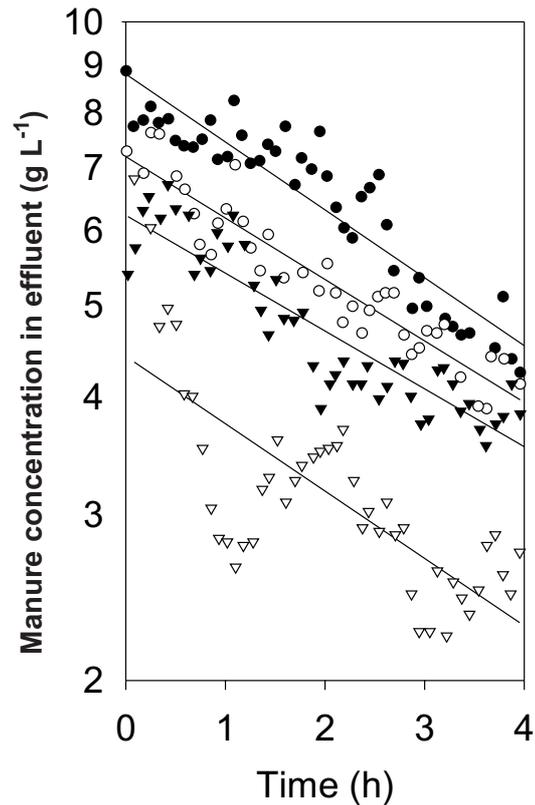

Fig. 1. Manure release curves obtained with different salinity of artificial raindrops applied to the miniature manure disks (after Bradford and Schijven, 2002). ○ - EC=0.3 dS m^{-1}, $\log(c_m) = 0.944-0.072t$, R^2 = 0.864; ● - electric conductivity of water EC = 5.0 dS m^{-1}, $\log(c_m) = 0.856-0.065t$, regression determination coefficient R^2 = 0.892; ▽ - EC=0.3 dS m^{-1}, $\log(c_m)=0.794-0.061t$, R^2 = 0.822; ▼ - EC = 14.8 dS m^{-1}, $\log(c_m) = 0.645-0.071t$, R^2 = 0.570.

$$C(X,T) = \exp(-\lambda^E T) G_1^E (X,T,\mu^E - R\lambda^E), \quad (9)$$

where: function G_1^E is:

$$G_1^E (X,T,\Omega) = \frac{1}{1+u} \exp\left[\frac{P(1-u)Z}{2}\right] erfc\left(\frac{RZ-uT}{\sqrt{4RT/P}}\right)$$

$$+ \frac{1}{1-u} \exp\left[\frac{P(1+u)Z}{2}\right] erfc\left(\frac{RZ+uT}{\sqrt{4RT/P}}\right)$$

$$- \frac{2}{1-u^2} \exp\left[PZ + \frac{P(1-u^2)T}{4R}\right] erfc\left(\frac{RZ+T}{\sqrt{4RT/P}}\right), \quad (10)$$

with $u = \sqrt{1 + 4\Omega/P}$. This expression is valid when $\Omega > -P/4$, *ie* $\mu^E > R\lambda^E - P/4$ and $\Omega \neq 0$. There exists also a solution for relatively small deposition and/or entrainment rates when $\mu^E > R\lambda^E - P/4$ (Van Genuchten, 1985). This solution is expressed in the form of integrals that need to be evaluated using quadratures.

Model parameters of θ, v, D, R, μ and Λ were estimated as follows. The values of θ and v were measured. The percentage of oocysts remained on the soil surface was used to compute values of Λ according to Eq. (7). The value of D was computed as $D = lv$, where the dispersivity, l, was estimated to be 1 and 0.5 cm in saturated and unsaturated samples, respectively (Pachepsky, 1990). The remaining two parameters R and μ were estimated by fitting the solution (9) to

the experimental data on percentages p_i (i=1,2,…5) of the total applied oocyst mass found in five 2 cm soil layers and the percentage p_6 recovered in leachate. The term $\mu C(X,T)$ was integrated over time to obtain the contents of oocysts accumulated in soil due to entrapment. Those contents were added to the contents in solution and adsorbed amounts in the end of the experiment to obtain total mass of oocysts in the 2 cm layers.

Values of R and μ were found that provided a minimum root-mean-square error:

$$RMSE = \sqrt{\frac{\sum_1^6 \left(p_i^{measured} - p_i^{simulated}\right)}{N - P}}.$$

Here $N = 6$ is the total number of available data and $P=2$ is the number of estimated parameters. The modified Marquardt-Levenberg algorithm was used to obtain non-negative parameter values that minimized RMSE in Eq. (6). We used a version of the algorithm published by Van Genuchten (1981). This version proved to be very efficient in multiparametric nonlinear optimization. The code provides both estimates of average values of parameters and the 95% confidence intervals of their values.

RESULTS AND DISCUSSION

Infiltration rates were relatively stable during the experiment (Fig. 2a). In saturated cores, infiltration rates were *ca.* 0.8 and 1.2 cm h^{-1} in sandy loam or clay loam soil
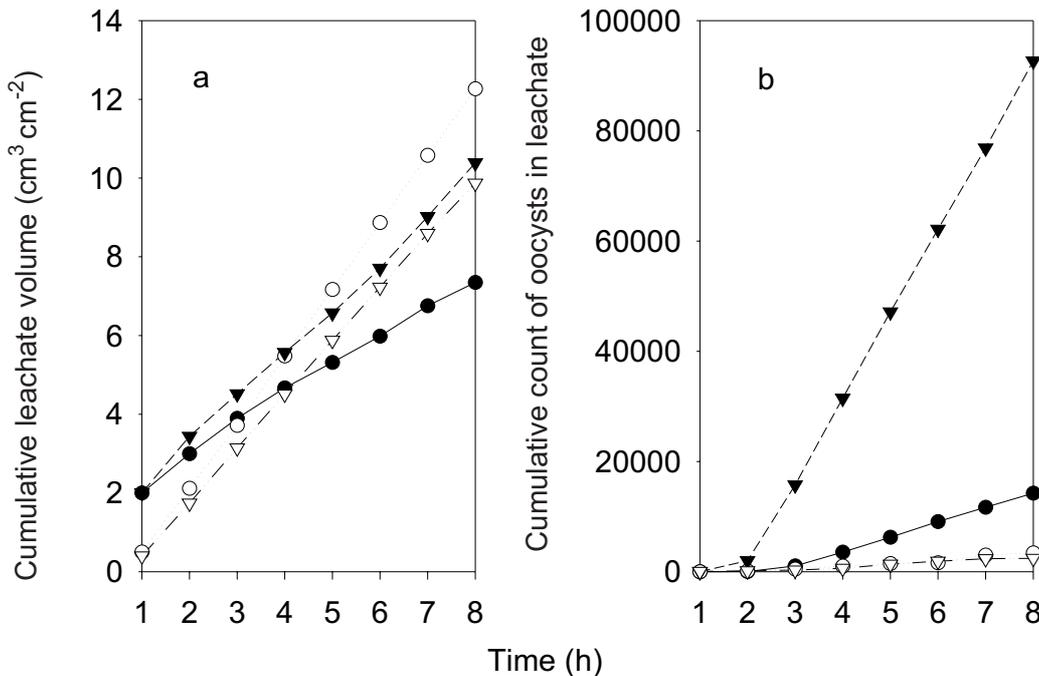

Fig. 2. Cumulative leachate volume (a) and count of leached oocysts (b); $\bigcirc$ - sandy loam, no suction applied; $\bullet$ - sandy loam, suction applied, $\bigtriangledown$ - no suction applied; $\blacktriangledown$ - clay loam, suction applied.

cores, respectively. Water accumulated in the tops of sandy loam or clay loam soil cores throughout the duration of the experiment. The difference in infiltration rates between sandy loam and clay loam soils could be attributed to differences in soil structure.

Saturated *vs*. unsaturated conditions and soil structure substantially affected the rates of oocyst leaching (Fig. 2b). Under unsaturated conditions in both sandy loam and clay loam cores, cumulative recoveries of oocysts in leachate were <0.1% of the applied amount. Under saturated conditions, oocyst breakthrough was observed in both sandy loam and clay loam soil cores after approx. 1 h. Cumulative recoveries of oocysts in leachate were 0.4 and 1.3% in sandy loam and clay loam soil cores, respectively. Again, higher rates of leaching observed in the clay loam soil were indicative of more aggregation in this soil.

The solution (10) provided a good fit to data in the top part of the profile (Fig. 3). The RMSE values were about 1.6% of applied amount for saturated columns and about 0.3% for unsaturated columns. Comparison of measured and simulated distributions in log-log scale reveals more details about the model performance (Fig. 4). The model does not predict well small amounts of oocysts found in lower layers of saturated columns and unsaturated sandy loam columns. The effect is more pronounced in sandy loam than in clay
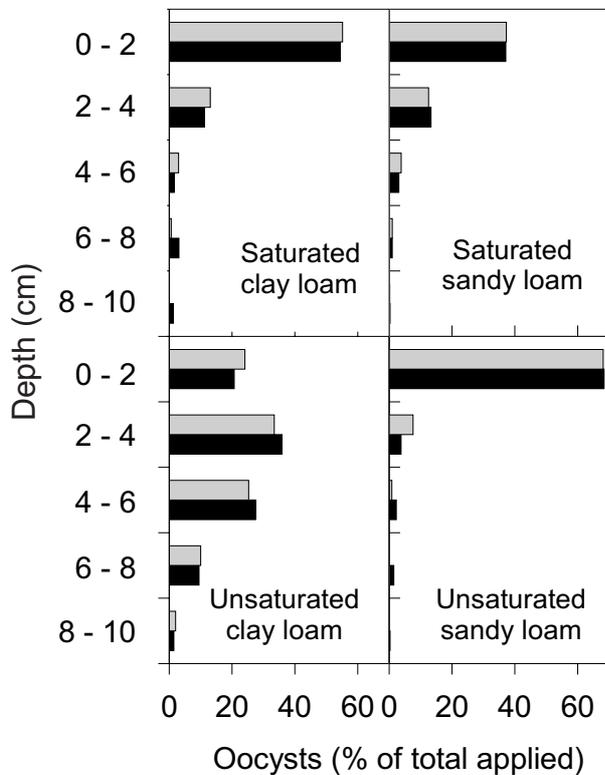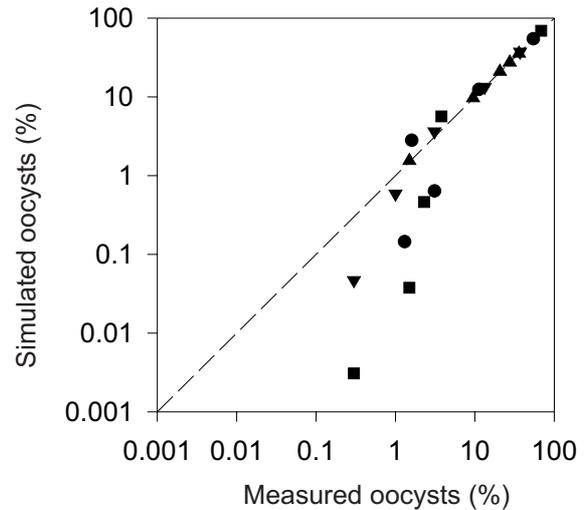

Fig. 4. One-to-one comparison of measured and simulated oocyst amounts in 2 cm soil layers. ● - saturated clay loam, ■ - saturated sandy loam, ▲ - unsaturated clay loam, ▼ - unsaturated sandy loam.

loam. The model seems to fail to simulate transport of small amounts of oocysts with a preferential flow-type water movement that cannot be mimicked in the convective-dispersive transport framework.

Model parameters are presented in Table 1. Water movement was relatively fast and about 2 pore volumes passed columns in about 2 h. Values of oocyst release rates, Λ, are comparable with sparse literature data on dissolving manure release rates. We computed the manure release rates from the data of Bradford and Schijven (2002) and found values ranging from 0.15 to 0.19 h^{-1}. Shelton *et al*. (2003) reported release rates of manure colloids of 0.32 h^{-1}. The difference between release rates in this and other works may be related both to the differences in water application to manure and to the quality of manure used.

Retention of manure-borne oocysts in columns was controlled by parameters R and μ, the former quantifying reversible adsorption and exclusion of the unavailable pore space according to Eq. (4), and the latter quantifying irreversible removal from pore solution. Values of R were most probably less than unity in saturated columns. That means that the available pore space was less than the total porosity, and no tangible reversible adsorption had occurred. In unsaturated columns, values of R were larger than unity, thus indicating that some reversible adsorption did occur.

Values of the removal rate parameter, μ, differed substantially between the saturated and unsaturated columns (Table 1). Saturation created much stronger removal with rate constants between 2.8 and 10 h^{-1} corresponding to the removal T_{90} between 0.23 and 0.8 h. Values of μ were much smaller in unsaturated columns. That could be interpreted as the result of water and oocyst transport occurring in small

Fig. 3. Measured (grey) and simulated (black) contents of oocysts in 2 cm layers of soil columns.

part of pore space with fairly high velocity, so that the total flux in unsaturated columns remained the same as in saturated. This part of pore space could be constituted by large well conducting pores where opportunities for oocyst removal were limited. Jin *et al.* (2000) observed differences in virus removal in saturated and unsaturated sand columns, and found larger removal in unsaturated sand that could be attributed to the increased sorption for one organism and to the increase in inactivation in the presence of air-water interfaces. The effect of the latter on oocyst transport is unknown and presents an interesting issue to explore as the colloid scavenging by air-water interfaces may be a dominating colloid mobilization (DeNovio *et al.*, 2004).

Data on oocyst removal rates are available in literature from column transport experiments with purified oocysts in water-saturated porous media that were more homogeneous than soils. We estimated oocyst removal rates from data of Harter *et al.* (2000) for sands and obtained values in the range from 2 to 30 h^{-1} with pore water velocities in the range 6 to 60 cm h^{-1}. These values were slightly larger than values in this work where saturated pore water velocities were 2.6 cm h^{-1}. Brush *et al.* (1999) experimented with glass beads, sands and shale aggregates and reported much higher values of the removal rates in the range from 1 500 to 3 000 h^{-1}. The pore water velocity in the latter experiments was about 40 000 cm h^{-1}. The removal rate parameter seems to demonstrate dependence on the pore water velocity. This dependence should be also affected by the structure of the porous media as discussed by Harter *et al.* (2000), Korsunskaia *et al.* (2004), and Mawdsley *et al.* (1996). The removal rates were consistently higher in sandy loam soil than in clay loam soil (Table 2).

axes. We cannot exclude cases when a plausible experimental noise will shift values of optimized parameters quite far within this valley.

Assuming an extreme scenario in which all retention in the columns has actually occurred because of adsorption, we still found a large difference between K_d estimated from batch experiments with the same manure and soil (Kuczynska *et al.*, 2004) and from the column experiments in this work. Equation (4) shows that the value of the soil partition coefficient K_d cannot be larger than $\theta(R-1)/\rho$ because the value of the available porosity, θ_a, cannot be larger than the total porosity, θ. Figure 4 shows that values of optimized R have to be between 4 and 22 if the point of optimum is on the axis R. Therefore the K_d has to be between 1.4 and 7.7. Such values are two orders of magnitude smaller than values of K_d between 100 and 700 found in batch experiments with the same soils (Kuczynska *et al.*, 2004). Very slow adsorption kinetics during the oocyst transport can be one reason for such differences. Another reason can be that oocysts are able to move only in large pores and do not reach the adsorbing surfaces. Yet another hypothesis is that the removal of oocysts from the solution in batch experiments is actually the first order attenuation by irreversible attachment to solid surfaces. This was suggested to explain large differences between K_d values in batch and column experiments with viruses (Jin *et al.*, 1997; Powelson and Gerba, 1994). Whatever is the reason of the differences in values of 'static' and 'dynamic' values of K_d, data of this work show that oocyst adsorption data from batch experiments may be inapplicable in transport models that employ the adsorption equilibrium hypothesis. We concur with Yin *et al.* (1997) who have worked with viruses and

T a b l e 2. Parameters of the oocyst transport in soil columns

Parameter	Saturated columns		Unsaturated columns	
	Clay loam	Sandy loam	Clay loam	Sandy loam
v (cm h^{-1})	2.7	2.6	3.3	1.5
D (cm^2 h^{-1})	2.7	2.6	1.7	0.7
Λ (h^{-1})	0.212	0.503	0.240	0.134
μ (h^{-1})	2.77-4.13	5.28-9.96	0.00-0.16	0.62-0.81
R*	0.00-0.97	0 .00-1.56	4.51-4.61	2.44-3.82

*Confidence intervals of the optimized values at the 5% significance level.

The data on oocyst retention in columns can present a challenge for determining both retardation coefficient and removal rate because both parameters control the retention of oocysts in column. This is illustrated in Fig. 5 where RMSE isolines are shown for the columns in this study. Lines of equal RMSE values are arching from the R to the μ axis implying that the same overall model error can be achieved with various combinations of R and μ. The valley of smallest RMSE is very shallow and also connects the two

have concluded that batch experiments may not be appropriate as a primary method for determining the extent of microorganism sorption in porous media because: (a) the mechanism of microorganism removal may be not a reversible sorption, (b) kinetics in flow systems is different from that of the flow system, and (c) differences in protocols of batch experiments affect results significantly, and no standard protocol exists. We note that using a nonequilibrium adsorption model, *ie* one utilized by Harter *et al.* (2000)
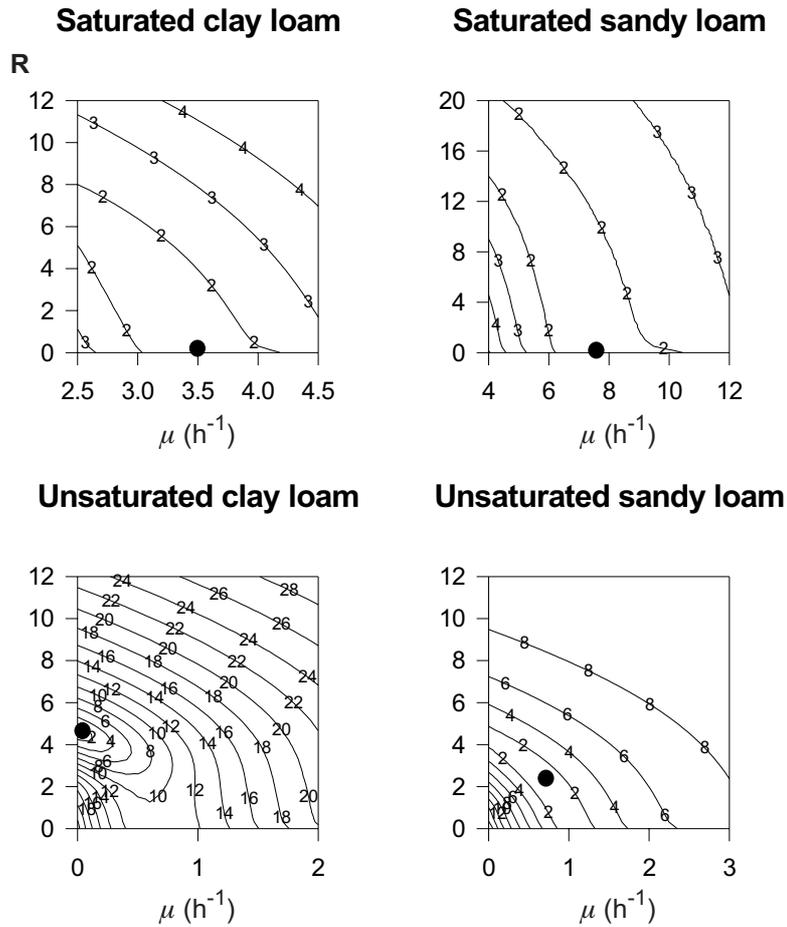
Saturated clay loam

Saturated sandy loam

Unsaturated clay loam

Unsaturated sandy loam

Fig. 5. Isolines of the model root-mean square error as dependent of the retardation factor R and the removal rate constant μ.

in oocyst transport studies in sand, would require estimation of additional parameters of adsorption and desorption kinetics. That becomes problematic with data on profile oocyst distributions owing to the dependence shown in Fig. 5. More needs to be learned about the physics and chemistry of oocyst transport in soils to develop a model with a small number of parameters identifiable from data on oocyst retention in soil profile.

CONCLUSIONS

1. Manure-borne *Cyptosporidium parvum* oocysts stayed mostly within the top 8 cm of soil columns after 8 h of manure dissolution and subsequent infiltration.

2. The cumulative contents of oocysts in leachates from unsaturated soils were less than 0.1% whereas the saturated column allowed breakthrough of 0.4 and 1.3% in sandy loam and clay loam soil cores, respectively.

3. The convective-dispersion model with exponential boundary release, instantaneous adsorption and kinetic removal of oocysts simulated the profile distributions of

oocysts reasonably well, but failed to simulate breakthrough of small amounts of oocysts occurring probably due to preferential flow mechanisms.

4. Values of retardation coefficient were most probably less than unity in saturated columns and greater than unity in unsaturated columns.

5. Values of the soil partition coefficient, K_d, derived from the retardation coefficient values were much less than values reported earlier from batch experiments with the same soils.

6. The removal rates were much higher in saturated columns as compared with unsaturated columns.

7. The effect of soil water saturation on the oocyst transport was substantial.

REFERENCES

Atwill E.R., Sweitzer R.A., M. Das Graças C. Pereira, Gardner I.A., van Vuren D., and Boyce WM., 1997. Prevalence of and associated risk factors for shedding *Cryptosporidium* oocysts and *Giardia* cysts within feral pig populations in California. Appl. Environ. Microbiol., 63, 3946-3949.

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

Bradford S.A. and Schijven J., 2002. Release of *Cryptosporidium* and *Giardia* from dairy calf manure: impact of solution salinity. Environ. Sci. Technol., 36, 3916-3929.

Brush C.F., Ghiorse W.C., Anguish L.J., Parlange J.Y., and Grimes H.G, 1999. Transport of *Cryptosporidium parvum* oocysts through saturated columns. J. Environ. Qual., 28, 809-815.

Darnault C.J.G., Steenhuis T.S., Garnier P., Kim Y.J., Jenkins M., Ghiorse W.C., Baveye P.C., and Parlange J.Y., 2004. Preferential flow and transport of *Cryptosporidium parvum* oocysts through the vadose zone: experiments and modeling. Vadose Zone J., 3, 262–270.

DeNovio N.M., Saiers J.E., and Ryan J.M., 2004. Colloid movement in unsaturated porous media: recent advances and future directions. Vadose Zone J., 3, 338-351.

Garber L.P., Salman M.D., Hurd H.S., Keefe T., and Schlater J.L., 1994. Potential risk factors for *Cryptosporidium* infection in dairy calves. J. Am. Vet. Med. Assoc., 205, 86-91.

Graczyk T.K., Fayer R., Trout J.M., Lewis E.J., Farley C.A, Sulaiman I., and Lal A.A., 1998. *Giardia* sp. and infectious *Cryptosporidium parvum* oocysts in the feces of migratory Canada geese (*Branta canadensis*). Appl. Environ. Microbiol., 64, 2736-2738.

Harter H., Wagner S., and Atwill E.R., 2000. Colloid transport and filtration of *Cryptosporidium parvum* in sandy soils and aquifer sediments. Environ. Sci. Technol., 34, 62-70.

Isensee A.R. and Sadeghi A.M., 1992. Laboratory apparatus for studying pesticide leaching in intact soil cores. Chemosphere, 25, 581-590.

Jin Y., Chu Y., and Li Y., 2000. Virus removal and transport in saturated and unsaturated sand columns. J. Contam. Hydrol., 43, 111-128.

Jin Y., Yates M.V., Thompson S.S., and Jury W.A., 1997. Sorption of viruses during flow through saturated sand columns. Environ. Sci. Technol., 31, 548-555.

Korsunskaia L.P., Shein E.V., and Pachepsky Y.A., 2004. Concurrent transport of chloride, sodium, and calcium ions in undisturbed soil columns. Int. Agrophysics, 18, 133-138.

Kouznetsov M.Y., Pachepsky Y.A., Gantzer C., Gillerman L., and Oron G., 2004. Microbial transport in soil caused by surface and subsurface drip irrigation with wastewater. Int. Agrophysics, 18, 239-247.

Kuczynska E. and Shelton D.R., 1999. Method for detection and enumeration of *Cryptosporidium parvum* oocysts from feces, manure, and soils. Appl. Environ. Microbiol., 65, 2820-2826.

Kuczynska E., Shelton D.R., and Pachepsky Y., 2004. Effect of bovine manure on *Cryptosporidium parvum* oocyst attachment to soil. Appl. Environ. Microbiol., (in press).

MacKenzie W.R., Hoxie N.J., Proctor M.E., Gradus M.S., Blair K.A., Peterson D.E., Kazmierczak J.J., Addiss D.A., Fox K.R., Rose J.R., and Davis J.P., 1994. A massive outbreak of *Cryptosporidium* infection transmitted through the public water supply. The New Eng. J. Med., 331, 161-167.

Mawdsley J.L., Brooks A.E., and Merry R.J., 1996a. Movement of the protozoan pathogen *Cryptosporidium parvum* through three contrasting soil types. Biol. Fertil. Soils, 21, 30-36.

Mawdsley J.L., Brooks A.E., Merry R.J., and Pain B.F., 1996b. Use of a novel soil tilting table apparatus to demonstrate the horizontal and vertical movement of the protozoan pathogen *Cryptosporidium parvum* in soil. Biol. Fertil. Soils, 23, 215-220.

Pachepsky Y., 1990. Mathematical Models of Physical Chemistry in Soil Science (in Russian). Nauka, Moscow.

Powelson D.K. and Gerba C.P., 1994. Virus removal from sewage effluents during saturated and unsaturated flow through soil columns. Water Res., 28, 2175-2181.

Shelton D., Pachepsky Y.A., Sadeghi A.M., Stout W.L., Karns J.S., and Gburek W.J., 2003. Release rates of manure-borne coliform bacteria from data on leaching through stony soil. Vadose Zone J., 2, 34-39.

Sigua G.C., Isensee A.R., and Sadeghi A.M., 1993. Influence of rainfall intensity and crop residue on leaching of atrazine through intact no-till soil cores. Soil Sci., 156, 225-232.

States S., Stadterman K., Ammon L., Vogel P., Baldigar J., Wright D., Conley L., and Sykora J., 1997. Protozoa in river water: sources, occurrence, and treatment. J. Am. Water Works Assoc., 74, 75-83.

Toride N., Leij F., and van Genuchten M.Th., 1999. The CXTFIT code for estimating transport parameters from laboratory or field tracer experiments. Version 2.0. Research Rep. 137. U. S. Salinity Laboratory, Riverside, CA.

Van Genuchten M.Th., 1981. Non-equilibrium transport parameters from miscible displacement experiments. Research Rep. 119. U.S. Salinity Laboratory, Riverside, CA.

Van Genuchten M.Th., 1985. Convective-dispersive transport of solutes involved in sequential first-order decay reactions. Computers in Geosciences, 11, 129-147.