Environ. Sci. Technol. **2002**, *36,* 3916−3923

Release of *Cryptosporidium* and *Giardia* from Dairy Calf Manure: Impact of Solution Salinity

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Studies were initiated to determine the release behavior of *Cryptosporidium* oocysts and *Giardia* cysts from dairy calf manure to waters of various salinities. Experiments were conducted by sprinkling a particular aqueous solution over a manure disk and collecting the runoff water. Effluent concentrations of manure and (oo)cysts were initially several orders of magnitude below their starting concentration in the manure, after continued application of water the concentrations gradually decreased, and then exhibited persistent concentration tailing. Solution salinity significantly affected the shape and magnitude of the manure and (oo)-cyst concentration curves. Increases in solution salinity tended to decrease the manure and (oo)cyst concentrations at a particular time. This was attributed to a stabilization of manure by compression of the double layer thickness between negatively charged components of the manure phase. Calculated release efficiencies of the (oo)cysts (relative to manure release) also decreased with increasing solution salinity. Experimental observations indicate that only the surface layer of manure was depleted of finer manure materials and (oo)cysts and that the manure will act as a long-term source of contamination. A conceptual model to describe and predict manure and (oo)cyst release rates and cumulative loading for the various solution salinities was proposed and applied to the experimental data. The calibrated model yielded a reasonable description of the experimental results.

Introduction

Cryptosporidium parvum and *Giardia duodenalis* are protozoan parasites that infect the intestines of a variety of wild and domesticated animals as well as man (*1*). The infectious stage of these parasites is biologically dormant (oo)cysts (*Cryptosporidium* oocysts and *Giardia* cysts) that are shed in high numbers within the feces of infected animals. Cattle, especially young calves, have been recognized as significant sources of the parasites, because of the high prevalence of infections with these pathogens, and the high number of (oo)cysts that are shed within the feces of these animals, as much as 2.6×10^{10}/kg (*2*−*7*). Ingestion of contaminated water containing as few as 10 (oo)cysts can lead to infection (*8*). Hence, drinking water contamination by these protozoan parasites is a serious concern for public health.

Over 300 million tons of manure were produced by confined beef and dairy cows in the United States in 1997 (*9*). Due to the application of animal waste to agricultural land, large numbers of pathogenic protozoa may be released into the environment. Both *Cryptosporidium* and *Giardia* (oo)cysts are ubiquitous in surface water (*10*−*12*). Many outbreaks of cryptosporidiosis and giardiasis have been reported in industrialized countries (*13*−*14*). In these outbreaks, (oo)cysts were present in drinking water due to contamination of the source water, failure in treatment of surface water (*Cryptosporidium* oocysts, and to a lesser extent *Giardia* cysts, are highly resistant to chlorination), and leakage into the distribution system. A single outbreak of cryptosporidiosis in 1993 caused illness in 370 000 individuals from Milwaukee, WI. Outbreaks of waterborne cryptosporidiosis, including the 1993 Milwaukee outbreak, have been attributed (without conclusive evidence) to contamination by bovine wastes from pasture areas and drainage from slaughterhouses (*15*).

The above literature indicates that animal waste is a potentially important source of parasite pathogens in the environment. Surprisingly, little research to date has explored the release behavior of pathogens from animal wastes (*16*−*19*). The few published studies have presented temporal changes in surface water runoff concentrations of *Cryptosporidium* (oo)cysts (*16*, *17*) or indicator bacteria (*18*, *19*) following precipitation events or simulated rainfall. Runoff concentrations were observed to gradually increase to a maximum value and then decrease over several orders of magnitude to persistently low concentration levels. These studies did not, however, attempt to mechanistically model the pathogen release process. This information is needed to estimate pathogen release rates and cumulative loading to surface and groundwaters as well as to assess contamination potential and persistence under various hydrologic conditions and animal management practices.

Animal waste will likely be exposed to a wide range of solution salinity as a result of mixing of animal urine (high salinity) and rainfall or drinking water (low salinity). Site specific hydrologic conditions and manure management practices will also influence the salinity of solutions on farms. The transport and fate of colloid particles in the subsurface has been found to be affected by solution salinity (*20*), because increasing the solution salinity diminishes electrostatic interactions between charged particles. It is therefore logical to anticipate that solution salinity will also influence the release and subsequent transport of pathogenic microorganisms (biocolloids).

The object of this research is to investigate the influence of solution salinity on the release behavior of naturally occurring *Cryptosporidium* and *Giardia* (oo)cysts from Holstein dairy calf manure. Experiments consisted of dripping waters of various salinities at a constant rate on top of calf manure disks and determining effluent concentrations of the parasites as a function of time. The temporal release of (oo)cysts from the calf manure was also simulated using a calibrated linear driving force model to describe manure release. A complementary study presented by Schijven et al. (*21*) examines the influence of physical factors (water application intensity and rate, manure type, and temperature) on the short- and long-term release of these parasites from dairy calf and cow manure.

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10.1021/es025573l CCC: $22.00
Published on Web 08/09/2002

Materials and Methods

Holstein dairy calf manure used in the experimental studies reported herein was collected from a farm in Chino, CA. Consistent with typical animal management practices in this area, 1-day to 3-month old Holstein dairy calves are placed in wooden crates until the animals are weaned. Manure and urine drops directly under the crates. Manure samples were collected directly under the crates of 2.5−3 month old calves. The manure samples were placed in a bucket and thoroughly mixed with a stick and then stored at 4° C. The calf manure was infected with naturally occurring *Cryptosporidium* and *Giardia* (oo)cysts, so that there was no need to artificially spike the manure.

Various aqueous solution salinities were used in the experimental studies discussed below. The reference solution in the experiments consisted of a low salinity 0.001 M NaCl solution with its pH buffered to 6.98 using 5×10^{-5} M $NaHCO_3$. Higher salinity solutions were prepared by adding either 2.8, 5.6, or 8.4 g/L of NaCl to this reference solution. The electrical conductivity for the various solutions was measured to be 0.3, 5.0, 9.5, and 14.8 dS m^{-1} using an Orion Conductivity Meter Model 126. These concentrations were chosen to encompass a wide range in solution electrical conductivities that could be found on a farm. The 0.3 dS m^{-1} solution is a surrogate for precipitation or drinking water, while the 14.8 dS m^{-1} solution is a surrogate for aqueous solution equilibrated with animal urine and manure; i.e., an electrical conductivity of around 15 dS m^{-1} was measured for ponded aqueous solution in contact with manure on a dairy farm.

Experiments were conducted to investigate the influence of solution salinity on the release behavior of *Cryptosporidium* and *Giardia* (oo)cysts from dairy calf manure. The experiments were conducted at 23 °C in a constant temperature room. Replicate experiments were conducted using 0.3 and 14.8 dS m^{-1} solutions. Calf manure was packed into a 1.75 cm height by 5 cm diameter aluminum ring. A 5 cm diameter plastic disk was then used to gently push the manure disk on top of a 105 micrometer stainless steel screen that rested on a 14 cm diameter ceramic filter funnel. The aqueous solution was then dripped at a constant rate for 250 min directly above the manure disk using a Masterflex L/S multihead drive pump (Barnant Company, Barrington, IL 60010). The pumped solution was connect to a 1/8 in. stainless steel tube placed on top of an upside down funnel covering the manure pat. Figure 1 provides a schematic of the experimental setup. Table 1 presents the initial manure density (ρ_i, M L^{-3}), manure volume (V_m, L^3), and the average aqueous phase flow rate for the various release experiments (Q, L^3T^{-1}). Throughout the manuscript, parameter dimensions are given in terms of length (L), mass (M), time (T), and number (N). Fifty effluent samples were collected for each experiment in 20 mL glass scintillation vials directly below the funnel. Each sample was gathered during a five minute time interval, capped, and stored at 4 °C before analysis. The manure disks were vertically sliced and visually examined at the end of the experiments.

Effluent sample volumes were determined by weight, and the optical density of each effluent sample was measured at 660 nanometers (OD_{660}) using a Turner SP 830 spectrophotometer. A calibration curve was established between the aqueous phase manure concentration (C_m, M L^{-3}) and the solution optical density at 660 nanometers:

$$C_m = 7.83\ OD_{660} \quad r^2 = 0.982 \qquad (1)$$

Here C_m has units of g L^{-1}. A highly linear relationship was observed between C_m and OD_{660} over the considered concentration range (C_m ranged from 12.4 to 0 g l^{-1}, and OD_{660} ranged from 1.539 to 0).

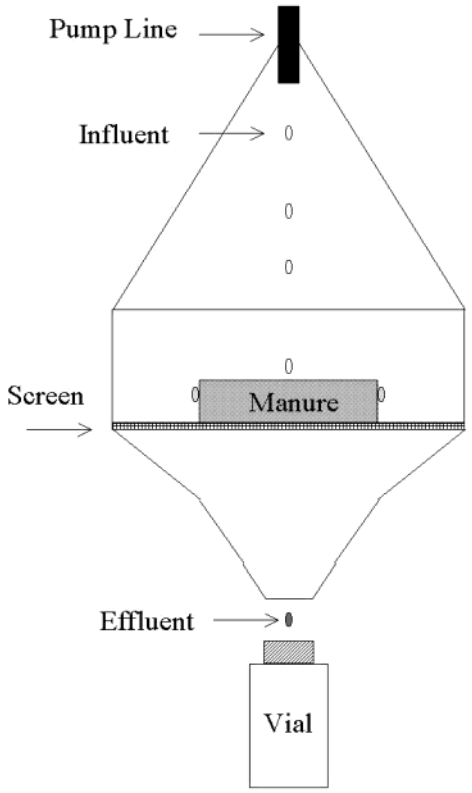


FIGURE 1. Schematic of the experimental setup used to study manure and (oo)cyst release.

TABLE 1. Experimental Conditions

EC (dS m^{-1})	Q (mL min^{-1})	ρ_i (g cm^{-3})
0.30	2.60	1.10
0.30[a]	2.03	1.12
5.00	2.47	1.12
9.50	1.95	1.13
14.8	2.09	1.11
14.8[a]	2.52	1.13

parameter	value
V_m (cm^3)	34.36
Giardia m_{ip} (N g^{-1})	9.5×10^4
Crypto. m_{ip} (N g^{-1})	7.1×10^4

[a] Denotes replicate experiment.

Concentrations of *Cryptosporidium* and *Giardia* (oo)cysts in some of the manure effluent samples (vials 3, 10, 20, 30, 40, and 50) were determined using the following protocol. To obtain a similar manure content and (oo)cyst recovery efficiency in each sample, manure effluent samples were diluted or concentrated to achieve an OD_{660} value of 0.2 in 5 mL. A half a milliliter of concentrated (10x) PST solution (phosphate buffered saline solution containing 2% - mass/volume- sodium dodecyl sulfate, and 2% -volume/volume-Tween 80) was then added to this manure effluent to facilitate the liberation of (oo)cysts and to minimize sorption losses. This solution was gently mixed and then centrifuged (Beckman Coulter, Allegra 25R Centrifuge, Fullerton, CA 92834-3100) for 10 min at 2600 rpm (1150 x acceleration due to gravity). The supernatant was pipetted down to a final volume of approximately 300 microliters, and the pellet was resuspended. (Oo)cysts (*Giardia* and *Cryptosporidium*) in the suspension were then stained with 100 μL of Aqua-glow (Waterborne Inc., New Orleans, LA 70118-6129) FITC monoclonal antibody and incubated (Revco Technologies, Inc.,

Asheville, NC 28804) in the dark for 30−45 min at 37 °C. After staining, a suspension was washed with 2 mL of (1x) PST, centrifuged, and pipetted down to approximately 100 μL, and the pellet was resuspended. Final volumes of the stained suspensions were determined by weight. A 10 μL aliquot of the suspension was then placed in a microscope well, air-dried using a hot air gun, and fixed to the slide well using 10 μL of DAPCO/glycerol mounting medium. A cover slip was then placed on the slide, and counts of *Giardia* and *Cryptosporidium* (oo)cysts were made at 100x and 600x magnification, respectively, using a Leica DM IRB epifluorescent microscope (Leica Microsystems Inc., Bannockburn, IL 60015). Identification criteria for counts were based upon size, shape, fluorescence, and comparison with positive controls; i.e., *Cryptosporidium* oocysts are spherical in shape with a diameter of around 4−6 micrometers, whereas *Giardia* cysts tend to be more oval in shape with a diameter of around 8−12 μm. The concentration was determined from the count, the well volume, the stained suspension volume, and the initial volume of manure effluent.

The initial concentrations of *Giardia* and *Cryptosporidium* (oo)cysts in the calf manure were determined from batch experiments as follows. First, 2.83 g of manure were placed in 20 mL of PST solution. The contents were then continuously mixed for 3 h using a magnetic stirrer. A 10 mL aliquot of this solution was then vacuum filtered through a 105 μm stainless steel wire mesh; all of the (oo)cysts were assumed to be in the effluent. The above (oo)cyst enumeration protocol was then followed to determine the concentration in this effluent and to achieve a similar (oo)cyst recovery efficiency. According to this protocol, the initial concentrations of *Giardia* and *Cryptosporidium* (oo)cysts in the calf manure (m_{ip}, N M^{-1}) were 9.5×10^4 (12 replicates, with a standard error of 3.0×10^4) and 7.1×10^4 (6 replicates, with a standard error of 1.7×10^4) per gram, respectively (cf. Table 1). These values agree well (within the 95% confidence interval) with previously reported data from 7 to 12 week old calves in The Netherlands (*10*).

Theory

The release behavior of manure will be modeled herein using the following manure mass balance equation

$$\frac{d\rho}{dt} = k_{wm}[\rho_e - \rho] \tag{2}$$

where ρ (M L^{-3})is the manure phase density, ρ_e (M L^{-3}) is the manure phase density at the water-manure interface in equilibrium with the aqueous phase, k_{wm} (T^{-1}) is the lumped manure mass transfer coefficient between the aqueous and manure phases, and t (T) denotes time. Equation 2 is developed under the assumption that no manure decay or additions occur during the course of the experiment and that the mass transfer can be described using a quasi steady-state approximation of Fick's first law of diffusion (a linear driving force model, with a boundary layer in the manure phase). Similar linear driving force models are also commonly used to describe rate-limited sorption, volatilization, and dissolution processes (*22*). The value of k_{wm} is hypothesized to be a complex function of the manure surface area accessible to flowing water, the solution chemistry, and the aqueous phase flow velocity. In analogy to dissolution of a nonaqueous phase liquid (*23*), k_{wm} will be generalized using a simple power function of the normalized manure density as

$$k_{wm} = \alpha\left(\frac{\rho}{\rho_i}\right)^{\beta} \tag{3}$$

where α (T^{-1}) and β are fitting parameters. The value of α controls the initial manure release rate, whereas β determines the shape of the manure release curve.

Note in eq 2 that the driving force for manure release is maximum when no manure is in the aqueous phase ($C_m = 0$) and ρ_e (ρ_e equals the product of C_m and a partition coefficient) equals zero. Under these conditions, eq 2 reduces to

$$\frac{d\rho}{dt} = -k_{wm}\rho = -\alpha\rho_i^{-\beta}\rho^{1+\beta} \tag{4}$$

The solution to eq 4 is given as

$$\rho(t) = \rho_i(1 + \alpha\beta t)^{\left(\frac{-1}{\beta}\right)} \tag{5}$$

The cumulative manure mass that has been released to the aqueous phase (M_w, M) as a function of time can be related to ρ_i and $\rho(t)$ (eq 5) by mass balance as

$$M_w(t) = V_m(\rho_i - \rho(t)) \tag{6}$$

The aqueous manure concentration follows from the temporal derivative of eq 6 as

$$C_m(t) = \frac{dM_w}{Q dt} = \frac{\rho_i \alpha V_m}{Q}(1 + \alpha\beta t)^{-((\beta+1)/\beta)} \tag{7}$$

The manure release rate is the product of Q and $C_m(t)$.

Since direct enumeration of (oo)cysts is labor intensive, expensive, and time-consuming, an alternative approach was sought to estimate (oo)cyst concentrations in manure effluent. If the initial (oo)cyst concentration in the manure is measured (cf. Table 1), then a relationship between the aqueous (oo)cyst concentration (C_p, NL^{-3}) (the subscript p is used herein to denote parasite) can also be estimated from the aqueous manure concentration and the (oo)cyst release efficiency (E_{rp}) as

$$C_p = m_{ip}C_mE_{rp} \tag{8}$$

Recall that the aqueous manure concentrations were determined according to eq 1 from OD660 measurements. The (oo)cyst release efficiency, E_{rp}, describes the partitioning behavior of (oo)cysts into water relative to that of manure. Efficiency in the (oo)cyst release from the manure is hypothesized to depend on the (oo)cyst size and charge as well as the solution salinity. An estimate of the release efficiency of a particular system can be obtained from the slope of a linear regression curve (zero intercept) between measured (oo)cyst and manure concentrations according to eq 8. The cumulative number of *Giardia* or *Cryptosporidium* (oo)cysts (N_p, N) in the aqueous phase can be determined as

$$N_p(t) = Q\int_0^t C_p(t^*)\, dt^* \tag{9}$$

where t^* (T) is a dummy time variable of integration. The (oo)cyst release rate is given as the temporal derivative of eq 9.

Results and Discussion

Manure. Figure 2 presents a plot of representative effluent manure concentrations as a function of time for the various solution salinities. Replicate manure effluent curves for the 0.3 and 14.8 dS m^{-1} systems were consistent with the behavior shown in this figure and are therefore not presented. Values of C_m presented in this figure can be converted to manure mass transfer rates by simply multiplying by the average

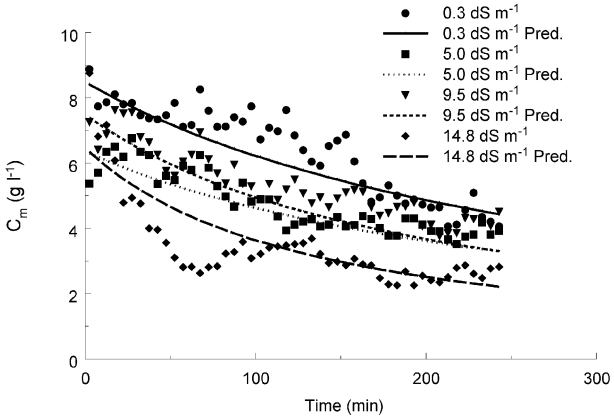


FIGURE 2. A plot of representative observed and predicted manure effluent curves for the various solution salinities. The predictions were obtained using eq 7 in conjunction with eqs 10 and 11.

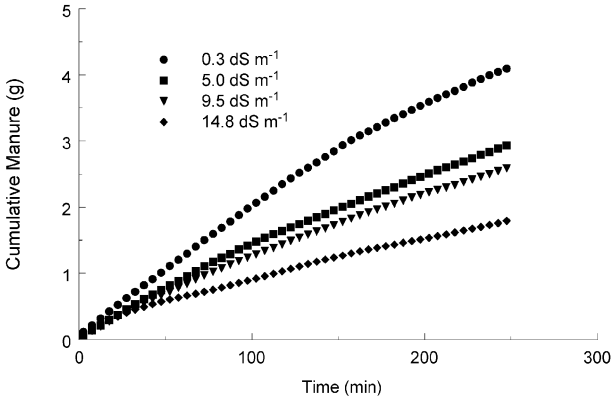


FIGURE 3. A plot of representative cumulative manure mass released into the aqueous phase as a function of time for the various solution salinities.

water flow rate given in Table 1. Hence, plots of the aqueous manure concentration (Figure 2) and release rate as a function of time were very similar. The initial aqueous manure concentrations were several orders of magnitude below (diluted approximately 141 times) the initial manure density, ρ_i. The effluent manure concentrations and manure transfer rates also tended to decrease with increasing time; i.e., C_m decreased from around 7.8 to 3.9 g/L after 250 min. The shape of the various manure effluent curves, however, was dependent on the solution salinity. The lowest salinity solution (0.3 dS m^{-1}) produced a consistently higher manure concentration, and the highest salinity solution (14.8 dS m^{-1}) yielded the lowest manure concentration at a given time. The solutions of intermediate salinity (5 and 9.5 dS m^{-1}) tended to exhibit manure concentrations between these two extreme values.

Figure 3 presents the cumulative manure mass in the aqueous phase as a function of time for the various solution salinities. Trends in the manure effluent data are more apparent in Figure 3 than in Figure 2. The cumulative manure mass in the aqueous phase increased with decreasing solution salinity. Table 2 summarizes the cumulative manure mass released from the various experimental systems. In comparison to the 0.3 dS m^{-1} system, the 5.0, 9.5, and 14.8 dS m^{-1} systems released 28.4, 36.7, and 56.2% less manure mass to the aqueous phase, respectively.

Figures 2 and 3 indicate that solution salinity had a pronounced influence on the partitioning behavior of manure to water. An explanation for these observations was obtained by considering the influence of solution salinity on charged particles. Increasing the solution salinity is known to decrease

TABLE 2. Cumulative Released Manure and (Oo)Cyst

EC (dS m^{-1})	mass (g) manure	% total manure	Nx10^5 *Giardia*	%total *Giardia*	Nx10^5 *Crypto.*	%total *Crypto.*
0.30	4.09	10.8	4.90	13.6	2.57	9.6
0.30[a]	4.97	13.0	3.70	10.0	0.55	2.1
5.00	2.93	7.7	1.58	4.4	1.84	6.7
9.50	2.59	6.7	1.64	4.4	1.51	5.4
14.8	1.79	5.7	1.08	2.9	0.30	1.1
14.8[a]	2.13	5.5	1.59	4.3	0.25	0.9

[a] Denotes replicate experiment.

TABLE 3. Parameters Fitted to Manure Effluent Curves and Statistical Measures of the Goodness of Fit

EC (dS m^{-1})	$\alpha \times 10^{-4}$ (min^{-1})	$SE_\alpha \times 10^{-4}$	β	SE_β	MSE	r^2 [b]	r^2 [c]
0.30	6.07	0.16	4.55	0.42	0.351	0.817	0.757
0.30[a]	6.59	0.19	7.46	0.49	0.537	0.815	0.815
5.00	4.18	0.10	6.72	0.54	0.156	0.823	0.733
9.50	3.72	0.07	8.25	0.54	0.146	0.871	0.604
14.8	3.72	0.22	26.4	2.63	0.546	0.694	0.657
14.8[a]	4.34	0.17	22.2	1.52	0.252	0.840	0.618

[a] Denotes replicate experiment. [b] Goodness of fit between observed data and fitted model output. [c] Goodness of fit between observed data and predicted model output (cf. Figure 2).

the electric potential between charged particles (*24*). Organic matter is reported to have a highly negative net charge (*25*). The manure is also expected to possess a negative net charge because it is composed of partially digested organic matter (feed) and microbial biomass. Increasing the solution salinity was hypothesized to lower the repulsive forces between negatively charged particles in the manure and thereby stabilize the organic manure matrix.

Figures 2 and 3 also reveal that the aqueous manure concentration and manure mass transfer rate tended to decrease with increasing time. One explanation for this behavior is that the exposed surface area of manure to flowing water becomes depleted of the finer colloidal materials with time. The remaining large sized fraction of manure is believed to shield the underlying manure components from flowing water, making it progressively more difficult for this fraction to partition into the water. Temporal spikes in the manure concentration and mass transfer rate may occur when fresh manure surface area becomes accessible to flow water as a result of slow disintegration of the manure disk. Visual inspection of a vertical slice of the manure disk at the end of the elution experiments revealed that only the exposed surface area of the manure disk had been depleted of manure components. These observations collectively suggests that the manure will act as a long-time source of contamination to flowing water.

Temporal changes in the aqueous phase manure concentration were modeled using eq 7. Parameter values for α and β were fitted to the aqueous manure concentration data using a nonlinear least squares optimization routine based upon the Levenberg−Marquardt algorithm (*26*). Table 3 summarizes best fit values of α and β as well as statistical parameters for the goodness of fit (*27, 28*); i.e., the coefficient of linear regression (r^2), the mean square error (MSE), and the standard error (SE). The fitted values of α are consistent with the initial aqueous manure concentrations shown in Figure 2 and tend to decrease with increasing salinity. In contrast, fitted values of β tend to increase with increasing salinity. Higher values of β produce an initial rapid decrease in mass transfer rate followed by a persistent low level of mass transfer. The values of α and β for replicate experiments conducted at the same solution salinity were comparable,

suggesting that the manure dissolution experiments exhibit good reproducibility.

For simulation purposes, the following correlations were established between the measured solution electrical conductivity (EC, dS m^{-1}) and the fitted values of α and β:

$$\alpha = 5 \times 10^{-4}\mathrm{EC}^{-0.127} \quad r^2 = 0.904 \qquad (10)$$

$$\beta = 4.95 \exp(0.097\mathrm{EC}) \quad r^2 = 0.829 \qquad (11)$$

The values of α and β are also likely functions of other physical (e.g., manure type and age, temperature, precipitation rate and intensity, and solution application method) and chemical (e.g., pH and surfactants) factors. Additional experimental studies are necessary to quantify the dependence of α and β on other system variables. Hence, application of eqs (10) and (11) to other aqueous solution−manure systems should be conducted with caution.

Figure 2 also shows the predicted (eqs 7, 10, and 11) manure effluent curves for the various solution salinities. The reasonable agreement between the observed and predicted data (cf. Table 3) suggests that the use of eq 7 in conjunction with eqs 10 and 11 can provide an adequate description of the manure effluent curves. Temporal spikes are not accounted for in the manure dissolution model (eq 7), but Schijven et al. (*21*) found that such spikes had a minimal impact on the long-term (5017 min) manure dissolution behavior and that the proposed model adequately described their data. The predicted behavior for the 14.8 dS m^{-1} system tended to overestimate the measured initial aqueous manure concentration. Recall that the lower aqueous manure concentration for the 14.8 dS m^{-1} system was attributed to a stabilization of the manure by the higher solution salinity. It is likely that the stabilization process does not occur instantaneously. The model (eq 7) attempts to account for this behavior by utilizing a high value of β.

(Oo)cysts. Figure 4a,b present plots of representative *Giardia* cyst and *Cryptosporidium* oocyst release rates as a function of time, respectively, for the various solution salinities. The 0.3 dS m^{-1} system exhibited a high initial release rate (4030 cysts per minute, and 1744 oocysts per minute) that rapidly decreased with increasing time to low values (223 cysts per minute, and 108 oocysts per minute) after 250 min. In contrast, the highest salinity system (14.8 dS m^{-1}) had a much lower initial release rate (512 cysts per minute, and 100 oocysts per minute), that gradually decreased (333 cysts per minute, and 50 oocysts per minute) with increasing time. In the case of *Giardia* cyst release (Figure 4a), the rates for the 5.0 and 9.5 dS m^{-1} systems were similar to the 14.8 dS m^{-1} system but with slightly higher initial values. In contrast, *Cryptosporidium* oocyst release rates for the 5.0 and 9.5 dS m^{-1} systems (Figure 4b) exhibited intermediate behavior to the low and high salinity systems, with a distinct trend of decreasing initial oocyst release rate with increasing solution salinity. Differences in the magnitude of the cyst and oocyst release rates can be attributed in part to the difference in the initial manure concentrations of these parasites (cf. Table 1). Comparison of Figures 2 and 4 reveal many similarities between the manure and (oo)cyst release rates as a function of time and solution salinity, suggesting a strong correlation between manure and (oo)cyst release.

If the (oo)cyst release rates in Figure 4 are dividing by the average aqueous phase flow rates (cf. Table 1), then the corresponding aqueous (oo)cyst concentrations can be obtained. Hence, plots of the (oo)cyst concentration and release rate (Figure 4) as a function of time were very similar and, therefore, concentration curves were not shown. An inspection of the aqueous (oo)cyst concentration data revealed that initial concentrations were several orders of magnitude below their manure phase values. The initial

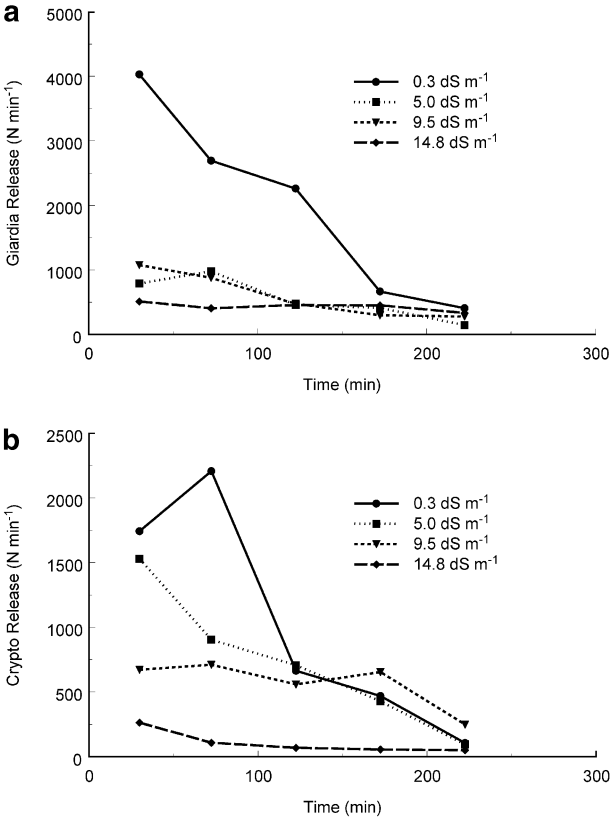
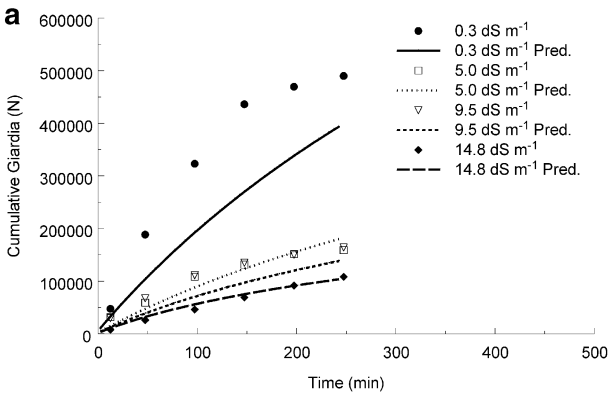


FIGURE 4. A plot of representative *Giardia* (Figure 4a) and *Cryptosporidium* (Figure 4b) (oo)cyst release rate as a function of time for the various solution salinities.

aqueous *Giardia* cyst concentration was diluted approximately 67 times for the 0.3 dS m^{-1} system and 427 times for the 14.8 dS m^{-1} system. In comparison, the initial aqueous *Cryptosporidium* oocyst concentration was diluted approximately 101 times for the 0.3 dS m^{-1} system and 545 times for the 14.8 dS m^{-1} system. Trends in (oo)cyst concentration data as a function of time and solution salinity were similar to those for the (oo)cyst release rates discussed above.

Figure 5a,b present representative plots of the cumulative *Giardia* cyst and *Cryptosporidium* oocyst numbers in the aqueous phase, respectively, for the various salinity solutions. The cumulative number of (oo)cysts released for the various systems is summarized in Table 2. Notice the trend of decreasing (oo)cyst number with increasing solution salinity (cf. Table 2). In comparison to the 0.3 dS m^{-1} system, the 5.0, 9.5, and 14.8 dS m^{-1} systems release 67.6, 66.5, and 78.0% fewer *Giardia* cysts and 28.3, 41.4, and 88.3% fewer *Cryptosporidium* oocysts to the aqueous phase, respectively. This result indicates a dramatic impact of salinity on the cumulative number of (oo)cysts released into the aqueous environment. In Table 2 the percentage of total (oo)cysts and manure that partitioned to the aqueous phase for a particular solution salinity were quite similar (an indication that the oocyst recovery efficiency was consistently high). Comparison of Figures 3 and 5 also reveals similar cumulative loading behavior for manure and (oo)cysts. These observations further indicate a strong correlation between the aqueous manure and (oo)cyst concentrations.

Replicate release behavior for cysts in the 0.3 and 14.8 dS m^{-1} systems and oocysts in the 14.8 dS m^{-1} systems were consistent with that shown in Figures 4 and 5. The replicate release behavior for oocysts in the 0.3 dS m^{-1} systems, however, was at a lower rate than that shown in Figures 4b and 5b. Since the manure and cyst effluent curves were quite



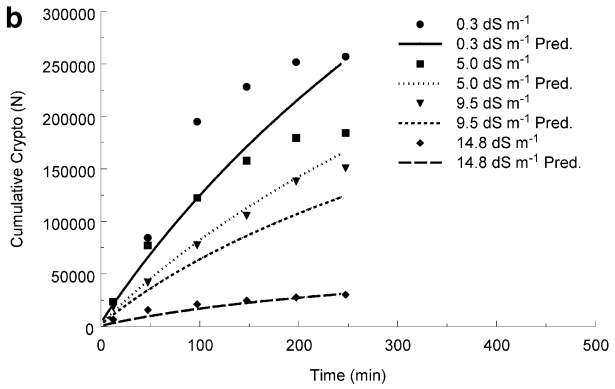
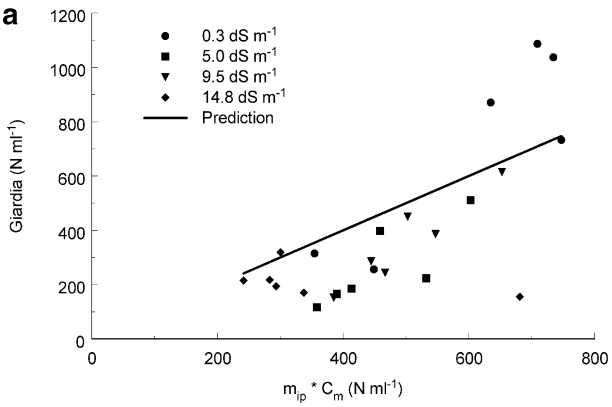


FIGURE 5. Plots of representative observed and predicted cumulative number of *Giardia* (Figure 5a) and *Cryptosporidium* (Figure 5b) (oo)cysts released into the aqueous phase. Predictions were obtained according to eq 8 using the predicted aqueous manure concentrations (cf. Figure 2), and the release efficiency obtained from eqs 12 or 13.



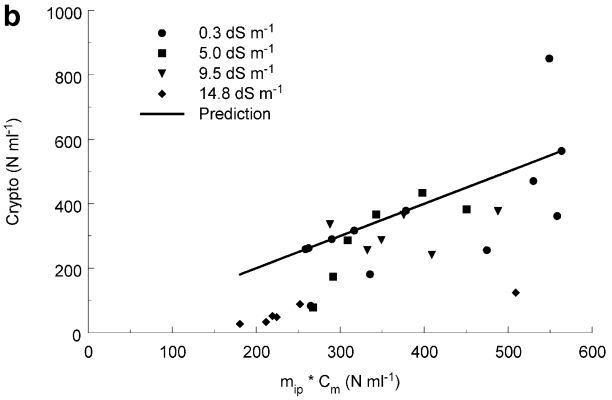


FIGURE 6. A plot of representative aqueous *Giardia* (Figure 6a) and *Cryptosporidium* (Figure 6b) (oo)cyst concentrations as a function of the product of the initial (oo)cyst manure concentration and the aqueous manure concentration (m_{ip}*C_m). Also plotted in the figure is the predicted (oo)cyst concentrations according to eq 8 assuming a perfect (oo)cyst release efficiency ($E_{rp} = 1$).

similar between replicate samples, this difference is believed to be due to variability in the initial oocyst concentration. The 95% confidence interval for initial *Cryptosporidium* oocyst (10.5×10^4–3.7×10^4 N g^{-1}) concentration in the manure indicates that concentrations may vary spatially by a factor of 2.8. Schijven et al. (*21*) presented replicate (two data sets) oocyst release data for 0.3 dS m^{-1} systems (experiments conducted at 5 °C) that were much more consistent with the behavior shown in Figures 4b and 5b. For additional information on the reproducibility of (oo)cyst release and loading behavior the interested reader is referred to Schijven et al. (*21*) for a detailed discussion.

The release behavior of *Giardia* and *Cryptosporidium* (oo)cysts shown in Figures 4 and 5 can be explained by considering the influence of solution salinity on manure stability. Recall that increasing solution salinity increased the manure stability (cf. Figures 2 and 3). Increased manure stability is hypothesized herein to account for the observed decreasing release rate and cumulative loading of *Giardia* and *Cryptosporidium* (oo)cysts with increasing solution salinity. The observed decrease in (oo)cyst release rate with increasing time can also be explained by a depletion of manure and (oo)cysts near the manure disk surface and subsequent shielding of the underlying manure material from flowing water by the remaining larger manure material. Differences between *Giardia* and *Cryptosporidium* (oo)cyst release rates and cumulative loading are hypothesized to be due to differences in the size of the two organisms; 8–12 micrometers for *Giardia* compared with 4–6 micrometers for *Cryptosporidium*. Stabilization of the manure at intermediate solution salinities (5 and 9.5 dS m^{-1}) apparently inhibited the release of the larger *Giardia* cysts (cf. Figures 4a and 5a) more effectively than the release of smaller *Cryptosporidium* (cf. Figures 4b and 5b) oocysts.

Figures 6a,b presents a plot of representative aqueous *Giardia* and *Cryptosporidium* (oo)cyst concentrations, respectively, as a function of the product of the initial (oo)cyst manure concentration and the aqueous manure concentration (m_{ip}*C_m). Also plotted in the figure is the predicted (oo)cyst concentration (solid line) according to eq 8 assuming a perfect (oo)cyst release efficiency ($E_{rp} = 1$); i.e., one to one correspondence between of C_p and m_{ip}*C_m. Although there was considerable deviation between the observed and predicted (oo)cyst concentrations, eq 8 provided a rough approximation to the measured data. Some of this deviation is hypothesized to occur as a result of spatial variability in the initial (oo)cyst concentration distribution in the manure and/or a temporally variable efficiency in the (oo)cyst release from the manure. The measured (oo)cyst concentrations were generally lower than the predicted concentrations (below the solid line). This suggests an imperfect efficiency in the (oo)cyst release.

Release efficiencies, E_{rp}, for *Giardia* and *Cryptosporidium* (oo)cysts were determined as the slope of the line, eq 8, fitted to each data set. Table 4 presents the (oo)cyst release efficiencies for the various solution salinities as well as the r^2 values for the goodness of fit. Some of the fits are quite poor. As mentioned above, some of this deviation is believed to occur as a result of spatial variability in the initial (oo)cyst concentration (distribution within the manure) and/or temporal variability of the (oo)cyst release efficiency (as the manure surface layer becomes depleted of oocysts and cysts the efficiency of release decreases). Attempts to characterize the time dependency of E_{rp} were unsuccessful due to the

TABLE 4. (Oo)Cyst Release Efficiencies

EC (dS m^{-1})	*Giardia* E_{rp}^{g}	*Giardia* r^2 [b]	*Giardia* r^2 [c]	*Crypto.* E_{rp}^{c}	*Crypto.* r^2 [b]	*Crypto.* r^2 [c]
0.30	1.23	0.67	0.496	0.86	0.54	0.780
0.30[a]	0.96	0.60	0.978	0.18	−0.45	0.133
5.00	0.61	0.45	0.901	0.86	0.43	0.657
9.50	0.74	0.61	0.564	0.81	−0.40	0.823
14.8	0.48	−3.76	0.974	0.23	0.80	0.796
14.8[a]	0.69	−3.60	0.869	0.22	−1.43	0.992

[a] Denotes replicate experiment. [b] Goodness of fit of the release efficiency (eq 8). [c] Goodness of fit between observed data and predicted model output (Figure 5).

superposition of these two confounding factors.

Table 4 indicates that the 0.3 dS m^{-1} systems exhibited an *Giardia* cyst release efficiency sometimes greater than unity, suggesting that *Giardia* cysts may be more easily released to the aqueous phase than other components in the manure at low solution salinities. The *Giardia* cyst release efficiency was much lower for the highest salinity system (14.8 dS m^{-1}), suggesting a preferential release of other manure constituents compared to *Giardia* cysts. Both *Giardia* and *Cryptosporidium* (oo)cysts exhibited a trend of decreasing release efficiency with increasing solution salinity. This is believed to occur as a result of stabilization of the manure.

Table 4 also presents (oo)cyst release efficiencies for replicate 0.3 and 14.8 dS m^{-1} systems. The cyst release efficiency exhibited reasonable agreement in replicate experiments. The oocyst release efficiency for the 14.8 dS m^{-1} system also had good reproducibility. In contrast, the oocyst release efficiency for the 0.3 dS m^{-1} systems were quite different (0.86 and 0.18), presumably due to variability in the initial oocyst concentration in the manure. Schijven et al. (*21*) also determined replicate oocyst release efficiencies for 0.3 dS m^{-1} systems (experiments conducted at 5 °C) that were more consistent and intermediate to the values determined herein, 0.61 and 0.53.

The following correlations were established between the observed *Giardia* and *Cryptosporidium* (oo)cyst release efficiencies and the solution electrical conductivity (dS m^{-1}):

$$E_{rp}^{g} = 0.894\mathrm{EC}^{-0.155} \quad r^2 = 0.75 \tag{12}$$

$$E_{rp}^{c} = 0.167 \ln\left(1 - \frac{\mathrm{EC}}{15}\right) + 0.918 \quad r^2 = 0.99 \tag{13}$$

The superscripts *c* and *g* are used here to denote *Cryptosporidium* and *Giardia*, respectively. Equation 13 did not consider the low oocyst release efficiency for the replicate 0.3 dS m^{-1} system, because this value is likely due to an overestimate of m_{ip} for this experiment. As for α and β, the value of E_{rp} is also likely a function of other physical and chemical variables. Hence, caution is warranted when applying eqs 12 and 13 to other aqueous solution−manure systems.

Figure 5a,b also present plots of the predicted cumulative number of *Giardia* and *Cryptosporidium* (oo)cysts released into the aqueous phase, respectively. Predictions were obtained according to eq 8 using the predicted aqueous manure concentration discussed above and the release efficiency obtained from eqs 12 or 13. The predictions provide a reasonable description of the observed trends in the data (salinity effects on release) as well as the cumulative number of (oo)cysts released during the experiment (cf. Table 4). Deviations between the observed and predicted cumulative loading curves are attributed to the use of a single value of E_{rp} and m_{ip} for a particular solution salinity and parasite. Spatial variability in the oocyst concentration in the manure disk and/or temporal variability in the release efficiency likely occurred in the experimental system but could not be quantified due to the superposition of these two factors during parasite release. Despite these acknowledged limitations, the modeling approach does provide a good first approximation to the observed release data.

Results from the studies presented herein indicate that solution salinity is an important factor to consider when describing the release rates and cumulative loading of manure and (oo)cysts to the aqueous phase. On the farm, higher release and loading rates for manure and (oo)cysts are anticipated when manure is exposed to low salinity rain or drinking water. Conversely, lower release and loading rates for manure and (oo)cysts are expected when manure is exposed to only animal urine. Temporal changes in aqueous manure and (oo)cysts concentrations are also expected during the rainy season. Decreasing concentrations of manure and (oo)cysts are projected with increasing water application duration. Many other chemical (solution composition, pH, surfactants, etc.) and physical factors (manure composition, wetting and drying cycles, etc.) will also likely influence the release rates and cumulative loading of (oo)cysts. These issues are topics of ongoing research. The release rates and cumulative loading of other viral and bacterial pathogens to aqueous solutions also needs to be systematically investigated as well as the extension of laboratory scale pathogen release and loading rates to the farm scale.

Acknowledgments

We would like to thank Mehdi Bettahar, Christine Lee, and Shihui Yang for their help in determining (oo)cyst concentrations.

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Received for review February 8, 2002. Revised manuscript received June 26, 2002. Accepted July 8, 2002.

ES025573L