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 × 10⁹/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.
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 samples were collected directly under the crates and urine drops directly under the crates. Manure and urine 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. Thereference solution in the experiments consisted of a low salinity 0.001 M NaCl solution with its pH buffered to 6.98 using 5 × 10^-3 M NaHCO3. 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, 3.0, 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 multihthead drive pump (Barnant Company, Barrington, IL 60010). The pumped solution was connected 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, g cm^-3), manure volume (V_m, L^3), and the average aqueous phase flow rate for the various release experiments (Q, mL min^-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, g L^-1) and the solution optical density at 660 nanometers:

\[ C_m = 7.83 \cdot OD_{660} \cdot r^2 = 0.982 \]  

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).

Table 1. Experimental Conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC (dS m^-1)</td>
<td>0.30</td>
</tr>
<tr>
<td>Q (mL min^-1)</td>
<td>2.60</td>
</tr>
<tr>
<td>ρ (g cm^-3)</td>
<td>1.10</td>
</tr>
<tr>
<td>Giardia mio (N g^-1)</td>
<td>9.5 × 10^4</td>
</tr>
<tr>
<td>Crypto. mio (N g^-1)</td>
<td>7.1 × 10^4</td>
</tr>
</tbody>
</table>

*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-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.,

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The following manure mass balance equation

\[ \frac{d \rho}{dt} = -k_{\text{wm}} \left[ \rho_{\text{e}} - \rho \right] \]

where \( \rho \) (M L\(^{-3}\)) is the manure phase density, \( \rho_{\text{e}} \) (M L\(^{-3}\)) is the manure phase density at the water-manure interface in equilibrium with the aqueous phase, \( k_{\text{wm}} \) (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 \( k_{\text{wm}} \) 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), \( k_{\text{wm}} \) will be generalized using a simple power function of the normalized manure density as

\[ k_{\text{wm}} = \alpha \left( \frac{\rho_{\text{e}}}{\rho} \right)^{\beta} \]

where \( \alpha \) (T\(^{-1}\)) and \( \beta \) are fitting parameters. The value of \( \alpha \) controls the initial manure release rate, whereas \( \beta \) determines the slope 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 \( (C_{\text{wm}} = 0) \) and \( \rho_{\text{e}} \) (\( \rho_{\text{e}} \) equals the product of \( C_{\text{wm}} \) and a partition coefficient) equals zero. Under these conditions, eq 2 reduces to

\[ \frac{d \rho}{dt} = -k_{\text{wm}} \rho \]

The solution to eq 4 is given as

\[ \rho(t) = \rho_1 (1 + \alpha \beta t)^{1/\beta} \]

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

\[ M_w(t) = V_m (\rho_1 - \rho(t)) \]

The manure release rate is the product of \( Q \) and \( C_{\text{wm}}(t) \).

Recall that the aqueous manure concentrations were determined according to eq 1 from OD660 measurements. The (oo)cyst release efficiency, \( E_{\text{wp}} \), 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

\[ N_p(t) = Q \int_{0}^{t} C_{\text{wn}}(t^*) \, dt^* \]

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

The concentration of (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 \( \mu \)m 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 \((m_p, N \text{ M}^{-1})\) were 9.5 \( \times \) 10\(^4\) (12 replicates, with a standard error of 3.0 \( \times \) 10\(^3\)) and 7.1 \( \times \) 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).
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 suggest 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 \( \alpha \) and \( \beta \) 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 \( \alpha \) and \( \beta \) 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 \( \text{MSE} \), and the standard error \( \text{SE} \). The fitted values of \( \alpha \) and \( \beta \) for replicate experiments conducted at the same solution salinity were comparable.

**TABLE 1. Parameters Fitted to Manure Effluent Curves and Statistical Measures of the Goodness of Fit**

<table>
<thead>
<tr>
<th>EC (dS m(^{-1}))</th>
<th>( \alpha \times 10^{-4} ) (min(^{-1}))</th>
<th>( SE_{\alpha} \times 10^{-4} )</th>
<th>( \beta )</th>
<th>( SE_{\beta} )</th>
<th>( r^2 )</th>
<th>MSE</th>
<th>( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.30</td>
<td>6.07</td>
<td>0.16</td>
<td>4.55</td>
<td>0.42</td>
<td>0.351</td>
<td>0.817</td>
<td>0.757</td>
</tr>
<tr>
<td>0.30(^a)</td>
<td>6.59</td>
<td>0.19</td>
<td>7.46</td>
<td>0.49</td>
<td>0.537</td>
<td>0.815</td>
<td>0.815</td>
</tr>
<tr>
<td>5.00</td>
<td>4.18</td>
<td>0.10</td>
<td>6.72</td>
<td>0.54</td>
<td>0.156</td>
<td>0.823</td>
<td>0.733</td>
</tr>
<tr>
<td>9.50</td>
<td>3.72</td>
<td>0.07</td>
<td>8.25</td>
<td>0.54</td>
<td>0.146</td>
<td>0.871</td>
<td>0.604</td>
</tr>
<tr>
<td>14.8</td>
<td>3.72</td>
<td>0.22</td>
<td>26.4</td>
<td>2.63</td>
<td>0.546</td>
<td>0.694</td>
<td>0.657</td>
</tr>
<tr>
<td>14.8(^a)</td>
<td>4.34</td>
<td>0.17</td>
<td>22.2</td>
<td>1.52</td>
<td>0.252</td>
<td>0.840</td>
<td>0.618</td>
</tr>
</tbody>
</table>

\(^a\) Denotes replicate experiment.
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 \(\alpha\) and \(\beta\):

\[
\alpha = 5 \times 10^{-4} EC^{-0.127} \quad r^2 = 0.904 \quad (10)
\]
\[
\beta = 4.95 \exp(0.097EC) \quad r^2 = 0.829 \quad (11)
\]

The values of \(\alpha\) and \(\beta\) 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 \(\alpha\) and \(\beta\) on other system variables. Hence, the 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 \(\beta\).

\((\text{oocyst})\) 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 dSm\(^{-1}\) 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 reveals many similarities between the manure and (oocyst) release rates as a function of time and solution salinity, suggesting a strong correlation between manure and (oocyst) release.

If the (oocyst) release rates in Figure 4 are divided by the average aqueous phase flow rates (cf. Table 1), then the corresponding aqueous (oocyst) concentrations can be obtained. Hence, plots of the (oocyst) 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 (oocyst) concentration data revealed that initial concentrations were several orders of magnitude below their manure phase values. The initial 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 (oocyst) concentration data as a function of time and solution salinity were similar to those for the (oocyst) 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 (oocyst) released for the various systems is summarized in Table 2. Notice the trend of decreasing (oocyst) 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 (oocyst) released into the aqueous environment. In Table 2 the percentage of total (oocyst) 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 (oocyst). These observations further indicate a strong correlation between the aqueous manure and (oocyst) 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
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 \( \times 10^4 - 3.7 \times 10^4 \) Ng-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.

Release efficiencies, \( E_{\text{rp}} \), 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 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, \( E_{\text{rp}} \), 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 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.

**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 \( (m_{\text{ip}} C_m) \). Also plotted in the figure is the predicted (oo)cyst concentrations according to eq 8 assuming a perfect (oo)cyst release efficiency \( (E_{\text{rp}} = 1) \).
TABLE 4. (Oo)Cyst Release Efficiencies

<table>
<thead>
<tr>
<th>EC (dS m$^{-1}$)</th>
<th>Giardia $E_p^g$</th>
<th>Giardia $p^b$</th>
<th>Giardia $c^c$</th>
<th>Crypto. $E_p^c$</th>
<th>Crypto. $p^b$</th>
<th>Crypto. $c^c$</th>
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</thead>
<tbody>
<tr>
<td>0.30</td>
<td>1.23</td>
<td>0.67</td>
<td>0.496</td>
<td>0.86</td>
<td>0.54</td>
<td>0.780</td>
</tr>
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<td>0.30</td>
<td>0.96</td>
<td>0.60</td>
<td>0.978</td>
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<td>-0.45</td>
<td>0.133</td>
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<tr>
<td>0.30</td>
<td>0.61</td>
<td>0.45</td>
<td>0.901</td>
<td>0.86</td>
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<tr>
<td>0.50</td>
<td>0.74</td>
<td>0.61</td>
<td>0.564</td>
<td>0.81</td>
<td>-0.40</td>
<td>0.523</td>
</tr>
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<td>14.8</td>
<td>0.48</td>
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<td>0.974</td>
<td>0.23</td>
<td>0.80</td>
<td>0.796</td>
</tr>
<tr>
<td>14.8</td>
<td>0.69</td>
<td>-3.60</td>
<td>0.869</td>
<td>0.22</td>
<td>-1.43</td>
<td>0.992</td>
</tr>
</tbody>
</table>

* Denotes replicate experiment.  
* Goodness of fit of the release efficiency (eq 8).  
* Goodness of fit between observed data and predicted model output (Figure 5).

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.

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Literature Cited


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