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Release of *Cryptosporidium* and *Giardia* from Dairy Cattle Manure: Physical Factors

Jack F. Schijven,* Scott A. Bradford, and Shihui Yang

ABSTRACT

Various physical factors affecting the release rate of naturally occurring *Cryptosporidium parvum* oocysts and *Giardia duodenalis* cysts from dairy manure disks to sprinkled water were studied. The investigated factors included temperature (5 or 23°C), manure type (calf manure, a 50% calf and 50% cow manure mixture, and a 10% calf and 90% cow manure mixture), and water application method (mist or drip) and flow rate. Effluent concentrations of manure and (oo)cysts were always several orders of magnitude below their initial concentration in the manure, decreased gradually, and exhibited persistent concentration tailing. Release of manure and (oo)cysts were found to be related by a constant factor, the so-called release efficiency of (oo)cysts. A previously developed (oo)cyst release model that included these release efficiencies provided a satisfactory simulation of the observed release. An effect of temperature on the release of manure and (oo)cysts was not apparent. The manure and (oo)cyst release rates from cow manure decreased faster than those from calf manure, and (oo)cyst release efficiencies from cow manure were higher than those from calf manure. In comparison with mist application, dripping water resulted in higher release rates of manure and (oo)cysts and in higher (oo)cyst release efficiencies due to the increased mechanical forces associated with droplet impact. Mist application at a higher flow rate resulted in faster release, but did not affect the (oo)cyst release efficiencies. The data and modeling approach described herein provide insight and an enhanced ability to describe the influence of physical factors on (oo)cyst release.

Cryptosporidium and *Giardia* are protozoan parasites that infect the intestines of a variety of animals and man. The infectious stage of these parasites is biologically dormant (oo)cysts (*Cryptosporidium* oocysts and *Giardia* cysts). Cattle, especially young calves, have been recognized as a significant source of (oo)cysts because of the high prevalence of infection, the high numbers of (oo)cysts that are shed within their feces, and the large volume of manure generated by confined beef and dairy cow operations (Garber et al., 1994; Ongerth and Stibbs, 1989; Schijven et al., 1999; Scott et al., 1994; Xiao et al., 1993; Xiao and Herd, 1994). Manure-contaminated runoff water from farms and the application of animal waste to agricultural land may therefore result in the release of large numbers of (oo)cysts into the environment.

(Oo)cysts are ubiquitous in surface water (Hoogenboezem et al., 2001; LeChevallier et al., 1991; Rose, 1988). Surface water is an important source for drinking water production and for recreational purposes. Ingestion of contaminated water containing as few as 10 (oo)cysts can lead to infection (Olson et al., 1999). Many outbreaks of cryptosporidiosis and giardiasis have been reported in industrialized countries (Craun, 1990; Craun et al., 1998). In these outbreaks, (oo)cysts were present in drinking water due to contamination of the source water, failure in treatment of surface water, and leakage into the distribution system.

Release rates of (oo)cysts from manure to water are needed to evaluate the transport and fate of (oo)cysts in surface and ground water. Unfortunately, few studies have examined the release behavior of (oo)cysts and other pathogens from animal manure. Published studies have presented temporal changes in surface water concentrations of *Cryptosporidium* or indicator bacteria following precipitation events or simulated rainfall (Thelin and Gifford, 1983; Kress and Gifford, 1984; Mawdsley et al., 1996; Tate et al., 2000). Runoff concentrations were observed to gradually increase to a maximum value and then decrease over several orders of magnitude to persistently low concentration levels. Bradford and Schijven (2002) recently investigated the release behavior of (oo)cysts from dairy calf manure to waters of various salinity. (Oo)cyst and manure release rates were found to decrease with increasing solution salinity. A conceptual model was presented to describe and predict manure and (oo)cyst release behavior.

The aim of this research was to determine release rates of naturally occurring (oo)cysts from dairy cattle manure under various physical conditions. Experiments were designed to identify the magnitude and sensitivity of key physical factors that influence the release of manure and (oo)cyst release from farms. The following factors were considered: temperature (5 or 23°C), manure type (calf manure, 50% calf and 50% cow manure mixture, and a 10% calf and 90% cow manure mixture), and water application (mist or drip) and flow rate. Variations in temperature affect the activity of microorganisms in manure and consequently manure physical and chemical properties. Manure composition experiments encompassed a range of manure types typical of various manure management strategies. The water application and flow rate experiments provided insight on the role of

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J.F. Schijven, National Institute of Public Health and the Environment, Microbiological Laboratory for Health Protection, P.O. Box 1, 3720 BA Bilthoven, the Netherlands. S.A. Bradford, George E. Brown, Jr. Salinity Laboratory, USDA-ARS, 450 West Big Springs Road, Riverside, CA 92507. S. Yang, Microbiology Program, 241 Boyce Hall, University of California, Riverside, CA 92521. Received 29 Jan. 2003. *Corresponding author (jack.schijven@rivm.nl).

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677 S. Segoe Rd., Madison, WI 53711 USA

Abbreviations: C_m, aqueous-phase manure concentration; C_p, aqueous-phase (oo)cyst number concentration; E, (oo)cyst release efficiency; E_{Crypto}, oocyst release efficiency; $E_{Giardia}$, cyst release efficiency; f_{cow}, fraction of cow manure; m_{ip}, initial (oo)cyst number concentration in the manure; Q, water flow rate.

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precipitation intensity, amount, and duration. The release data were characterized using the model developed by Bradford and Schijven (2002). Interpretation of the data was aided by analyzing trends in the fitted model parameters, and by conducting statistical tests to determine significant differences among fitted parameters.

MATERIALS AND METHODS

Manure

Manure from holstein dairy cows and calves was collected at a dairy farm in Chino, California. The holstein calves are kept in wooden crates until weaned (the first 3 mo). Approximately 8 kg of manure from 2.5- to 3-mo-old calves were collected from under the wooden crates with a shovel into a bucket. This sample was denoted as Calf 1. About 2 mo later, approximately 8 kg of manure from 2.5- to 3-mo-old calves (denoted as Calf 2), as well from adult cows, were collected. The latter was collected from the concrete floor of the milking parlor. The manure in each bucket was thoroughly homogenized with a stick and stored at 5 ± 3°C. The electrical conductivity and pH of the manure samples were measured to be about 15 dS m^{-1} and 9, respectively. Four types of manure were considered in the experiments: Calf 1, Calf 2, a mixture of 50% Calf 1 and 50% cow manure, and a mixture of 10% Calf 1 and 90% cow manure. Cow and calf manure are frequently combined on farms and the mixtures of Calf 1 and cow manure are used to represent this manure management scenario. The manure mixtures were created by thoroughly mixing known manure mass fractions in a beaker with a spatula.

Experimental Setup and Conditions

A particular manure type was packed into an aluminum ring that was 5 cm in diameter and 1.75 cm thick. A 5-cm-diameter plastic disk was then used to gently push the manure disk on top of a 105-μm stainless steel screen that rested on a 14-cm-diameter ceramic filter funnel. Figure 1 presents a schematic of the experimental setup. A plastic funnel was placed upside down over the ceramic funnel to form a hood. A stainless steel tube was inserted at the top of this hood directly above the manure disk. Aqueous solution was dripped from this tube at a constant rate using a Masterflex L/S multihead drive pump (Barnant Company, Barrington, IL). Some of the experiments were conducted using the aqueous solution applied as a mist instead of droplets. In this case, an nozzle was attached to a constant air pressure source at the end of the stainless steel tube. Air and pumped aqueous solution were combined in the nozzle to create a mist that fell onto the manure disk. The aqueous solution always consisted of 0.001 *M* NaCl with its pH buffered to 7 using 5×10^{-5} *M* $NaHCO_3$. The electrical conductivity of this solution was measured to be 0.12 dS m^{-1}.

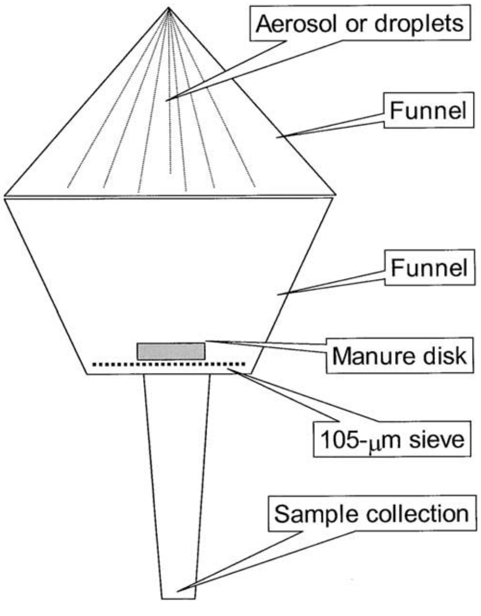

Fig. 1. Experimental setup to elute (oo)cysts from a disk of manure.

Table 1 summarizes the experimental conditions for all the release experiments. Information is provided about the manure type (Calf 1, Calf 2, mixture of 50% Calf 1 and 50% cow manure, and a mixture of 10% Calf 1 and 90% cow manure), water application method (drip or mist), and the water flow rate (Q). All experiments were conducted in constant-temperature rooms at the indicated temperature. The initial manure bulk concentration (ρ_i) provided in the table was determined from the measured manure volume (V_m) and weight of the manure disk. Experiments were designated as Experiments 1 through 9 and are described in Table 1. The duration of the experiments was always at least 250 min, although some were conducted 5017 min to deduce the long-term release behavior.

Aqueous Concentration of Manure

Effluent samples from the release experiments were collected directly below the funnel as 10-mL aliquots in borosilicate vials, as 50-mL aliquots in polypropylene tubes, or as 1.5-L samples in Erlenmeyer flasks and stored at 5 ± 3°C. The optical density (OD) of effluent samples was measured at 660 nm using a Turner SP 830 spectrophotometer (Barnstead/Thermolyne Corporation, Dubuque, IA). The measured sample OD was then related to an aqueous-phase manure concentration, C_m, using the following linear calibration curve (Bradford and Schijven, 2002):

$$C_m = 7.8298\text{OD};\ r^2 = 98\% \quad [1]$$

This calibration curve was established when C_m ranged from 0 to 12.4 g L^{-1} and OD from 0 to 1.54.

(Oo)cyst Concentrations

Concentrations of *Cryptosporidium* oocysts and *Giardia* cysts in the manure and the manure effluent samples were determined by staining (oo)cysts with FITC monoclonal antibody and enumeration of (oo)cysts using an epifluorescent microscope as described in detail by Bradford and Schijven (2002).

Release Model

Bradford and Schijven (2002) developed a conceptual model for release of manure and (oo)cysts into the aqueous phase. This approach describes mass transfer from the manure to the aqueous phase 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). A simple power function of the manure-phase density was used to characterize the manure mass transfer coefficient. For steady-state water flow and influent solutions that do not contain dissolved manure, the following analytic expression for the aqueous manure concentration as a function of time was derived (Bradford and Schijven, 2002):

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Table 1. Experimental conditions during water application to calf manure disks.

Experiment	Manure type	Water application	Water flow rate (Q)	ρ_i†	Temperature
			mL min^{-1}	g mL^{-1}	°C
1	Calf 1	drip	2.42	1.11	5 ± 3
2	Calf 1	drip	2.82	1.07	5 ± 3
3	Calf 1	drip	2.60	1.10	23 ± 3
4	Calf 1	drip	2.03	1.12	23 ± 3
5	Calf 2	drip	2.21	1.11	5 ± 3
6	50% Calf 1 + 50% cow	drip	2.96	1.08	5 ± 3
7	10% Calf 1 + 90% cow	drip	2.69	1.09	5 ± 3
8	Calf 1	mist	2.89	1.14	5 ± 3
9	Calf 1	mist	10.48	1.12	5 ± 3

† Initial manure bulk concentration determined from manure volume (0.03436 L) and initial weight of disk (g).

$$C_m(t) = \frac{V_m}{Q}\rho_i\alpha(1 + \alpha\beta t)^{-\left(1+\frac{1}{\beta}\right)} \qquad [2]$$

where α and β are fitting parameters and t is time. The value of α controls the initial manure release rate and β determines the shape of the manure release curve. Manure release curves were quantified in this work by fitting Eq. [2] parameters (values of α and β) to measured C_m values. The nonlinear regression algorithm of Levenberg–Marquardt in Mathematica 4.2 (Wolfram Research, 2002) was used to optimize the fit between observed and modeled log-transformed manure concentrations. A log-transformation was applied to the concentration data to improve the long-term sensitivity of model fits. The long-term concentration data were lower in value. By applying log-transformation of the data a greater weight was placed on these low concentrations compared with the untransformed data.

A strong correlation between release of manure and (oo)cysts was expected because the (oo)cysts were components of the manure phase. The aqueous (oo)cysts concentration [$C_p(t)$] was therefore predicted from the aqueous manure concentration (Eq. [2]), the measured initial pathogen concentration in the manure (m_{ip}), and an (oo)cysts release efficiency (E) as (Bradford and Schijven, 2002):

$$C_p(t) = m_{ip}C_m(t)E \qquad [3]$$

The values of m_{ip} (Table 2) and C_m (Eq. [2]) were measured, whereas E was estimated using linear regression from measured C_p values as $E = C_p(t)/[m_{ip}C_m(t)]$. A release efficiency of one implies that manure and (oo)cyst partition into the aqueous phase at equal rates. When $E < 1$, (oo)cysts are released into the aqueous phase at a lower rate than other manure components. The opposite occurs for $E > 1$.

Statistical Analysis

Log likelihood estimates of manure release functions (Eq. [2]) and release efficiency functions (Eq. [3]) were made to investigate effects of physical factors on the release of manure and (oo)cysts. Assuming normally distributed errors, a log likelihood function, L_f, which includes parameters a, b, and s (standard error), can be formulated for each set of n observations O_i (C_m or C_p) at time t_i from an experiment (Hogg and Craig, 1995):

$$L_f(a,b,s) = 2\sum_{i=1}^{n}\left\{\ln\left(s\sqrt{2\pi}\right) + \frac{[\ln O_i - f(t_i,a,b)]^2}{2s^2}\right\} \qquad [4]$$

where i is the ith of n observations and $f(t_{i,a,b})$ is the objective function for manure release or release efficiency of (oo)cysts (e.g., ln[C_m] or ln[C_p], where C_m and C_p are determined from Eq. [2] and [3], respectively). In the case of manure release, parameter a equals α and parameter b equals β. In the case of release efficiency, parameter a equals E and b equals zero. Values for parameters a, b, and s for the data of the individual Experiments 1 through 9 (separate data sets) or for combinations of the data from two or more of these experiments (pooled data sets) were obtained by maximizing this log likelihood function (equivalent to least squares solution) using numerical optimization in Mathematica 4.2. In the case of pooled data sets, a common value of a and/or b was evaluated by summing the right hand side of Eq. [4] over all the data sets. Likelihood ratio tests (Cox and Hinkley, 1974) were applied to parameters a and/or b for different release experiments to determine whether differences were significant. The sum of the log likelihoods of the separate data sets (Eq. [4]) was compared with that of the pooled data. Equation [4] was applied to all separate data sets as well as pooled data sets. The difference was interpreted as a χ^2 deviation with number of degrees of freedom equal to the difference in the number of parameters in the pooled data set and the total number of parameters of all separate data sets (Teunis et al., 1996). If the log likelihood of the pooled data was found to be significantly higher than that of the sum of the log likelihoods of the separate data sets, then significant differences existed between the data sets. Unless specifically noted, all parameter values were found to be significantly different according to the likelihood ratio test.

Distribution of (oo)cysts in the manure was investigated by fitting microscopic counts in manure samples to Poisson distribution and negative-binomial distributions. The Poisson and negative-binomial distributions are a nested pair and can be used in a likelihood ratio test (Cox and Hinkley, 1974). According to this test, if the Poisson distribution describes the counts as well as the negative-binomial distribution then the counts are homogeneously distributed, otherwise the counts are heterogeneously distributed. The potential influence of (oo)cyst variability on calculated release efficiencies was tested by including either the Poisson or negative-binomial distribution counts for the maximum likelihood estimate of m_{ip} in Eq. [3].

As a measure of the goodness of fit between observed and fitted data, the coefficient of determination r^2 was calculated (Spiegel, 1980):

Table 2. Manure-phase concentrations of (oo)cysts, m_{ip}.

	Calf 1	Calf 2
	Cryptosporidium oocysts	
m_{ip}, number g^{-1}	7.1 × 10^4	1.3 × 10^5
SD, number g^{-1}	1.7 × 10^4	6.9 × 10^3
Number of observations	6	2
	Giardia cysts	
m_{ip}, number g^{-1}	9.5 × 10^4	3.9 × 10^4
SD, number g^{-1}	3.0 × 10^4	1.4 × 10^4
Number of observations	12	2

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$$r^2 = 1 - \frac{\sum_{i=1}^{N}(O_\mathrm{i} - F_\mathrm{i})^2}{\sum_{i=1}^{N}(O_\mathrm{i} - \overline{O})^2} \qquad [5]$$

where $\overline{O}$ is the average of the observations, O_i is the observed value, and F_i is the fitted value. This goodness of fit value, which is also known as the Nash and Sutcliffe efficiency (Nash and Sutcliffe, 1970), was calculated to evaluate fitted manure release and predicted (oo)cyst release using log-transformed data and for evaluation of the fitted (oo)cyst release efficiencies. In the latter case, no data transformation was applied.

RESULTS AND DISCUSSION

General

Table 2 summarizes the measured initial concentrations of (oo)cysts in the Calf 1 and Calf 2 manure. The (oo)cyst concentrations were well within the range (95% confidence interval) previously reported for 7- to 12-wk-old calves in the Netherlands (Schijven et al., 1999). Concentrations differed between both calf samples. In the Calf 2 manure, oocyst and cyst concentrations were found to be about twice as high and low as in the Calf 1 manure, respectively. The cow manure did not contain detectable numbers of (oo)cysts. (Oo)cyst concentrations in the mixtures of Calf 1 and cow manure were therefore attributed only to the calf manure.

The oocyst counts in Calf 1 manure were found to be Poisson distributed whereas the cyst counts were slightly better described by the negative-binomial distribution. According to the likelihood ratio test this implies a homogeneous distribution of oocysts and a slightly heterogeneous distribution of the cysts. The 10 highest of the 12 counts were found to be Poisson distributed. The (oo)cyst counts in Calf 2 manure are within a small range and were found to be within a Poisson distribution. Based on this analysis the assumption that the (oo)cysts in the manure were homogeneously distributed appears reasonable. In addition, microscopic slides always showed single (oo)cysts and aggregates were never found.

Manure and (oo)cyst release behavior for the various experiments are presented in Fig. 2 through 6. The manure release rate was determined as the product of water flow rate (Q) and $C_\mathrm{m}(t)$. The (oo)cyst release rate was determined as the product of Q and $C_\mathrm{p}(t)$. The release rates can be converted to concentration values by dividing by the water application rate given in Table 1. Hence, aqueous concentrations of manure and (oo)cysts follow the same trends as the release rates. In all the release experiments the effluent concentrations of manure and (oo)cysts were initially several orders of magnitude below the corresponding initial manure bulk concentration (ρ_i) and m_ip values. The manure and (oo)cyst release rates decreased with time, due to depletion of manure components in the exposed surface area. The manure release curves sometimes exhibited fluctuations over time, because of cycles of manure depletion and disintegration that exposed fresh surface area. Initially, the manure disks had a low intrinsic permeability, and water was observed to flow over the surface of the disks. The water flow behavior was more complex as components of the manure were released to flowing water and the intrinsic permeability increased, especially for the cow manure disks. A detailed analysis of the water flow behavior in the manure disk was not undertaken.

Table 3. Parameter values from fitting manure release model to measured aqueous-phase manure concentration (C_m) values.

Experiment	α ($\times 10^{-4}$†) Value	SD	β Value	SD	r^2
	—— min^{-1} ——				%
1	7.1	0.43	5.2	0.58	93
2	5.4	0.21	1.4	0.33	91
3	6.3	0.21	5.4	0.42	83
4	6.4	0.31	7.1	0.61	78
5	9.7	0.66	1.9	0.46	92
6	7.0	0.47	7.7	0.71	94
7	6.0	0.86	13	2.0	86
8	1.0	0.19	40	21	40
9	5.2	0.44	4.9	1.3	67

† Multiply the reported numbers by 10^{-4} to obtain the actual numbers.

The simulated release behavior in Fig. 2 through 6 was obtained by fitting (α, β, and E) Eq. [2] and [3] to the experimental data. Table 3 summarizes the manure release model (Eq. [2]) parameters (α and β) that were fitted to the experimental data (C_m as a function of t). Table 4 summarizes (oo)cyst release efficiency values (Eq. [3]) that were fitted to the experimental data (C_p, C_m, and m_ip). No differences in estimates of release efficiencies were found when assuming constant, Poisson-distributed or negative-binomially distributed m_ip.

The goodness of fit between observed and simulated data is also presented in Tables 3 and 4, as well as the standard deviation for fitted model parameters. Note

Table 4. Correlation between release of (oo)cysts and manure, release efficiency (E) of (oo)cysts, and goodness of fit values.

Experiment	Correlation coefficient	E Value	SD	r^2 LR†	RM‡
	%			—— %	——
		Cryptosporidium			
1	87	0.53	0.043	75	80
2	83	0.61	0.039	69	77
3	75	0.81	0.18	42	28
4	27	0.18	0.036	−2.5	−45
5	79	0.76	0.066	61	80
6	78	1.0	0.059	86	84
7	96	5.9	0.32	93	68
8	16	0.55	0.092	−62	−21
9	−47	0.29	0.064	−1.7	−184
1 + 2		0.53	0.033	66	
8 + 9		0.40	0.067	−9.0	
		Giardia			
1	98	1.2	0.040	94	78
2	90	1.4	0.072	81	58
3	88	1.2	0.13	67	39
4	46	0.96	0.073	60	−153
5	87	0.99	0.068	75	68
6	88	1.6	0.11	80	59
7	68	1.7	0.24	68	63
8	82	0.83	0.079	66	−55
9	75	0.83	0.085	51	25
1 + 3		1.2	0.042	91	
8 + 9		0.83	0.061	58	

† Goodness of fit from linear regression (release efficiency function).

‡ Goodness of fit of simulated values from release model to observed values.

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Table 5. Cumulative release (CR) of manure mass and (oo)cysts.

	Experiment								
	1	2	3	4	5	6	7	8	9
					Manure				
Total disk weight, g	**37.99**	**36.69**	**37.83**	**38.53**	**38.00**	**37.09**	**37.45**	**39.16**	**38.31**
CR after 247.5 min, g	**4.51**	**4.24**	**4.05**	**4.97**	**6.87**	**3.92**	**3.01**	**0.67**	**3.55**
CR after 247.5 min, %	**12**	**12**	**11**	**13**	**18**	**11**	**8.0**	**1.7**	**9.3**
CR after 5017 min, g	**17.75**	**20.96**			**20.36**	**11.12**	**7.79**		
CR after 5017 min, %	**47**	**57**			**54**	**30**	**21**		
					Cryptosporidium				
Total number in disk, no. × 10^6	**2.70**	**2.60**	**2.68**	**2.68**	**4.94**	**1.32**	**0.27**	**2.72**	**2.78**
CR after 247.5 min, no. × 10^6	**0.15**	**0.16**	**0.23**	**0.055**	**0.69**	**0.11**	**0.095**	**0.036**	**0.089**
CR after 247.5 min, %	**5.6**	**6.2**	**8.6**	**2.1**	**14**	**8.3**	**35**	**1.3**	**3.2**
CR after 5017 min, no. × 10^6	**0.52**	**1.27**			**2.23**	**0.27**	**0.21**		
CR after 5017 min, %	**19**	**49**			**45**	**20**	**78**		
					Giardia				
Total number in disk, no. × 10^6	**3.61**	**3.49**	**3.59**	**3.59**	**1.48**	**1.76**	**0.36**	**3.64**	**3.72**
CR after 247.5 min, no. × 10^6	**0.46**	**0.48**	**0.45**	**0.37**	**0.25**	**0.26**	**0.051**	**0.054**	**0.26**
CR after 247.5 min, %	**13**	**14**	**13**	**10**	**17**	**15**	**14**	**1.5**	**7.0**
CR after 5017 min, no. × 10^6	**0.99**	**1.92**			**0.51**	**0.28**	**0.061**		
CR after 5017 min, %	**27**	**55**			**34**	**16**	**17**		

that in Table 4 some of the goodness of fit values are negative. This is the case when:

$$\sum_{i=1}^{N}(O_{\mathrm{i}} - F_{\mathrm{i}})^2 > \sum_{i=1}^{N}(O_{\mathrm{i}} - \overline{O})^2$$

(see Eq. [5]).

The release model typically provided a reasonable description of the experimental data, but did not capture the periodic fluctuations that occurred as a result of manure disintegration. Cumulative release values of manure and (oo)cysts for the various release experiments are given in Table 5.

Temperature

This section examines the influence of ambient temperature on the release of manure and (oo)cysts. Duplicate experiments were conducted at both 5 and 23°C. Figures 2a, 2b, and 2c present observed and simulated manure, oocyst, and cyst release rates, respectively, at 5°C (Experiments 1 and 2) and 23°C (Experiments 3 and 4). Observed manure release rates coincided for most of the experiments (Fig. 2a), although one of the 5°C experiments (Experiment 2) had a lower manure release rate initially. Parameter values for α were also quite similar at 5 and 23°C (Table 3). Three of the four experiments exhibited similar values of β (5.2–7.1), but one of the 5°C experiments (Experiment 2) had a significantly lower value of β (1.4). Differences in manure release behavior between replicate disks (Experiments 1 and 2) were apparently larger than disks at different temperatures (Experiment 1 versus Experiments 3 and 4). Cumulative manure release after 250 min was also very similar at 11 to 13% (Table 5) for the experiments. This analysis suggested that manure release was independent of temperature for the considered short-term experiments.

Release data for cysts at 5 and 23°C were also quite similar (Fig. 2c). The likelihood ratio test indicated a common cyst release efficiency (E_{Giardia}) value of 1.2 for one of the 5°C (Experiment 1) and 23°C (Experiment 3) studies. The release data for oocysts at 5 and 23°C

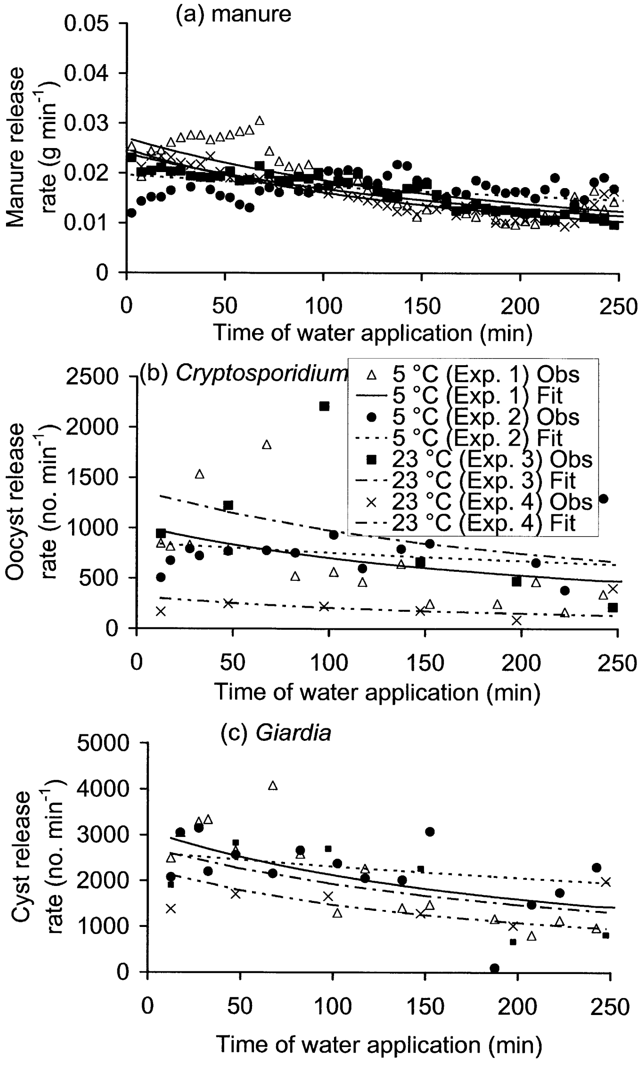

Fig. 2. Observed and simulated manure (a), oocyst (b), and cyst (c) release rates as a function of time at 5°C (Experiments 1 and 2) and 23°C (Experiments 3 and 4).

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exhibited more variability, especially for early times (Fig. 2b). The replicate oocyst release efficiencies for the 5°C systems had a common value of 0.53 according to the likelihood ratio test, whereas those for the 23°C systems were significantly different (0.18 and 0.81). These differences in the oocyst release efficiency possibly result from variability in manure texture among the manure disks (Table 2), rather than the effect of temperature. At longer time scales the role of temperatures will probably be more important due to microbial degradation of manure and (oo)cysts. Because of the similarity in parameter values of α and β between Experiments 3, 8, and 9 it was decided to use Experiment 3 as a reference experiment for comparison with the data from the other experiments.

Reproducibility

This section examines the reproducibility of manure and (oo)cyst release experiments. Two calf manure samples (Calf 1 and Calf 2) were employed (Table 1).

Figures 3a, 3b, and 3c present observed and simulated manure, oocyst, and cyst release rates, respectively, from Calf 1 (Experiment 1) and Calf 2 (Experiment 5) disks. The Calf 2 disk had a higher manure release rate (Fig. 3a) and a corresponding higher value of α (9.7×10^{-4}) (Table 3). The cumulative manure released from the Calf 2 disk (18%) was higher than the Calf 1 disk (12%) after 250 min. After 5000 min the cumulative manure released was much more similar (47 and 54%).

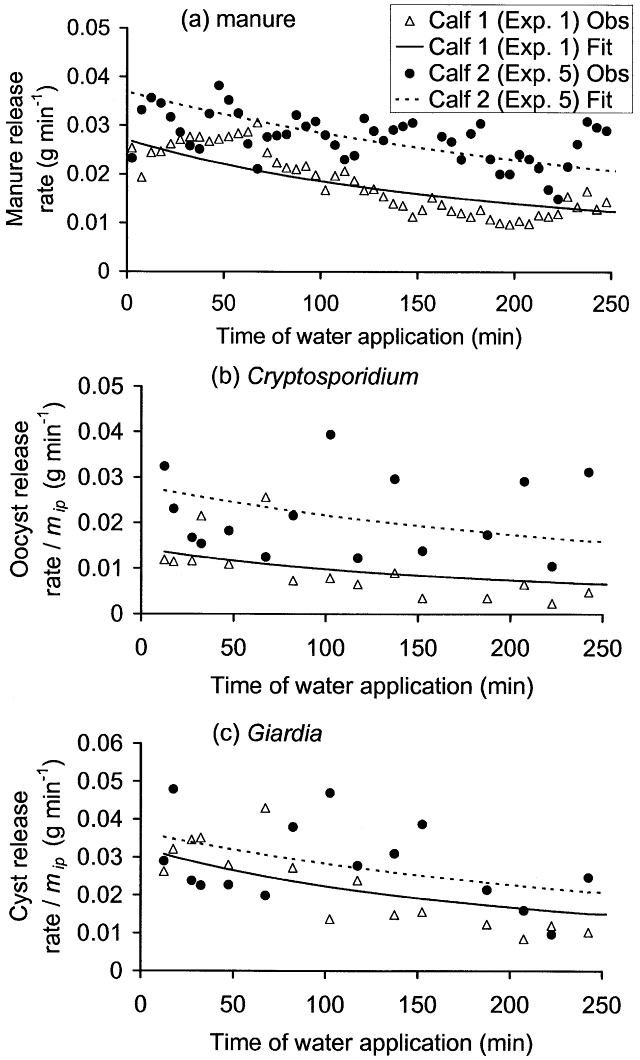

Fig. 3. Observed and simulated manure (a), normalized oocyst (b), and normalized cyst (c) release rates as a function of time for Calf 1 (Experiment 1) and Calf 2 (Experiment 5) manure.

The observed oocyst release rates from the Calf 2 disk were much higher than the Calf 1 disk due to the higher oocyst concentration in this manure (Table 2) and the higher value of C_m released into the aqueous phase. In contrast, the observed cyst release rates from the Calf 2 disk were lower than from the Calf 1 disk. This was due to the fact that the Calf 2 disk had a lower cyst concentration in the manure than the Calf 1 disk (Table 2). Because of the differences in m_{ip} of (oo)cysts between Calf 1 and Calf 2 manure, the (oo)cyst release rates were normalized by m_{ip} in Fig. 3b and 3c, thereby revealing a similar difference in release as the manure. The (oo)cyst release efficiencies were significantly different for Calf 1 and Calf 2 manure. Considering the difference in m_{ip} values (Table 2), however, the release efficiencies for a particular (oo)cyst for Calf 1 and Calf 2 manure were within 30% of each other (oocyst release efficiency [E_{Crypto}] equaled 0.53 and 0.76 for Calf 1 and Calf 2 manure, respectively; E_{GI} equaled 1.2 and 0.99 for Calf 1 and Calf 2 manure, respectively).

Manure Type

This section examines the influence of manure type on the release of manure and (oo)cysts. To encompass a range of manure management strategies, three combinations of Calf 1 and cow manure were considered in the experiments: Calf 1, a mixture of 50% Calf 1 and 50% cow manure, and a mixture of 10% Calf 1 and 90% cow manure. The manure mixtures were designated below by their cow manure fraction (f_{cow}). The experimental duration was 5017 min to provide insight into short- as well as long-term release behavior of manure and (oo)cysts.

Figures 4a, 4b, and 4c present observed and simulated manure, oocyst, and cyst release rates, respectively, for disks consisting of f_{cow} equal to 0 (Experiment 1), 0.5 (Experiment 6), and 0.9 (Experiment 7). There was a decrease in the initial manure release rate with increasing f_{cow} (Fig. 4a). The values of α were found to be similar for the various manure types, with values of α not significantly different when f_{cow} equaled 0 and 0.5 (Table 3). The value of β (Table 3) increased with increasing f_{cow} according to the following trend:

$$\beta = 8.8 f_{\mathrm{cow}} + 4.6;\ r^2 = 92\% \qquad [6]$$

When using the β value of Experiment 2 instead of that from Experiment 1, a steeper slope of 13, an intercept of 1.4, and a r^2 of 100% would be found. According to this analysis the increase of β with increasing f_{cow} is even stronger. Higher values of β reflect a faster initial rate of decrease in the manure release curve and therefore lower release rates for a particular α value and

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time. Due to the low number of observation points (three), Eq. [6] is likely to provide only a qualitative description of data for other systems.

The cumulative manure release (Table 5) also reflected the decrease in manure release rate with increasing f_{cow}. After 250 min, manure release was similar (12 and 11%, respectively) when f_{cow} equaled 0 and 0.5, but was lower (8%) when f_{cow} equaled 0.9. After 5000 min, the difference in cumulative manure release was even more apparent, with 47, 30, and 21% of the initial manure mass released when f_{cow} equaled 0, 0.5, and 0.9, respectively.

The above results indicated that manure release was strongly dependent on the manure composition. Cow manure was composed of larger particles of incompletely digested hay and grain materials that do not readily partition into the aqueous phase. In contrast, the calf manure was probably much finer and partitions relatively easily to the aqueous phase (Fig. 4a). This difference in manure composition is hypothesized to be due to differences in animal diet (e.g., the calves are still drinking milk). Manure texture also probably influenced water flow through the manure as well as the release behavior of manure.

Release efficiencies (E_{Crypto} for oocysts and $E_{Giardia}$ for cysts) appeared to increase with an increasing f_{cow} (Table 4), according to the following trends:

$$E_{Crypto} = 5.7f_{cow} - 0.21;\ r^2 = 76\% \qquad [7]$$

$$E_{Giardia} = 0.57f_{cow} + 1.2;\ r^2 = 93\% \qquad [8]$$

When using the release efficiencies of Experiment 2 instead of those from Experiment 1, similar trends are found. The cumulative number of oocysts released (Table 5) also reflected the increased release efficiency with an increased f_{cow}. After 250 min, 5.6, 8.3, and 35% of the oocysts were released from the disks when f_{cow} equaled 0, 0.5, and 0.9, respectively. After 5000 min, 19, 20, and 78% of the oocysts were released when f_{cow} equaled 0, 0.5, and 0.9, respectively. In contrast, $E_{Giardia}$ increased much less with an increase in f_{cow} than E_{Crypto}. The cumulative percentage of cysts released was quite similar for the various manure types at a particular time, reflecting the relative insensitivity of f_{cow} on $E_{Giardia}$. After 250 min, 13, 15, and 14% of the cysts were released when f_{cow} equaled 0, 0.5, and 0.9, respectively. After 5000 min, 27, 16, and 17% of the cysts were released when f_{cow} equaled 0, 0.5, and 0.9, respectively.

The finer calf manure particles were probably released more efficiently than the larger cow manure particles in the manure mixtures. This occurs as a result of differences in the size of the particles and the accessibility of the particles to flowing water, since water flows more easily through the larger textured cow manure. Increasing f_{cow} further accentuates the difference of smaller calf and larger cow manure particle release. Once the accessible manure surface area has been depleted of finer calf manure particles, then the manure release rates decreased to a relatively constant low value. This hypothesis was confirmed by the higher value of β with increasing f_{cow} (Table 3). The trend of increasing (oo)cyst release efficiency with increasing f_{cow} also supported this hypothesis (Table 4). For a given manure type, the higher release efficiency for the small oocysts (4–6 μm) compared with the larger cysts (8–12 μm) also suggested that finer manure particles were more readily released than larger particles (Table 4). The pronounced difference in cumulative oocyst and cyst release when f_{cow} equaled 0.9 may be due to the difference in the size of the protozoa (Table 5). The release of the larger cysts was apparently hindered by the presence of the cow manure matrix. The very high cumulative oocyst release values from the experiment with the excess of cow manure (f_{cow} equaled 0.9) suggested that finer particles in the calf manure were rapidly leached from the entire manure disk.

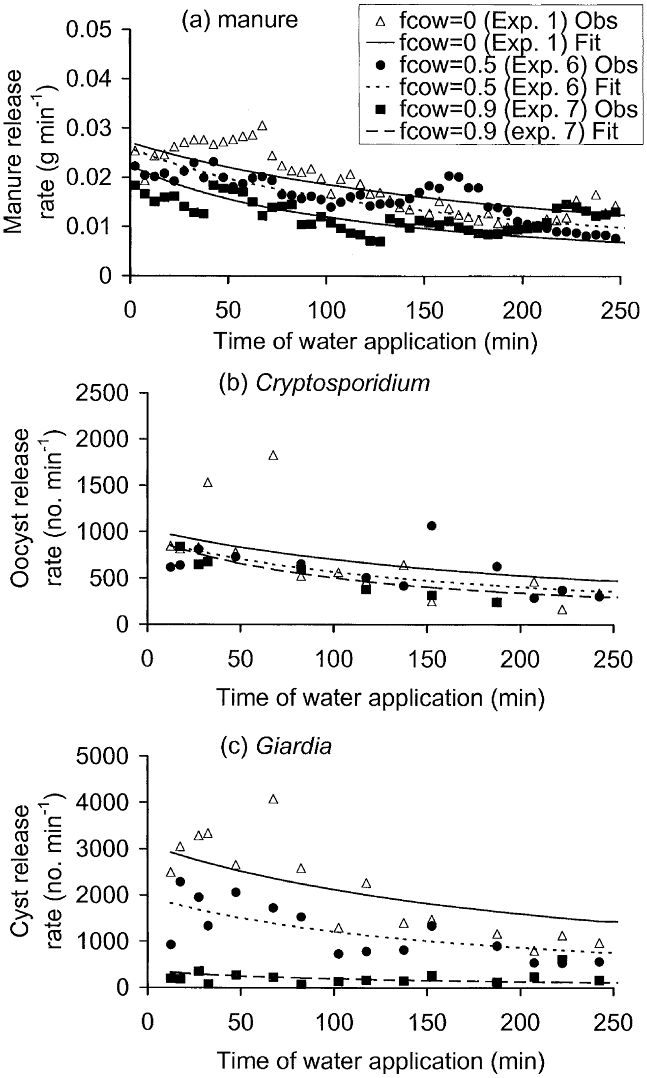

Fig. 4. Observed and simulated manure (a), oocyst (b), and cyst (c) release rates as a function of time for manure composed of various cow manure mass fractions (fraction of cow manure, f_{cow}, equals 0, 0.5, and 0.9 for Experiments 1, 6, and 7, respectively).

Water Application Method

This section examines the influence of water application (drip and mist) on release of manure and (oo)cysts.

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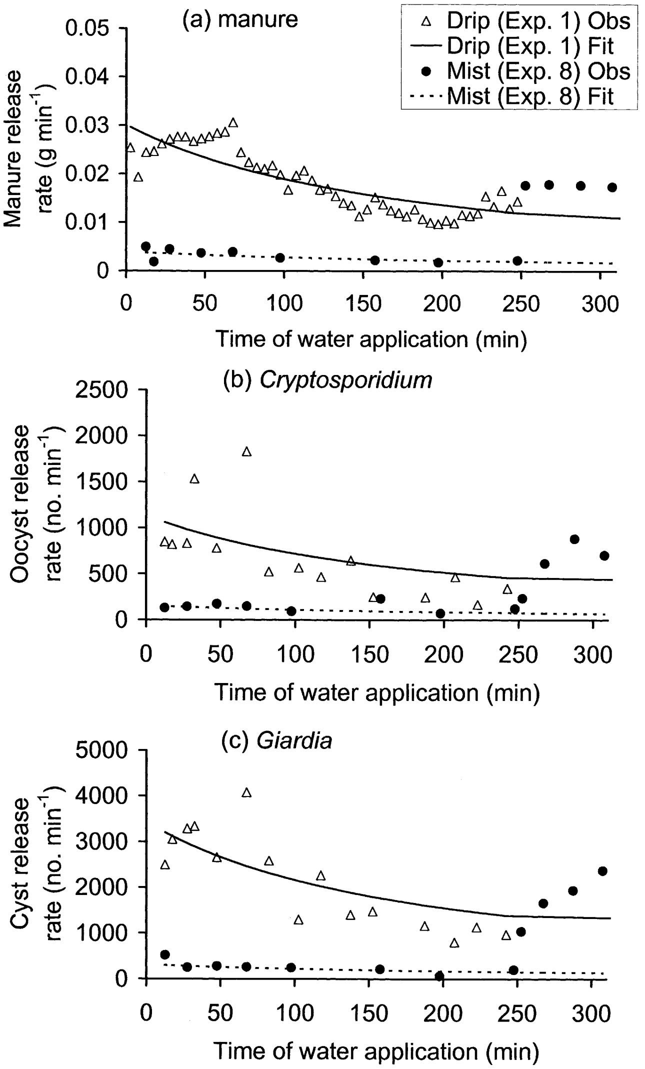

Fig. 5. Observed and simulated manure (a), oocyst (b), and cyst (c) release rates as a function of time for drip (Experiment 1) and mist (Experiment 8) experiments. After 250 min the mist application was stopped for 41 h and switched to drip application for 50 min.

Drip application was used to mimic a rain shower, whereas mist application was used as a surrogate for a mist or light drizzle. These application methods produced different physical (mechanical) forces on the manure disks. Water was applied over a smaller area of manure (greater intensity), and water drops were larger in size during drip application. Figures 5a, 5b, and 5c present observed manure, oocyst, and cyst release rates, respectively, for mist (Experiment 8) and drip (Experiment 1) experiments. Drip application resulted in a higher release rate (about seven times greater) for manure and (oo)cysts compared with mist application, due to the greater mechanical forces on the manure in this system. Mist application produced consistently lower release rates for manure and (oo)cysts with time than drip application, suggesting that the manure surface was not depleted of components during the experiment. The cumulative release of manure, oocysts, and cysts after 250 min of water application (see Table 5) was about seven, four, and nine times higher, respectively, for the drip than the mist system. To verify that differences in the release behavior were due to the water application method, the mist system (Experiment 8) was briefly (50 min) switched from mist to drip application after 250 min. As expected, the manure and (oo)cyst release rates increased to levels observed initially in the drip system (see Fig. 5).

Figures 5a, 5b, and 5c also present simulated manure, oocyst, and cyst release rates, respectively, for mist (Experiment 8) and drip (Experiment 1) experiments. The value of α (see Table 3) was about seven times higher for drip than mist application. The value of β (see Table 3) for mist (Experiment 8) was eight times higher than for the drip experiment. In all experiments, the release efficiency for cysts was higher than for oocysts (see Table 4). The value of E for cysts was also higher for drip than for mist systems (see Table 4).

When using the data from Experiment 2 instead of those from Experiment 1 similar conclusions could be drawn.

Water Application Rate

This section examines the influence of water application rate on release of manure and (oo)cysts. Water was applied as a mist at high (10.5 mL min^{-1}) and low (2.9 mL min^{-1}) flow rates to represent different rainfall intensities. The release experiments were conducted using the same manure type (Calf 1) and temperature (5°C). The water application intensity for the high and low flow rates was estimated to be 0.53 and 0.15 cm min^{-1}, respectively.

Figures 6a, 6b, and 6c present observed and simulated manure, oocyst, and cyst release rates, respectively, for high (Experiment 9) and low (Experiment 8) flow rates. Initial manure and (oo)cyst release rates were much higher for the higher flow rate system. The higher flow rate system resulted in a five-times-higher value of α (Table 3) and in a prolonged higher release rate, as reflected by an eight-times-lower value of β (Table 3). Cumulative release of manure, oocysts, and cysts after 250 min was about five, three, and five times higher at the higher mist application rate, respectively (Table 5).

The mist application rate did not appear to affect the (oo)cyst release efficiencies, and no significant differences between efficiency values were found in high and low flow rate systems (Table 4). According to the log likelihood ratio test, common values of the release efficiencies for high and low flow rate systems were found as 0.40 for oocysts and 0.83 for cysts.

CONCLUSIONS

Experiments were conducted to identify the magnitude and sensitivity of manure and (oo)cyst release behavior to key physical factors that occur on dairy farms. The release data were characterized using the model developed by Bradford and Schijven (2002). Data interpretation was aided by analyzing trends in the fitted model parameters and conducting statistical tests to determine significant differences among fitted parameters.

The effluent concentrations of manure and (oo)cysts

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were always several orders of magnitude below their concentration in the manure. The manure and (oo)cyst release rates decreased with time, due to depletion of manure components in the exposed surface area. The release model typically provided a reasonable description of the experimental data.

Differences in manure release behavior between replicate disks were larger than disks at different temperatures. Hence, manure and (oo)cyst release data indicated a temperature independence over the considered range of temperatures and time scales. Over longer time scales the role of temperatures will probably be more important due to microbial degradation of manure and (oo)cysts.

Manure composition experiments encompassed a range of manure types typical of various manure management strategies. The manure and (oo)cyst release rates tended to decrease and the (oo)cyst release efficiency increased with increasing cow manure fraction. These observations were attributed to differences in manure texture (cow manure is composed of larger particles than calf manure) and water permeability (water flows more readily through cow manure). Hence, management strategies that mix cow and calf manure probably promote more efficient release of the pathogens. In contrast, separation of cow and calf manure will minimize the release of pathogens from cow manure and produces less efficient (oo)cyst release from the calf manure. Reproducibility of (oo)cyst release rates from different manure samples was enhanced when the (oo)cyst release rates were normalized by the measured (oo)cyst concentration in the manure.

Manure and (oo)cyst release rates were higher when water was applied using the drip application method (instead of a mist) and for higher application rates. In both of these cases, applied water exerted greater mechanical forces on the manure disk due to increases in the drop size and/or the application intensity. Increasing the application intensity by 3.5 times resulted in about five-, three-, and five-times-higher cumulative release of manure, oocysts, and cysts, respectively, after 250 min. Increases in precipitation intensity on a farm are therefore expected to similarly increase manure and (oo)cyst release.

The release model may play a useful role in determining loading rates of pathogens from manure to the environment, but still many other variables such as precipitation intensity and amount, availability of manure surface, age of manure, and variability in m_{ip} values are needed. This model can also be helpful when testing various hypotheses about manure management and predicting strategies that will minimize pathogen release. Additional experiments are warranted to verify model predictions and further quantify key processes affecting pathogen release.

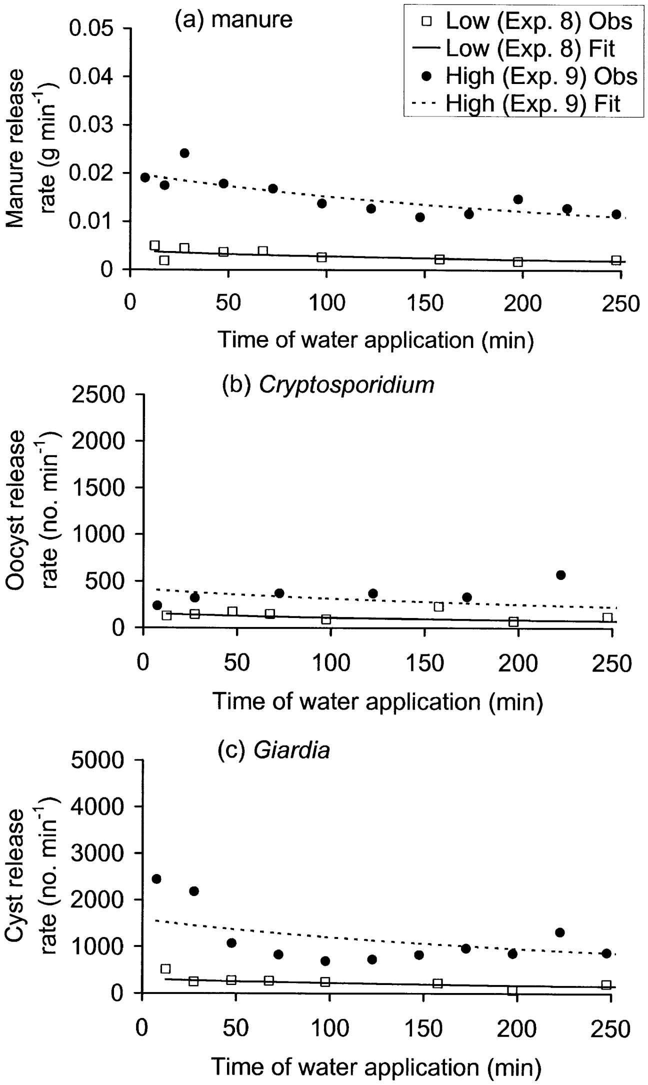

Fig. 6. Observed and simulated manure (a), oocyst (b), and cyst (c) release rates as a function of time for high (10.5 mL min^{-1}; Experiment 8) and low (2.9 mL min^{-1}; Experiment 9) flow rates.

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