Field scale quantification of microbial transport from bovine faeces under simulated rainfall events

Christobel M. Ferguson, Cheryl M. Davies, Christine Kaucner, Martin Krogh, Jörg Rodehutskors, Daniel A. Deere and Nicholas J. Ashbolt

ABSTRACT

The dispersion and transport of *Cryptosporidium parvum* oocysts, *Escherichia coli* and PRD1 bacteriophage seeded into artificial bovine faecal pats was studied during simulated rainfall events. Experimental soil plots were divided in two, one sub-plot with bare soil and the other with natural vegetation. Simulated rainfall events of 55 mm.h^{-1} for 30 min were then applied to the soil plots. Each experimental treatment was performed in duplicate and consisted of three sequential artificial rainfall events ('Runs'): a control run (no faecal pats); a fresh faecal pat run (fresh faecal pats); and an aged faecal pat run (one week aged faecal pats). Transportation efficiency increased with decreasing size of the microorganism studied; *Cryptosporidium* oocysts were the least mobile followed by *E. coli* and then PRD1 phage. Rainfall events mobilised 0.5 to 0.9% of the *Cryptosporidium* oocysts, 1.3–1.4% of *E. coli* bacteria, and 0.03–0.6% of PRD1 bacteriophages from the fresh faecal pats and transported them a distance of 10 m across the bare soil sub-plots. Subsequent rainfall events applied to aged faecal pats only mobilised 0.01–0.06% of the original *Cryptosporidium* oocyst load, between 0.04 and 15% of the *E. coli* load and 0.0006–0.06% of PRD1 bacteriophages, respectively.

Key words | buffer, catchment, *Cryptosporidium*, manure, pathogen, transport

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INTRODUCTION

Although previous studies have quantified catchment input concentrations of pathogens (Ong *et al.* 1996; Xiao *et al.* 2001; Jellison *et al.* 2002) few have quantified the relationship between pathogen inputs and the catchment processes that influence their fate and transport (Tate *et al.* 2000; Davies *et al.* 2004; Trask *et al.* 2004). Animal faecal deposits to land are a significant source of pathogenic protozoa in catchments (Olson *et al.* 1997; Atwill *et al.* 2003) and have been linked to rainfall event-related increases in pathogen concentrations in surface waters (Atherholt *et al.* 1998).

A review of catchment processes that control pathogen fate and transport identified the need to quantify pathogen transport processes at a range of scales, noting that most studies to date have relied on observations made from laboratory experiments (Ferguson *et al.* 2003). Laboratory experiments usually examined microbial transport using either repacked soils (Atwill *et al.* 2002) or intact soil columns or blocks (Mawdsley *et al.* 1996*a*; Davies *et al.* 2004; Trask *et al.* 2004). However, there is a need to verify that observations made at laboratory-scale can be extrapolated to field-scale with respect to the mobility and transport of a range of pathogens. The majority of microbial transport studies have examined either indicator bacteria or *Cryptosporidium* oocysts. Tate *et al.* (2000) studied *Cryptosporidium* oocyst transport at field scales and determined that slope was an important factor, with oocyst transport

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increasing significantly with slope. Slope is also significant as a predictor of *Escherichia coli* loads delivered to streams following rainfall events, as is time elapsed since livestock grazing (Collins *et al.* 2005).

The potential transport of enteric viruses in surface water runoff has received relatively little attention, although the migration of viruses through subsurface soil and into groundwater has been studied extensively (Jin *et al.* 2000; Schijven and Hassanizadeh 2002; Schijven *et al.* 2003). The main risks/sources of human enteric viruses in catchments are sewage discharges and septic seepage rather than animal faecal deposits (Charles *et al.* 2003), since most enteric viruses tend to be very host-specific. However, a previous study on the detection of animal enteroviruses in surface waters has highlighted the difficulty in distinguishing human and animal enterovirus contamination (Rothwell *et al.* 2004).

The objective of the current study was to quantify pathogen transport using bacterial, protozoan and viral index organisms seeded into bovine faecal pats, under simulated rainfall events, similar to those employed in the laboratory-scale experiments of Davies *et al.* (2004). The bacteriophage PRD1 was included in this study for two reasons: first, because it is similar in size (60–80 nm) and characteristics to many animal enteric viruses, particularly adenoviruses, and may therefore be an appropriate model to examine the transport of animal viruses from faecal deposits. Second, the transport of PRD1 phage is relevant as a model for surface transport of human viruses from septic seepage (Nicosia *et al.* 2001) with regard to general persistence (Gerba *et al.* 2003). The present study also examined the potential for the cumulative release of microorganisms using multiple artificial rainfall events. Collection of samples from the subsurface was not considered feasible in this field-scale experiment without severely affecting the physical characteristics of the plot. The current study therefore, examined microbial transport in surface water, ignoring infiltration processes.

MATERIALS AND METHODS

Location

Field experiments were conducted *in situ* at Arthursleigh Farm, Marulan, New South Wales, adjacent to the location where the intact soil blocks used in the study by Davies *et al.* (2004) were collected. Livestock had not grazed the location for at least 12 months but it had been accessible to wildlife, and care was taken to remove any faecal deposits on the surface of the soil prior to the experiments. The soil texture was a fine, sandy, clay loam (49% sand, 27% silt, 24% clay) (Davies *et al.* 2005), classified as a non-calcic brown soil (Great Soil Group) or chromosol (Australian classification system). The mean hydraulic conductivity (K_o) ($n = 5$) and the mean bulk density ($n = 10$) for soil at the study location were $15.0 \pm 2.1\,\mathrm{mm\,h^{-1}}$, and $1.032 \pm 0.11\,\mathrm{g\,cm^{-3}}$, respectively (Davies *et al.* 2004). The extent of vegetation cover on the surface of the plots was $67 \pm 8\%$ (Davies *et al.* 2004) and the predominant species (>70%) was the introduced grass *Phalaris aquatica* L. The grasses *Avena barabata* Pott. Ex Link. and *Carex inversa* R. Br., and an unidentified daisy species were also present, but at much lower densities (<10%). The smallest slope that could be found at this location was 18 degrees, which unfortunately is higher than the slopes of 10 and 5 degrees used in the previous study (Davies *et al.* 2004). However, other factors including the availability of water to perform rainfall simulations (during drought conditions), and the amenability of the owners to the use of microorganisms, dictated that the research station was the most suitable location for this study.

Preparation of field plots

Field experiments were performed using two separate plots prepared adjacent to each other. To enhance similarity with the previous laboratory-scale experiments of Davies *et al.* (2004), the plots were constructed adjacent to the location from which the laboratory-scale soil plots had been excavated. Each test plot was constructed by inserting metal borders directly into the soil to a depth of 10 cm. A central dividing panel was also inserted, creating two separate sub-plots each 1 m across and 10 m in length (downslope). Three submerged sample collection wells were constructed at distances of 2.5 m, 5 m and 7.5 m distances to enable collection of surface water runoff at different distances downslope on the sub-plots (sub-plot samples). Each well accommodated a 500 ml sterile sample container. Replicate samples at each distance allowed

assessment of the potential for preferential surface flow. At the base of each 10 m sub-plot was a submerged trapezoidal cross-section shaped metal channel (Replogle Bos Clemmens flume; Bos *et al.* 1993), suitable for measuring flow in open channels (Bos *et al.* 1993). One week before the start of each experiment the vegetation was removed from one sub-plot using a turf cutter (Ryan® Jr. Sod Cutter, Jacobsen, North Carolina). The loose soil on the surface of the bare sub-plot was lightly packed down using a turf roller to minimise the loss of loose surface soil. On the vegetated sub-plot the surface vegetation was made uniform by mowing the grass to a height of 2 cm and removing all loose clippings by hand. Any missed grass stalks were trimmed using scissors and removed. Each experimental treatment was performed in duplicate and consisted of three sequential artificial rainfall events (or 'Runs'): a control run (no faecal pats on plots, Runs 1 & 4); a fresh faecal pat run (fresh faecal pats on plots, Runs 2 & 5); and an 'aged' faecal pat run (one week old faecal pats on plots, Runs 3 & 6).

The rainfall simulator

A field-scale rainfall simulator capable of producing rainfall over an area of 19 m^2, was erected over the prepared soil plots (Phillips *et al.* 2002). Rigorous calibration determined that the mean artificial rainfall simulation event delivered uniformly across the plots was 55.1 ± 1 mm ($n = 6$) (Rodehutskors 2003). Water was pumped from the Wollondilly River and mixed with natural rainwater in a 1:2 ratio in a closed storage tank. Water from the storage tank was pumped into a 2,000-litre header tank through an in line filter that removed particles ≥ 5 μm (CUNO Pacific Pty Ltd, Blacktown, Australia). The header tank was connected *via* hoses to the rainfall simulator, and the intensity of rainfall delivery controlled by adjusting the pressure gauges for each stand. The water used for rainfall was disinfected with sodium hypochlorite (>1 ppm) for a minimum of 18 h and neutralised with an excess of sodium thiosulphate until no chlorine residual remained. Disinfection was verified by examining water from the header tank for the absence of *E. coli* for each run.

The rainfall intensity and duration of the simulated events were chosen from event average recurrence interval (ARI) curves (Sydney Catchment Authority), for a wide geographical spread of locations within the Sydney catchment, and represented a 1 in 1 year ARI for the majority of locations. These rainfall characteristics are relevant to conditions in previous (North American) rainfall simulation studies (Thelin & Gifford 1983; Trask *et al.* 2001; Atwill *et al.* 2002), and identical to those employed by Davies *et al.* (2004).

Experimental design

The experiment consisted of a series of artificial rainfall events, each referred to as a 'Run' and the main effects treatments were examined sequentially (control, fresh and aged faecal pats) with a 1-week break between. Sub-plot samples (500 ml) were collected at three distances (2.5 m, 5 m and 7.5 m) every 6 min over the duration of the rainfall event (30 min). Flume samples were collected at the 10 m distance every minute, and for 5 min following cessation of the event. Due to low runoff volumes (particularly on the vegetated plot) composites of some sub-plot and flume samples were made before measuring microbial concentrations (see Tables 1 and 2). The logistics and resources required to undertake such a large field-scale experiment were the main reasons influencing the timing and sequence of these treatments.

Preparation and characterisation of artificial faecal pats

Cow faeces was collected in bulk (approximately 11 kg) from a dairy farm (Corstorphine Dairy, Camden, NSW, Australia) and sterilised by gamma-irradiation at 90 kGy using the ^{60}Co source (Davies *et al.* 2004). For each experiment a batch of five artificial bovine faecal pats were prepared by inoculating and mixing the seed micro-organisms into 1 kg portions of sterilised cow faeces (200 mm diameter, 30 mm deep) prepared in spring-sided baking trays. The size and weight of the faecal pats were based on the reported average wet weight of 23 kg of faeces excreted by cattle (Dorner *et al.* 2004) and an estimated frequency of 12 excretion events per animal per day (Thelin & Gifford 1983; Larsen *et al.* 1994). Since Larsen *et al.* (1994) noted that cattle defecated several deposits per event, it was estimated that two faecal pats of 1 kg wet weight might be deposited per event. This is in close agreement

Table 1 | Mean concentrations and SNK groupings for *Cryptosporidium, E. coli* and PRD1 bacteriophage for sub-plot samples (microbial transport in surface runoff from bare and vegetated sub-plots after simulated rainfall of 55 mm.h^{-1} for 30 min)

			Cryptosporidium parvum **(log_{10} oocysts.l^{-1})**				***E. coli*** **(log_{10} mpn.ml^{-1})**				**PRD1 (log_{10} pfu.ml^{-1})**			
			Bare sub-plots		**Vegetated sub-plots**		**Bare sub-plots**		**Vegetated sub-plots**		**Bare sub-plots**		**Vegetated sub-plots**	
	n (bare sub-plots)	**n (vegetated sub-plots)**	**Mean**	**SNK**	**Mean**	**SNK**	**Mean**	**SNK**	**Mean**	**SNK**	**Mean**	**SNK**	**Mean**	**SNK**
Age														
Fresh	30	18	3.20	A	1.09	A	3.84	A	0.98	A	1.85	A	0.23	A
Aged	30	18	1.80	B	0.88	A	3.09	B	0.71	A	1.15	B	0.08	A
Time														
0–6	12	–	1.81	A	–	–	2.30	A	–	–	0.49	A	–	–
6–12	12	–	2.69	B	–	–	3.81	B	–	–	1.57	B	–	–
12–18	12	12	2.80	B	0.83	A	3.80	B	0.54	A	1.96	B	0.08	A
18–24	12	12	2.73	B	0.60	A	3.71	B	0.90	A	1.60	B	0.11	A
24–30	12	12	2.48	B	1.52	B	3.72	B	1.08	A	2.01	B	0.27	A
Distance														
2.5 m	20	12	3.04	A	1.34	A	3.85	A	1.12	A	1.69	A	0.35	A
5.0 m	20	12	2.46	B	1.11	AB	3.56	A	0.85	A	1.45	A	0.08	A
7.5 m	20	12	2.00	C	0.51	B	2.99	A	0.55	A	1.37	A	0.06	A

– = not tested.
n = number of samples included in the analysis.
mpn = most probable number.
pfu = plaque forming units.
SNK = Student Newman Keuls test; values with different letters are significantly different at the $p < 0.05$ level.

with the average wet weight per deposit of 920 g reported by Davies-Colley *et al.* (2002). After preparation, all artificial faecal pats were placed in an incubator at 20°C for 6 h.

Faecal pats were transported to the field site and stored overnight at ambient temperature (ranging from 5 to 20°C). Immediately prior to each fresh faecal pat treatment, two faecal pats were placed 10 cm from the top edge of each of the bare and vegetated sub-plots. The fifth faecal pat was stored at 4°C and returned to the laboratory where analysed for initial (time zero) concentrations of each microorganism. Triplicate 5 to 10 g portions were analysed for moisture content (Davies *et al.* 2004). Ambient and faecal pat temperatures were recorded at 15-minute intervals for the duration of the experiments using iButtons™ (Maxim/Dallas Semiconductor Corp., Dallas, Texas). To quantify changes in aged faecal pats during ambient field-storage, two random 1 g samples were removed from each aged faecal pat immediately prior to the aged faecal pat rainfall simulations (Runs 3 and 6). Each replicate was analysed for moisture content, *Cryptosporidium, E. coli* and PRD1 phage concentrations.

Table 2 | Mean concentrations and SNK groupings for *Cryptosporidium, E. coli* and PRD1 bacteriophage for flume samples (microbial transport in surface runoff at 10 m distance from bare and vegetated sub-plots after simulated rainfall of 55 mm.h^{-1} for 30 min)

			Cryptosporidium parvum **(log_{10} oocysts.l^{-1})**					***E. coli*** **(log_{10} mpn.ml^{-1})**			**PRD1 (log_{10} pfu.ml^{-1})**	
			Bare sub-plots		**Vegetated sub-plots**			**Bare sub-plots**			**Bare sub-plots**	
	n (bare sub-plots)	**n (vegetated sub-plots)**	**Mean**	**SNK**	**Mean**	**SNK**	**n (bare sub-plots)**	**Mean**	**SNK**	**n (bare sub-plots)**	**Mean**	**SNK**
Age												
Control	10	–	0.20	B	–	–	14	1.13	A	–	–	
Fresh	12	6	1.53	A	0.39	A	21	3.26	B	12	1.77	A
Aged	11	2	0.36	B	0.72	A	22	2.95	B	9	0.86	B
Time												
4–5	5	–	0.62	A	–	–	6	1.75	A	3	1.34	A
7–8	–	–	–	–	–	–	5	2.77	A	–	–	–
9–10	6	–	1.00	A	–	–	6	2.80	A	4	1.54	A
12–13	–	–	–	–	–	–	5	2.82	A	–	–	–
12–18	–	2	–	–	0.00	A	–	–	–	–	–	–
14–15	6	–	0.65	A	–	–	6	2.60	A	3	1.57	A
17–18	–	–	–	–	–	–	5	2.59	A	–	–	–
18–24	–	3	–	–	0.77	A	–	–	–	–	–	–
19–20	6	–	0.69	A	–	–	6	2.66	A	4	1.42	A
22–23	–	–	–	–	–	–	4	2.48	A	–	–	–
24–25	5	–	0.85	A	–	–	5	2.92	A	3	1.31	A
24–30	–	3	–	–	0.50	A	–	–	–	–	–	–
27–28	–	–	–	–	–	–	4	2.97	A	–	–	–
29–30	5	–	0.61	A	–	–	5	2.65	A	4	1.11	A

– = not tested.
n = number of samples included in the analysis.
mpn = most probable number.
pfu = plaque forming units.
SNK = Student Newman Keuls test; values with different letters are significantly different at the $p < 0.05$ level.

Preparation of inocula

The fresh (Runs 2 and 5) and aged (Runs 3 and 6) faecal pat treatments required fresh artificial faecal pats to be inoculated with *Cryptosporidium* oocysts, *E. coli* and PRD1 bacteriophages. The *Cryptosporidium parvum* oocysts were purified from defatted fresh calf faeces by density gradient flotation in sucrose solution (Upton 1997). The stock oocyst suspension was sterilised by gamma-irradiation at 90 kGy using a ^{60}Co source (Steritech Pty Ltd,

Wetherill Park, NSW, Australia). This dosage was expected to effectively inactivate any *Cryptosporidium* oocysts that may have been present (Yu & Park 2003). Oocyst stock suspension concentrations were determined by diluting and staining on membranes (Davies *et al.* 2003). The irradiated suspension (1 ml) containing approximately 8×10^7 oocysts was used to inoculate each faecal pat.

An overnight culture of *E. coli* (isolated from bovine faeces) was grown up in 50 ml of Tryptone Yeast Extract Glucose broth (Oxoid, Australia; 50 ml) centrifuged at 2,500 *g* for 10 min and the pellet resuspended in 50 ml of sterile ultra pure water. One millilitre of this suspension was used to inoculate each faecal pat with approximately $10^9 - 10^{10}$ organisms. A phage stock suspension was prepared using an infected *Salmonella typhimurium* LT2 host culture (Adams 1959). The suspension was left overnight and approximately 3.6×10^9 PRD1 virions were collected per ml from the supernatant after centrifugation (2,500 *g*). The stock suspension (1 ml) was used to inoculate each faecal pat.

Simulated rainfall experiments

Each experimental run was performed early in the day (approximately 7 a.m.) to avoid the effect of convective wind (characteristic of daylight hours), which may blow rainfall away from the plot. All runoff that reached the 10 m distance and exited the flume was collected in 10 l plastic bladders (EB407; Entapak Pty Ltd, Dandenong, Victoria, Australia). The flow rate was measured every minute by reading the silting well. At the end of each run, the rainfall gauges were checked to ensure that the rainfall event was of the required intensity and had fallen evenly over the plot. The faecal pats were left undisturbed on the plot until the following week when the next run (aged faecal pat treatment) was undertaken.

The nearest meteorological rainfall gauge (approximately 5 km distant) was used to monitor natural rainfall throughout the six-week experimental period. The maximum rainfall recorded on an experimental day was 3.5 mm (recorded after the experiment on the day of Run 1). The maximum rainfall recorded on any day was 7.5 mm, 2 days before Run 5. Most days recorded 0 mm natural rainfall.

Sample analysis

Sub-plot samples (500 ml) were stored in ice-chests during transit to the laboratories (< 3 h). Large volume flume samples were stored in large, opaque plastic containers at ambient temperature during transport (< 3 h). At the laboratory, all samples were refrigerated at 4°C until analysis. Where sample volume was sufficient, samples were analysed individually.

Cryptosporidium oocysts in surface runoff samples were concentrated by sequential centrifugation at 2,500 *g* for 10 min to a maximum packed pellet of 1 ml, with the exception of two samples of large volume from the vegetated sub-plot that were concentrated using Hemoflow hollow fibre ultrafiltration cartridges (HF80S; Fresenius Medical Care AG, Bad Homburg, Germany) (Simmons *et al.* 2001). The packed pellets were processed using immunomagnetic separation and immunofluorescence (2004). Faecal samples were processed and analysed for *Cryptosporidium* using the method reported by Davies *et al.* (2003) using 100 ColorSeed™ *Cryptosporidium parvum* oocysts (BTF Decisive Microbiology, North Ryde, NSW, Australia) to determine recovery. Since enumeration of *Cryptosporidium* oocysts in raw water samples is known to be more variable than for treated or 'finished waters' (Ferguson *et al.* 2004), turbidity measurements were used as a rough index to group water samples. ColorSeed™ oocysts were spiked into 10% of samples, ensuring that each group with similar turbidity contained at least one spiked sample. Recovery efficiency was calculated as (NC/NI * 100) where NC was the number of ColorSeed™ oocysts counted in the sample and NI was the number of ColorSeed™ inoculated into the sample.

To desorb *E. coli* and phage from faecal particles, faecal samples were diluted by placing 1 g in 20 ml of sterile 0.002 M sodium pyrophosphate. Sample suspensions were mixed on a vortex mixer for 2 min and allowed to stand for 30 min at room temperature. After further mixing on a vortex for 15 s, the suspensions were allowed to settle for 10 min at room temperature and 2 ml of the supernatant withdrawn from 2 mm below the surface. This aliquot was serially diluted and assayed for *E. coli* and PRD1 phage using the Quantitray™ format of Colilert®-18 (IDEXX, USA), and the double agar layer technique (Adams 1959)

with the *Salmonella typhimurium* LT2 (ATCC 19585) host, respectively. Water matrices were serially diluted if required and then assayed as described above. Bacteria and phage analyses were carried out within 24 h of sample collection.

Data analysis

Statistical analysis was performed using analysis of variance (ANOVA) and analysis of covariance (ANCOVA) using the SAS Generalised Linear Model (GLM) procedure (Version 8.1, SAS Institute Inc., Cary, North Carolina). The Student-Newman-Keuls Test (SNK) was used to test for significant differences between means at the $\alpha = 0.05$ level. Factors (treatment, time, distance and bare *versus* vegetated) were assumed to be fixed, and significance was determined using a Type 1 error rate of $\alpha = 0.05$. Residuals were examined to assess normality and homogeneity. Microbial concentrations that were less than the detection limit were assigned the value of half of the detection limit. *Cryptosporidium* oocysts counts were adjusted for recovery efficiency calculated as $(100/R * C)$ where R was the percentage recovery efficiency and C was the number of oocysts counted in the sample. Statistical analysis was performed separately for sub-plot samples and flume samples.

For sub-plot samples only the fresh (2 and 5) and aged (3 and 6) runs could be compared statistically because of differences in the way in which samples were chosen and pooled together for analysis during the control runs (1 and 4). *Cryptosporidium* oocysts, *E. coli* and PRD1 bacteriophage concentrations were log-transformed ($Log_{10}(X + 1)$) and then analysed using either a two or three factor ANOVA with factors time, faecal pat age and distance (sub-plot samples); or time and faecal pat age (flume samples). When examining the effect of distance, the means of the three sub-plot samples collected at each distance for each treatment (bare vs. vegetated) were calculated and used in all subsequent analyses. ANCOVA was also used with the same factors, but included runoff volumes as the covariate to determine whether differences in runoff volumes affected the decision on significance of factors.

RESULTS

Rainfall simulation

E. coli results for tank water tested from each run were all < 0.01 mpn.100 ml^{-1} indicating the effectiveness of the water disinfection procedure. For all rainfall events there was considerably more surface runoff produced from the bare sub-plots compared with the vegetated sub-plots, presumably due to greater infiltration and/or interception on the vegetated sub-plots. The reduced volume of surface runoff from the vegetated sub-plots necessitated sample compositing to provide sufficient volume for analysis, and consequently limiting the number of statistical comparisons between bare and vegetated sub-plots. On the bare sub-plots, average flume runoff volumes were significantly higher ($p < 0.0001$) on the aged faecal pat runs (8,995 ml.min^{-1}, $n = 60$) compared with the control (4,408 ml.min^{-1}, $n = 57$) and fresh faecal pat runs (5,165 ml.min^{-1}, $n = 60$). This was not surprising since rainfall events were performed sequentially (Runs 1 to 3 and Runs 4 to 6) but it indicates that allowance needs to be made for differing runoff volumes when attempting statistical comparison of experimental treatments.

Characterisation of artificial faecal pats

For the first experiment the arithmetic mean concentrations of *Cryptosporidium* oocysts, *E. coli* and PRD1 phage in each artificial faecal pat at time zero were 6.4 ± 5.7 log_{10} oocysts.kg^{-1}, 10.1 ± 9.2 log_{10} mpn.$kg^{-1}$and 8.9 ± 8.3 log_{10} pfu.kg^{-1}, respectively. For the second experiment the arithmetic mean concentrations at time zero were 6.5 ± 6.0 log_{10} oocysts.kg^{-1}, 9.7 ± 9.5 log_{10} mpn.$kg^{-1}$and 9.2 ± 8.6 log_{10} pfu.kg^{-1} (all n = 3), respectively. The study was carried out during early winter, and ibutton™ measurements indicated that, although ambient temperatures ranged from -2°C to 25°C, temperatures within the faecal pat only varied from 1°C to 14°C (Davies *et al.* 2005). The moisture content of fresh faecal pats was 90% and after one week at ambient temperature decreased to between 65% and 85%, which is similar to the values reported by Wang *et al.* (2004). The variation in moisture content of aged faecal pats is likely to be related to variations in air temperature, solar radiation and relative humidity during the experiments.

Cryptosporidium oocyst concentrations remained unchanged after one-week field-storage at ambient temperature while PRD1 bacteriophage concentrations in the faecal pats decreased during the one-week of field-storage by 2 to 3 $\log_{10}$ pfu.kg^{-1} (dry weight) (data not shown). Six of the eight faecal samples analysed for *E. coli* concentrations showed decreases of 1 to 2 $\log_{10}$ cfu.kg^{-1} (dry weight). However, two samples from the repeat experiment (one each from the bare and vegetated sub-plots) showed an increase in *E. coli* concentrations of approximately 1 $\log_{10}$. Wang *et al.* (2004) reported that concentrations of bacteria in cow faeces could increase in the first 3 days following excretion (Wang *et al.* 2004). However, since the bacteria in this study were spiked, the observed increases were likely to be due to heterogeneity within the faecal pats.

Cryptosporidium oocysts

The mean recovery efficiency of *Cryptosporidium* oocysts was 55.3% ± 21.3 ($n = 54$) for water samples, and 40.0% ± 16 ($n = 10$) for faecal samples (Davies *et al.* 2003), which is comparable to those reported in other studies examining similar matrices (McCuin *et al.* 2001; Atwill *et al.* 2003). Table 1 shows the adjusted mean *Cryptosporidium* concentrations in surface runoff collected from the sub-plot samples. Adjusted mean concentrations in surface runoff from fresh faecal pats were higher than from the aged faecal pats on the bare sub-plots ($p = <0.0001$) but not on the vegetated sub-plots. On both bare and vegetated sub-plots *Cryptosporidium* concentrations in surface runoff increased over time during the rainfall simulations. The effect of distance was significant on both bare and vegetated sub-plots ($p = 0.0002$, $p = 0.035$, respectively). Mean *Cryptosporidium* concentrations in the surface runoff from the bare sub-plots decreased from 3.0 $\log_{10}$ at the 2.5 m distance to 2.0 $\log_{10}$ at the 7.5 m distance. On the vegetated sub-plots the mean concentrations decreased from 1.3 $\log_{10}$ to 0.5 $\log_{10}$ over the same distance. Thus once mobilised the decrease in concentration was similar on both sub-plots.

On the vegetated sub-plots, the mean *Cryptosporidium* oocyst concentrations at the 2.5 m distance were less than half the mean concentrations on the bare sub-plot, indicating that initial release was much lower than on the bare sub-plots. Table 2 shows the mean $\log_{10}$ *Cryptosporidium* oocyst concentrations in surface runoff from the bare and vegetated sub-plots collected in the 10 m flume samples. Significant differences over time in *Cryptosporidium* oocyst concentrations were identified on the bare soil sub-plots at the 10 m distance. However, because there were fewer analyses for vegetated sub-plots no significant effects (among times or among treatment levels of control/fresh/aged) were detected. For bare soil sub-plots the flume samples from the fresh faecal pat runs had higher mean concentrations of oocysts compared with the control and aged faecal pat runs ($p = 0.0001$).

Figure 1 shows the cumulative adjusted total load of *Cryptosporidium* oocysts exported from the bare sub-plots for both experiments. The total export from the control runs

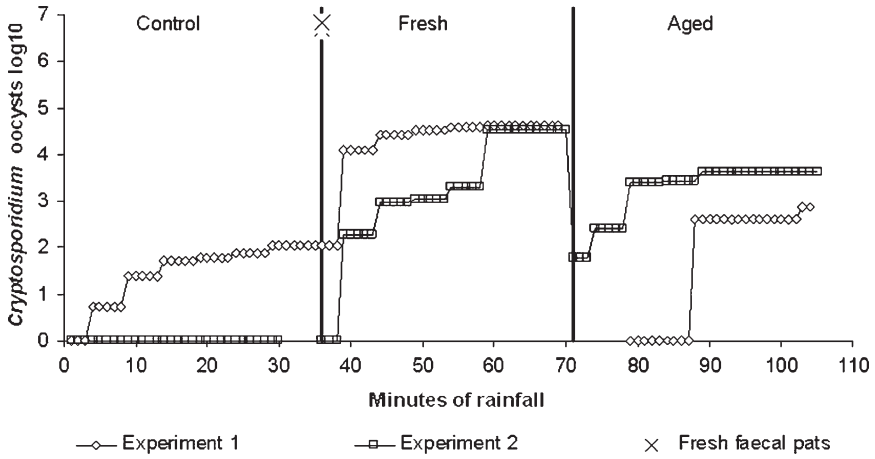

Figure 1 | Cumulative loads of *Cryptosporidium* oocysts exported from 10 m bare soil sub-plots for experiments 1 and 2. Samples collected at one minute intervals; control run = samples 1–35, fresh run = samples 36–70, aged run = samples 71–105.

(1 to 35 min of rainfall) were 2 $\log_{10}$ and <0 $\log_{10}$ oocysts, respectively. The cumulative loads for the fresh runs (36 to 70 min of rainfall) were 4.6 and 4.5 $\log_{10}$ oocysts, respectively. The loads from the aged runs (71 to 105 min of rainfall) were one to two orders of magnitude lower (2.9 and 3.6 $\log_{10}$ oocysts, respectively). The net transport of *Cryptosporidium* oocysts from the two experiments was reasonably consistent. The proportion of material being exported in the fresh runs was 0.5–0.9% of the total load of oocysts deposited onto the bare sub-plots in the faecal pats. The subsequent aged runs released a much lower proportion (0.01–0.06%).

E. coli

Table 1 shows the mean $\log_{10}$ *E. coli* concentrations for the bare and vegetated sub-plot samples. The mean *E. coli* concentrations from the bare sub-plots was significantly higher for the fresh compared with the aged runs ($p = 0.029$). On the bare sub-plots there was also significant variation in *E. coli* concentrations over time during the rainfall event; samples collected during the first time interval (0 to 6 min) had significantly lower concentrations of *E. coli* than any of the other time intervals in the rainfall simulation ($p = 0.026$). Although mean *E. coli* concentrations decreased slightly with increasing distance from the faecal pat, the differences were not significant, indicating that *E. coli* were easily mobilised across the surface of the bare sub-plot. Mean *E. coli* concentrations from the vegetated sub-plots (0.98 and 0.71 $\log_{10}$ mpn.ml^{-1} for fresh and aged pats, respectively) were considerably lower than from the bare sub-plots (3.84 and 3.09 $\log_{10}$ mpn.ml^{-1}, respectively). Table 2 shows that the mean *E. coli* concentrations in flume samples from the bare sub-plots were significantly higher for both the fresh and aged runs compared with the control run ($p = 0.0002$). There was also no significant trend in *E. coli* concentrations over time during the rainfall event.

Figure 2 shows the cumulative total loads of *E. coli* exported from the bare sub-plots for both experiments. The total export from the control runs (1 to 35 min of rainfall) were 6.7 and 5.0 $\log_{10}$, respectively. The cumulative load for the fresh runs (36 to 70 min of rainfall) was 8.5 and 8.2 $\log_{10}$, respectively, two to three orders of magnitude higher than the control runs. In experiments 1 and 2 the proportion of *E. coli* load exported from the fresh faecal pats across the bare soil sub-plots was 1.3 and 1.4% of the initial load deposited. The proportion of the initial load exported from the aged runs (71 to 105 min of rainfall) was 0.04 and 15.3%, respectively.

PRD1 bacteriophage

The mean PRD1 phage concentrations in the surface runoff produced from the bare sub-plots were significantly higher for the fresh compared with the aged runs ($p = 0.001$). There was also significant variation in PRD1 phage concentrations over time during the rainfall event; samples

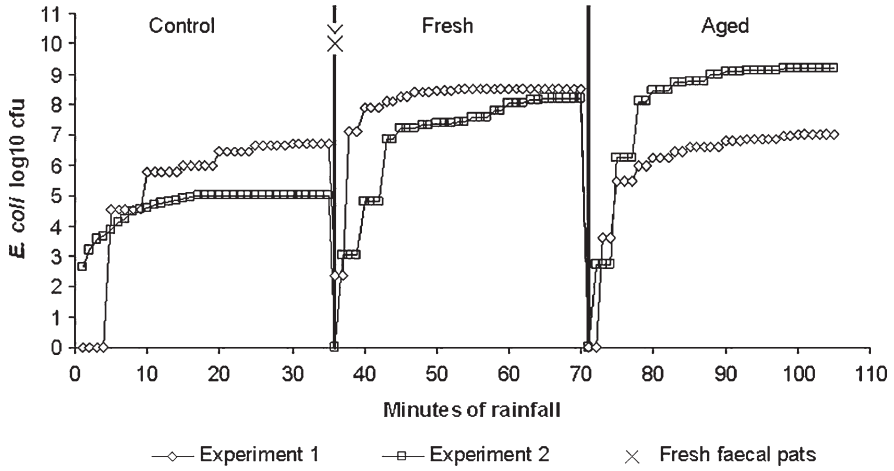

Figure 2 | Cumulative loads of *E. coli* exported from 10 m bare soil sub-plots for experiments 1 and 2. Samples collected at one minute intervals; control run = samples 1–35, fresh run = samples 36–70, aged run = samples 71–105.

collected during the first time period (0 to 6 min) had much lower concentrations of PRD1 phage than later samples ($p = 0.009$). Although PRD1 phage concentrations appeared to decrease slightly with increasing distance down the bare sub-plots the decrease was not statistically significant, indicating that PRD1 was highly mobile in the surface runoff (Table 1). On the vegetated sub-plots the mean PRD1 phage concentrations in the surface runoff were not significantly different between the fresh and aged runs ($p = 0.262$) nor were there any significant differences over time or with distance. However, the mean concentrations from the vegetated sub-plots were considerably lower than from the bare soil sub-plots. Table 2 shows the mean $\log_{10}$ PRD1 phage concentrations for flume runoff collected from the bare sub-plots indicating that concentrations from the fresh runs were significantly higher than from the aged runs ($p = 0.049$). As with *Cryptosporidium* and *E. coli* there was no significant trend in PRD1 phage concentrations over time during a rainfall event.

Figure 3 shows the cumulative total loads of PRD1 phage exported from the bare sub-plots for experiments 1 and 2. The total exports from the control runs (1 to 35 min of rainfall) were 3.4 $\log_{10}$ and <0 $\log_{10}$, respectively. The cumulative loads for both fresh runs (36 to 70 min of rainfall) were 6.9 and 6.0 $\log_{10}$, respectively. The loads from the aged faecal pat runs (71 to 105 min of rainfall) were 6 and 4.3 $\log_{10}$ respectively. The fraction of material exported from the fresh faecal pats ranged from 0.03 to 0.6% of the total PRD1 phage deposited onto the sub-plots. However the load exported one-week later from the aged faecal pats were much lower and more variable ranging from 0.0006 to 0.06% of the deposited PRD1 phage.

DISCUSSION

Comparatively few studies have attempted to quantify pathogen transport in surface runoff (Mawdsley *et al.* 1996*b*; Tate *et al.* 2000; Atwill *et al.* 2002; Trask *et al.* 2004). Davies *et al.* (2004) examined the release of *Cryptosporidium* oocysts from faecal pats and their transport through and over intact soil blocks under different simulated catchment characteristics of slope, vegetation status and rainfall event intensity/duration. In laboratory (Davies *et al.* 2004) and field (current study) experiments, the volumes of surface runoff from the vegetated sub-plots were much lower than from the bare sub-plots. However, the extent of runoff reduction due to the presence of vegetation in this study compared with the laboratory-scale study was much higher than anticipated, making statistical comparisons difficult due to the reduced volumes of sample available for analysis. This highlights the important role of vegetation cover as a mechanism for increasing infiltration and retarding pathogen transport.

Other effects of vegetation related to microbial transport are probably not yet fully understood. For example, in this study, visual observations indicated that faecal pat dispersion

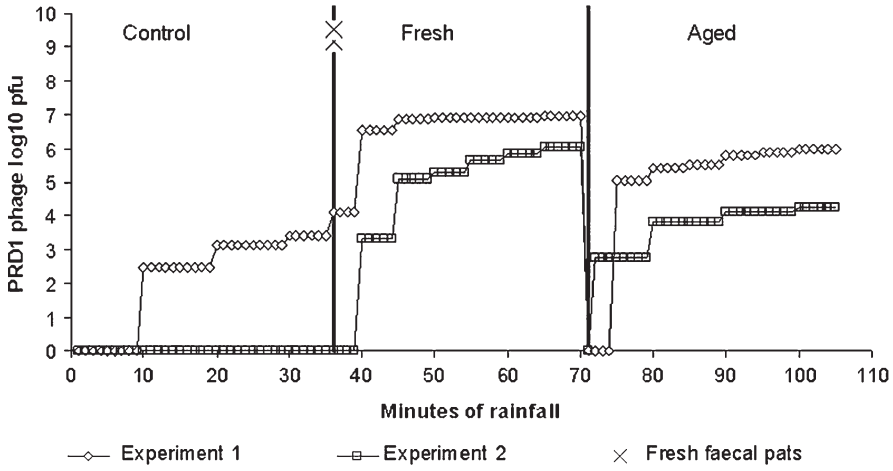

Figure 3 | Cumulative loads of PRD1 phage exported from 10 m bare soil sub-plots for experiments 1 and 2. Samples collected at one minute intervals; control run = samples 1–35, fresh run = samples 36–70, aged run = samples 71–105.

was much more rapid on the bare sub-plots compared with the vegetated sub-plots. Concentrations of each microorganism on the grassed sub-plots at the 2.5 m distance were less than half that of the bare soil sub-plots suggesting that vegetation may also affect the initial rate of release of microorganisms. Although Bradford & Schijven (2002) have investigated some of the factors controlling the initial release of microorganisms from faecal pats, particularly salinity, our results indicate that further investigation of release mechanisms and specifically the role of vegetation are warranted.

On both bare and vegetated sub-plots, *Cryptosporidium* was the only organism that showed significant reductions in mean concentrations with increasing distance from the faecal pats. Both *E. coli* and PRD1 phage were easily mobilised in the surface runoff and showed no significant decrease in concentration downslope from the faecal pats on either bare or vegetated sub-plots. These results are consistent with field experiments by Bales *et al.* (1995), which showed that the bacteriophage PRD1 could travel considerable distances (12 m) over prolonged periods of time (25 d) without losing infectivity. Although PRD1 phage was highly mobile, the concentration of PRD1 phages in runoff from the vegetated sub-plots was at least one log lower than the concentrations on the bare sub-plots. This is probably due to the reduction in actual runoff volume, rather than a decrease in the mobilisation rate, since it is clear that the viruses move easily. These results have major implications for the effective use of vegetated riparian buffer zones as a means of reducing pathogen transport in surface waters. The results suggest that once mobilised *E. coli* and PRD1 phage are only weakly attenuated by contact with soil and vegetation. Since many studies only examine bacterial and protozoan transport, it is likely that these studies will underestimate the potential for the transport of enteric viruses to surface waters. Situations where human enteric viruses may be transported in surface runoff include failing septic or on-site systems or surface disposal of effluent (e.g. spray irrigation).

Although it is well known that buffer strips play an important role in the reduction of pollutant transport to waterways (Olley & Deere 2003), this study demonstrates the considerable differences in surface flow and pathogen transport over unvegetated areas. This is particularly relevant to the current drought conditions being experienced in Australia, where much of the vegetation has either died or been heavily grazed. The remaining vegetation subsequently increases in its importance as a contaminant buffer. When loss of vegetation is complete the contaminant/pathogen buffering capacity is largely lost and runoff increases considerably.

CONCLUSIONS

Both *E. coli* and PRD1 phage may be transported considerable distances in surface water runoff. This has significant implications for the use of vegetated buffer strips as a means of reducing bacterial and viral transport to waterways. In contrast, the mobilisation of *Cryptosporidium* oocysts was significantly reduced with increasing distance and mean concentrations were much lower on vegetated than bare soils. These results imply that, while vegetated riparian buffers of the order of 10 m distance will be successful in reducing *Cryptosporidium* oocyst transport to waterways, vegetation buffers to retard bacterial and viral pathogens may need to be significantly wider.

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