The effect of flow impedance on deposition of *Cryptosporidium parvum* oocysts with or without a vetiver buffer strip

Hossein Ghadiri^a, Janet Hussein^{a*}, Mavourneen Lutton^b, Andrew Smolders^b and Peter Schneider^b

^a Australian Rivers Institute, Griffith University,, 170 Kessels Rd., Nathan, Brisbane 4111, Australia

^b SEQWater, Level 2, 240 Margaret St., Brisbane City East 4002, Australia

* Corresponding author Email address: janethussein@bigpond.com

Abstract

Cryptosporidium parvum oocysts can be transported in overland flow from faecal deposits of grazing animals causing potential water contamination problems. Deposition of oocysts can occur when overland flow encounters vegetative buffers and its transport capacity is decreased in the upslope backwater region. Deposition of oocysts was investigated using a large rainfall simulator, with and without a vetiver buffer strip. Oocysts-spiked slurries were applied to two contrasting soils and the resultant oocyst/sediment depositions from the flow were compared. The buffers substantially reduced suspended sediment loads from the two soils and increased the number of oocysts retained in the soil with >99% of the soil-oocysts being deposited upslope of the buffer.

Keywords: *Cryptosporidium parvum* oocysts; Pathogen deposition from overland flow; Vegetative buffer

Introduction

Transport of oocysts of the protozoan parasite, *Cryptosporidium parvum*, from faecal deposits of grazing animals into adjacent water storage reservoirs poses potential public health risks in Australia and elsewhere (SEQWater, 2002-2003; Wohlsen et al., 2004). The oocysts are primarily transported by overland flow (Atwill et al., 2002; Trask et al., 2004) which can be intercepted by vegetative buffers (Tate et al., 2004; Ferguson et al., 2007).

When flow encounters a buffer, a backwater (ponded area) is created upslope of the buffer and flow velocity and concomitant transport capacity are reduced, causing deposition of sediments (and therefore sorbed-oocysts). The backwater may extend for several metres upslope of the buffer depending on slope, flow and type of vegetation (Ghadiri et al., 2001; Hussein et al., 2007a,b) and may remove up to 98% of inflow sediment deposited (Meyer et al., 1995; Dorioz et al., 2006). The oocyst load reduction is dependent on release from faecal deposits (Tyrrel and Quinton, 2003; Atwill et al., 2006), soil type/density (Atwill et al., 2002; Tate et al., 2004) as well as vegetation, rainfall intensity/duration and slope (Davies et al., 2004; Trask et al., 2004; Ferguson et al., 2007).

In most previous studies, oocysts were applied at the upslope edge of, or in the buffers, and so the researchers did not effectively simulate the presence of the backwater and assess its effect on oocyst transport. There are therefore few representative studies on which to base an understanding of the entrapment of oocysts by backwater development. This communication compares the oocyst deposition from flow with and without the presence of a vetiver (*Vetiveria zizaniodes* L.) buffer strip. The work formed part of a larger project examining the effect of transport of oocysts over different soil types under applied rainfall.

Methods & Materials

Soils from two contrasting grazing sites near Wivenhoe Dam in Queensland Australia (27° 8' 26" S; 152° 39' 11" E) were used for the experiments: AFS (above full-supply – never inundated) and BFS (below full supply - frequently inundated). The BFS soil was more erodible due to its higher silt (Si=41%) and lower total carbon (C=1.1%) contents compared to the sandier AFS soil (Si=25%, C=2.7%).

The flow experiments were carried out in the Griffith University Tilting-Flume Simulated Rainfall Facility (GUTSR). Three experiments were firstly conducted for each soil without a buffer. A 6x0.5 m section of the flume was lined with new black plastic for each experiment and filled with 0.06 m depth of homogenised soil(<10 mm). Soils were saturated, equilibrated for 1 hour, and then random samples were taken to determine pre-test concentrations of *Cryptosporidium* oocysts using a sterile 10 ml syringe. All soil samples were chilled immediately (4°C) and transported to the Queensland Health Laboratory for analysis (Lacovski et al., 2004).

Ten minutes before rainfall application, the flume was raised to 5% slope and a purified, oocyst-spiked, suspension (100 ml of RO water with 1×10^8 of gamma-irradiated oocysts, BTF Precise Microbiology) was applied to the soil surface, 3 m from the flume exit. Simulated rainfall (average drop diameter of 2.2 mm) was applied for 40 min at a rate of $138 \pm 5 \text{ mm hr}^{-1}$ (Ghadiri and Rose, 1993). After cessation of rainfall, further soil samples were taken at designated positions from the flume (Fig. 1a) and tested for oocyst concentration as previous. The soil/plastic sheeting was then removed and the flume repeatedly washed before the next experiment.

A second set of experiments was performed with a 1 m-long vetiver grass strip inserted into the soil 1 m from the outflow. Due to funding limitations only one replicate was carried out for each of the two soils. The spiking was performed 1 m upstream of the buffer. Photographs were taken through the Perspex side of the flume and later digitised to provide vertical water depths upslope of the buffer.

Results and discussion

Pre-inoculation oocyst counts (24 measurements) for all but one BFS sample (38 g^{-1}) were zero. Oocysts measured in the soil after the simulations were therefore primarily from the spiked oocysts and were transported from the spiked site by splash action of raindrops and/or by overland flow. The soil-oocysts concentrations at the end of the simulation, for the six without-buffer experiments, were variable with distance (Fig. 1a). Overall mean concentrations were $10^{2.84}$ (688.2) and $10^{1.77}$ (59.5) g^{-1} for AFS and BFS respectively. The AFS soil thus retained about eleven times more oocysts than the BFS soil, probably due to increased deposition of the AFS's coarser particles. As distance from the spiked site increased, so the sorbed-oocyst concentration decreased (Fig. 1a), due to progressive "dilution" over the soil volume in the flume.

The oocyst concentrations for the with-buffer experiments were also variable with distance (Fig 1b), giving an average of $10^{3.31}$ (1626) and $10^{2.61}$ (146) per g soil for the AFS and BFS soils, respectively. While there was a general decrease in the oocyst concentration downstream from the spiked sites for the without-buffer runs (Fig. 1a), the with-buffer runs show a different trend (Fig. 1b), with concentration generally increasing towards the buffer. This is most likely due to the differences in water depths between the two sets of

experiments (Fig. 1a,b). Water depths upstream of the buffer are considerably larger (3-38 mm, Fig 1b) and its velocity lower than in the without-buffer runs (~3 mm).

Thus, the sorbed-oocysts entrained in the flow have been deposited due to the slow flow velocity in the backwater, rather than being continually flushed through the outflow. Flow velocities in the backwater region (Fig 1b) were calculated using the Griffith University Buffer model (Hussein et al., 2007a) as 0.08 ms^{-1} at the start of the backwater to 0.005 ms^{-1} just upstream of the buffer. Thus time taken by a suspended particle to transverse a ~0.6 m length backwater, at these velocities, would vary between 7.5 and 120 s. An oocyst sorbed to a coarse sand particle (diameter ~1000 μm) would have a settling velocity of $100 \mu\text{m s}^{-1}$, in contrast to a non-sorbed(free) oocyst (diameter ~5 μm), with a settling velocity of $0.76 \mu\text{m s}^{-1}$ (Searcy et al., 2005). Maximum settling distance would therefore be $120 \times 100 = 12\,000 \mu\text{m}$ for the sorbed-oocyst as opposed to $120 \times 0.76 = 91.2 \mu\text{m}$ for a free oocyst. As the backwater depths were 3000 - 38 000 μm , therefore it is likely that only the sorbed-oocysts would fully settle out of suspension within the backwater while the free oocysts would remain suspended in the flow passing into the buffer. Organic-sorbed oocysts (e.g. those released from faeces) would be intermediate between these two extremes.

These preliminary results suggest that a single vetiver buffer strip enhanced deposition of sediment-sorbed oocysts but not of non-sorbed oocysts upslope of the buffer. Further replicated experimentation is required to examine this effect in more detail.

Acknowledgements

Analyses of pathogens and TSS were funded by SEQWater and the buffer work by ARC Discovery Project (DP0451654). The authors thank Simon Rotherham from SEQWater, and Joe McMahon of Griffith University, for assistance with the experiments.

Fig 1. Variation in mean water depths and oocyst concentrations in soils at the end of the experiments at different distances downstream from the spiked site for (a) without-buffer and (b) with-buffer experiments. Oocysts concentrations were plotted as log (no.+1.1) oocysts per g of saturated soil to facilitate column display.

References

- Atwill, E.A., Hou, L., Karle, B.M., Harter, T., Tate, K.W., Dahgren, R.A., 2002. Transport of *Cryptosporidium parvum* oocysts through vegetated buffer strips and estimated filtration efficiency. *Applied and Environmental Microbiology* 68, 5517-5527.
- Atwill, E.A., Tate, K.W., das Gracas Cabral Pereira, M., Bartolome, J., Nader, G., 2006. Efficacy of natural grassland buffers for removal of *Cryptosporidium parvum* in rangeland runoff. *Journal of Food Protection* 69, 177-184.
- Davies, C.M., Ferguson, C.M., Kaucver, C., Krogh, M., Altavilla, N., Deere, D.A., Ashbolt, N.J., 2004. Dispersion and transport of *Cryptosporidium* oocysts from fecal pats under simulated rainfall events. *Applied and Environmental Microbiology* 70, 1151-1159.
- Dorioz, J.M., Wang, D., Poulenard, J., Trevisan, D., 2006. The effect of grass buffer strips on phosphorus dynamics – a critical review and synthesis as a basis for application in agricultural landscapes in France. *Agricultural Ecosystems & Environment* 117, 4-21.
- Ferguson, C.M., Davies, C.M., Kauchner, C., Krogh, M., Rodehutsors, J. Deere, D., Ashbolt, N.J. 2007. Field scale quantification of microbial transport from bovine faeces under simulated rainfall events. *Journal of Water and Health*, 5, 83-95.
- Ghadiri, H., Rose, C.W., 1993. Water erosion processes and the enrichment of sorbed pesticides in the eroded sediment. Part 2: Enrichment under rainfall dominated erosion process. *Journal of Environmental Management* 37: 35-50.
- Ghadiri, H., Rose, C.W., Hogarth, W.L., 2001. The influence of grass and porous barrier strips on runoff hydrology and sediment transport. *Transactions of the American Society of Agricultural Engineers* 44, 259-268.
- Hussein, J., Ghadiri, H., Yu, B., Rose, C.W., 2007a. Sediment Retention by a Stiff Grass Buffer under Subcritical Flow Conditions. *Soil Science Society of America Journal* 71 (5), 1516-1523.
- Hussein, J., Yu, B., Ghadiri, H., Rose, C.W., 2007b. Prediction of surface flow hydrology and sediment retention upslope of a vetiver buffer strip. *Journal of Hydrology* 338, 261-272.
- Lacovski, R.B., Barardi, C.R., Simoes, C. M.O., 2004. Detection and enumeration of *Cryptosporidium* sp. Oocysts in sewage sludge samples from the city of Florianopolis

(Brazil) by using immunomagnetic separation combined with immunofluorescence assay. *Waste Management and Research* 22, 171-176.

Meyer, L.M., Dabney, S.M., Harmon, W.C., 1995. Sediment trapping effectiveness of stiff grass hedges. *Transactions of the American Society of Agricultural Engineers* 38, 809-815.

Searcy, K.E., Packman, A.I., Atwill, E.R., Harter, T., 2005. Association of *Cryptosporidium parvum* with suspended particles: Impact on oocyst sedimentation. *Applied and Environmental Microbiology* 71, 1072-1078.

SEQWater, 2002-2003. 2002/2003 Annual Environmental Report. SEQWater, Queensland, Australia. <<http://www.seqwater.com.au/files/pdf/SEQWATER2002-03.pdf>> accessed on 9 January 2008.

Tate, K.W., das Gracas, M., Pereira, C., Atwill, E.R., 2004. Efficacy of vegetated buffer strips for retaining *Cryptosporidium parvum*. *Journal of Environmental Quality* 33, 2243-2251.

Trask, J.R., Kalita, P. K., Kuhlenschmidt, M.S., Smith, R. D. Funk, T.L., 2004. Overland and near-surface transport of *Cryptosporidium parvum* from vegetated and nonvegetated surfaces. *Journal of Environmental Quality* 33, 984-993.

Tyrrel, S.F., Quinton, J.N., 2003. Overland flow transport of pathogens from agricultural land receiving faecal wastes. *Journal of Applied Microbiology* 94, 875-935.

Wohlsen, T., Bates, J. Gray, B., Katouli, M., 2004. Evaluation of Five Membrane Filtration Methods for Recovery of *Cryptosporidium* and *Giardia* Isolates from Water Samples. *Applied and Environmental Microbiology* 70, 2318–2322.