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Researchers study
Cryptosporidium parvum
containment

using vegetated buffer strips for CONTAINING PARASITES

Photo courtesy of USDA



Cryptosporidium parvum is a ubiquitous protozoal parasite, with specific genotypes able to be transmitted between domestic animals and humans. Vegetated buffers are widely advocated as a management practice to minimize the likelihood that animal agricultural operations contaminate surface water with enteric microorganisms prevalent in fecal matter, such as generic *E. coli*. Considerably less work has been conducted on the ability of vegetated buffers to remove pathogenic microorganisms from overland and shallow subsurface flow, such as the protozoa *C. parvum*. Larger-diameter pathogens such as *C. parvum* are likely to be more susceptible to filtration compared to the smaller enteric bacteria. The situation is less clear for adsorption given the physicochemical processes governing attachment of a biological colloid onto a solid substrate and the ability of *C. parvum* to desorb from sediments in a time-dependent manner.

When enteric pathogens are excreted by a host in a matrix of fecal material onto the terrestrial component of a watershed, at least three events need to occur for *C. parvum* to reach surface water to become a waterborne hazard. First, *C. parvum* oocysts need to be released from the fecal matrix; second, the entrained oocysts need to avoid such processes as filtration, adsorption and settling while being transported in overland and subsurface flow to a receiving body of water; and

third, oocysts need to remain infective during the first two processes.

We have determined previously that a meter of vegetated buffer can function to remove 1 to 3 log₁₀ of waterborne *C. parvum* oocysts entrained in overland flow. The objective of this project was to assess the log₁₀ reduction per meter of vegetated buffer when *C. parvum* oocysts are instead placed into a fecal matrix, as occurs naturally on watersheds with vertebrate hosts, and to determine if land slope (percent) influences the ability of a buffer to retain oocysts. For this experiment, we simulated the vegetation, soil, land slope and rainfall conditions of annual grasslands found in California's central and southern Sierra Nevada foothills. These grasslands occupy more than 1.8 million ha in California and are a critical source of the state's surface drinking water supply. Approximately two-thirds of the state's drinking water reservoirs are located within annual grassland landscapes.

Materials and methods

Overall Study Design The experimental unit was a soil box designed to allow collection of overland and subsurface flow. Twelve soil boxes were filled with soil and planted with annual grass typical of south Sierra Nevada foothill rangelands. Three land slope treatments (5 percent, 12 percent and 20 percent) were tested across

four trials (reps). Bovine feces spiked with *C. parvum* oocysts was placed 1.0 m up-slope from the bottom of the soil box and rainfall were applied via one of three stationary nozzle rainfall simulators for 120 minutes. Thus, during each trial three rainfall simulators (A, B and C) individually and simultaneously applied rainfall to three soil boxes, each box set to one of the land slope treatments. Overland and subsurface flow volume from each box was measured and subsampled for oocyst concentration throughout the 120-minute rainfall-runoff trial.

Soil box dimensions were 0.5 m wide × 1.1 m long × 0.3 m deep, allowing us to determine the filtration or retention efficiency of a meter of vegetated buffer while allowing for reasonable root development of planted vegetation.

Simulated Watershed Scenario

Landscape. Herbaceous vegetation in southern Sierra Nevada foothills is composed of annual grasses and forbs found either as open grasslands or as understory for scattered blue oak, interior live oak or foothill pine. Soils are sandy loams derived from decomposing granite with soil depths typically ≤70 cm. Topography is rolling to steep uplands with significant swale areas formed from alluvial deposits. Swales are intermittent wetlands that become saturated with

lateral subsurface and saturated overland flow from surrounding uplands, serving as variable source areas for stream flow generation. Precipitation falls almost entirely as rainfall from October through April.

Vegetation Type. Annual ryegrass was selected for use in this experiment to simulate soil surface cover typical of open grasslands and understory in these rangelands. We used 150 to 200 g of seed per box, which resulted in ≥95 percent soil surface cover. Soil boxes were planted with annual ryegrass in October and allowed to mature over the winter growing season, with the experiment conducted the following May. To simulate moderately grazed conditions, the annual ryegrass was clipped to 10 cm one day before the application of feces and simulated rainfall. Grass roots extended the entire 0.3-m depth of the soil boxes. Herbaceous vegetation root zone depth on annual rangelands is approximately 0.2 to 0.4 m.

Soil Type. The Ahwahnee coarse sandy loam soil series was selected for use as a rangeland soil found extensively throughout this region. Soil in the boxes was compacted to a mean bulk density of 1.40 g/cm³ to simulate compaction typical of heavy grazing in the region.

Land Slope Treatment. Land slope treatment of 5 percent was chosen to simulate swale or variable source area conditions for the southern Sierra Nevada and a 12 percent and

20 percent land slope treatment was chosen to simulate upland or hill-slope conditions for this region of California. A further justification for choosing these land slope treatments was that these slope classes receive significantly different amounts of beef cattle fecal deposition and thus potential oocyst loading rates for grazed rangeland in the southern Sierra Nevada.

Rainfall Simulation Trial

For each trial (n = 4), rainfall was emitted from three stationary nozzle emitters (A, B, C). Each emitter was positioned 2 m above the surface of the soil box, inside a small shed to minimize lateral drift. In this configuration, the emitters generated a circular rainfall pattern with a diameter of approximately 1.1 m at the soil box surface. Thus, the rate of water emission did not equal the rate of water application to the surface of each soil box. Soil boxes were consistently placed so that the box center was exactly under the rainfall emitter. Variable amounts of simulated rainfall were actually received by each soil box due to variability in the pattern of water emission between rainfall emitters and some drift of small water drops. Each emitter was calibrated to produce approximately 53 mm/hour of rainfall, measured directly underneath the emitter

Parasitologist Ronald Fayer leads a project to determine the sources of cryptosporidium in surface waters and develop techniques for accurate detection of these organisms that infect humans, livestock, and wild animals. Photo by Keith Weller.

as the total volume produced in a 5-minute period at the start and finish of each rainfall simulation. We applied water to the soil boxes at this rate for 2 hours, simulating a storm event for the south Sierra Nevada foothills with a significantly greater than a 100-year return interval, a worst-case precipitation runoff event. Our previous work on this soil type indicates that due to inherently high infiltration capacity, extreme rainfall events and saturated antecedent soil moisture conditions are required to exceed infiltration capacity and generate overland flow.

Each land slope treatment was present in each trial, and the position of each land slope treatment was rotated among simulators (A, B and C) between trials. For each trial, all three soil boxes were irrigated for 2 hours immediately prior to fecal deposit application to create saturated soil conditions, and thus simulate worst-case antecedent soil moisture conditions for oocyst transport during storm events. After the simulators were calibrated and boxes saturated, a 200-g deposit of bovine fecal material spiked



Using vegetated buffer strips for containing parasites December Self-Study Examination

- A reason for this research study was to**
 - a. assess the health risks from bacteria in the environment and to livestock.
 - b. determine the best grass species to use in California pastures.
 - c. make an appraisal of the ideal grazing frequency to minimize the environmental impact.
 - d. guide buffer width recommendations for livestock operations.
- The grass species used to simulate native grasses in this trial was**
 - a. goatgrass.
 - b. annual rye.
 - c. red fescue.
 - d. Indiangrass.
- A characteristic of California grasslands is that they**
 - a. are often heavily wooded with dense undergrowth.
 - b. mostly grow on heavy clay soils.
 - c. experience dry conditions during the winter months.
 - d. are the source for most of California's drinking water reservoirs.
- The study measured the total number of *C. parvum* oocysts that discharged from each soil box traveling a distance of**
 - a. 1 m.
 - b. 3 m.
 - c. 5 m.
 - d. 7 m.
- A characteristic of this study is that it included**
 - a. four land slope treatments.
 - b. natural rainfall.
 - c. measurement of subsurface flow.
 - d. the use of manure from different animal species.
- To become a waterborne hazard in the environment, a pathogen from manure must do all of the following EXCEPT**
 - a. develop a viral ring sheath.
 - b. release from the fecal matrix.
 - c. avoid filtration and settling during movement.
 - d. remain infective.
- The rainfall amounts used in the trial were designed to mimic**
 - a. a typical summer rainfall episode.
 - b. a worst-case rainfall event for the Sierra foothills.
 - c. the season total precipitation that occurs in the area.
 - d. a month-long series of rainfall events followed by leaching and evaporation.
- The concentration of oocysts applied to the soil boxes in this study represented**
 - a. a typical beef cattle herd in California.
 - b. the infection level of week-old, partially decomposed feces.
 - c. cattle fed a diet high in grains and protein.
 - d. a highly infected calf or a worst-case scenario for cattle in California.
- Higher retention of *C. parvum* oocysts at 5 percent land slope was due to**
 - a. oocysts not releasing from the fecal matrix.
 - b. lower volumes of subsurface flow, resulting in less filtration and absorption by the soil.
 - c. higher volumes of subsurface flow.
 - d. less soil erosion in the soil box.
- Under the conditions of this study and relative to total *C. parvum* oocysts applied, each additional meter of grass vegetated buffer strip resulted in a**
 - a. 0.1 to 0.3 log₁₀ mean reduction in *C. parvum* oocysts.
 - b. 0.4 to 0.8 log₁₀ mean reduction in *C. parvum* oocysts.
 - c. 0.9 to 2.0 log₁₀ mean reduction in *C. parvum* oocysts.
 - d. 2.1 to 2.5 log₁₀ mean reduction in *C. parvum* oocysts.

with 1×10^6 *C. parvum* oocysts/g was placed 1.0 m up from the bottom of the box and on top of the 10 cm annual ryegrass stubble and rainfall simulation continued for 2 hours. Thus, the total oocyst spike per soil box was 2×10^8 . This concentration of oocysts represents a highly infected calf or a very worst-case scenario for fecal shedding by California beef cattle. Overland and subsurface flow was collected separately as 5-minute composite samples, with the total volume measured for each sample.

Source of *Cryptosporidium parvum* Oocysts

Naturally infected dairy calves from two local commercial dairies were the source of wild-type *C. parvum* oocysts. Approximately 2.4×10^9 oocysts were added to 2,400 g of fresh beef cattle fecal material negative for *C. parvum*.

Total Oocyst Discharge Calculation and Statistical Analysis

The total number of oocysts that discharged from each soil box during each trial and adjusted for the percent recovery of the assay (outcome variable) was calculated from our set of 26, 5-minute composite samples collected from each soil box for overland ($n = 13$ composite samples) and subsurface flow ($n = 13$ composite samples). An equation was used to calculate overland and subsurface oocyst discharge in each composite sample.

Results and discussion

A total of 2×10^8 oocysts were applied to each plot. The total number of oocysts discharged from each soil box (combined overland and subsurface flow) during the 120-minute simulation ranged from 1.5×10^6 to 23.9×10^6 oocysts. Examination of the mean breakthrough curves of each land slope treatment demonstrated that although mean concentration of oocysts/L in overland flow was not substantially

different for buffers set at different land slopes, mean rates of overland flow were positively correlated with land slope. This resulted in distinct breakthrough curves for the flux (flow \times concentration) of discharged oocysts for buffers set at 5 percent compared to buffers set at 12 percent and 20 percent.

These results suggest that each additional meter of grass vegetated buffer with ≥ 95 percent surface cover and under conditions of 5 to 20 percent land slope, sandy loam soil and precipitation rates of 30 to 47.5 mm/h for a 2-h duration could generate an additional 0.9 to 2.0 log₁₀ mean reduction in *C. parvum* oocysts relative to the total oocyst spike applied in a fecal matrix. These values are consistent with our previous estimate of 1.0 to 1.9 log₁₀ mean reduction in waterborne *C. parvum* oocysts using a meter long soil box with 85 percent to 99 percent grass cover and under conditions of 5 percent to 20 percent land slope, sandy soil, and rainfall rates of 1.5 or 4.0 mL/cm²/h, and similar to the range of values observed by researchers in 2004 for their vegetated buffers. The substantial release of oocysts from fresh fecal deposits and the association between land slope and overland transport of oocysts during storm events is also consistent with previous findings in the field under natural rainfall events for annual grasslands in the southern Sierra Nevada foothills, as well as under laboratory conditions. The higher retention of oocysts at 5 percent land slope is likely due to a higher volume of rainfall infiltrating the soil surface and becoming subsurface flow, resulting in a substantial portion of the *C. parvum* load being removed via filtration and absorption within the soil matrix.

Conclusions

We found that a meter of grass vegetated buffer with ≥ 95 percent surface cover and under experimental conditions of 5 percent to 20 percent land slope, sandy

loam soil, and 2-hour precipitation rates of 30 to 47.5 mm/h produced a 0.9 to 2.0 log₁₀ mean reduction in *C. parvum* oocyst flux relative to the total oocyst load applied in a fecal matrix. The observed overall mean log₁₀ reduction of total *C. parvum* flux per meter of grass vegetated buffer was 1.44, 1.19 and 1.18 for buffers at 5-, 12- and 20-percent land slope, respectively. These levels of log₁₀ reduction per meter of vegetated buffer can be matched to a mammal's specific rate of *C. parvum* loading to guide buffer width recommendations. For example, we calculated that adult beef cattle on California rangeland shed on average between 3,800 and 9,200 oocysts per cow per day, or approximately 6.5×10^5 oocysts per day for a herd of 100 beef cattle with no calves or yearlings. Based on an estimated range of 1.2 to 1.4 log₁₀ reduction per meter of grass buffer, ≥ 5 m of equivalent buffer would be needed to adequately reduce the translocation potential of this herd's 24-hour cumulative load of freshly deposited oocysts. Such estimates remain crude approximations at this time, but these results in combination with previous research on vegetated buffer filtration for this protozoal pathogen provide insight into the inherent capacity of grasslands with sufficient infiltration rates to attenuate *C. parvum* oocysts deposited by cattle and other mammals. Moreover, these studies illustrate the potential for vegetated buffers to function as one of a suite of beneficial management practices that can minimize the risk of waterborne cryptosporidiosis in humans from livestock production systems.

Editor's Note: Content was adapted from the paper "Efficacy of Vegetated Buffer Strips for Retaining Cryptosporidium parvum," which was published in the Journal Environmental Quality, Vol. 33, November-December 2004, and is courtesy of the authors Kenneth W. Tate, Maria Das Gracas C. Pereira, and Edward R. Atwill.

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