CHARACTERIZATION OF PATHOGEN TRANSPORT IN OVERLAND FLOW

BY

PAUL CURTIS DAVIDSON

DISSERTATION

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Doctoral Committee:

Professor Prasanta K. Kalita, Chair
Professor Mark S. Kuhlenschmidt
Research Scientist Theresa B. Kuhlenschmidt
Associate Professor Richard A. Cooke
Extension Specialist Ted L. Funk
Assistant Professor Michelle L. Soupir, Iowa State University

ABSTRACT

Overland transport kinetics of pathogens are controlled, in large part, by soil and vegetation. With an increasing number of concentrated animal operations, there is becoming a greater need to dispose of a vast amount of manure in a single, localized area. Animal manure contains a substantial amount of microbial pathogens, including *Cryptosporidium parvum* and rotavirus that may pose a threat of potential contamination of water resources. This study examines the kinetics of *C. parvum* and rotavirus in overland transport, with an overall objective of optimizing the design of best management practices, especially vegetative filter strips. Three soil types are tested (Catlin silt-loam, Alvin fine sandy-loam, Darwin silty-clay), spanning the entire spectrum of typical Illinois soils, in terms of soil texture. A 20-minute rainfall event is produced using a small-scale (1.07 m x 0.66 m) laboratory rainfall simulator over a soil box measuring 0.67 m x 0.33 m. Each soil type is tested for pathogen transport kinetics with bare and vegetated surface conditions. Surface runoff, soil cores, and near-surface runoff are each analyzed for infective *C. parvum* oocysts and infective rotavirus particles using cell-culture infectivity assays. Results show that vegetation reduces the recovery of infective oocysts in surface runoff by an average of 62% and rotavirus particles by an average of 73%, in addition to delaying the time to the peak recovery. Recovery of infective rotavirus particles from surface runoff of bare Alvin (high sand content) soil is seven times higher than that of infective oocysts. Recovery from surface runoff of bare Darwin (high clay content) soil is nearly one and a half times more for *C. parvum* than for rotavirus, with the recovery of *C. parvum* oocysts approaching 40%. Recovery from the soil cores was slightly higher for *C. parvum* (0.06%) than for rotavirus (0.05%) in the case of the bare Alvin soil, but was slightly higher for rotavirus (0.92%) than *C. parvum* (0.19%) for the bare Darwin soil condition.

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CHAPTER 1: INTRODUCTION

Preventing pathogens from entering drinking water supplies is of great importance worldwide. Eighty-eight percent of diarrheal disease is attributed to unsafe water supply, inadequate sanitation and hygiene. In addition, it is estimated that 1.8 million people die every year from diarrheal diseases worldwide, 90% of whom are children under the age of five (WHO, 2004). As reported by the American Society of Microbiology, illnesses related to waterborne infectious disease affect up to 900,000 individuals each year in the U.S. It has also been estimated that up to 900 Americans die each year from waterborne diseases (American Public Health Association, 2001).

There are various ways microbial organisms, including pathogens, can reach watercourses, including direct leakage of a drainage system from an animal waste storage facility or livestock barn, application of manure as a fertilizer to agricultural fields, direct runoff from animal production facilities, direct contamination by animals (Mawdsley et al., 1995; Kuczynska and Shelton, 1999; Graczyk et al., 2000), human waste-disposal systems, and wildlife.

Since low levels of *Cryptosporidium* oocysts have been detected in 65%-97% of surface-water supplies in the United States, there is a concern that most populations may be at risk for waterborne infection (Juranek, 2007). The primary environmental concern with *C. parvum* is its resistance to many environmental stresses for long periods of time. The oocyst has evolved to survive in harsh environmental conditions, which is the key factor for its transport and ability to survive within the environment. From 1991 to 2002, *Cryptosporidium* was responsible for 15 outbreaks, or 7% of all waterborne disease outbreaks in the United States. Perhaps more importantly, *Cryptosporidium* was the cause of 94% of all reported waterborne illnesses during that same time period, accounting for 408,371 cases (Craun et al., 2006).

Rotaviruses have been identified as the major causative agents of diarrheal disease in humans and a wide variety of animals (Estes et al., 1984; Kapikian and Chanock, 1985). It has been estimated that rotaviruses account for over a million human deaths annually in developing countries (Snyder and Merson, 1982; De Zoysa and Feachem, 1985; Kapikian and Chanock, 1985), and at least 100 deaths each year in the U.S. (CDC, 2005). Rotavirus infections are also of high agricultural importance because of the impact diarrheal disease can have on neonatal and post-weaning animals, especially pigs and calves. There is a lack of recent literature on the economic burden of rotavirus on livestock, but studies (Bohl, 1979; Dodet et al., 1997; Dhama et al., 2009) have found that morbidity due to rotavirus infections in pigs and calves can be as high as 80% with mortality reaching 60%. High morbidity and mortality in livestock animals leads to economic losses from the loss of animals, treatment costs, and reduced growth rates.

The size of pathogens contained in agricultural runoff can range from as small as rotavirus (70 nanometers) to as large as *C. parvum* (5 micrometers). Low settling velocities prevent both of these pathogens from settling out of watercourses when they travel at the same velocity as the water (Graczyk et al., 2000; Brush et al., 1999). Therefore, there is a need to implement practices that reduce surface runoff rates.

Vegetative filter strips (VFS) have been successfully used for sediment and nutrient control in surface runoff, with recent research showing efficient reduction of pathogens with filter strips. There are a number of components in a VFS that are in contact with surface runoff. The plant stem, the decaying plant material and the underlying soil are all potential adsorption sites. Adsorption of microorganisms to plant or soil particles can be due to many factors that include particle size, mobility, surface charge and surface area. Currently, very little work has been done in this area to characterize microorganism adsorption to these particles.

For advancing the relationship between soil and *C. parvum* transport, small-scale transport studies have been conducted to determine the ability of grass buffers to remove pathogens (Stout et al., 2005; Tate et al., 2004; Trask et al., 2004; McLaughlin, 2003; Mawdsley et al., 1996b). The studies by McLaughlin (2003) and Tate et al. (2004) both show that properly designed vegetated filter strips can reduce the risk of waterborne *C. parvum* attributable to extensive cattle grazing on annual grassland watersheds. In addition, McLaughlin (2003) found that a small soil chamber produced results accurately reflecting the overland transport kinetics of *C. parvum* as may be seen in a slightly larger soil chamber studied by Trask et al. (2004). The ability to achieve consistent results using a small, lab-bench soil chamber provides the opportunity to refine methodologies to more accurately detect and monitor *C. parvum* (and other microbial pathogens), and then extrapolate results to larger-scale situations. This study involves the design and construction of a small-scale soil box and tilting chamber to use in conjunction with the rainfall simulator developed by Davidson (2007). *C. parvum* and rotavirus are examined in overland transport studies with the aim to better characterize the interactions between pathogens, soil particles, and vegetation.

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CHAPTER 2: OBJECTIVES

The overall objective of this research is to characterize the overland transport of *Cryptosporidium parvum* and rotavirus, identifying factors that are critical for their survival in a natural soil-water environment. This research aims to provide a foundation for developing a field and watershed-scale model for microbial fate and overland transport.

The specific objectives of this research are to:

1. Investigate the infectivity of *C. parvum* oocysts and rotavirus particles in the surface runoff, soil cores, and near-surface runoff from three different soil types.
2. Examine the ability of two different types of vegetation to inhibit overland transport of both infective *C. parvum* oocysts and infective rotavirus particles.
3. Develop basic models to identify parameters affecting *C. parvum* and rotavirus transport in overland flow that can be used to design vegetative filter strips for controlling microbial pathogen transport to surface water sources.

CHAPTER 3: REVIEW OF LITERATURE

The transport of microbial pathogens from agricultural land into waterways has become an increasing concern. Vegetative filter strips (VFS) have been proposed as a measure that can limit the transport of microbes to waterways within overland flow. However, studies measuring their effectiveness have often produced variable results (Crane et al., 1983; Fajardo et al., 2001). One of the primary hurdles in studying pathogen transport is the difficulty in detecting and monitoring such small particles on the watershed scale. Therefore, this research has narrowed the scope. Small, watershed-representative soil beds are used to study the transport through an area of either bare soil, or soil with vegetative cover. The results from this research should provide the necessary information for expanding pathogen transport to the field and watershed level and eventual creation of a predictive model.

The amount of available information on the ability of a VFS to control pathogen transport is limited, and somewhat contradictory. Therefore, previous published research on this topic is detailed below. In addition, while both *C. parvum* and rotavirus are causative agents of gastrointestinal illness in humans and animals, there are some basic differences between the two organisms. *C. parvum* sporozoites are protozoa, or single-celled eukaryotes, present in the environment in the form of an oocyst and measuring 4-6 μm in size. Rotavirus, on the other hand, is a triple-layered, or double-shelled, virus particle measuring approximately 70 nm in diameter. The specific properties of each pathogen are outlined in this section as well.

3.1 Vegetative filter strips

A Vegetative filter strip (VFS) is an area of vegetation usually located down slope of cropland or an animal production facility. A VFS is designed to reduce the runoff volume and

velocity of water by increasing surface roughness (Borin et al., 2005). This reduced flow volume and velocity allow more surface contact of the water. This promotes infiltration into the soil profile, deposition of suspended solids, adsorption to vegetation and sediment, and uptake of soluble pollutants by plants (Misra et al., 1996; Blanche et al., 2003). These mechanisms have identified a VFS as a best management practice (BMP) to help control the movement of solids and nutrients into receiving waters. A VFS has also shown effectiveness in removing microorganisms from runoff (Kalita et al., 2002). Microbes contained in agricultural runoff can range from something as small as a virus (nanometers) to something as large as a protozoan (micrometers). *Cryptosporidium parvum* oocysts have a low settling rate (approx. 0.5 μm/s), which prevents them from settling out of watercourses when they travel at the same velocity as the water (Graczyk et al., 2000; Brush et al., 1999). For this reason, vegetation becomes very important. Several laboratory experiments have examined the overland transport of *C. parvum* oocysts. Trask et al. (2004) used a large-scale laboratory horizontal tilting chamber with simulated rainfall to monitor overland and shallow subsurface transport of *C. parvum* oocysts. The tilting bed was split into two chambers, bare soil and vegetation. The vegetated side reduced *C. parvum* oocyst concentrations by ten fold when compared to the bare soil. These results show that a VFS is effective in reducing concentrations of a pathogen as large as *C. parvum*. Using a much smaller soil chamber, Tate et al. (2004) confirmed the results of Trask et al. (2004), showing that a strategically placed VFS can serve as a management strategy to reduce the risk of waterborne *C. parvum* attributable to extensive cattle grazing on annual grassland watersheds.

There are a limited number of components in a VFS that are in contact with surface runoff. The plant stem, the decaying plant material and the underlying soil are all potential adsorption sites. Adsorption of microorganisms to plant or soil particles can be due to many

factors that include particle size, mobility, surface charge and surface area. Very little work has been done in this area to characterize microorganism adsorption to particles. Mawdsley et al. (1996a) found that transport of *C. parvum* oocysts occurred in clay and silt soils with higher organic matter content, and not in the sandy soils. However, a study by Kuczynska and Shelton (1999) found that of the three columns inoculated with oocysts (i.e. sandy loam, silty clay loam, clay loam) the proportion of oocysts recovered decreased as the clay content increased. They concluded that lower rates of recovery were due to oocysts adhering to soil particles, specifically to clay. This is in agreement with previous work by McLaughlin (2003), who used fluorescent microscopy to visualize the strong affinity of clay to bind with oocysts while little or no aggregation was seen between oocysts and the sand or silt fractions. Results of these studies indicate that more research is needed in this area because many questions remain unanswered.

3.2 Effect of soil on protozoa transport

Research pertaining to adsorption of protozoa to soil is not extensive and can be contradictory. Protozoan cysts are larger than bacterial cells, so adsorption may be expected to be similar to those of bacteria: filtration, sedimentation and adsorption. However, Mawdsley et al. (1996a) reported that transport of *Cryptosporidium* occurred in clay and silt soils with higher organic matter content, and not in the sandy soils. Their soil column studies demonstrated that pathogen leaching 30 cm below the soil surface was possible, but the amount of leaching was likely to depend on soil characteristics. They attributed the increased leaching from silty loam and clay loam soils to rapid flow through macro pores. This is in agreement with research by Harter et al. (2008) that showed that although oocysts are too large to be transported in the matrix pore space of soils with a significant fraction (>10%) of silts and clays, the macro pore network in a wide range of soils is sufficiently well developed to transport measurable amounts

of oocysts into the subsurface. However, when average cell diameter exceeds 10% of the average grain diameter in a matrix, straining becomes an important factor limiting entry and transport in internal soil drainage (McDonald and Kay, 1981).

A study by Dai and Boll (2003) concluded that oocysts do not adhere to soil particles based solely on surface charges. Furthermore, Tufenkji et al. (2004) have found that both straining and physiochemical filtration are expected to control the removal of *C. parvum* oocysts in settings typical of riverbank filtration, soil infiltration, and slow sand filtration. Their findings show that irregularity of sand grain shape contributes considerably to the straining potential of the porous medium. The variation in the shape of the individual sand grains can contribute significantly, trapping *C. parvum* oocysts in the spaces that are too small to allow passage. Since natural subsurface environments are highly heterogeneous with respect to grain size and shape, straining is expected to be a dominant removal mechanism for *C. parvum* oocysts in such settings.

A study by Walker and Montemagno (1999) showed that oocysts were bound to Al_2O_3, concluding that clay and silt sized particles that are highly enriched with oxides and hydroxides may increase adsorption. Clay and silt that are enriched with oxides and hydroxides have an abundance of iron and aluminum, increasing adsorption. The adsorption to Al is also shown in water treatment. When the proper dose of alum is added to water, over 99% of oocysts are removed from water as compared to below 94% removal of oocysts when alum doses are not correct. These data are assuming that every other process is operating correctly (Rose, 1997).

In the study by Kuczynska and Shelton (1999), looking at detection methods of oocysts in soil, they found that of the three columns inoculated with oocysts (i.e. sandy loam, silty clay loam, clay loam), the proportion of oocysts recovered decreased as the clay content increased.

The authors concluded the lower rates of recovery were due to oocysts adhering to soil particles, specifically to clay. This is in agreement with research performed by McLaughlin (2003) that found *C. parvum* oocysts preferentially bind to clay particles.

Results from Kato et al. (2002) showed a significant oocyst inactivation in soil and water after a single freeze-thaw event, with oocyst inactivation exposed to repeated freeze-thaw events being faster in soil than in water. The oocysts that survived the initial freezing appeared to survive longer than 7 days as observed by Fayer and Nerad (1996) and continued to undergo a decline in viability because they remained frozen or underwent freeze-thaw cycles. In contrast to very cold temperatures, research by Fayer (1994) indicates that oocysts may remain infectious when exposed to water temperatures of 67.5 ºC in 1 min but were rendered noninfectious upon exposure to temperatures of 72.4 ºC or higher. *C. parvum* oocysts were also rendered noninfectious when temperatures reached 64.2 ºC or higher for 2 min or longer.

Jenkins et al. (2002) performed a laboratory pot study to determine if soil type, temperature, and soil water potential affected oocyst survival. Their results indicated that the range of soil water potentials that they tested had no significant effect on oocyst inactivation, however, survival was significantly greater in the silt loam compared to the silty clay loam and loamy sand, and temperature appeared to have the greatest effect on inactivation, with the greatest survival associated with 4 ºC. Peng et al. (2008) agree that soil texture, a static property of the soil, has an effect on oocyst survival in the soil environment. They further explain that soil particles probably do not directly affect oocyst survival, but they may modify oocyst metabolic activity through changing other physiochemical and biological soil properties and their attachment properties.

3.2.1 Previous small-scale *C. parvum* transport studies

Interactions between *C. parvum* and soil particles explain the most basic form of transport in a simulated natural environment. Advancing the relationship between soil and *C. parvum*, small-scale transport studies have been performed to look at the ability of grass buffers to remove pathogens through use of small soil chambers (Stout et al., 2005; Tate et al., 2004; Trask et al., 2004; McLaughlin, 2003; Mawdsley et al., 1996b). Stout et al. (2005) did not find a statistically significant difference in runoff rates as a function of either box length or slope. While the study looked at different slope lengths, the lengths only varied from 1 to 3 m, which may not be enough to show a difference. Mawdsley et al. (1996b) performed experiments at an angle of 7.5% and found that movement of *C. parvum* in runoff continued for at least 21 days, and potentially as long as 70 days from the time of inoculation. They also made the connection that oocysts contained in runoff stay in the aqueous phase and do not precipitate out onto the soil surface, suggesting that even if the distances traveled are increased there may still be a significant pollution threat. However, it is quite possible that the oocysts failed to settle out onto the soil surface because the rainfall was applied using a watering can, an approach that would likely provide a high enough flow rate to cause flooding. In addition, there may have been some error in the data collection as the leachate (subsurface) volume was as much as 10 times higher than the surface runoff volume.

Studies by McLaughlin (2003), Trask et al. (2004), and Tate et al. (2004) all show that properly designed vegetated filter strips can reduce the risk of waterborne *C. parvum* attributable to extensive cattle grazing on annual grassland watersheds. In addition, McLaughlin (2003) found that the small soil chamber produced results accurately reflecting the transport kinetics of *C. parvum* as seen in a slightly larger soil chamber studied by Trask et al. (2004). Trask et al. (2004) found that the amount of *C. parvum* oocysts in both surface and subsurface runoff was

considerably less from vegetated surfaces than those from bare soil. The ability to achieve consistent results using a small, lab-bench soil chamber provides the opportunity to more accurately detect and monitor *C. parvum* (and other microbial pathogens), and then extrapolate results to larger-scale situations. In addition, the results from Trask et al. (2004) indicate that a vegetated filter strip can be a best management practice for controlling *C. parvum* in runoff from animal production facilities.

3.3 Effect of soil on virus transport

Since rotavirus has been shown to aggregate in suspended solids, and because of the increasing emphasis placed upon land application as a means of wastewater disposal, it is important to evaluate the influences of different factors affecting virus survival in soil.

Due to the smaller size of viruses, transport is influenced by the adsorption to soil particles. Unlike other microbes, pore size will have little effect on movement of viruses because of their size (Mawdsley et al., 1995). Virus adsorption is also affected by the pH of the soil-water system and surface properties of the virus (Williamson et al., 1998). As the soil pH increases, adsorption of viruses to soil becomes more of a factor. The mobility of viruses is determined by the properties of the amphoteric protein coat. It has been accepted that the formation of virus-cation-clay bridges increase with an increase in the positive charge on the virus protein coat (Mawdsley et al., 1995). Other researchers have shown that viruses have been adsorbed by clays, glass, iron oxides, silica, and aluminum (Burge and Enkiri, 1978a).

Both virus and bacteria transport through soil is enhanced in the presence of water or other transporting agents (Gannon et al., 1991a; Madsen and Alexander, 1982). Movement slows or ceases when water drains out of soil; therefore, there is increased movement and faster flow rates of virus and bacteria in saturated soils (Mawdsley et al., 1995). Viral and bacterial

transport has been shown to increase in coarse sandy soil as opposed to fine clay soil (Mawdsley et al., 1995; Mawdsley et al., 1996a). These differences can be attributed to the adsorptive properties of soil, properties of soil particles and the size of micro and macro pores within soil. The degree of water saturation has been found to have significant effects on virus transport through porous media due to enhanced virus sorption or inactivation during unsaturated transport. Sobsey et al. (1980) found that although sandy and organic soil materials were poor adsorbents for poliovirus when suspended in wastewater, they removed at least 95% of viruses from intermittently applied wastewater through unsaturated columns. Lance and Gerba (1984) found that movement of poliovirus during unsaturated flow of sewage through soil columns was much less than during saturated flow. The increased virus removal under unsaturated flow conditions has been attributed to either increased inactivation or enhanced sorption. During unsaturated flow, viruses move through the soil in thinner films of water and will be drawn nearer to the soil particles than during saturated flow (Chu et al., 2003).

Viruses contained in wastewater that are applied to soil can persist in the environment for prolonged periods of time. As determined by Hurst et al. (1980), temperature was a significant predictor of virus survival under all sets of conditions tested. In general, it has been demonstrated that temperature, solution chemistry, clay composition, metal oxides, degree of saturation of the solid media, and virus strains are primary factors influencing virus survival and transport in the subsurface environment (Jin and Flury, 2002).

Studies have shown that soils with high clay content have a high sorption capacity for viruses (Gerba et al., 1975; Bitton et al., 1978). The association of viruses with clay minerals has been attributed to the large surface area and high cation exchange capacity (CEC) of clays. The mechanisms and sites of sorption differ for different viruses and are influenced by characteristics

of the clays such as anion exchange capacity (AEC), CEC, and AEC to CEC ratios (Schiffenbauer and Stotzky, 1982). Some researchers believe both positively and negatively charged sites on clay minerals are involved in virus sorption.

In contrast, however, Blanc and Nasser (1996) found that, in general, virus adsorption to sandy soil was greater than to loamy soil. They attribute the lower sorption of viruses to loamy soil to the high content of organic material in the soil. Natural organic matters are notoriously heterogeneous because they contain different amounts and types of functional groups, and therefore, vary in their hydrophilic and hydrophobic properties. Organic matter sorbed on soil particles can provide additional negative charges that repulse viruses or cover positively charged sites, which may decrease the electrostatic interactions between viruses and soil particles. Davis et al. (2006) are in agreement with Blanc and Nasser (1996). Davis et al. (2006) performed batch and column studies and found that dairy manure wastewater (high organic matter content) decreased viral adsorption to sandy soil. Furthermore, they found that dairy manure wastewater increased the release of viruses attached to soil. These findings support those by Davidson (2007) that showed that rotavirus has a high affinity for sand particles. Some sorption of rotavirus to clay particles was observed, but the extent was minor compared to that of sand particles. Zhuang and Jin (2003) found that as a general trend, the effect of organic matter was dominated by electrostatic rather than hydrophobic interactions. In no case did changes in pH influence virus adsorption or desorption.

Chu et al. (2003) found that soils composed primarily of finer sand particles (rather than large sand particles and thus having a larger specific surface area) removed more viruses through soil columns. Chu et al. (2003) also found a higher outflow concentration of viruses in soils with higher clay content. Other factors that could potentially affect virus adsorption to and

inactivation in soil are the phosphorus and calcium content. Some studies have shown increased virus adsorption in the presence of increased Ca concentrations (Yates et al., 1985; Burge and Enkiri, 1978b; Goyal and Gerba, 1979). However, more recent research shows little affect of P or Ca concentration on virus adsorption or inactivation (Gerba et al., 1981; Moore et al., 1981; Chu et al., 2003). Chu et al. (2003) additionally found high outflow virus concentrations in both saturated and unsaturated conditions for a soil with high organic matter content. They concluded that the overall effect of organic matter as part of the soil composition and in the dissolved form was to lower the retention capacity of the soil for viruses.

3.4 Effect of soil on bacteria transport

While studies concerning the fate and transport of bacteria in soil are not comprehensive, they are more extensive than those concerning the fate and transport of protozoa or viruses. Therefore, lessons learned from bacteria transport may be applied to scenarios involving protozoa and viruses.

The major factor governing the transport of bacteria may be the size. With a size larger than that of viruses, filtration and sedimentation play more of a role in the environment. Studies have shown a significant statistical relationship between cell lengths and the percentage of cells transported through soil, showing that the soil acts as a filter to the larger bacteria (Gannon et al., 1991a; 1991b). Other factors that impede or enhance the movement of bacteria through soil are starvation, predation, lysis, parasitism, sorption to soil particles or solid surfaces, and the presence of roots and earthworm tunnels (Mawdsley et al., 1995). Adsorption of bacteria to soil solids can be influenced by a number of factors including cell hydrophobicity, presence of extracellular polysaccharides and the net electrostatic charges of the surfaces of the bacteria and the solid. Gannon et al. (1991a) showed that adsorption to particles smaller than those of the

bacteria actually enhanced transport rather than retarding it. It was only through adsorption to a larger particle that transport was stopped. Results from another study by Gannon et al. (1991b) showed that transport was low for bacteria that had high sorption capacity to soils. Their results showed that bacteria may adhere to soil particles because of the presence of extracellular polysaccharides.

Zhang et al. (1997) found that the depth of soil involved in contaminant transport from soil to runoff (mixing zone) is approximately 1-2 cm. Furthermore, Gessel et al. (2004) found that *Salmonella*, fecal coli, and male-specific coliphages died-off quickly in the runoff mixing zone and survival time did not relate to manure application rate. They concluded that the short persistence times for these particular organisms indicate a low risk of transport off-site by runoff and may be the result of environmental conditions in the shallow runoff mixing zone. A study by Johnston et al. (1996), however, showed that *Salmonella anatum* in land-applied manure could survive for at least 27 days in soil. Gessel et al. (2004) concluded that traditional indicators of fecal pathogens may overlook the presence and survival of some classes of pathogens, specifically enteric viruses.

In a study by Stamm et al. (2001), the greatest concentrations of bacteria found in subsurface drainage were after the first flush. Their study also showed rapid vertical movement of bacteria through the soil, suggesting that contamination of either groundwater or subsurface drainage is a fast process. Abu-Ashour et al. (1998) reported that *E. coli* traveled at a maximum speed of 17 cm/min. Rapid movement in the soil is dependant on the structure of the macro pores of the soil as well as the initial moisture content of the soil. Results also showed that when the top layer of soil was disturbed to remove the macro pores, transport through the soil column did not occur.

3.5 Soil particle properties

Since previous research indicates that both particle types and sizes seem to have an effect on microbial transport, it is necessary to briefly discuss soil particle properties.

The mineral particles present in soils are extremely variable in size. With the exception of rock fragments, soil particles range in size over four orders of magnitude. Sand particles are round or angular in shape, ranging from 0.05 to 2 mm in size. Most sand particles are composed of a single mineral, usually quartz (SiO_2) or another primary silicate, and as a result are very low in nutrient levels. Sand also has very low specific surface area, so water retention is very low, pore size is larger and sand is considered noncohesive. Silt is essentially microsand particles dominated by the mineral quartz. They range in size from 2 to 50 μm. Silt exhibits some water retention and little plasticity due to a film of clay particles adhering to the surface of the silt particle. Clay particles are typically less than 2 μm but they have a very large specific surface area. For example, a spoonful of clay may have a surface area the size of a football field. The large surface area gives clay the unique ability to absorb tremendous amounts of water. Clay is considered a colloid; in water it does not readily settle out. Clay particles are shaped like tiny flakes or platelets, so water and air movement between them is very slow. Soil properties such as shrink-swell, plasticity, water holding capacity, soil strength and chemical adsorption are dependent on the kind of clay as well as the amount present in the soil (Brady and Weil, 2002). The proportion of each of these three particles in soil defines the type of soil and the properties it exhibits and the potential for the soil to absorb or retain bacteria, viruses and perhaps other microbes.

The major soil components affecting the adsorption of bacteria and viruses are the clay and organic matter contents. This is attributable to the large surface area, the negative charge

these particles exhibit and because the micro pores are smaller between particles in these soils (Mawdsley et al., 1996a).

3.5.1 Adsorption capacity of soil

Soil colloids contain two major sources of charges: those created by hydroxyls and other functional groups on the surfaces of the colloidal particles that by releasing or accepting H+ ions can provide negative and positive charges, and those due to the charge imbalance brought about by the isomorphous substitution in some clay crystal structures of one cation by another of similar size but differing in charge.

All colloids, organic or inorganic, exhibit surface charges associated with OH- groups, or those that are primarily pH dependent. Most charges associated with humus, 1:1 clays, and iron- and aluminum-oxides are pH dependent. With 2:1 clays, however, the charges are complemented by a much larger number of charges produced by isomorphous substitution on one cation for another in the octahedral or tetrahedral sheets. Since the charges produced by isomorphous substitution are not pH dependent, they are termed permanent or constant charges (Brady and Weil, 2002).

3.5.2 Adsorption of organic molecules to soil

The large surface area and high number of charged sites on clay particles attract and bind many types of organic molecules. These molecules include substances such as DNA, enzymes, toxins, and even microorganisms. Adsorption of organic materials in believed to take place rapidly (in minutes) and the amount bound related to the type of clay, since sand and silt particles lack the sorption capacity of clay minerals.

The binding of organic molecules to clay minerals typically protects them from enzymatic attack, allowing the molecules to persist in the soil for long periods of time. It has additionally been found that organic molecules retain their biological activity in the bound state. Toxins remain toxic to susceptible organisms, enzymes continue to catalyze reactions, viruses can lyse cells or transfer genetic information to host cells, and DNA strands retain the ability to transform the genetic code of living cells, even while bound to clay minerals and protected from decay (Brady and Weil, 2002).

3.6 *Cryptosporidium parvum*

Cryptosporidium was first documented by Ernest E. Tyzzer in 1907, when he found the parasite in the peptic glands of laboratory mice and considered it to be an extracellular species related to the coccidian protozoa (Tyzzer, 1907).

C. parvum is a protozoan parasite that belongs to the phylum Apicomplexa. Organisms in this phylum are also referred to as coccidian, or single-celled organisms that infect the intestine of both invertebrate and vertebrate animals. While some organisms within this group are considered cyst-forming coccidian because they require extraintestinal development (Fayer et al., 1997), *Eimeria*, *Isospora*, *Cyclospora*, and *Cryptosporidium* complete their life cycle entirely within the gastrointestinal tract of vertebrates. . Among the family of *Cryptosporidium*, there are eight known species of which only *C. parvum* is known to infect both animals and humans (zoonotic).

The structure of *Cryptosporidium*, in general, is unique in comparison to other microorganisms. It is transported in the form of an oocyst, which contains a two-layered membrane. This inner and outer layer membrane provides the organism with great resistance to many environmental factors unlike other microorganisms. The oocyst structure of *C. parvum* is

similar to other coccidian, but also contains a suture at one end of the inner layer. Upon ingestion of mature oocysts, the suture dissolves allowing the life cycle to begin within the small intestine of the host (Fayer et al., 1997).

When Ernest Tyzzer first discovered this protozoan parasite in 1907, he proposed the genus name *Cryptosporidium*. In 1912, he noticed a smaller protozoan parasite that developed only in the small intestine of laboratory mice, which he named *Cryptosporidium parvum*. Its life cycle and presence in the small intestine of other animals were studied for the next several decades (Current and Garcia, 1991; Fayer et al., 1997), but it was not until 1976 when the first cases of human infection by *C. parvum* were reported.

In 1982, there were reports of *C. parvum* infection by 21 males in six large cities across the United States. This was the first major human infection by *C. parvum*. Since the first infection, there have been thousands of reports of human cryptosporidiosis spanning 95 countries including Algeria, Bangladesh, India, Brazil, Austria, Ireland, Egypt, New Zealand, Cuba, Canada, and the United States. Cases have been reported on every continent except Antarctica (Fayer et al., 1997). The outbreaks of cryptosporidiosis have varied dramatically, with each case and the source of contamination being unique.

3.6.1 *Cryptosporidium* life cycle

The *Cryptosporidium* life cycle begins when a host ingests the oocyst. The four sporozoites contained in an oocyst escape, or excyst, and adhere to host epithelial cells that line the gastrointestinal or respiratory tract. For most coccidia, excystation requires reducing internal conditions, exposure to pancreatic enzymes, and bile salts. However, *Cryptosporidium* is unique in that it can survive in normal physiological conditions, with an enhanced excystation in the presence of reducing conditions, exposure to pancreatic enzymes, and bile salts.

One end of each excysted sporozoite adheres to the luminal surface of an epithelial cell until it becomes encapsulated by microvilli, making it intracellular but extracytoplasmic. Each of the four sporozoites from the oocyst form spherical trophozoites and undergo asexual reproduction (merogony) when the trophozoite nucleus divides. Upon completion of the asexual reproduction cycle, six to eight nuclei are formed and mature into a type I meront, a similar structure to the sporozoite. Approximately 30 percent of type I meronts infect another epithelial cell and repeat asexual reproduction, causing the first of two autoinfections, while the remaining 70 percent undergo sexual reproduction to form a type II meront. Type II meronts are the product of sexual reproduction and can either become microgamonts or macrogamonts. Microgamonts, approximately 30 percent of the type II meronts, are thin-walled oocysts that maintain the parasite's survival within the host. They are responsible for the second autoinfection in the gastrointestinal tract. It is the fertilized macrogamonts that develop into thick-walled oocysts and are eventually excreted as mature oocysts in the host's feces (Current and Garcia, 1991; Fayer et al., 1997). Each mature oocyst has a two-layered outer wall, containing four sporozoites, and is approximately 5-µm in diameter (Clark, 1999). Once the oocysts are excreted into the environment, they are viable, completely sporulated, infective, and await a new suitable host. It is at this time that other mammals such as cattle, sheep, and humans become infected with *C. parvum* via fecal-oral route.

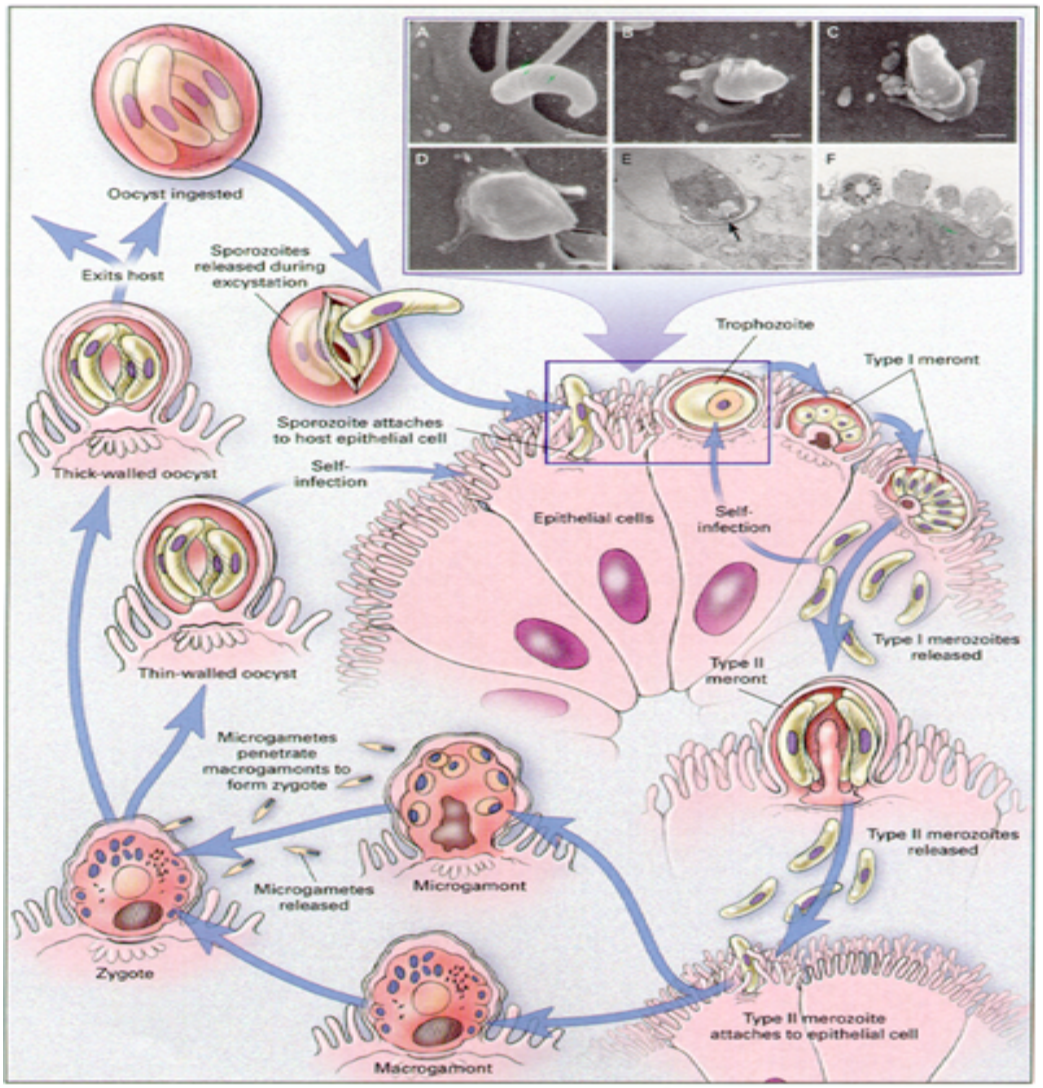


Figure 3.1. Major features of *C. parvum* life cycle (Modified from Heyworth, 1992).

3.6.2 Major sources of *C. parvum*

Currently, there are about 80 mammalian species that can be infected with *C. parvum*. These species include cattle, lambs, goats, water buffalo, horses, dogs, cats, primates, and humans. The number of oocysts necessary to cause infection and cryptosporidiosis varies with species. In calves and lambs, experimental infectious doses of 1.5×10^6 oocysts have caused illness, whereas the average human dose for infection is 132 oocysts (DuPont et al., 1995; Fayer et al., 1997). *C. parvum* oocysts can be transmitted through direct fecal contact with an infected

mammal, animal-to-animal contact, water sources contaminated by animal or human fecal pollution, and infected food sources. There have been many reported cases of each type of transmission. However, disease transmission through environmental contamination of source waters is a major concern because of the ability of *C. parvum* to spread quickly and over a large area in water.

Cattle are commonly considered to be the likely source of *C. parvum* in livestock because of the potential to excrete large amounts of oocysts at a time. However, other livestock animals are susceptible to infection as well. Infection rates and shedding numbers of other livestock animals such as sheep and horses have not been as exhaustively reported as that of cattle. Therefore, the significance of oocyst contributions to watershed contamination by these animals is not completely known. Cryptosporidiosis in lambs, horses, and goats has been reported worldwide while infection in pigs has been limited to 10 countries including the U.S. Similar to cattle, it is the young, often within the first several weeks of life, which experience infection by *Cryptosporidium* (Fayer et al., 1997).

There has also been a concern that non-livestock animals as well as wild animals play a major role in source water contamination. Mechanical carriers capable of infection by *C. parvum* such as birds and insects can infect livestock and contaminate water sources (Fayer et al., 1997). Canada Geese have been examined as carriers for both *Giardia* cysts and *C. parvum* oocysts in the environment. Average concentrations of oocysts found in feces from Canada Geese have been 370 oocysts per gram of feces around the Chesapeake Bay area (Graczyk et al., 1998).

Wildlife in many geographic locations has been suspected to contaminate surface waters. Wildlife poses a direct health risk not only to humans but also to livestock. Infection of livestock

by wildlife may lead to an increased number of cryptosporidiosis cases in both calves and adults, thus increasing the risk of human infection from source water contamination.

3.6.3 Transmission

Cryptosporidium parvum is a protozoan parasite that infects the intestines of humans as well as a variety of wild and domesticated animals (Current, 1986). The infectious form of this parasite is biologically dormant oocysts that are shed in high numbers within the feces of infected animals. Oocysts are resistant to almost all natural environmental conditions (Current, 1986). The resistance of the oocyst allows it to survive for extended periods of time in the environment, giving it the potential to travel long distances, most commonly in water.

Cattle, especially young calves, have been recognized as significant sources of the parasite, because of the high prevalence of infections with this pathogen, and the high number of oocysts that are shed [as much as 2.6×10^{10} per kg feces (Ongerth and Stibbs, 1989; Xiao et al., 1993; Xiao and Herd, 1994; Scott et al., 1994; Schijven et al., 1999)]. One national survey determined that the probability of detecting *Cryptosporidium* oocysts on medium and large (>100 and <200 cows) dairy and beef farms approaches 100% as the number of calves sampled exceeds 15 (Garber et al., 1994). On small farms (up to 100 cows), the probability of finding infected and shedding livestock approaches 80% as the herd size increases from 15 to 100 head. Shedding appears to increase from 4 days of age, peak at day 12, and then decrease. The average number of days for shedding is approximately 3 for natural infections, while it increases to 6 to 9 days for experimentally induced infections (Nydam et al., 2001). A study conducted with a single strain isolated from a calf and propagated by infection of other calves estimated that the infectious dose for 50% of apparently antibody-negative, healthy, human hosts is approximately 132 oocysts (Dupont et al., 1995). However, other research shows that ingestion of

contaminated water containing as few as 10 oocysts can lead to human infection (Olson et al., 1999).

3.6.4 Cryptosporidiosis

The first recorded case of human illness by *Cryptosporidium* was reported in 1976 in a three year old girl from Nashville who had symptoms of vomiting, watery diarrhea, and abdominal pains (Nime et al., 1976). She lived on a small farm and had been in excellent health prior to the onset of symptoms. A rectal biopsy confirmed an infection with *Cryptosporidium*, and she recovered without complications using typical supportive treatment.

Cryptosporidium causes the disease cryptosporidiosis. Transmission can occur directly via the fecal-oral route or through waterborne transmission (Current and Garcia, 1991; Fayer and Unger, 1986; Widmer et al., 1996). Infections occur almost exclusively in the gastrointestinal tract, and with symptoms beginning anywhere from 2 to 10 days following infection and persisting for one to two weeks (Current and Garcia, 1991; Fayer et al., 1997).

In most cases, people experience multiple symptoms including watery diarrhea, nausea and vomiting, abdominal pain, mild fever, anorexia, malaise and fatigue. Frequent bowel movements usually result in water loss causing weight loss and dehydration. Also, feces often contain water and mucus, but not blood. Other symptoms of the illness include headaches, abdominal cramps, fever, and nausea. The severity of the illness is mainly dependent on the immune response of the individual.

For immunocompetent individuals, the illness is usually self-limiting with mild to moderate symptoms for about 7 days, but may last anywhere from 5 to 28 days (Fayer et al., 1997; Marshall et al., 1997). Immunocompromised (immune suppressed) individuals, however, may experience more severe symptoms for a longer duration leading to chronic symptoms of

cryptosporidiosis. Immunocompromised individuals include those with recurring infectious diseases (chicken pox, AIDS, measles), organ or tissue transplants, inherited immune deficiencies, and those receiving chemotherapeutic or other immunosuppressive drugs (Fayer et al., 1997). If the parasite continues an autoinfection process in addition to the already suppressed immune system, the illness can become life threatening (Current and Garcia, 1991).

Transmission of *Cryptosporidium* can continue for one to four weeks after most symptoms have subsided, as oocyst excretion may continue during this time. The overall length of illness and severity of the disease depends on the health of the individual (Mawdsley et al., 1996a; Current and Garcia, 1991; Fayer et al., 1997; Rose, 1997; Goldstein et al., 1996).

3.6.4.1 Treatment of cryptosporidiosis

One of several difficulties with *Cryptosporidium* and its associated illness is the lack of a vaccine or medical cure at this time. Unlike similar species (i.e. *Taxoplasma gondii* and *Eimeria*), there is no curative therapy for cryptosporidiosis, and *Cryptosporidium* is resistant to antimicrobial drugs. One reason for this resistance may be due to the fact that the pathogen creates a unique compartment (intracellular but extracytoplasmic) within the host cell sheltering it from treatment drugs (Griffiths et al., 1998). For immunocompetent individuals, oral or intravenous rehydration is the current treatment to maintain water balance within the body. Hydration is necessary to maintain with any diarrheal illness. For immunocompromised individuals, antiparasitic and antimicrobial drugs have proven ineffective (Current and Garcia, 1991).

3.6.4.2 Infectious dose

The infection of *C. parvum* leading to cryptosporidiosis in humans is based on several characteristics of the pathogen and individual. The immune response of the individual plays an important role in the infectious dose necessary to cause illness. Studies on dose response have shown that the average number of *C. parvum* oocysts needed for infection is 132 with no prior infection; however, as few as 10 oocysts can lead to human infection (Olson et al., 1999).

Considering large outbreaks of cryptosporidiosis have resulted from contamination of source waters, it is important to understand the consequences of a repeated infection to contaminated drinking water. A study investigating the immune response of healthy individuals exposed to *C. parvum* two times, separated by 1-year duration, was performed to simulate the seasonal occurrence of *C. parvum* infection and to determine the development of protective immunity in humans against *C. parvum* after initial infection. Even though individuals shed lower numbers of detectable oocysts and the clinical severity of the illness was reduced, individuals were still susceptible to *C. parvum* infection upon a second exposure. It was noticed that there was a shift in IgM (temporary) response to IgG (long-term) reactivity, but one exposure was not enough to develop complete immunity against *C. parvum* (Okhuysen et al., 1998). Therefore, the risk of infection and re-infection by *C. parvum* is a considerable health risk to the public.

3.6.5 *Cryptosporidium* in the environment

The primary environmental concern with *C. parvum* is its resistance to many environmental stresses for long periods of time. The oocyst has evolved to survive in harsh environmental conditions, which is the key factor for its transport and ability to survive within the environment. Its resistance to temperature and various chemicals has made it a major water

quality concern. Studies have shown that oocysts remain infectious in both cold and warm water temperatures. As studied by Nasser et al. (2003a), the persistence of *Cryptosporidium* in stream water was not influenced by temperature and no change was observed in the concentration of the oocysts over a 30 day incubation at 15 ºC and 30 ºC. Likewise, Olson et al. (1999) found that oocysts could survive in water and soil for more than 12 weeks at -4 ºC and 4 ºC, with an accelerated degradation at 25 ºC.

With the ability to survive environmental stresses for extended periods of time, it is no surprise that *Cryptosporidium* is commonly found in water and wastewater treatment plants. While conventional treatment practices are designed to eliminate solids, nutrients, and some microbial organisms, *C. parvum* oocysts have an inherent resistance to current practices such as chlorination, flocculation and sedimentation (Mawdsley et al., 1995; Goldstein et al., 1996). This has led to the search for more efficient removal methods. LeChevallier (1991) studied the effects of newly designed slow sand filter treatments and found that oocysts were still present in the effluent. In addition, a study by Logan et al. (2001) showed that the property that affects the removal of oocysts in sand bed filtration is particle size. Logan et al. (2001) concluded that sand filters, when designed properly, are adequate in removing oocysts from wastewater at relatively high concentrations.

Another key survival characteristic of *C. parvum* is the size and structure of the oocyst. Due to the small size and near neutral buoyancy, oocysts can remain suspended in water for extended periods of time with the potential to travel long distances (Walker et al., 1998). The oocyst also contains a negative surface charge indicating low hydrophobicity and the potential to be carried in surface water (Drozd and Schwartzbrod, 1996). A study by Medema et al. (1998) found that both free and attached oocysts remained in suspension (water) due to their relatively

slow settling velocities compared to larger particle, thus increasing the chances of transport in water.

The outer membrane structure of the oocyst wall may change in chemical composition over time, increasing its survival rate in the environment (Brush et al., 1998). The structure and composition of the oocyst also prevents inactivation by various chemical treatments. Many chlorine-based chemicals such as chlorine, monochloramine, and chlorine dioxide at typical applied concentrations and conventional treatment disinfection times have little effect on oocyst inactivation (Fayer et al., 1997). Zimmer et al. (2003) found that *C. parvum* was very sensitive to low doses of UV irradiation, but because of its high cost, it is not typically used. Therefore, conventional drinking water treatment alone cannot guarantee the complete removal of *C. parvum* oocysts from finished drinking water supplies, increasing the risk of disease transmission.

3.6.5.1 Cryptosporidium in water sources

There are various ways *Cryptosporidium* can reach watercourses, including direct leakage of a drainage system from an animal waste storage facility or livestock barn, application of manure as a fertilizer to agricultural fields, direct runoff from animal production facilities, or by direct contamination by animals (Mawdsley et al., 1995; Kuczynska and Shelton, 1999; Graczyk et al., 2000). Livestock, especially cattle, are thought to be a major source of oocysts because of their numbers, distribution, incidence of infection, and because they are capable of producing up to 10 billion oocysts per gram of feces (Olson et al., 1999; Kuczynska and Shelton, 1999).

In 1997, confined beef and dairy cows produced over 300 million tons of manure in the U.S. (Bradford and Schijven, 2002). With this type of production and the potential for just a few

infected calves to contaminate a large water supply, the potential for human infection is enormous. Open range animals such as cattle provide a concern for direct oocyst contamination in locations containing rivers, lakes, and streams. Indirect contamination to surface water is caused by surface runoff carrying agricultural or human pollutants. Graczyk et al. (2000) studied dairy and beef cattle in Pennsylvania that were allowed to wade and graze near streams and found that 64% of the farms tested positive for the presence of oocysts in at least one manure sample and 44% were positive in all samples. In addition, Hansen and Ongerth (1991) found that the concentration and production rates of the downstream area influenced by dairy farming were nearly 10 times higher than rates at upstream stations, and that watershed character and management affected surface water oocyst concentrations significantly.

Hansen and Ongerth (1991) found that oocyst concentrations in watersheds of appreciable size were continuous as opposed to intermittent and that seasonal factors, including runoff of land drainage, may affect oocyst concentrations by 10-fold, with concentrations in drier periods being significantly lower than those in wetter periods. In addition, the character and intensity of both human and domestic animal activities in a watershed may affect oocyst concentrations in the surface water by as much as 10- to 15-fold.

C. parvum has been known to travel long distances, therefore making surface runoff a major concern for source water contamination. Some studies have indicated that 97 percent of surface waters that serve as water for treatment plants and 54 percent of filtered water contain some low numbers of oocysts (Goldstein et al., 1996). The presence of *C. parvum* in surface waters is ubiquitous. Reducing and preventing contamination of surface water sources is therefore a primary concern for public health.

While surface water is the quickest and most direct route of contamination of drinking water supplies, there may also be potential for groundwater contamination. Shallow groundwater can potentially be contaminated directly from surface waters, or contamination can occur with seepage into the ground through permeable soils. A study showed that of 463 groundwater samples from across the United States, a low level of *Cryptosporidium* is frequently detected (Harter et al., 2000). Laboratory experiments testing the transport of *C. parvum* through soil columns, however, have had mixed results. Mawdsley et al. (1996a) conducted a study to examine the transport of oocysts through three different soil types and detected trace levels in shallow subsurface drainage. However, Kalita et al. (2002) examined oocyst transport through soil columns and found that oocysts reaching tile or groundwater sources is unlikely. Oocysts were only detected in the discharge of the columns (3 ft. depth) when an exceptionally high concentration (10^{11}/mL) of oocysts was applied to the surface. Additionally, Nasser et al. (2003b) found that the highest percentage of *Cryptosporidium* oocysts was recovered from the upper 3 cm of soil, indicating a low probability of oocysts reaching groundwater when water table levels are typically several meters below the soil surface. The same study showed that a high microbial activity led to the destruction of oocysts in the soil matrix. And, even though *Cryptosporidium* oocysts might survive for prolonged periods in saturated soil, they are efficiently retained in loamy soil, hindering their access to groundwater. Nasser et al. (2003b) attributes the strong removal of *Cryptosporidium* in the soil columns to physical sieving of the oocysts (by clay perhaps) because of poor adsorption to loamy soil,. Further understanding of the mechanisms that govern the movement of oocysts through soil is needed to develop best management practices (BMP) to prevent the migration of oocysts from agricultural areas to watercourses and subsequent drinking water sources.

3.6.6 Water quality concerns

Water contamination by microbial agents has been associated with waterborne disease for over a century. The first studied waterborne disease was cholera in the 1860's in England, which was the result of a bacterial agent (Rose, 1997). Since that time, there have been other waterborne diseases by microbial agents, including pathogenic organisms. As reported by the American Society of Microbiology, illnesses related to waterborne infectious disease affects up to 900,000 individuals each year in the U.S. It has also been estimated that up to 900 Americans die each year from waterborne diseases (American Public Health Association, 2001).

Intense research on the fate and transport of *C. parvum* in water sources was initiated after the largest waterborne outbreak of cryptosporidiosis in U.S. history in Milwaukee, WI. After this outbreak, there was a movement to develop measures for detection, recovery, destruction, prevention, and treatment to reduce the number of outbreaks and cases of *C. parvum* infection nationwide (Fayer et al., 1997).

Because low levels of *Cryptosporidium* oocysts have been detected in 65%-97% of surface-water supplies, there is concern that most populations may be at risk for waterborne infection (Juranek, 2007). However, some researchers believe that reducing low-dose waterborne exposures may increase rather than decrease the risks of diarrheal and gastrointestinal illnesses. Frost et al. (2005) support this claim by explaining that serological responses appear to develop after exposure to *Cryptosporidium* oocysts, even in the absence of clinical illness (Chappell et al., 1999). They also found that sources other than drinking water may commonly transmit *Cryptosporidium*. In fact, they found that food and other modes of parasite transmission may be at least as important as drinking water and may be more likely to transmit higher dose exposures. They believe that the complete removal of pathogens from

drinking water may decrease the risk of waterborne enteric illnesses but increase the risks from non-waterborne exposures to the same pathogens.

3.6.7 Diagnosis of cryptosporidiosis

An additional difficulty that arises when dealing with *Cryptosporidium* is the difficulty of properly identifying cases of cryptosporidiosis. Laboratory identification of infection is hampered because a large number of oocysts per gram of stool are needed to reliably confirm infection (Weber et al., 1991). During the large waterborne outbreak in Milwaukee, there were an estimated 403,000 cases of cryptosporidiosis, 4,400 hospitalizations, and 100 deaths, but only 285 laboratory-confirmed infections (Mackenzie et al., 1994; Hoxie et al., 1997). The uncertainty about the importance of various modes of transmission is largely due to the difficulties in diagnosing infection. Large waterborne outbreaks that infect many people, however, may be more likely to be detected than small foodborne or person-to-person outbreaks, because of an increased chance of correct identification.

3.6.8 Outbreaks of cryptosporidiosis

Throughout the last decade, outbreaks of *C. parvum* have increased dramatically introducing thousands of individuals to cryptosporidiosis. The sources of the outbreaks vary from direct contact with infected livestock to contamination of source waters.

From 1991 to 2002, *Cryptosporidium* was responsible for 15 outbreaks, or 7% of all waterborne disease outbreaks in the United States. Perhaps more importantly, *Cryptosporidium* was the cause of 94% of all reported waterborne illnesses during that same time period, accounting for 408,371 cases (Craun et al., 2006). This number does not include those cases where individuals experienced major symptoms of cryptosporidiosis but tested negative for

oocyst fecal contamination. However, since reports of cryptosporidiosis are voluntary, not all cases are reported many cases are not even identified by the general public due to the unfamiliarity of the pathogen and illness symptoms. Documentation of previous outbreaks, however, shows that *C. parvum* is geographically widespread throughout the United States (Dietz and Roberts, 2000).

The high prevalence of *C. parvum* in water sources has led to an increase in the number of infectious outbreaks worldwide. From the first identified cases of cryptosporidiosis in 1976, there has been a dramatic increase in *C. parvum* infection through many transmission routes. Even though there have been cases of outbreaks in daycare facilities and nursing homes through possible fecal contact, the majority of outbreaks are waterborne, and primarily due to consumption of drinking water that can be traced to a variety of water sources. Recently, the number of outbreaks due to recreational waters has also increased, indicating the deposition of human oocysts into the environment and transmission from person to person.

Many outbreaks of cryptosporidiosis go unreported because individuals are not aware of the symptoms associated with the illness. Therefore, the reported cases of waterborne cryptosporidiosis underestimate the total number of *C. parvum* infections that have taken place in the U.S. and worldwide.

3.6.8.1 Drinking water outbreaks

The majority of *Cryptosporidium* outbreaks have occurred from drinking water that has been contaminated by *C. parvum*. Depending on the source, the number infected may range from a few to several thousand individuals.

The largest waterborne outbreak of cryptosporidiosis took place in the spring of 1993 in Milwaukee, WI when approximately 400,000 people were infected and 100 individuals died

from infection by *C. parvum*. The source of the outbreak was found to be surface water from Lake Michigan contaminated by cattle located along two rivers that flow into the Milwaukee Harbor, slaughterhouses, and human sewage (MacKenzie et al., 1994). This outbreak was the catalyst for government-mandated initiation of prevention and treatment of *C. parvum* in water supplies.

Between 1990 and 1995, there were multiple reports of cryptosporidiosis in Las Vegas, Nevada. The number of cases varied from 6 to 100, with the primary outbreak between January and April of 1994. In 1994, 78 individuals became infected with *C. parvum*, 63 of whom were HIV-positive. Samples from the drinking water facility as well as Lake Mead were examined for the presence of *C. parvum* oocysts, but oocysts were not detected during any of the reported cases. Regardless, the CDC concluded the public water supply was the source of the outbreak based on epidemiological data (Roefer et al., 1996).

Another outbreak took place in Clark County, Nevada between January 1993 and November of 1994. In this outbreak, 148 individuals were infected and 20 died as a result of cryptosporidiosis. Even though turbidity values at the treatment plant never exceeded the standards, it was concluded that surface water contamination must have taken place at some time before entering the water treatment plant (Goldstein et al., 1996).

In 1991, an outbreak occurred in Berks County, PA in which 551 individuals were infected with *Cryptosporidium* at a picnic facility. Raw samples of the water tested positive for fecal coliform and various surface water indicators of contamination. In addition, stool samples from the infected individuals tested positive for *C. parvum* (Solo-Gabriele and Neumeister, 1996).

Other *C. parvum* outbreaks related to drinking water have been reported in locations throughout the U.S. including Florida, Wyoming, New Mexico, Ontario, and Washington from contaminated surface water (Gallaher et al., 1989; CDC, 1996; Solo-Gabriele and Neumeister, 1996).

3.6.8.2 Recreational water outbreaks

Over the past several years, there has been an increase in outbreaks in recreational waters due to human infection of *C. parvum*. Recreational water outbreaks have confirmed that *C. parvum* transmission is not only zoonotic, but can also take place from person to person. In most cases, infection from recreational waters has resulted from individuals ingesting water that has been contaminated with human fecal material.

In the summer of 2000 alone, there were 5 reported outbreaks of cryptosporidiosis related to swimming pools in which two of the outbreaks resulted in approximately 1,000 cases of *C. parvum* infection (CDC, 2001). The increase in outbreaks associated with recreational waters indicates the prevalence of *C. parvum* infection within the human population that can potentially be transmitted between individuals. It also indicates the lack of oocyst inactivation by simple chlorine treatment in recreational waters.

One of the most recent outbreaks took place in Peoria, Illinois where 51 individuals were infected with *C. parvum* from a water park (Splashdown Aquatic Center) at the beginning of August, 2001. During this time, there were also a few reported cases of cryptosporidiosis in Rockford, Illinois that may have been associated with Magic Waters water park. The number of cases for the state of Illinois has increased over the last few years with 84 cases in 1998 to 90 cases in 1999, and 126 cases in 2000. However, the Peoria outbreak is the first reported case that has been traced to recreational waters (Smothers, 2001).

In the summer of 2005, after numerous reports by patrons of gastrointestinal upset, a water park at Seneca Lake State Park, in the Finger Lakes region of upstate New York, was found to be the source of a waterborne outbreak. There were nearly 4,000 cases in 35 New York counties reported, with over 600 confirmed cases with symptom onset dating back to June. An apparent irony of this outbreak is that while the Seneca Lake facility was just three years of age, there appears to have been little to no consideration of *C. parvum* control in its design. Investigators found *C. parvum* oocysts present in two underground water tanks that recycle water within the spray park. In addition to using recycled water, the facility lacked the high-level filtration and water-sanitizing systems necessary to limit proliferation of *Cryptosporidium*. Many victims of the outbreak visited the park several times during the outbreak period and suffered re-infection with crypto while remaining oblivious to the nature of their gastrointestinal problem. State health investigators believe the original vector for crypto in this setting was an infected patron (Clark, 2007).

The Utah Department of Health (2008) received reports of 1,902 laboratory-confirmed cryptosporidiosis cases through passive surveillance from June through December 2007. Cases were identified through reports to local health departments or laboratory reports to UDOH. Onset dates were known for 1,601 (84%) patients and ranged from May 23 through November 11. Patients were residents of all 12 local health districts, and the highest age-specific rates were among children aged <5 years. Recreational water exposures were reported by 80% of patients, and multiple venues were named. As the outbreak progressed, the proportion of patients reporting contact with a person ill with similar symptoms increased; overall, 55% of patients reported exposure to ill contacts during their exposure period. Evidence did not support the occurrence of other modes of transmission (e.g., drinking water, contaminated food or drink, or animal-to-person).

Throughout the Summer of 2008, many public swimming areas, water parks, and public pools in the Dallas/Fort Worth Metroplex of Texas suffered an outbreak of Cryptosporidiosis. Burger's Lake in Fort Worth was the first to report such an outbreak. This prompted some if not all city-owned & private pools to close and hyperchlorinate. To date, there have been 400 reported cases of *Cryptosporidium* (St. James, 2008).

3.7 Rotavirus

Rotaviruses were first identified in humans in 1973, when characteristic particles were observed in the cytoplasm of duodenal (beginning portion of small intestine) epithelial cells obtained from young children admitted to the hospital for treatment of acute diarrhea. Since then, numerous epidemiological studies have confirmed that rotaviruses are the major cause of severe acute diarrhea in children throughout the world (Bishop, 1994; Kapikian and Chanock, 1990).

Rotaviruses are wheel-shaped viruses classified as a genus within the family *Reoviridae*. They contain double stranded RNA (dsRNA) that can be separated into eleven distinct bands by gel electrophoresis. Each of the eleven distinct bands represents a rotavirus gene that codes for a single structural or non-structural viral protein (Estes, 1990). The complete infectious particle is composed of six structural proteins assembled as a triple layer (Figure 3.2). The three layers comprise an inner core of VP1 (viral protein), VP2, and VP3, and an inner capsid of VP6 and an outer capsid of a glycoprotein VP7 that is penetrated by 60 spikes of VP4. Rotaviruses have been subdivided into five groups (Groups A, B, C, D and E) based on antigens present on VP6. Group A rotaviruses have been further subdivided on the basis of antigens on VP6 (subgroup I, II), on VP7 (G-types), and on VP4 (P types). Human infections with Groups A, B, and C viruses

have been recorded. Most severe acute diarrhea in children is caused by rotaviruses of Group A, subgroups I or II, G types 1-4, and P types 1A (Bishop, 1994) or 1B (Bartlett et al., 1985a).

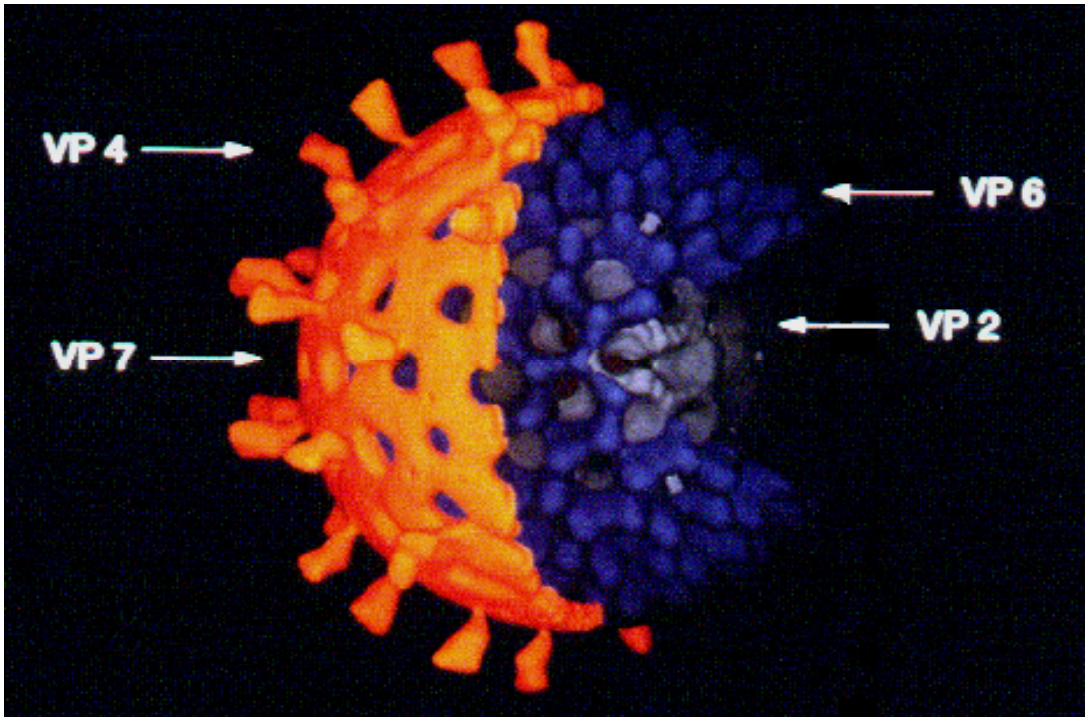


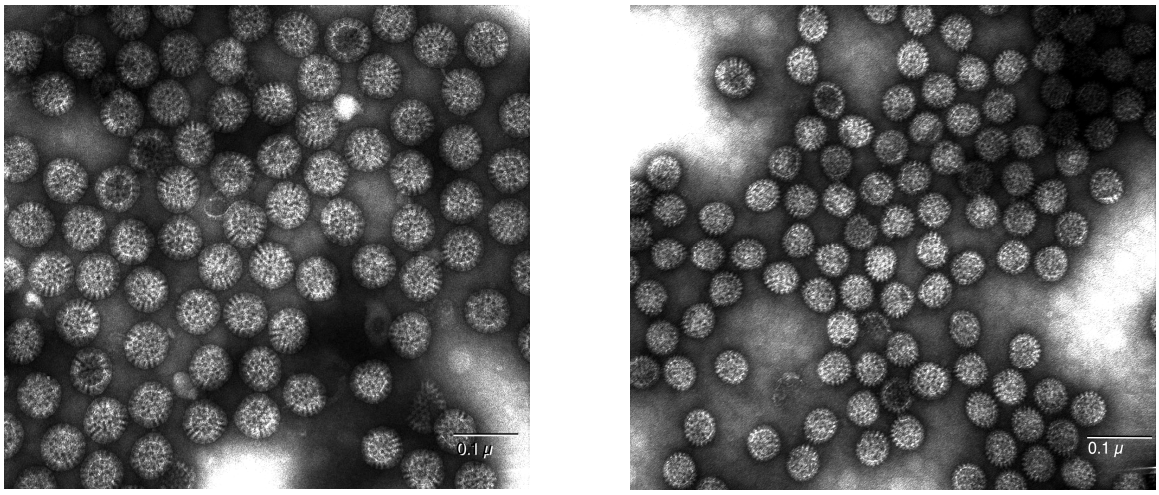
Figure 3.2. Rotavirus capsid structure. The three layers of the viral particle are denoted by orange, blue, and black. Major viral proteins are labeled accordingly (From Prasad et al., 1990).

3.7.1 Rotavirus replication cycle

The replication of rotavirus takes place entirely in the cytoplasm of the cell, in contrast to *Cryptosporidium* which is extracytoplasmic. Rotaviruses tend to affect gastrointestinal epithelial cells that are at the tip of the villus. Their triple-layer protein coats make them very resistant to the normally prohibitive pH of the stomach, and also digestive enzymes (lipases and proteases) in the gastrointestinal tract.

The replication cycle begins with the adsorption of rotavirus to cellular receptors, allowing receptor-mediated endocytosis, or direct penetration into a vesicle known as an endosome. Proteins in the third layer (VP7 and the VP4 spike) disrupt the membrane of the endosome, creating a difference in the Ca^{2+} concentration. This facilitates the breakdown of VP7

trimers into single protein subunits, leaving only the VP2 and VP6 coats around the viral dsRNA, forming a double-layer particle (DLP) (Figure 3.3).



(a) **(b)**

Figure 3.3. Photomicrographs of rotavirus particles. (a) Triple-layered rotavirus particle (TLP). (b) Double-layered rotavirus particle (DLP). The TLP is infectious while the DLP is not, lacking the outermost layer responsible for binding to the host epithelial cell. (Pictures contributed by M. Kuhlenschmidt, Department of Pathobiology).

Messenger RNA (mRNA) is then produced in the cytoplasm from double-layered subviral particles. The mRNA is translated to synthesize the 11 proteins, six of which are structural and five which are nonstructural. Assembly of double-layered particles containing VP1, VP2, VP3, and VP6, and a full complement of 11 single-stranded RNAs (ssRNAs) begins. Double-stranded RNA is then formed through the process of replication. Rotavirus undergoes a maturation process to form triple-layered particles. The envelope is removed, and the virus is liberated by cell lysis. The rotavirus particles are once again infectious and capable of beginning the replication cycle over again. Figure 3.4 shows the rotavirus replication process.

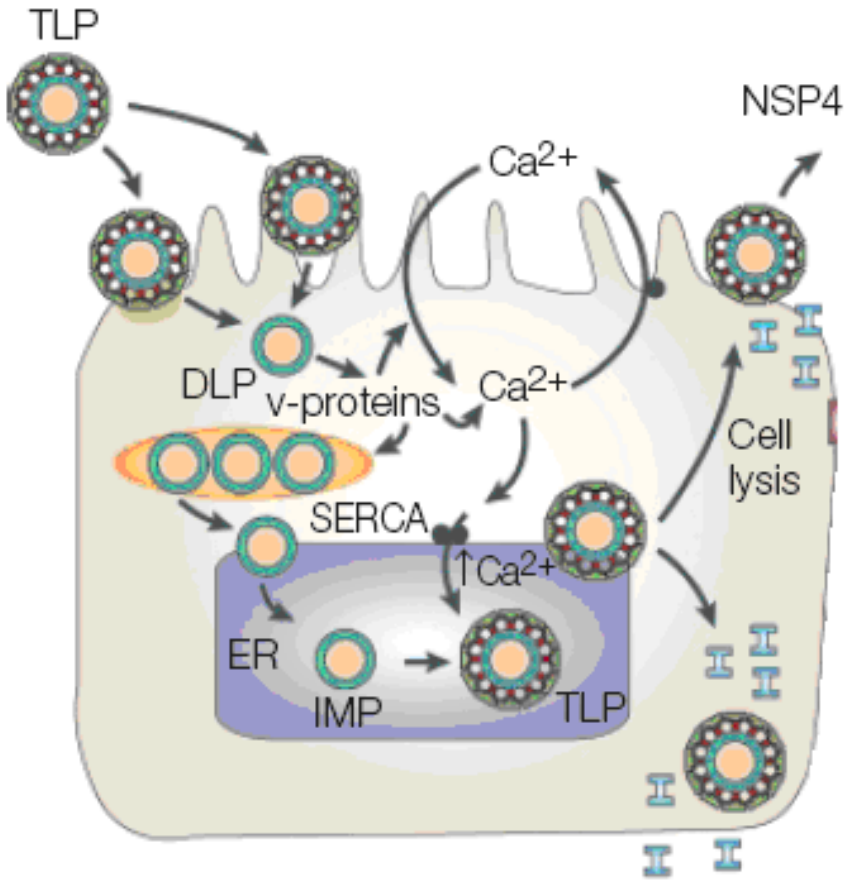


Figure 3.4. Major features of rotavirus replication cycle (Mod. from Bomsel and Alfsen, 2003).

3.7.2 Transmission

Rotaviruses are transmitted mostly by the fecal-oral route, meaning the rotavirus is passed in the feces of one host, and then ingested orally by another. A high degree of resistance to physical inactivation, the large number of virus particles shed, and the very low infectious dose required ensure that transmission easily occurs through environmental sources. This is apparent by widespread nosocomial infections once a hospital ward has been contaminated. In addition, animals infected by various rotavirus types may act as a reservoir for human rotavirus infections.

Rotaviruses are highly infectious when transmitted within the same species. Replication within the intestinal tract can result in shedding of 10^{10} plaque forming units (PFU) per milliliter

of feces. Rotaviruses are very durable in the environment and can survive for weeks in potable and recreational water (Ansari et al., 1991).

The infectious dose for the human small intestine has been calculated as approximately 10 PFU per milliliter (Graham et al., 1987). Rotaviruses show loss of infectivity at a pH less than 4, so the number that must be ingested to ensure passage of an infectious dose through the stomach is not known.

Transmission of rotavirus infection in a susceptible community can occur extremely rapidly. For example, during a multi-island outbreak in the islands of Truk Atoll in the Pacific, rotavirus infections spread from the individuals initially infected to at least 31% of the population on one island within one week (Foster et al., 1980). Additional "explosive" outbreaks have also been observed in Native American and Brazilian communities (Kapikian and Chanock, 1990; Santosham et al., 1985).

High nosocomial infection rates have been recorded in most pediatric hospitals and daycare centers (Bartlett et al., 1985a; Pickering et al., 1988). Although respiratory spread has been suspected, it appears more likely that infections result from airborne spread after aerosol formation of particles, such as from diapers or toilet flushing. Circumstances have occurred where waterborne viruses have been presumed to have caused wide-spread infection, such as after the floods in Bangladesh (Ahmed et al., 1991).

There is now evidence that transmission of animal rotavirus to humans is possible, perhaps as whole virions, but more commonly by a process of genetic reassortment (Nakagomi and Nakagomi, 1993). One of the most common sources of interspecies infections appears to be domestic animals. Gene segments derived from canine or feline strains have been detected in human rotaviruses from infections in Israel and Japan (Nakagomi and Nakagomi, 1991). Early

experimental attempts at cross-species infection noted the apparent ease with which human rotaviruses could be adapted to infect dogs (Smith and Tzipori, 1979). The close physical contact that is common between human infants and domestic animals could promote this exchange, and has been identified as a risk factor for rotavirus diarrhea in some conditions (Engleberg et al., 1982).

Human infection with reassortants possessing bovine (or occasionally porcine or avian) genes has been recorded in Italy, India, Indonesia, Finland, Thailand and the U.S. (Nakagomi and Nakagomi, 1993). Most strains have appeared as single isolates and have not spread within communities where they were identified. It is possible that these represent sporadic interspecies transmissions, where the resulting reassortant has either no, or only limited, capacity for secondary transmission and hence does not result in production of an outbreak. An exception to this may have occurred in India where infections with a human-bovine reassortant strain have become endemic in a neonatal nursery (Das et al., 1993).

In summary, current data support the belief that interspecies transmission of whole viruses or reassortant viruses occurs, can be responsible for severe disease in some infants, but is unlikely to involve strains that have the capacity to cause significant outbreaks of disease. By contrast, reassortment between human genogroups may occur more commonly than is realized, and may sometimes result in strains able to cause epidemiologically important outbreaks.

3.7.3 Rotavirus pathogenesis

Rotavirus infects primarily the mature absorptive enterocytes in the proximal two-thirds of the ileum (lower portion of small intestine), and is thought to cause diarrhea by several mechanisms. Virus-associated cell death followed by sloughing of the villus epithelium and proliferation of the secretory crypt cells can result in reduced absorptive capacity of the gut,

leading to fluid and electrolyte loss into the lumen. Epithelial dysfunction can lead to reduced expression of certain digestive enzymes such as sucrase and isomaltase, causing the osmotic pull of accumulated sugars in the small intestine to further exacerbate fluid loss. A non-structural protein (NSP4) expressed by rotavirus is thought to trigger an intracellular calcium-dependent signaling pathway, which leads to an increased membrane permeability to electrolytes. Furthermore, rotavirus has been shown to activate the secretormotor neurons of the neteric nervous system that stimulate secretion of fluids and solutes, an effect recently found to be mediated via vasoactive intestinal peptide (Kordasti et al., 2004).

After a short incubation period of 24-48 hours, the onset of illness is sudden, with watery diarrhea, vomiting, and rapid dehydration. Effective treatments include oral or parenteral rehydration with oral rehydration solution formula. In addition, some drugs are an effective treatment for rotavirus illness (Desselberger, 1999). The disease process takes from five to seven days (Greenberg et al., 1994).

Acute rotavirus infection is followed by a virus-specific, humoral immune response comprising immunoglobulin IgM, IgG, and IgA antibodies, and by a cell-mediated immune response of rotavirus-specific cytotoxic T-cells in the lamina propria of gut tissue.

Rotavirus infection has traditionally been thought to be limited to the gastrointestinal tract, but several recent case reports have challenged this paradigm. Evidence of rotavirus RNA has been found by polymerase chain reaction in the cerebrospinal fluid of rotavirus-infected children who have seizures (Iturriza-Gomara et al., 2002) and in liver and kidney sections of immunocompromised children (Gilger et al., 1992). The clinical significance of these findings remains unclear but remains under investigation.

3.7.3.1 Rotavirus seasonality

Clinical rotavirus disease in humans can be accompanied by shedding of more than 10^{12} rotavirus particles per gram of feces. The virus is highly infectious and appears to retain infectivity over many months. In temperate climates, disease is most common during the colder months, when it is likely that rapid spread within families and communities occurs. Nosocomial (hospital-acquired) infections are frequent, and rotaviruses can become endemic within obstetric hospital nurseries for the newborn.

Climatic factors exert a major influence on the incidence of rotavirus disease (and infections) in areas where there are distinct seasonal changes. Rotavirus infections are noticeably more common in the cooler months of the year, with peak months varying from country to country and from year to year (Cook et al., 1990). In countries with tropical climates, rotavirus disease is present throughout the year. The differing seasonal patterns in tropical and temperate climates may point to differing patterns of transmission and perhaps to differing reservoirs of infection (Cook et al., 1990). In general, tropical countries are also the most populous, with high birth rates, crowded living conditions, and climates optimal for rotavirus survival. The seasonality observed in temperate climates may result more from the occurrence of unfavorable conditions for transmission in summer months (with higher ambient temperatures, low relative humidity and less crowding) rather than from a direct effect of cooler weather favoring transmission. A study conducted for one season across the U.S. noted that the peak rotavirus month varied from southwest to northeast, suggesting that areas to the south served as the source of annual epidemics once the hot dry summer weather had ceased (LeBaron et al., 1990). It is also possible that cold weather can have physiological effects that exacerbate the

symptoms of rotavirus infection. For example, inducing hypothermia in piglets enhances the severity of symptoms of rotavirus disease (Woode, 1982).

There is abundant evidence that the occurrence of rotavirus disease is influenced by seasonal factors, and that in temperate climates the disease is influenced by seasonal factors and is more prevalent in the cooler months. The mechanism underlying this seasonal variation is complex and probably involves the interplay of many factors including survival of virus in the environment, physiological effects on the host, and degree of crowding of potential hosts. It is worth noting that rotavirus disease in infant laboratory mice and in premature newborn babies has also been observed to be seasonal (Bishop, 1994; Woode, 1982), even though both live in controlled environments that appear unaltered year-round. It is not clear if the controlled-environment specimen had exposure to people or animals from external sources, which could introduce rotavirus during high-prevalence seasons.

3.7.4 Rotavirus disease

Rotaviruses, members of the *Reoviridae* (Matthews, 1979), have been identified as the major causative agents of acute infantile gastroenteritis in humans and a wide variety of animals (Estes et al., 1984; Kapikian and Chanock, 1985). It has been estimated that these viruses account for over a million human deaths annually in developing countries (Snyder and Merson, 1982; De Zoysa and Feachem, 1985; Kapikian and Chanock, 1985).

Prior to 2000, it was believed that rotaviruses caused approximately 22% of childhood diarrhea hospitalizations worldwide (Parashar et al., 2003). However, from 2000 to 2004, this proportion increased to 39% (Parashar et al., 2006). Application of this proportion to the recent World Health Organization estimates of diarrhea-related childhood deaths gave an estimated 611,000 rotavirus-related deaths. It is further explained that this likely reflects a relatively

slower rate of decrease in hospitalizations for rotavirus compared with other causes of severe childhood diarrhea. This slower rate of decrease could be due to improvements in hygiene and sanitation that are likely to have a greater impact on diarrhea caused by bacterial and parasitic agents, which are transmitted primarily through contaminated food or water, unlike rotavirus, which is often spread from person-to-person through either direct physical contact, touching infected surfaces, or sharing items of personal use (Parashar et al., 2006).

Each year in the U.S., rotavirus infection results in the hospitalization of an estimated 70,000 children, 160,000 emergency room visits in children younger than five, and half a million visits to doctor's offices. It is estimated that 100 children die each year in the U.S. from complications of rotavirus infection (CDC, 2005). Also, the Centers for Disease Control and Prevention (CDC, 2005) explains that rotavirus affects populations in all socioeconomic groups and is equally prevalent in industrialized and developing countries.

3.7.4.1 Age Range of Human Rotavirus Infection

Rotavirus infection of humans can occur from birth to old age, with the vast majority occurring from 2-24 months of age for humans. Rotavirus infection is endemic among newborn babies in obstetric hospital nurseries in many countries, including Australia, U.K., U.S., Sweden, India, and Venezuela (Bishop, 1994).

Neonatal infections appear to be nosocomial in origin because they are rarely seen in babies born at home. Results of serological surveys show that most children have experienced a rotavirus infection by 24 months of age (Bishop, 1994; Simhon et al., 1990). Sources of infection of young children include siblings and adult family members as well as children and adults within the community (Koopman et al., 1989).

There is strong evidence from serological studies and longitudinal surveillance studies that rotavirus infections pass from member to member of a family, regardless of age. Studies of children (0-3 years of age) in daycare illustrate the frequency with which young children can be sporadically infected (Bartlett et al., 1985a; Bartlett et al., 1985b). The broadening of the spectrum of serum neutralizing antibody with age (to include antibody to more than one G type) also implies repeated rotavirus infections (Brussow et al., 1990).

By contrast with the capacity of rotaviruses to cause infection at any age, the clinical consequences of infection appear to be strongly influenced by age. For example, most neonatal infection is asymptomatic or produces only mild symptoms in healthy full term neonates (Bishop, 1994). This cannot be entirely due to the influence of breast feeding, because asymptomatic rotavirus infections have been observed in babies fed artificial milk formula (Duffy et al., 1986).

The severity of neonatal infection is likely determined by a number of factors including the neutralizing antibodies in serum and breast milk, maturity of the infant gut and immune system, presence of non-specific rotavirus inhibitory factors in the lumen, and virulence of rotavirus strains endemic in neonatal nurseries. Breast feeding is associated with a decrease in severity of symptoms and with a decreased incidence of rotavirus infection. A recent case-control study in rural Bangladesh has concluded that the latter effect of breast feeding is mainly attributable to postponement of rotavirus infection, and that the majority of infants have no permanent immunity once breast feeding ceases (Clemens et al., 1993).

The most severe symptoms caused by rotavirus infection are experienced by children between the age of 6 and 23 months (Oyejide and Fagbami, 1988; Rodriguez et al., 1987). The resistance to clinical symptoms that is observed in older children and adults is likely not due

solely to age-altered physiological status of the gut. Severe symptoms have been observed in adults in community outbreaks and in aged people in nursing homes (Hrdy, 1987; Kapikian and Chanock, 1990). It is more likely that age-related resistance to severe rotavirus is due to active immunity, reinforced by repeated infection throughout life.

3.7.5 Rotavirus vaccination

Rotavirus infection remains the most common cause of severe, dehydrating gastroenteritis among children worldwide. Almost every child in the world, in both developed and developing countries, will be infected with rotavirus in the first five years of life. Globally, more than 500,000 children die every year from rotavirus gastroenteritis, with the vast majority of these deaths occurring in the poorest countries. In developed nations, rotavirus infection rarely results in death but remains the most common cause of hospitalizations for acute gastroenteritis in children and leads to major medical and societal costs. At the current manufacturer's price of $62.50 per dose, or $217.50 per child, vaccination may not be a feasible option for persons in developing nations. However, the reduced burden on the healthcare system may be a large enough benefit to administer the vaccine to the masses in developed nations.

3.7.5.1 Rationale for vaccination

Review of the natural history of rotavirus infection shows that a child contracts its first infection with rotavirus early in life. This first infection is usually the most clinically severe and results in immunity against subsequent illness. In addition, this protective immunity against severe disease is boosted by subsequent infections. The rationale for vaccination, then, is that administration of an attenuated rotavirus strain early in life will mimic the initial natural infection and induce immunity. An additional reason for this vaccine approach is that while oral

rehydration therapy may be effective in the prevention of severe dehydration and death in poorer countries (Kosek et al., 2003), it has had limited impact on diarrheal hospitalizations in the U.S. Furthermore, the economic burden of rotavirus illness is huge.

In the United States every year, 3 million children <5 years of age develop rotavirus disease, of whom approximately 700,000 seek health care. This produces a total cost of approximately $300 million to the health care system and nearly $1 billion to society (Widdowson et al., 2007; Tucker et al., 1998). In addition, Widdowson et al. (2007) conclude that a mature rotavirus immunization program would prevent almost two-thirds of all serious rotavirus disease but, at the current manufacturer's list price, would almost certainly result in net costs to the health care system and would be unlikely to be cost-saving from the societal perspective, realizing that few children die as a result of rotavirus disease in the United States.

3.7.5.2 Tetravalent Rhesus-based Rotavirus Vaccine (RRV-TV)

In 1998, the first vaccine against rotavirus, tetravalent, rhesus-based rotavirus vaccine (RRV-TV), was approved by the U.S. Food and Drug Administration and recommended for inclusion in the 1999 U.S. schedule for routine childhood immunizations. In July 1999, this vaccine was withdrawn in the United States following reports of cases of intussusception among recently vaccinated children.

RRV-TV (Rotashield, Wyeth Laboratories), was composed of the parent rhesus strain with G3 specificity and three human-rhesus reassortants, each with the VP7 capsid gene of either G1, G2, or G4 specificity, and 10 other rhesus genes. Trials of this vaccine demonstrated efficacies of up to 91% against severe rotavirus diarrhea.

Between September 1998 and July 1999, more than 1 million doses of RRV-TV were administered to approximately 500,000 children, or approximately 13.4% of all eligible children

(Smith et al., 2003). In July 1999, the vaccine was withdrawn after 15 cases of intussusception were reported among individuals recently vaccinated. Subsequent epidemiologic studies confirmed an association between receipt of the vaccine and the development of intussusception during the two weeks after the first and second doses of vaccine.

3.7.5.3 New-generation live oral rotavirus vaccines

Two vaccines have recently been licensed for use against rotavirus illness. Each vaccine has been tested in very large phase III clinical trials (more than 60,000 children) that are designed to assess safety with respect to intussusception.

One vaccine is a pentavalent vaccine (Rotateq, Merck) composed of five human-bovine reassortant strains containing single-gene reassortants expressing human VP7 genes with G1, G2, G3, G4 specificity and one strain with a human VP4 gene with P1 specificity, all in the parent bovine rotavirus strain. A trial of a prototype quadrivalent vaccine found the vaccine to be 75% effective at preventing all episodes of rotavirus gastroenteritis and 100% at preventing severe disease (Clark et al., 2004). Moreover, the vaccine was well tolerated by infants, with no increase in fever, vomiting, diarrhea, or irritability in recipients of vaccine compared with placebo. This contrasts with RRV-TV, a more reactogenic vaccine that provoked side effects, including fever (Joensuu et al., 1997).

A live attenuated, monovalent P[8]G1 human rotavirus strain (Rotarix, GSK Biologicals, Rixensart, Belgium) has been developed and recently licensed in Mexico, the Dominican Republic, and Kuwait. Rationale for development of this vaccine includes the observation that rotaviruses of G1 or P [8] serotypes account for more than 75% of rotavirus infections worldwide. It is also believed that compared with multivalent reassortant vaccines, vaccine manufacture of a monovalent, human strain may be simpler and less expensive.

This vaccine strain has been found to have virtually no side effects while eliciting a strong immune response when administered orally at a high titer (Vesikari et al., 2004). A trial of more than 6,000 children in Mexico, Brazil, and Venezuela demonstrated an efficacy of more than 70% against severe rotavirus diarrhea and protected not only against rotavirus disease caused by the homologous serotype (G1) but also against a single heterologous serotype (G9). Protection against the full range of rotavirus strains in circulation remains to be demonstrated (Perez-Schael et al., 2002).

3.7.6 Rotavirus impact on livestock

Rotaviruses have been detected worldwide in the feces of humans, pigs, cattle, sheep, horses, and deer. Rotavirus infections are of high agricultural importance because of the impact diarrheal disease can have on neonatal and post-weaning animals, especially pigs and calves. Morbidity due to rotavirus infections in pigs and calves can be as high as 80% with mortality reaching 60% (Bohl, 1979).

High morbidity and mortality in livestock animals also leads to economic losses. Economic loss is not only incurred from the loss of animals, but from treatment costs and reduced growth rates. Snodgrass et al. (1986) found rotavirus by itself to be responsible for 46% of the scours cases in dairy calves. And, similar to rotavirus infection in humans, animals typically become infected at early stages of life. For instance, the greatest risk of diarrhea for a calf is during the first two weeks of life (Bendali et al., 1999).

It is very difficult, and few studies have investigated the actual economic loss due to disease agents in livestock animals. However, one study by House (1978) determined that the estimated average annual loss due to disease agents in calves was nearly $100 million per year. Rotavirus was estimated to account for nearly 10% of this economic loss. It is also important to

note that this study was conducted in the early 1970s, so the current economic impact would likely be much higher.

3.7.7 Rotavirus in the environment

When considering disease transmission, it is important to understand that more than 120 different virus types are known to be excreted in human feces by infected persons, some of which are capable of causing illness. In addition, a number of human enteric viruses may be present even in treated wastewater effluents (Gerba et al., 1975; Hoadley and Goyal, 1976), making it essential to determine the survival and fate of viruses after the application of wastewater on land.

Factors affecting virus removal during land treatment of wastewater, such as adsorption to soils, have not been studied extensively, and little is known about the survival or fate of human viruses applied to the soil. If viruses are not retained by the soil, they may migrate vertically, resulting in groundwater contamination. Enteroviruses have, in fact, been found in groundwater after land treatment (Schaub and Sorber, 1977; Vaughn et al., 1978; Wellings et al., 1974; 1975). Abbaszadegan et al. (2003) tested groundwater samples from 448 sites in 35 states and found that about one-third of the groundwater drinking wells used by utilities contain human pathogenic enteric viruses.

Once wastewater is applied to land, the primary source of removal is by the soil. Sagar and Gerba (1979) found that virus adsorption to soil is highly dependent on the strain of virus. The differences in adsorption between different strains of the same virus type could be due to the variability in the configuration of proteins in the outer capsid of the virus, since this can influence the net charge on the virus (Gerba et al., 1975). The net charge on the virus would

affect the electrostatic potential between virus and soil, and thereby could influence the degree of interaction between the two particles.

Sagar and Gerba (1979) also found that a low soil pH (below 5) appeared to favor virus adsorption of all the viruses studied. In addition, they found that percent clay, percent sand, percent organic matter, total phosphorus, resin-extractable phosphorus, total iron, total aluminum, exchangeable aluminum, and exchangeable magnesium all influenced virus adsorption.

A high percentage of viruses are associated with the solids in sewage effluents and are thereby protected from chlorine inactivation (Stagg et al., 1978). Viruses associated with large particles (>6 μm) quickly settle into bottom sediments, while viruses adsorbed on smaller particulates or colloids (<3 μm) tend to stay suspended in the water for a longer time. When suspended solids-associated viruses settle out of a water column, they accumulate in a loose, fluffy layer over the compact bottom sediment. This fluffy layer then serves as a reservoir from which virus can be released into a water column by storm action, dredging, and boating.

In a study of enteric viruses adsorbed to estuarine sediment, LaBelle and Gerba (1979) found that once most enteroviruses are attached to a sediment particle, they are dependent upon the particle for transport. This study concentrated primarily on clay sediment particles because clay sediments are the slowest to settle, and are therefore on the top layer, and also because clay particles are more easily transported due to the smaller size and mass. However, in previous work (Davidson, 2007), it was apparent that rotavirus preferentially interacts with sand particles. Some interaction was seen with clay particles, but not nearly to the extent as with sand. Therefore, if rotavirus is bound to the much larger sand particles, the opportunity for transport is greatly reduced.

Rao et al. (1984) conducted a study in Galveston Bay to determine and compare the quantitative distribution of naturally occurring enteroviruses and rotaviruses in water, suspended solids, fluffy sediment, and compact sediment. They found that 72% of suspended solids samples were positive for enteroviruses compared to only 14% of the water samples. In addition, rotaviruses were associated with one-half of the suspended solids samples, more than in any of the other samples.

3.7.8 Rotavirus detection

Until the 1970s, diagnostic techniques for infectious diarrhea were limited to bacteria and protozoa, and an etiologic agent could be identified in a limited proportion of cases. Investigators had hypothesized, however, that viruses might account for many of the cases of unknown etiology. In 1972, in the examination of stool specimens, electron microscopy identified the Norwalk agent, the most common viral cause of gastroenteritis outbreaks among adults. In 1978, the same technique was used to detect rotavirus, the most common cause of severe diarrhea in children. Since that time, knowledge about these and other more recently discovered pathogens has increased dramatically.

3.7.9 Rotavirus outbreaks

Rotavirus disease is endemic in almost every part of the world. Therefore, rotavirus outbreaks are not common. Outbreaks produce similar symptoms as would be seen with any rotavirus illness, but the cases are more concentrated in a given area. Also, consistent with the outbreaks described below, rotavirus disease follows a temporal pattern based on the area involved.

3.7.9.1 Nicaragua outbreak

During February and March of 2005, one of the largest recorded outbreaks of severe acute gastroenteritis occurred in Nicaragua, affecting more than 64,000 individuals and causing at least 56 deaths, with children under the age of five being most affected. Bucardo et al. (2007) observed that the outbreak was associated with a mutated G4P[8] virus not previously detected in Nicaragua and most likely introduced from South America. The source of transmission has not been documented at this time.

3.7.9.2 Waterborne outbreak

On Friday, March 13, 1981, several citizens independently alerted the Colorado Department of Health to a possible outbreak of gastrointestinal disease in Eagle-Vail and Avon, CO. Telephone contact with the principal medical facility serving the five communities described above showed that office and emergency-room visits for gastrointestinal disease had risen sharply in early March (Hopkins et al., 1984).

In early 1981, Eagle-Vail and Avon shared a common water supply and distribution system. Raw water was obtained either from a small stream in the mountains south of the area, or from an intake on the Eagle River downstream from its confluence with Gore Creek. The Eagle River was ordinarily an auxiliary water source, but dry warm conditions in early 1981 forced its use starting in January as the primary source when the usual source was insufficient to meet demand (Hopkins et al., 1984).

The epidemiologic and engineering evaluation together showed that this was a waterborne gastroenteritis outbreak whose immediate cause was a chlorinator breakdown during March 4 and 5. On March 4, 1981, the chlorinator was the only effective barrier between the toilets of Vail and the water taps of Eagle-Vail and Avon. The other two barriers designed to

ensure safe drinking water were compromised: raw water quality and inadequate pretreatment and filtration. The simultaneous existence of these defects opened the way for a waterborne outbreak (Hopkins et al., 1984).

On October 8, 2005, an earthquake struck Kashmir, India. Between October 14 and December 17, 2005, a total of 1,783 cases of acute diarrheal disease were reported in Tangdar, India (population 65,000). The overall attack rate was 20% in children under 4 years of age. Following the earthquake, drinking stream water or tap water without boiling or chlorination may have lead to a common source water-borne outbreak of rotavirus gastroenteritis. Other possible contributing factors include overcrowding, poor sanitation, open-air defecation, poor hygiene, and living in makeshift camps near streams. Person-to-person transmission may also have contributed to perpetuation of the outbreak (Karmakar et al., 2008).

3.7.9.3 Person to person outbreak

Between November 26 and December 6, 1998, 7 of 25 elderly residents in a privately owned, skilled-nursing facility in Hawaii experienced at least one episode of diarrhea, with or without fever and vomiting. These episodes lasted anywhere from 24 to 48 hours. All seven patients received medical care, and one patient was hospitalized for dehydration. No nursing staff or food handlers reported gastrointestinal symptoms during the outbreak, and the incubation period was estimated to be 48 hours. A stool specimen was obtained from each patient. Neither contaminated food nor water could be implicated in the spread of infection, and investigators from the Hawaii Department of Health concluded that transmission was from person to person (Dixie et al., 2002).

In a different outbreak in a Maryland nursing home in late November, 1999, 26 of 96 elderly residents of a long-term care facility experienced at least one episode of diarrhea, with or

without fever and vomiting. These episodes lasted from 2 to 168 hours. None of the patients were hospitalized or received medical attention, and several employees were ill with gastroenteritis before the outbreak. Ten stool specimens were obtained from nine patients, but none were obtained from employees. In the absence of an obvious food or water source, investigators from the Maryland Department of Health and Mental Hygiene concluded that the disease was transmitted from person to person (Dixie et al., 2002).

3.7.9.4 Foodborne outbreak

In April of 2000, 85 of 5453 students at a Washington, DC, university experienced at least three episodes of diarrhea and/or at least two episodes of vomiting within a 24 hour period. Some patients reported fever and nausea. The illness had a mean duration or 96 hours. Of the 85 students affected, 53 sought medical attention, and 9 of those received intravenous fluids. All but 8 of the affected students resided on campus. Because food was the suspected vehicle of infection, 23 of the 29 employees of the university dining hall were interviewed, and several reported having gastroenteritis during the outbreak. Six stool specimens from students and 21 from employees were received for testing. The investigators from the Washington, DC, Department of Health identified contaminated dining hall food as the source of infection (Dixie et al., 2002).

CHAPTER 4: METHODS AND MATERIALS

Studies investigating the overland transport of *C. parvum* and rotavirus in three different Illinois soils and two types of vegetation have been conducted at the University of Illinois during 2007-09. The methodology used to perform the necessary experiments is discussed in this section:

- Design and construction of small-scale rainfall simulator
- Design and construction of small-scale soil box assembly
- *Cryptosporidium parvum* and rotavirus transport experiments

The experiments were conducted to advance our understanding of the kinetics and fate of microbial pathogens (*C. parvum* and rotavirus) during overland transport. We hypothesize the interactions with sand and clay particles are the dominating factors governing the kinetics and fate of microbial pathogens during overland transport. Therefore, the three soil types used in this study span the range of sand and clay contents for soils typically found in Illinois. In addition, transport was studied in the presence of a Smooth Brome or Tall Fescue vegetative cover to determine the affect of vegetation on overland transport kinetics.

4.1 Construction and uniformity of a small-scale rainfall simulator

Previous research performed by Trask (2002) and McLaughlin (2003) investigated the transport of *Cryptosporidium parvum* using a laboratory rainfall simulator and soil bed. In addition, McLaughlin (2003) used a miniature-scale version of the laboratory soil bed, but applied the water, or "rainfall", using only a typical garden watering can. A more accurate small-scale rainfall simulator was developed by Davidson (2007) that is used in this study. This

study investigated both *C. parvum* and rotavirus transport, which are both handled more easily using a small-scale setup because detection is easier and more accurate.

4.1.1 Construction of small-scale rainfall simulator

The entire rainfall simulator, including the frame, was constructed using 12.7 mm (½") PVC pipe and measures 1.07 m (42 in) by 0.66 m (26 in) (Figures 4.1 and 4.2). PVC pipe was chosen because of its low weight and ease with construction.

For the rainfall simulation, mister nozzles (poly mist nozzles from ACF Greenhouses, Buffalo Junction, VA (http://www.littlegreenhouse.com)) were used because of their ability to supply a very low rate of water flow. Three different nozzles types were used; 1.05 mL/s (1 gph), 2.10 mL/s (2 gph), and 3.15 mL/s (3 gph). All three volume outputs were used; two groups of the three nozzles (one at each end) with an additional 1.05 mL/s nozzle in the center to increase the aerial coverage of the 1.05 mL/s nozzles. The 1.05 mL/s nozzle has a narrower spray pattern than the other two nozzle types. The three different nozzle types used in combination allows for smaller increments of rainfall intensity than if each nozzle type was used individually.

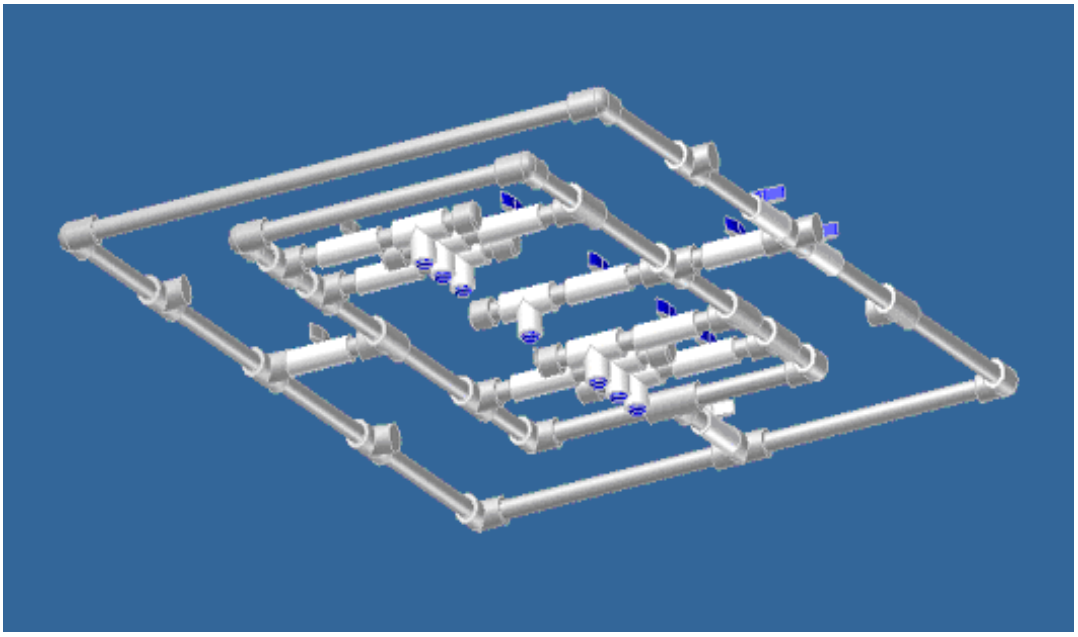


Figure 4.1. Bottom view schematic of the small-scale rainfall simulator (Davidson, 2007).

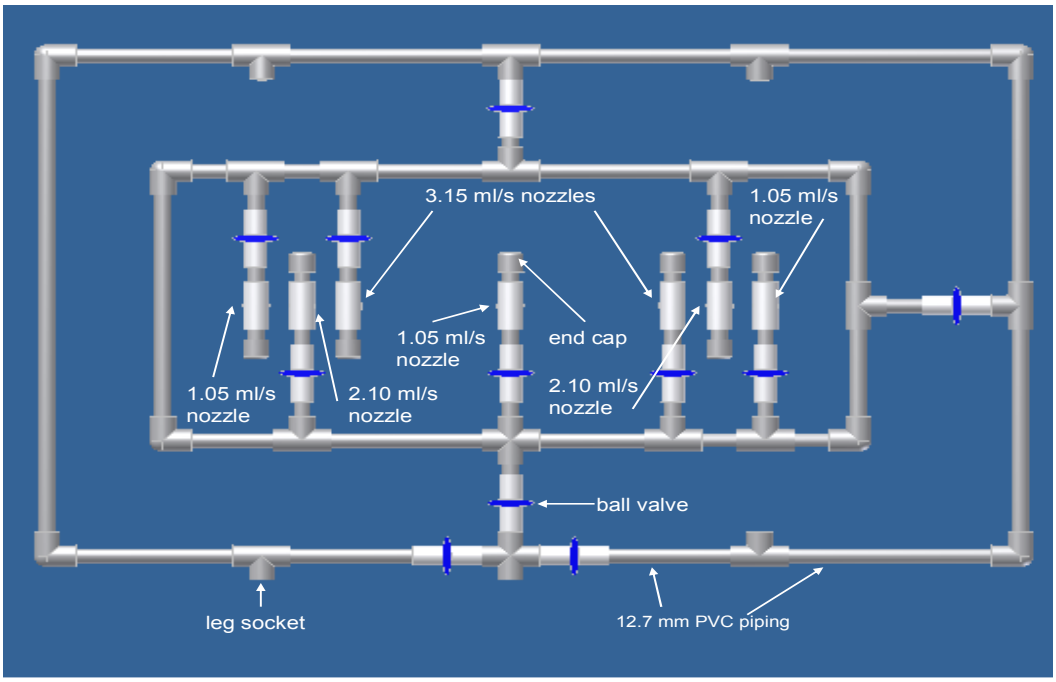
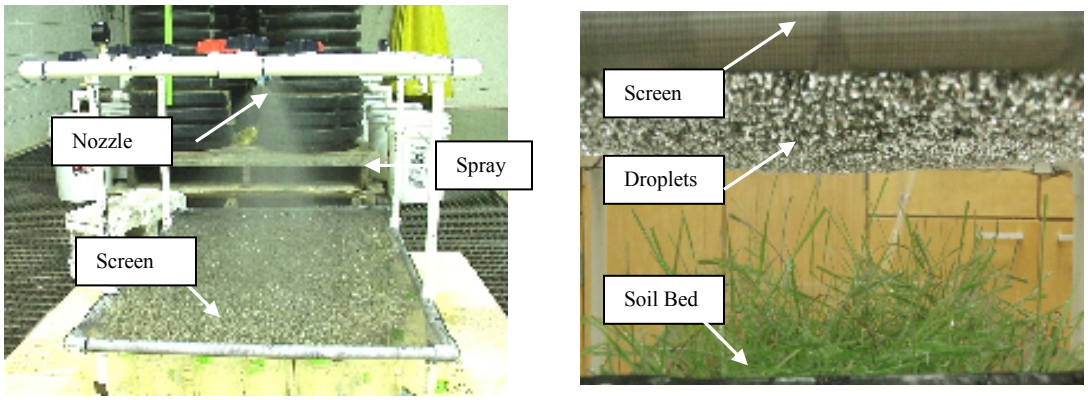


Figure 4.2. Top view schematic of the small-scale rainfall simulator, showing the position of the different types of mister nozzles (Davidson, 2007).

Since the nozzles are mister nozzles, adequate droplets were not produced by the nozzles alone. Therefore, a common fiberglass window screen (purchased at a local hardware

store) was placed 305 mm (12 in) below the nozzle assembly frame. The screen has 1 mm square openings. The nozzles spray a water mist onto the screen (Figure 4.3 a), and droplets are then formed and allowed to fall onto the surface below, more closely mimicking natural rainfall droplets (Figure 4.3 b).



(a) **(b)**

Figure 4.3. Photographs of small-scale rainfall simulator. (a) Full display of rainfall simulator as water is sprayed on the fiberglass droplet-generating screen. (b) Magnified photograph of the underside of the fiberglass screen, showing water droplets forming to produce simulated rainfal1.2

4.1.2 Uniformity of water distribution of the small-scale rainfall simulator

Specifications on water distribution characteristics of the mister nozzles were unavailable from the manufacturer, so they were determined experimentally.

A series of 500 mL beakers were carefully placed on a grid below the droplet generating screen, and the rainfall simulator was turned on. The rainfall continued for 15 minutes. The volume of water was measured for each beaker, and the distribution of water was determined by the following water-distribution efficiency relationship (Christiansen, 1942) as follows:

$$E_d = 100\left[1 - \left(\frac{y}{d}\right)\right] \quad (1)$$

where E_d = water-distribution efficiency

y = average numerical deviation in depth of water stored from average depth stored during the event

d = average depth of water stored during the event

The possible rainfall intensities that can be produced by this rainfall simulator range from 23 mm/hr (0.89 in/hr) to 90 mm/hr (3.54 in/hr) as shown in Table 4.1 along with corresponding distribution efficiencies. This range is more than adequate for simulating a typical Illinois rainfall event. There may not be a need to test an intensity lower than 23 mm/hr, and an intensity greater than 90 mm/hr would likely cause excessive soil erosion, an undesirable outcome when collecting pathogen fate and transport data.

Table 4.1. Distribution efficiencies and average rainfall intensities for small-scale rainfall simulator.

Nozzle(s)	Ed (%)	Average Intensity (mm/hr)
1.05 mL/s	40.1	34
2.10 mL/s	75.5	23
3.15 mL/s	62.5	25
all nozzles	74.5	90
1.05 & 2.10 mL/s	67.7	57
1.05 & 3.15 mL/s	57.9	65
2.10 & 3.15 mL/s	75.4	48

In this study, a constant 65 mm/hr (2.5 in/hr) rainfall intensity was used for all conditions in order to monitor the effect of soil and vegetation types. This intensity was one of several intensities used by Trask et al. (2004), representing a common rainfall event for central Illinois.

It should be noted that other usual considerations for a rainfall simulator (mean drop size, drop size distribution, and drop energy) are not deemed important for this work since the

objective of this study to evaluate surface runoff, not erosion. If surface sealing or erosion of soil particles is deemed important, further refinement of the simulator will be necessary to adequately represent the energy of natural falling raindrops (terminal velocity).

Upon completion of all transport studies, the rainfall intensity and distribution were again calculated to insure consistency of the rainfall events produced by the small-scale rainfall simulator. This post-calibration gave a rainfall intensity of 69 mm/hr (compared to 65 mm/hr originally) and a distribution efficiency of 55.4% (compared to 57.9% originally). The numbers for pre and post-calibration are very close, and the slight discrepancy between the two calibrations is likely due to wear of the nozzles and possible stretching of the droplet-generating screen.

4.2 Soil box construction

Soil boxes measuring 0.305 m (1 ft) wide by 0.610 m (2 ft) long by 0.015 m (6 in) deep were constructed of 6.35 mm (0.25 in) thick Plexiglas (Figure 4.5). An acrylic strip heater was used to form the bottom edges. All corners were sealed with epoxy and silicon caulk to prevent leaks. A series of 11 holes, 4.76 mm (3/16 in) in diameter were drilled 76.2 mm (3 in) above the bottom of the soil box (corresponding to soil surface location) to allow for collection of surface runoff. To collect near-surface flow samples, another set of 14 (2 rows of 7) holes of the same diameter were drilled in the down-slope half section of the soil box bottom. Twelve soil beds were constructed in this manner so that simultaneous experiments can be conducted with different slope, soil type, and surface cover conditions.

Figure 4.4. Completed soil box with holes for surface and near-surface runoff collection.

4.3 Tilting soil box assembly construction

A frame was constructed of 2" x 4" lumber (38 mm × 89 mm) (Figure 4.6 a). The frame was then mounted with hinges to a 9.5 mm thick plastic base (0.305 m by 0.610 m). A 6.35 mm (0.25 in) diameter steel rod was connected to a 9.5 mm (3/8 in) threaded nut on one side and the wood frame on the other by hinges. The nut was threaded on a 9.5 mm (0.375 in) diameter all-thread rod. A handle was welded to the all-thread rod which was then mounted on the plastic base. By simply turning this handle, the slope of the soil box frame can be adjusted. An aluminum tray was constructed and fastened to the down-slope end of the wood frame. A piece of rubber weather-stripping tape was attached to the top of the tray. When the soil bed is placed on the frame, the tape forms a seal with the bed, ensuring that all of the surface runoff will be collected in the tray (Figure 4.6 b). A vinyl tube (0.375 in. inside diameter) was attached to the aluminum tray so samples could be collected. A piece of Plexiglas was placed over the collection tray to prevent rainfall from entering the surface runoff collection system. A second aluminum tray was constructed and mounted in the wood frame to collect near-surface flow

samples. A vinyl tube (0.375 in. inside diameter) was also connected to the second tray to transfer the flow into sampling containers (Figures 4.6 a and b).

(a) (b)

Figure 4.5. Photographs of small-scale rainfall soil bed and tilting frame. (a) Picture of tilting soil bed frame. Note the collection trays for surface and near-surface runoff. (b) Photograph of the bare surface soil bed on tilting frame. Notice how surface runoff passes through the holes at the surface level and is collected in the upper tray and diverted to sampling containers via the connecting hose.

4.4 Soil collection and preparation

Three soil types, spanning the typical range of soil textures in Illinois, were collected and placed in the small-scale soil chambers. A moderately drained silt-loam (Catlin series: 24% sand, 50% silt, 26% clay) soil was collected from a site in Champaign, IL, and a well drained fine sandy loam (Alvin series: 60% sand, 25% silt, 15% clay) soil and poorly drained silty-clay (Darwin series: 5% sand, 50% silt, 45% clay) soil were collected from a farm near Newton, IL.

All vegetation and organic material were removed from the soil surface, and the top 7.6 mm (3 in) of soil was collected so that soil texture would remain constant. The top 3 inches of soil matches the depth of the soil in the small-scale soil chambers.

The soil was oven-dried at 105 ºC for 24 hours to reduce existing microbial contamination, to produce uniform antecedent moisture conditions, and to allow for easier separation of soil particles. The soil was sifted using a 3 mm (⅛") screen to break up the larger pieces into more uniform sizes and to remove large organic matter and rocks.

4.5 Packing of soil beds

The soil was packed in each individual soil bed in three one-inch (25.4 mm) layers. Each layer was moistened and compacted with a plate (Figure 4.7). After all three layers were placed in the chamber, rainfall was simulated to bring the soil back to uniform moisture conditions. Rainfall was continually applied to the chambers on a regular basis to simulate natural climatic conditions and to approach natural soil compaction (from the wetting-drying process) in the beds.

Figure 4.6. Preparation of soil beds. The soil beds were packed in one-inch layers, tamping and adding water to each layer to provide compaction similar to natural field conditions. After the addition of all three layers, the beds were watered regularly to bring the soil back to natural moisture conditions and approach natural compaction.

4.6 Establishing vegetation

Once the beds were packed and returned to natural soil compaction, grass was seeded accordingly. Two grass types were used (Smooth Brome and Tall Fescue, purchased at Illini FS, Urbana, IL). The chambers were seeded by hand until a uniform distribution of seed was achieved. The recommended seeding rate for each grass is approximately 15 lbs/acre. The seeding rate applied to our soil chambers was slightly higher than the recommended rate, but was necessary to achieve adequate vegetative cover in the given length of time. Approximately 3-6 months was necessary to achieve the desired maturity level of vegetation before use in transport studies (Figure 4.8).

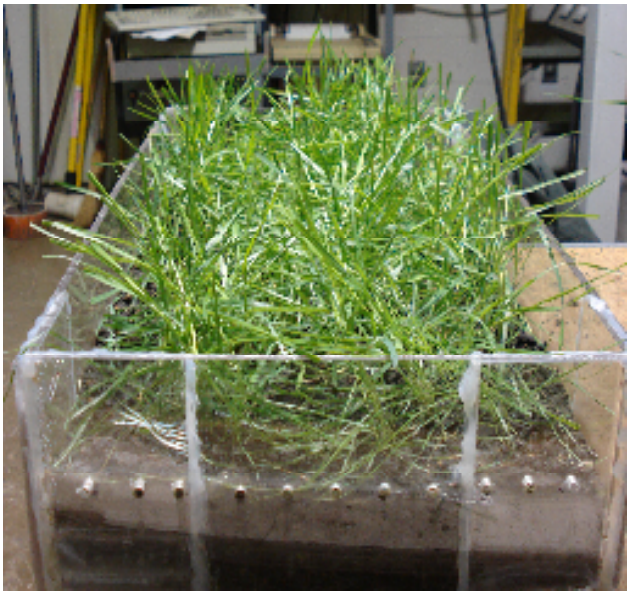


Figure 4.7. Established Smooth Brome vegetation in soil chamber before application of *C. parvum* and rotavirus for transport studies.

4.7 Estimation of vegetative cover

Before conducting a vegetated condition experiment, the grass of each bed was trimmed to a height of 6 inches and a digital photograph was taken of each bed from directly above, looking down. The digital photographs were then imported into a program (*Analyzing Digital*

Images 11.0, Global Systems Science) to calculate the percent vegetative cover. Since the photographs were taken from directly above the beds, vegetation canopy was estimated, rather than density of vegetation. This process was performed for each vegetated bed.

4.8 Overland transport experiments

Rotavirus survival in the presence of different soil fractions and at various temperatures was previously investigated (Davidson, 2007) and served as a prelude to overland transport studies. In these studies, survival of rotavirus was tested in three soil fractions (Catlin soil, pure sand, and pure clay) and three temperatures (4 ºC, 25 ºC, and 37 ºC). Briefly, a rotavirus suspension was mixed with soil, sand, or clay in test tubes and allowed to incubate for up to 18 days. Samples were collected daily to investigate the survival over time. The results of these earlier studies which showed variable survival of rotavirus in clay and sand prompted the decision to test soils with widely varying clay and sand contents in the overland transport experiments presented here.

By investigating a small-scale slope condition within the range of large-scale slope conditions, one can determine the adequacy of the small-scale system in representing the trends observed in the large-scale system. Previous research conducted by Trask et al. (2004) determined the effectiveness of vegetated filter strips (VFS) in reducing *C. parvum* and nutrient transport in surface runoff under various conditions. Trask et al. (2004) used a large-scale horizontal tilting soil chamber (3.6 m long, 1.5 m wide, and 0.3 m deep) containing vegetated (smooth brome) and bare soil beds and a large-scale rainfall simulator to investigate surface and near-surface transport of *C. parvum* for different slope, rainfall intensity, and soil type combinations. Three slope conditions (1.5, 3.0, and 4.5%) were used under two rainfall intensities (25.4 and 65 mm/hr) for 44 minutes. To determine the adequacy of the small-scale

soil bed/rainfall simulator system in representing field conditions, Koch (2009) used results from the present study to compare with the larger-scale results (Trask et al., 2004) to confirm that the small-scale system accurately reflected the overland transport kinetics of the large-scale system. The results from the small-scale 2.5% slope and 65 mm/hr intensity experiment were compared with those from the large-scale 1.5% slope and 65 mm/hr and 3.0%, 65 mm/hr experiments. Although the slope and rainfall intensity are not identical between the large and small-scale experiments, the slope tested in the small-scale experiment is within the boundary of the slopes in the large-scale experiments. This allows insight into the impact slope plays on pathogen transport.

To compare the runoff kinetics of different soil beds with and without vegetation and different slopes, the time scale was converted to a t/t_c time scale, where t represents the time at the end of sample collection and t_c represents the time of concentration (time needed to achieve maximum runoff rate). Lastly, the volumes of runoff were compared to determine if the small-scale experimental system accurately represents the hydrologic runoff events observed in the larger-scale condition (Figure 4.9).

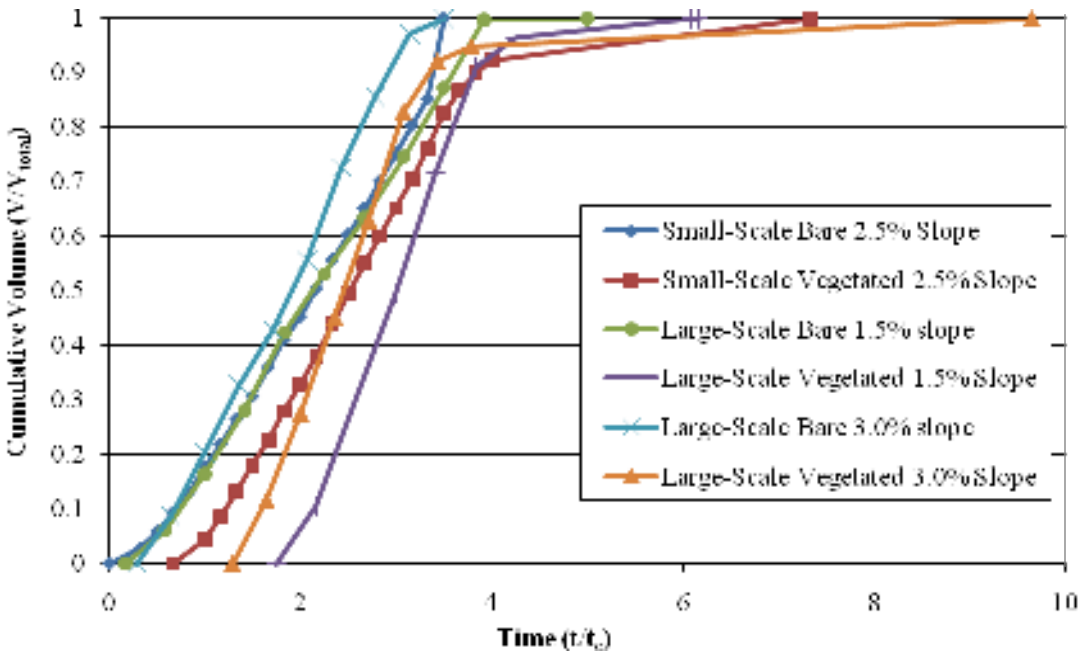


Figure 4.8. Cumulative runoff volume from large and small-scale experiments under 65 mm/hr rainfall event. Note that the axes are given in unit-less dimensions to allow comparison between the two scales (Koch, 2009).

Overland transport experiments presented in this work were performed on both vegetated and bare surface conditions to investigate the effect of different soil types and vegetation on microbial transport, at a simulated rainfall intensity of 65 mm/hr. This rainfall intensity was previously tested by Trask et al. (2004) and would therefore allow for comparison between the two data sets. A 2.5 % slope was chosen because it is between the slope conditions tested by Trask et al. (2004) (1.5% and 3.0% slopes). A rainfall duration of twenty minutes was used to allow the opportunity of observing the entire transport curve.

Experiments began after adequate grass cover was established on the vegetated soil beds, or after a somewhat natural soil compaction was achieved through repeated rainfall events for the bare soil conditions.

The soil bed was placed on the tilting frame and set to a 2.5 % slope. To ensure the initial surface moisture conditions were similar for each experiment, rainfall was simulated until

flow began. The soil bed was then allowed to drain until surface and near-surface flow ceased prior to application of fecal slurry. The moisture content of the soil was then near field-capacity. For all experiments, one slurry was applied containing a mixture of *C. parvum* oocysts and rotavirus as previous control experiments showed that detection of one pathogen was independent of the presence of the other (data not shown). The combined fecal slurry closely represented conditions of fecal deposits. Approximately 15 mL of slurry containing 10^7 oocysts/mL (*C. parvum*) and 10^8 FFU/mL (rotavirus) was applied in a band approximately 5 cm (2 in) from the up-slope end of the soil bed with an additional slurry aliquot taken for initial concentration analysis. For all experiments, surface and near-surface runoff samples were collected throughout the entire flow event. In addition, 7.6 cm (3 in) soil cores were taken with a 2.1 cm diameter cork borer to determine the transport of *C. parvum* oocysts and rotavirus particles in the soil. Each of the eight soil cores was then divided into three 2.54 cm (1 inch) depths and these in turn were halved to provide a section for each *C. parvum* extract and rotavirus extract. These cores were analyzed to determine the number of infective oocysts and rotavirus particles at each depth increment.

Figure 4.9. Complete laboratory-scale rainfall simulator and tilting soil bed system.

4.9 Source of *C. parvum*

C. parvum was obtained from a male neonatal calf (less than three days old) by experimental oral inoculation of 1 x 10^7 oocysts (Fayer et al., 1990, p. 41–42, 44–45) in the animal care isolation facilities of the Veterinary Medicine Basic Sciences Building at the University of Illinois. Feces containing oocysts were collected in plastic drop pans maintained over melting ice and contained 100 ml of Pen/Strep/Amphotericin (containing 6 g penicillin, 10 g streptomycin and 25 mg amphotericin per liter of 0.85% sterile saline) and 100 ml Nystatin (containing 1.98 g per liter sterile saline) per 1-1.5 liter of feces. The collection pans were

emptied twice daily. Feces containing oocysts were stored as a fecal slurry at 4 °C in the antibiotic-antimycotic suspension until use. As the feces aged, oocysts in the fecal slurry were concentrated by centrifugation at approximately 1500 x g. The age of cultured feces ranged from 2-6 months after experimental inoculation of the calf.

4.10 Concentration of runoff samples for *C. parvum* analysis

Surface runoff samples were collected every minute after runoff began. Near-surface was collected on longer time intervals (as needed) since near-surface runoff volume was much lower than surface runoff volume. These samples contained a dilute concentration of *C. parvum* oocysts, requiring concentration prior to use in infectivity assays. Briefly, runoff volume was measured for each collected sample. Then, 1.5 mL of runoff sample was added to a 2 mL microfuge tube and centrifuged at 10,000-15,000 x g for 1 minute. Any sediment and *C. parvum* oocysts were pelleted. The supernatant was removed and another 1.5 mL of the same sample was added to the tube and centrifuged. This process was repeated 2 more times until 6 mL of equivalent runoff fraction was contained in the pellet which was finally suspended in 0.5 mL PBS.

4.11 *C. parvum* extraction from soil cores

The large amount of soil in core samples prevented performing direct cell infectivity assays. Therefore, oocysts were extracted from soil cores using a sugar floatation procedure.

The sugar floatation procedure was used to remove soil particles and organic materials, leaving a sample of relatively pure oocysts. Soil core sections were placed in 15 mL conical tubes with 5 mL of phosphate buffer solution (PBS). Using a spatula, the cores were broken up and mixed with the PBS. Approximately 8 mL Sheather's sucrose solution (1.2 g/cc) and one

drop of Tween 20 was then added to the conical and mixed. The conical was centrifuged at 1260 x g for 10 minutes at 4 ºC. To recover the oocysts that floated to the top of the Sheather's solution, 1.5 mL of (0.2%) Tween 20 was added to the top of the conical tube with a Pasteur pipette and mixed gently (only the top section). With the same pipette, a portion (< 1.5 mL) of the top was skimmed off and placed in a fresh collection conical. The tube containing the soil core and Sheather's solution was mixed again. The collected oocysts were then washed twice with 0.2% Tween 20 centrifuging as before and removing the supernatant. The collected oocysts in the pellet were then stored at 4 ºC until assayed (usually within 2 days of the rainfall simulation).

4.12 Enumeration of *C. parvum* using most probable number method

The most probable number (MPN) method has traditionally been used for enumerating bacteria, but has been used in this study as a method to quantify the number of infectious *C. parvum* oocysts (Slifko et al., 1999). The method consists of making a series of dilutions of the oocyst sample, infecting confluent monolayers of HCT-8 cells, incubation of infected cells for 2-3 days followed by fixation and immunofluorescent detection of *Cryptosporidium* intracellular life forms. In this assay, fluorescent-stained clusters (products of infection) were observed in an inverted microscope equipped with a fluorescent detector. MPN (oocysts/mL of sample) was determined as described by Silfko (1999).

Briefly, upon completion of the sugar floatation process, 0.5 mL oocysts from soil core extracts were placed in a 2 mL polyethelene microcentrifuge tube and placed on ice, followed by the addition of 0.5 mL of a 40% bleach solution. The addition of bleach primarily serves to sterilize the solution, but it also "weakens" the oocysts' wall, facilitating excystation of sporozoiteswhen the oocysts encounter a cell. After 10 minutes on ice, each sample was

centrifuged at 2040 x g in a microcentrifuge. The supernatant was then suctioned off under sterile conditions. A 1 mL volume of phosphate buffered saline (PBS) was added to the pellet, mixed and centrifuged again, removing the supernatant (this step was performed a total of five times to ensure complete removal of bleach). The final pellet was resuspended in 1 mL (0.3 mL for soil cores) of sterile HCT-8 cell culture medium. This was used as the stock for each sample.

4.13 Immunodetection

A ten-fold serial dilution set was prepared for each sample. Once dilution sets were completed for all samples, a 96-well plate of confluent HCT-8 cells was prepared, removing the spent medium and adding 180 µL of fresh HCT-8 medium. A 20 µL volume of each sample in dilution series was added to each of three wells (in triplicate). The 96-well plate was placed in the incubator for 2-3 days at 37 °C.

At the end of the 2-3 day incubation of cells with oocysts, the medium was removed. A 0.2 mL volume of MeOH-glacial acetic acid (9:1) was added to each well and allowed to sit at room temperature for 2 minutes. Following removal of MeOH-glacial acetic acid, the now fixed HCT-8 cells were sequentially rehydrated using 0.2 mL of 70% ethanol followed by the addition of 0.2 mL of 50% ethanol. After rehydration, the wells were rinsed twice with 0.2 mL of a wash buffer (WB; 125 mM Tris, 350 mM NaCl, 0.25% Triton X-100, pH 7.6). Nonspecific primary antibody binding was blocked with 50 µL of 5% normal goat serum (Vector Laboratories, Burlingame, CA) diluted in WB for one hour. Each well was rinsed by adding 0.2 mL WB for 10 minutes. The contents were removed and 50 µL of a 1x FITC labeled primary antibody (Sporo-glo, cat. # A600FLR Waterborne) solution were added and allowed to sit overnight at 4 °C. The following day, after removal of the primary antibody, each well was rinsed by adding 200 µL WB for 10 minutes. The contents were removed and each well rinsed with 200 µL PBS

to remove any detergent. Each well was rinsed one final time with water. Each well was observed microscopically for the presence of fluorescent "clusters" representing various stages of newly synthesized *Cryptosporidium* life forms (infectivity).

4.14 Source of rotavirus

Group A Porcine rotavirus OSU strain (P9[7], G5) was obtained from the American Type Culture Collection catalog # VR-892 and passaged two additional times in cultured Ma104 cells (Rolsma et al., 1994). Infectious units of activity were determined in a focus forming assay (FFU) as described by Rolsma et al. (1998). Three colostrum-deprived newborn piglets were inoculated with 1 mL (3 $x10^6$ FFU) each and the feces were pooled and collected over a two day period. The combined fecal material was homogenized with a Dounce homogenizer with an equal volume of Vertrel XF organic cleaning agent (Miller-Stephenson) to enhance rotavirus separation from fecal debris. After centrifugation to separate layers, the aqueous top layer was centrifuged at 13,000 x g to pellet debris. The supernatant was centrifuged at 180, 000 x g to pellet the virus. The pellet was suspended in TNC (50 mM TRIS, 150 mM NaCl, and 10 mM $CaCl_2$, pH 7.4) buffer to the desired FFU/mL (approximately 10^7-10^8 FFU/mL). Alternatively, porcine OSU rotavirus was cultivated in Ma104 cells and purified as described by Rolsma et al. (1998), excluding the CsCl gradient step.

4.15 Extraction of rotavirus from soil cores

At the time of sampling, a volume of TNC approximately equal to the porosity of the soil core sample (1-2 mL) was added to the tube and the contents mixed thoroughly with a spatula. The tube was then centrifuged at 1260 x g for 10 minutes at 4 ºC. The aqueous supernatant was

then removed from the tube and saved. This process was repeated for a total of three extractions. All three extractions were combined.

Assuming 100% availability of the virus and no loss of infectivity after each addition of TNC and mixing, 3 extractions would yield 87.5% of the initial virus. With uniform mixing of 1 mL virus suspension in soil and 1 mL TNC, and soil, 50% of the virus would be removed during the first and subsequent extractions. A combination of the three extracts should, therefore, contain 87.5% of the original virus. Further extractions would yield more rotavirus but would substantially dilute the sample, making it more difficult to assay such a low concentration of rotavirus and introducing more error into the analysis. The data was normalized for the remaining 12.5% that was not extracted.

4.16 Preparation of samples for analysis of rotavirus infectivity

The surface and near-surface runoff samples were collected and analyzed for both *C. parvum* and rotavirus. Therefore the collection process is the same for both analyses. Surface runoff samples were collected every minute after runoff began. Near-surface was collected on longer time intervals (as needed) since near-surface runoff volume was much lower than surface runoff volume. These samples contained a relatively high concentration of rotavirus particles, requiring a dilution of the samples for analysis of infectivity. The procedure for diluting the samples involved measuring the total volume, shaking the samples and allowing soil particles to settle, and then taking a given amount from each sample. A 1/5 dilution was typically prepared by combining 200 µL of each sample with 800 µL of serum-free MEM and applying 500 µL directly to the wells of confluent Ma104 cells. Soil core extract samples (250 µL) were mixed with 750 µL of serum-free MEM. Again, 500 µL was applied directly to the plate wells of Ma104 cells.

4.17 Focus forming unit (FFU) assay

The focus forming unit (FFU) assay was performed following previously published methods (Rolsma et al., 1998). A summary of this method, as modified for analysis of rotavirus recovery for soil samples, is briefly described below.

Confluent monolayers of Ma104 cells in 24-well plates were rinsed twice with phosphate-buffered saline (PBS). Each plate well was then inoculated with the 500 µL of sample and allowed to incubate for 30 minutes at 37 ºC in a 5% CO_2 incubator. After the short incubation, the sample was aspirated from the cells, rinsed one time with 1 mL PBS, and allowed to incubate overnight (37 ºC in a 5% CO_2) with 1 mL MEM + antibiotics.

After overnight incubation, the plate wells were rinsed twice with 1 mL of PBS, and fixed using 1 mL MeOH-glacial acetic acid (9:1) per well. The monolayers were rehydrated using a 70% ethanol solution followed by the addition of a 50% ethanol solution. The rehydration was followed by rinsing with a wash buffer (WB; 125 mM Tris, 350 mM NaCl, 0.25% Triton X-100, pH 7.6). Endogenous peroxidase activity was inhibited by addition of 3% H_2O_2 diluted in WB. Nonspecific primary antibody binding was inhibited by addition of a 5% normal goat serum (Vector Laboratories, Burlingame, CA) diluted in wash buffer. After a short incubation of 20 minutes, the normal goat serum was removed and the primary antibody (rabbit anti-human rotavirus; Dako; catalog no. B218) was added to the monolayers. After rinsing, a secondary antibody of biotinylated goat anti-rabbit immunoglobulin G (Vector Laboratories, #BA1000) diluted in WB with normal goat serum was added. The monolayers were rinsed and incubated 20 minutes with an ABC reagent (Vector Standard Elite Kit, #PK6100). The ABC reagent is a horse radish peroxidase-conjugated biotin-strepavidin complex that binds to the biotinylated 2º antibody. After washing out unbound conjugate, peroxidase substrates DAB (Kit

#541000 from Kirkegaard and Perry, Gaithersburg, MD) and H_2O_2 were added and incubated 10 minutes at room temperature. The DAB is a precipitation substrate for the peroxidase that turns brown during the reaction (oxidation) to reveal the cytochemical location of the rotavirus antigen. Excess substrates were washed out and brown foci quantified.

4.18 Quantification of rotavirus infectivity

The rotavirus FFUs were quantified by counting the number of FFUs present in a given well. The FFUs (stained viral-antigen-positive cells) were observed and imaged at a magnification of ×100 using a Nikon TS 100 inverted microscope equipped with a computer-controlled electronic stage and Spot RT-slider CCD camera. Twenty five digital images were automatically collected from each plate well using Metamorph software (Molecular Devices, Inc) to develop and capture images within a scan grid which covered >80 % of the well surface area (Figure 4.11). The number of FFUs in each of these images were then counted either visually "by hand" or automatically using integrated morphometric parameters within the software to recognize FFUs. The ability to automatically count FFUs was dependent on the degree of background staining, or degree of interference of soil particles.

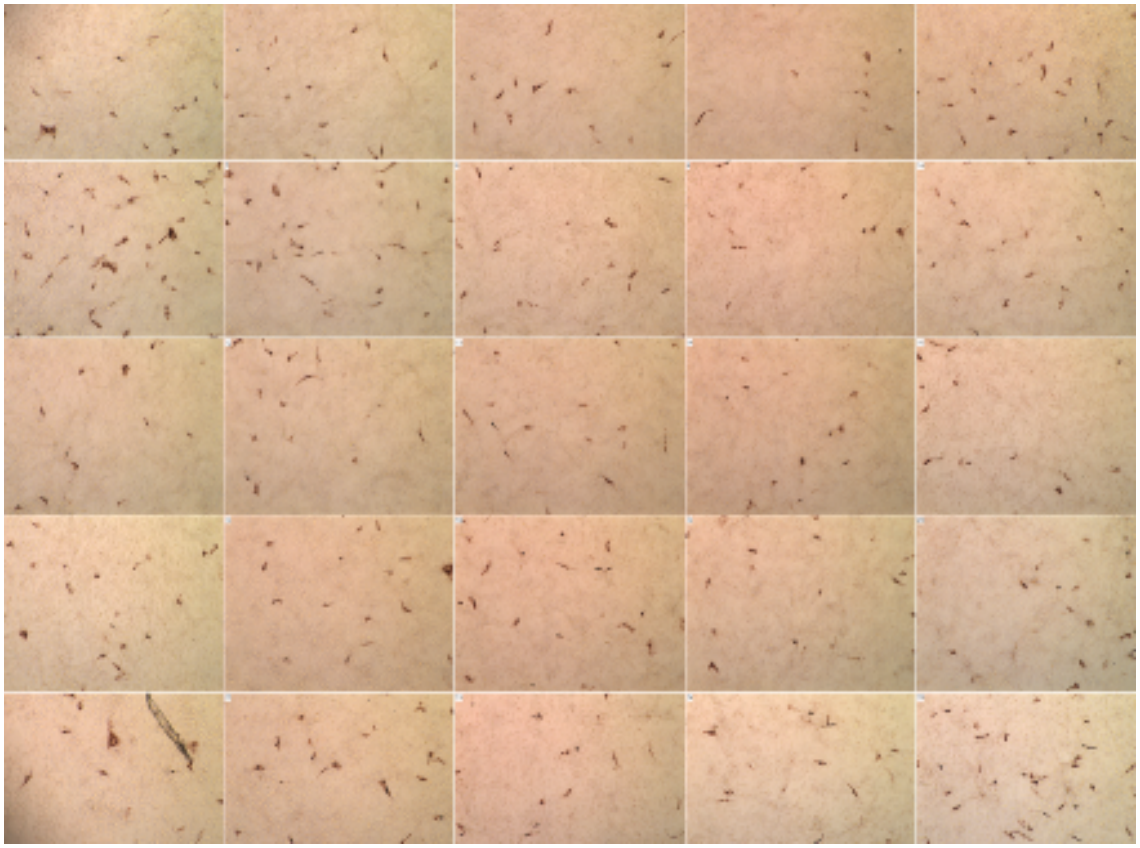


Figure 4.10. Photomicrograph collected using MetaMorph software displaying the result of the rotavirus focus forming unit (FFU) assay. The small dark-colored dots are the actual FFUs representing rotavirus particles that have actually infected the host cell. (Picture contributed by M. Kuhlenschmidt, Department of Pathobiology).

4.19 Overland transport kinetics model development

The SAS statistical analysis program was used to evaluate data recorded from the overland transport studies. Results from nine overland transport studies were used for the development of two empirical models, one each for *C. parvum* and rotavirus. The nine studies include three different soil types with varying textural composition as well as three surface conditions (bare soil, Brome vegetation, Fescue vegetation). The Proc Reg procedure was used to create regression models for all possible combinations of the seven parameters used in the nine studies (% sand, % silt, % clay, % vegetative cover, % organic matter, bulk density, and total surface runoff volume). The best 5 regression models, in terms of statistical measures, for

each pathogen and number of parameters (1-parameter models, 2-parameter models, etc.) were further analyzed for their performance in predicting independent sets of pathogen transport data. Therefore, each model was not only evaluated based on its ability to predict the original nine recoveries for each pathogen, but the ability to predict independent data sets for overland transport of either *C. parvum* or rotavirus. The statistical measures used to evaluate each model were r-squared, adjusted r-squared, mean squared error, sum of squares error, and residuals. Based on these statistical criteria and the ability to predict independent data, the best model was selected for each *C. parvum* and rotavirus.

CHAPTER 5: RESULTS AND DISCUSSION

Previous results from our laboratory showed that infective *C. parvum* oocysts preferentially bind to clay particles (McLaughlin, 2003) while infective rotavirus particles primarily interact with sand particles (Davidson, 2007). However, these findings were determined from static adsorption studies. To confirm their relevance to the design and use of VFS, it is necessary to conduct dynamic transport experiments using both infective *C. parvum* oocysts and rotavirus particles. McLaughlin (2003) and Koch (2009) verified the overland transport kinetics of small-scale soil beds accurately reflect the overland transport kinetics of the larger soil bed (1.5 m x 3.6 m) used by Trask et al. (2004). Accordingly, results from the current transport study using the small-scale soil beds and rainfall simulator can be used to provide a foundation for creating a transport model and develop recommendations for the design of field-scale best management practices.

The soil beds in this study were prepared by collecting soil from the field and then packing it in plexiglass soil boxes. The compaction was performed in one-inch layers with tamping. The soil was then allowed to reach a somewhat natural compaction by simulating rainfall over a period of several weeks. It is known that the compaction level may be slightly less than field conditions, but the compaction of our beds is comparable to the plow layer of a newly seeded field, mimicking conditions of a newly constructed filter strip. In addition, it is difficult to recreate the natural field compaction in the small-scale soil box, so for this study is was deemed more important to maintain consistency throughout all experiments by following a uniform procedure for all soil beds. Bulk density measurements were taken as an indication of compaction level.

Catlin, Darwin, and Alvin soils have been used to investigate the transport of *C. parvum* and rotavirus in overland and near-surface flow. The three soil types span the range of soil textures found in Illinois and collectively represent typical soils found in central Illinois, in terms of soil texture.

The results presented in this section compare and contrast the overland transport of *C. parvum* and rotavirus for three widely different soils. In addition, Smooth Brome and Tall Fescue grasses have been tested to confirm that vegetation is effective for reducing overland flow, and also overland transport of pathogens.

Preliminary experiments (Davidson, 2007) investigating the survival of rotavirus in the presence of different soil types (whole soil, sand, and clay) as well as a study by McLaughlin (2003) investigating the adsorption of *C. parvum* to soil particles prompted the current experimental setup for overland transport. The study by McLaughlin (2003) found that *C. parvum* oocysts strongly adsorb to clay particles with very little (if any) interaction with silt or sand particles. The results from Davidson (2007), however, found that rotavirus particles interact primarily with sand particles (summarized in Figures 5.1, 5.2, and 5.3).

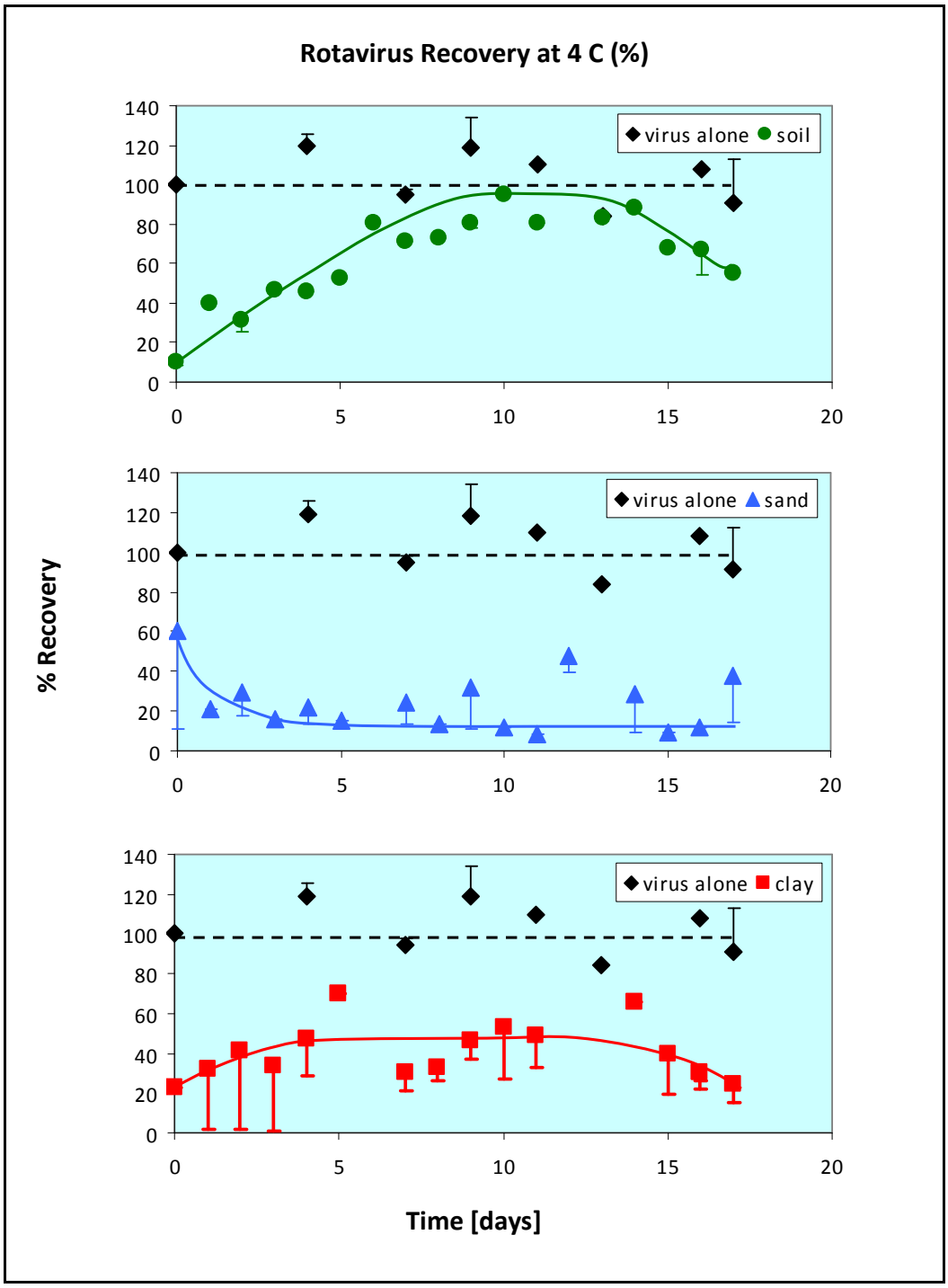


Figure 5.1. Percent recovery of virus alone and in the presence of soil particles at 4 ºC. The virus alone trend is shown as a best fit line (dotted line). Error bars represent +/- one standard deviation from the mean (Davidson, 2007).

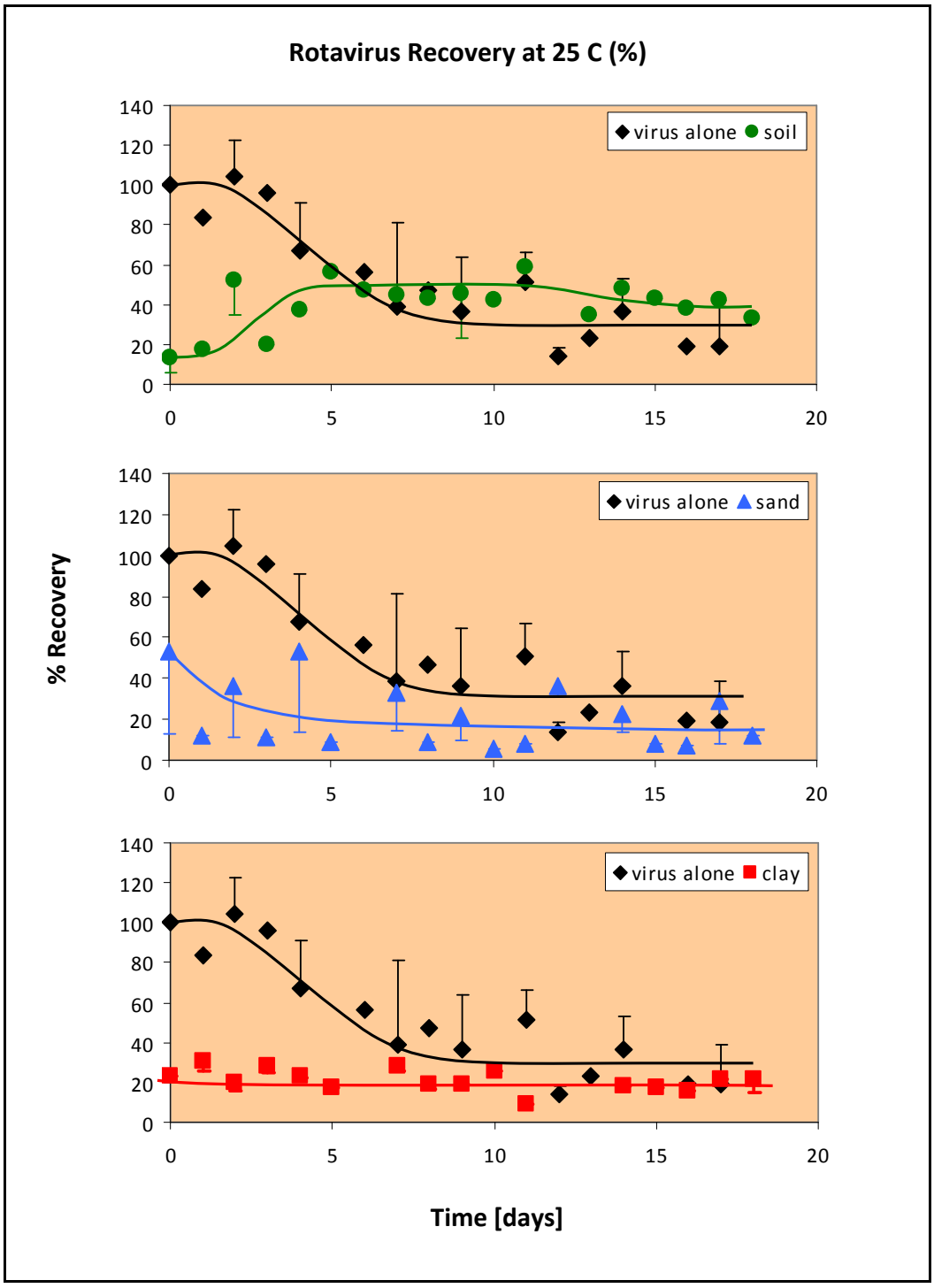


Figure 5.2. Percent recovery of virus alone and in the presence of soil at 25 ºC. Error bars represent +/- one standard deviation from the mean (Davidson, 2007).

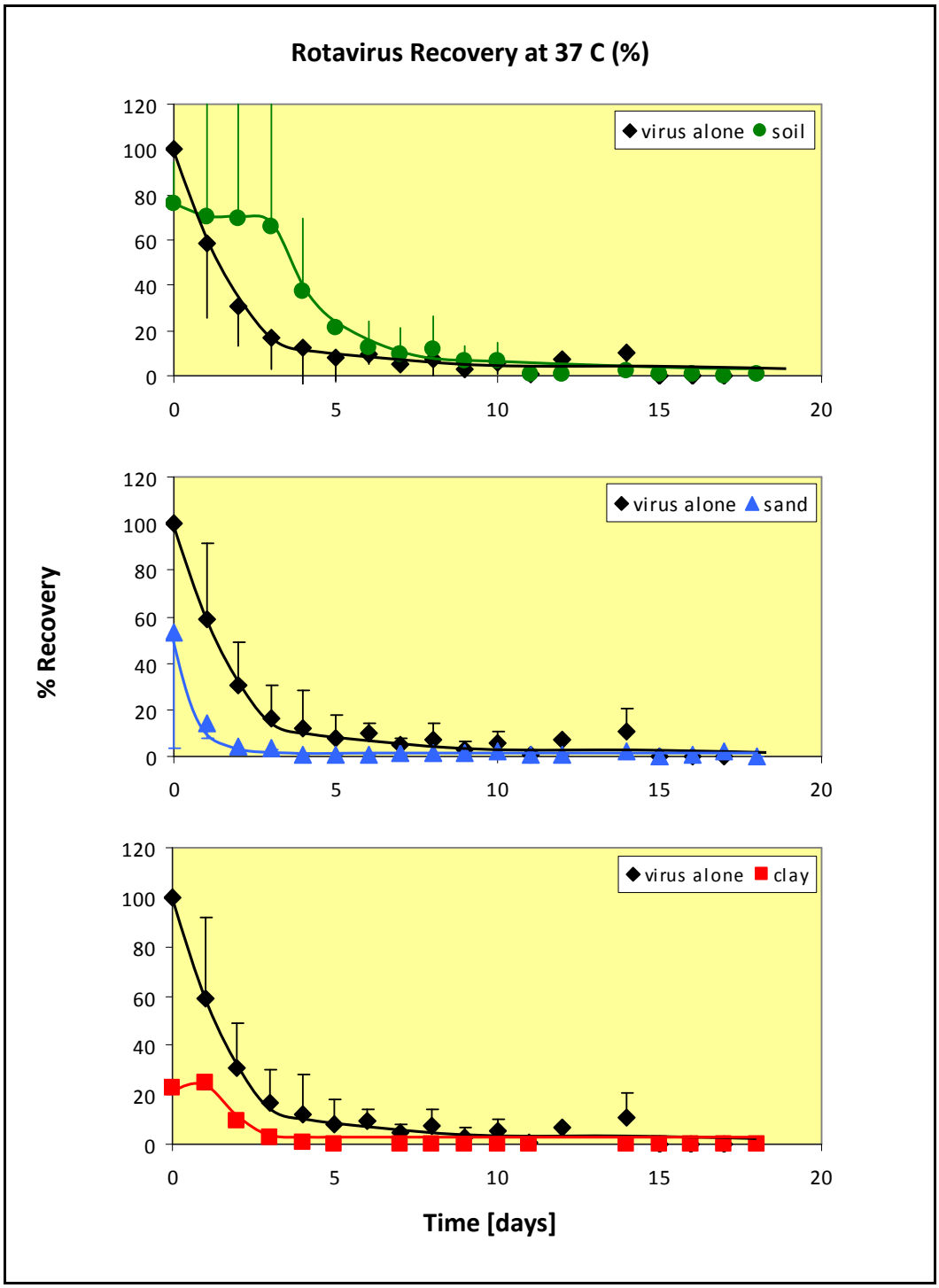


Figure 5.3. Percent recovery of virus alone and in the presence of soil particles at 37 ºC. Error bars represent +/- one standard deviation from the mean (Davidson, 2007).

The rotavirus survival study shows that rotavirus is capable of surviving and is extractable in whole soil, sand, and clay given appropriate conditions. Whole soil appears to have a protective characteristic that improves survival at relatively high temperatures (37 ºC). Both whole soil and clay showed a lower percent recovery of rotavirus in early time periods, probably due to an initial tight adsorption of the rotavirus to the clay particles, with the virus being somewhat desorbed as time progresses. After the initial low recovery, rotavirus was found to survive slightly better in soil than by itself at 25 ºC, therefore, further confirming soil is somewhat thermal protective for rotavirus.

Sand was found to inhibit rotavirus survival at every temperature tested. Only at early time points was any substantial amount of infective rotavirus able to be recovered sand. It is possible that the virus is being inactivated by sand, is being trapped among sand particles, or even that the viruses are not homogeneous, with some more resistant than others. From this study, it cannot be concluded that the sand is toxic or killing the virus. However, considering the large individual pore spaces in sand, the 15-20% of infective rotavirus that is recovered at 25 ºC may never actually come into direct contact with the sand particles. Even though it is currently not possible to determine the exact mechanism for the low recovery in sand, the majority of infective rotavirus is, in large part, incapable of escaping or being extracted from the sand. This reason alone may be enough to strongly consider sand as a filtering material for rotavirus.

The following sections examine the transport of *C. parvum* oocysts and rotavirus particles in three different soil types and two types of vegetation. The recoveries are first plotted against flow rates for comparison of recovery trends with flow rates. Then, to better understand the effects of soil type and vegetation on the reduction of infective *C. parvum* oocysts and rotavirus particles, the data is plotted according to soil type, and then again plotted according to

vegetation type. While this study was focused on the overland transport kinetics of *C. parvum* and rotavirus, a summary (Table 5.1) at the end of this section also shows the recoveries from near-surface flow, soil core extractions, and vegetation extractions for all conditions tested. Since not all infective particles were recovered in the surface runoff, it was deemed necessary to also determine the fate and transport in the soil and near-surface flow, as well as that portion that may have attached to surface vegetation.

5.1 Overland transport kinetics

Due to the nature of the overland transport studies, only one experiment was conducted for each combination of soil type and surface condition. However, the Catlin bare condition was repeated for rotavirus in order to verify that the experimental setup could be accurately reproduced from one experiment to another. Figure 5.4 shows both the hydrograph and rotavirus recovery trend for the two Catlin bare soil experiments. All experimental conditions were the same for the two experiments (slope, rainfall intensity, etc.).

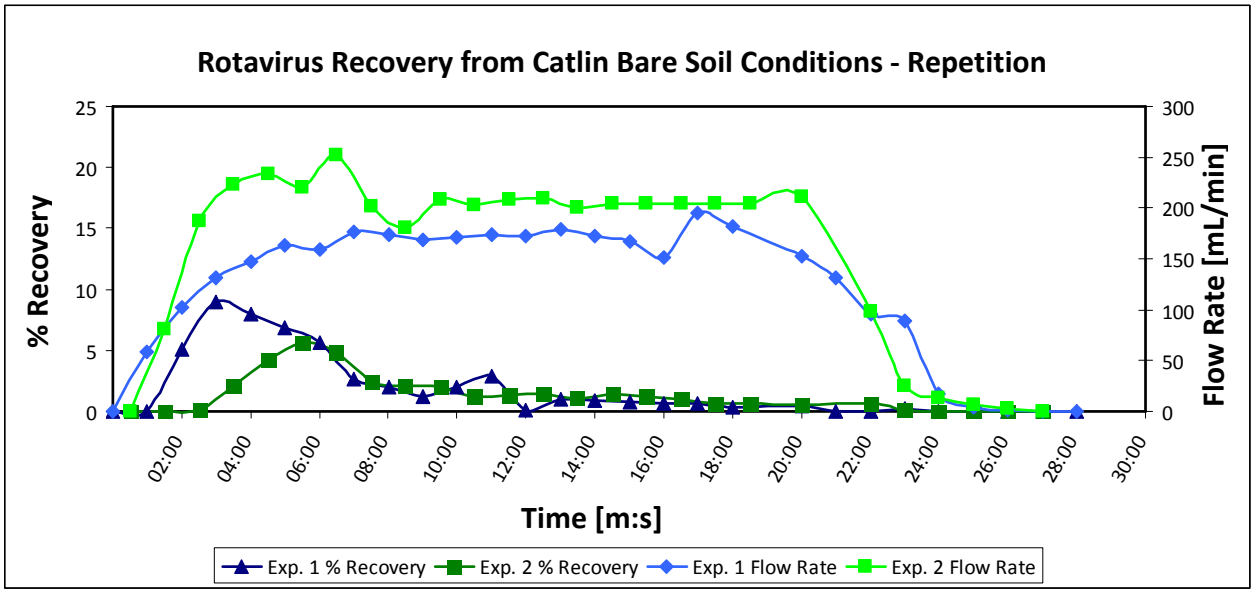


Figure 5.4. The Catlin bare soil condition was repeated for rotavirus to verify that the experimental setup was able to be accurately reproduced.

Since all conditions were the same for the two experiments in Figure 5.4, the experiments will be referred to as experiment 1 and experiment 2 for ease of clarification.

The hydrographs (flow rates for surface runoff) follow a similar trend for each experiment. The flow rate of surface runoff for experiment 2 is slightly higher than that of experiment 1, but this could be due to differences in compaction level. The two soil beds were compacted following the same procedure, but a slight difference in the degree of compaction may have occurred. Both hydrographs show a sudden peak and drop, even though the peaks occur at different times for the two experiments. Since it occurs in both hydrographs, it is likely caused by inconsistencies in the soil surface, or perhaps the collection tray becoming plugged momentarily. Likewise, the rotavirus recovery trends appear very similar for the two experiments. The trend in experiment 2 is delayed longer than in experiment 1, probably also due to compaction levels.

With verification that the small-scale soil box assembly and rainfall simulator could accurately reproduce overland transport kinetics for two experiments of the same conditions, it was deemed sufficient to perform one experiment for the remaining combinations of conditions.

The Catlin soil (24% sand, 50% silt, 26% clay) was used to investigate the transport of both *C. parvum* and rotavirus in bare soil conditions and in soil with Smooth Brome and Tall Fescue vegetative covers. The Catlin soil was collected from a site in Champaign, IL, and is a common soil type in central Illinois. The percent recovery of infective *C. parvum* oocysts and infective rotavirus particles recovered in the surface runoff from the Catlin soil experiments is presented in Figure 5.5 along with the plot of runoff rate over time.

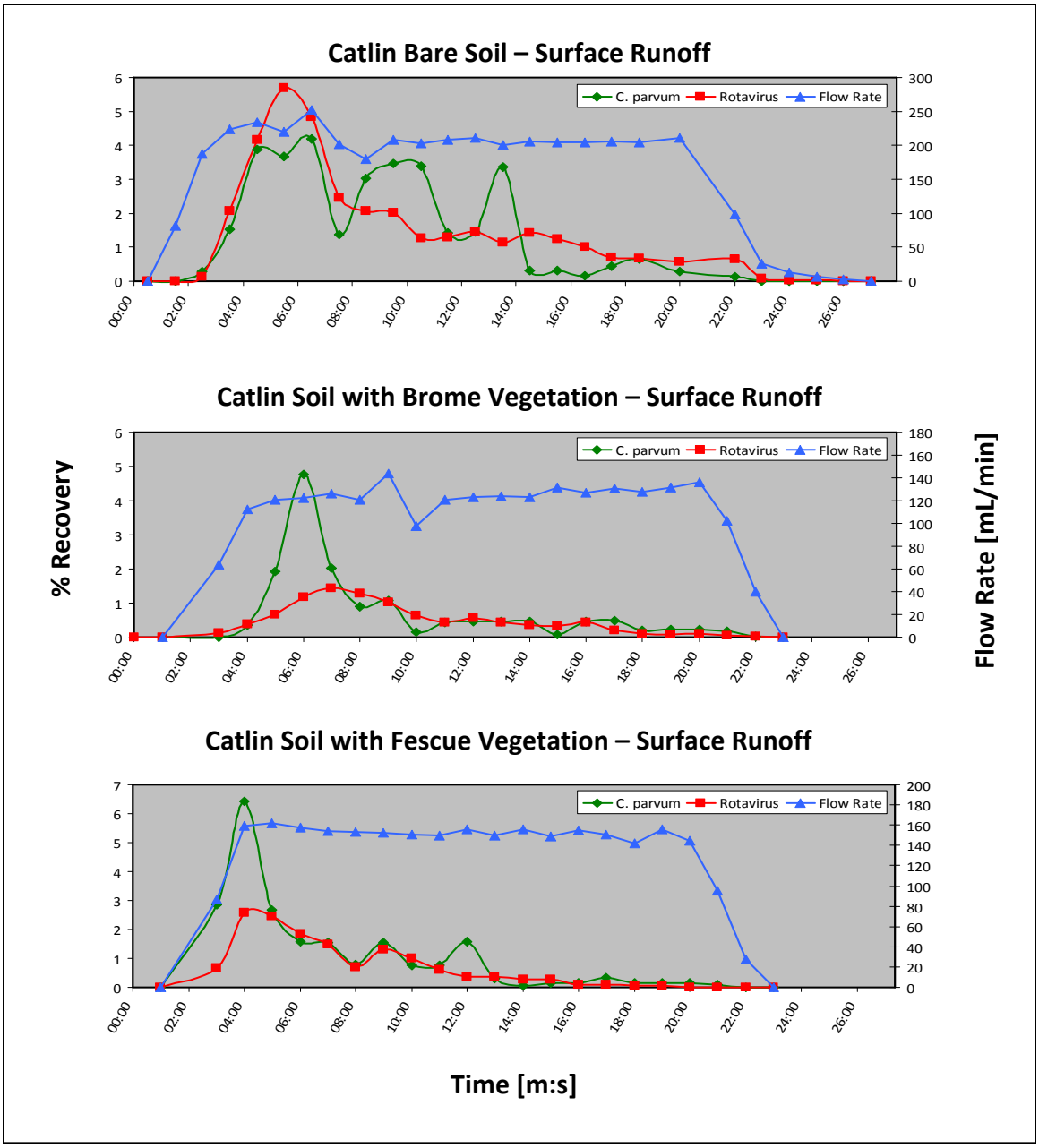


Figure 5.5. The percent recoveries (%) for infective *C. parvum* oocysts and infective rotavirus particles are plotted along with the flow rates (mL/min) of the surface runoff for Catlin soil conditions.

The recovery of infective oocysts from the bare Catlin experiment shows three distinct peaks, with the first peak (~ 4%) being the highest. It is also apparent that the peak recovery of infective rotavirus particles corresponds to the peak flow rate. In both vegetation conditions, the peak recovery is higher than with bare soil conditions. However, vegetation appears to reduce

the two subsequent peaks that occurred with bare soil. Overall, more infective oocysts are recovered in the surface runoff of the bare soil condition (33.38% for bare versus 14.91% for Brome and 22.07% for Fescue).

Less than 35% of the infective rotavirus particles applied is recovered in surface runoff samples for bare soil conditions. Furthermore, the recovery is greatly reduced with the establishment of vegetation. The initial peak is lower and delayed when vegetation is present. When Brome vegetation is present, the first peak occurs at least one minute later than with bare soil and the peak recovery of rotavirus is reduced by nearly 85% (from 6% to 1%) . When Fescue vegetation is present, the first peak occurs two minutes earlier than with bare soil, but the peak recovery is still reduced by nearly 50% (6% to 3%). The differences between the two grasses that are evident here are likely due to a difference in the percent of the surface covered by vegetation in addition to differences in the thickness of the grass stems and size of roots penetrating the soil profile. This is somewhat evident in the differences in flow rates between bare and vegetated conditions, as well as between the two vegetation types. The flow rate for the bare soil conditions is sustained at approximately 225 mL/min for most of the rainfall event, while only 140 mL/min for Brome vegetation and 160 mL/min for Fescue vegetation.

With a decreased number of infective oocysts and infective rotavirus particles being recovered in the surface runoff, it is essential to investigate if a higher number are entering the soil. Infective *C. parvum* oocysts recovered from Catlin soil cores are displayed in Figure 5.6 and infective rotavirus particles recovered from Catlin soil cores are displayed in Figure 5.7. The scale of the y-axis for each plot varies, depending on the magnitude of infective oocysts or infective rotavirus particles recovered from each condition. Soil cores were collected along two transects of the bed, one 5 cm (2 inches) from the upslope end of the bed and one 30.5 cm (12

inches). Four cores were collected along each transect and separated into three equal layers. All soil core plots show the average recovery from each transect, separated by depth.

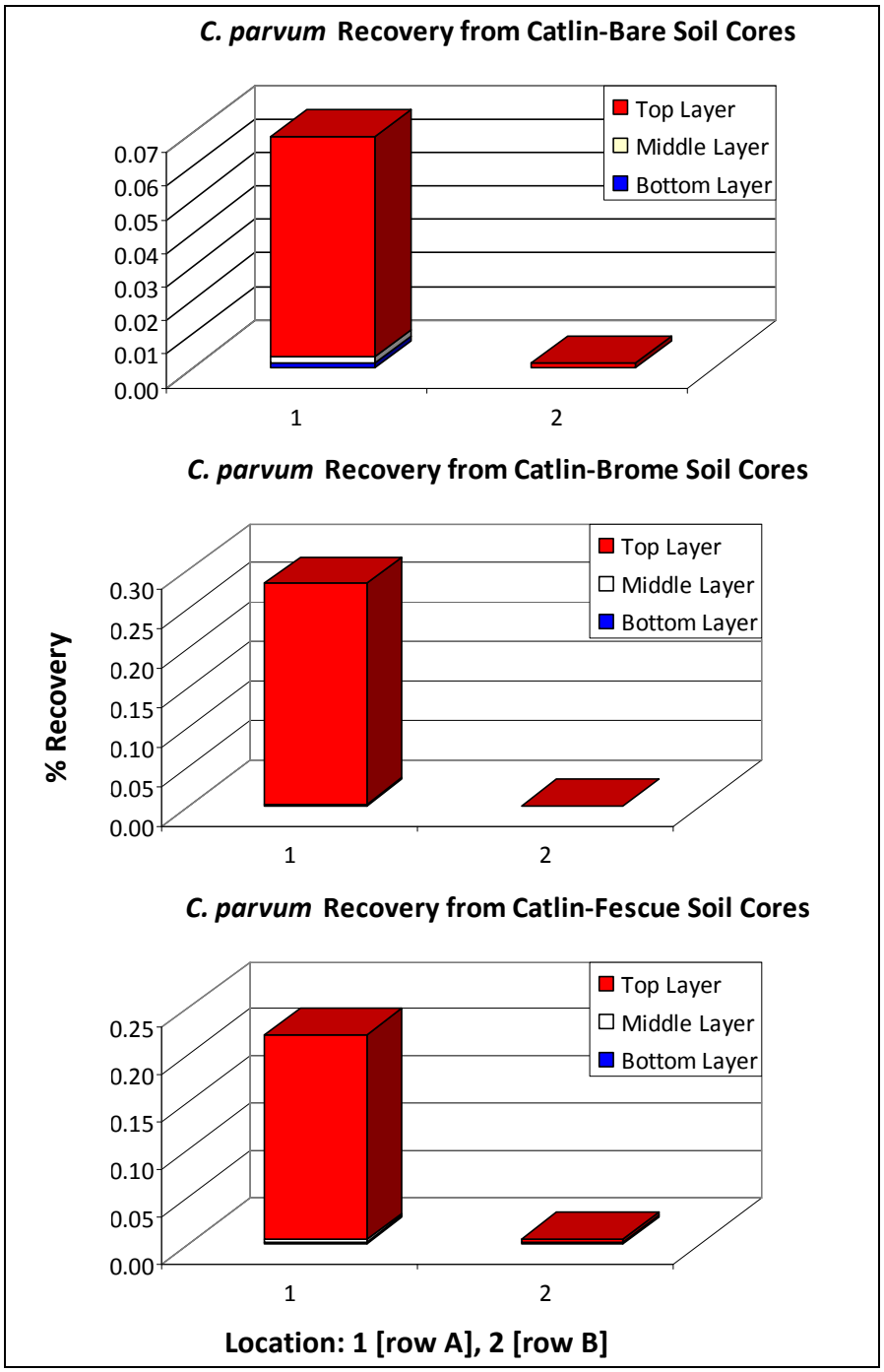


Figure 5.6. The percent recovery of infective *C. parvum* oocysts from Catlin soil conditions is shown as a bar graph. Location 1 is 5 cm (2 inches) and location 2 is 30.5 cm (12 inches) from the upslope edge of the soil bed. Each layer is 2.54 cm (1 inch) in depth, but displayed according to the percent recovered in each layer.

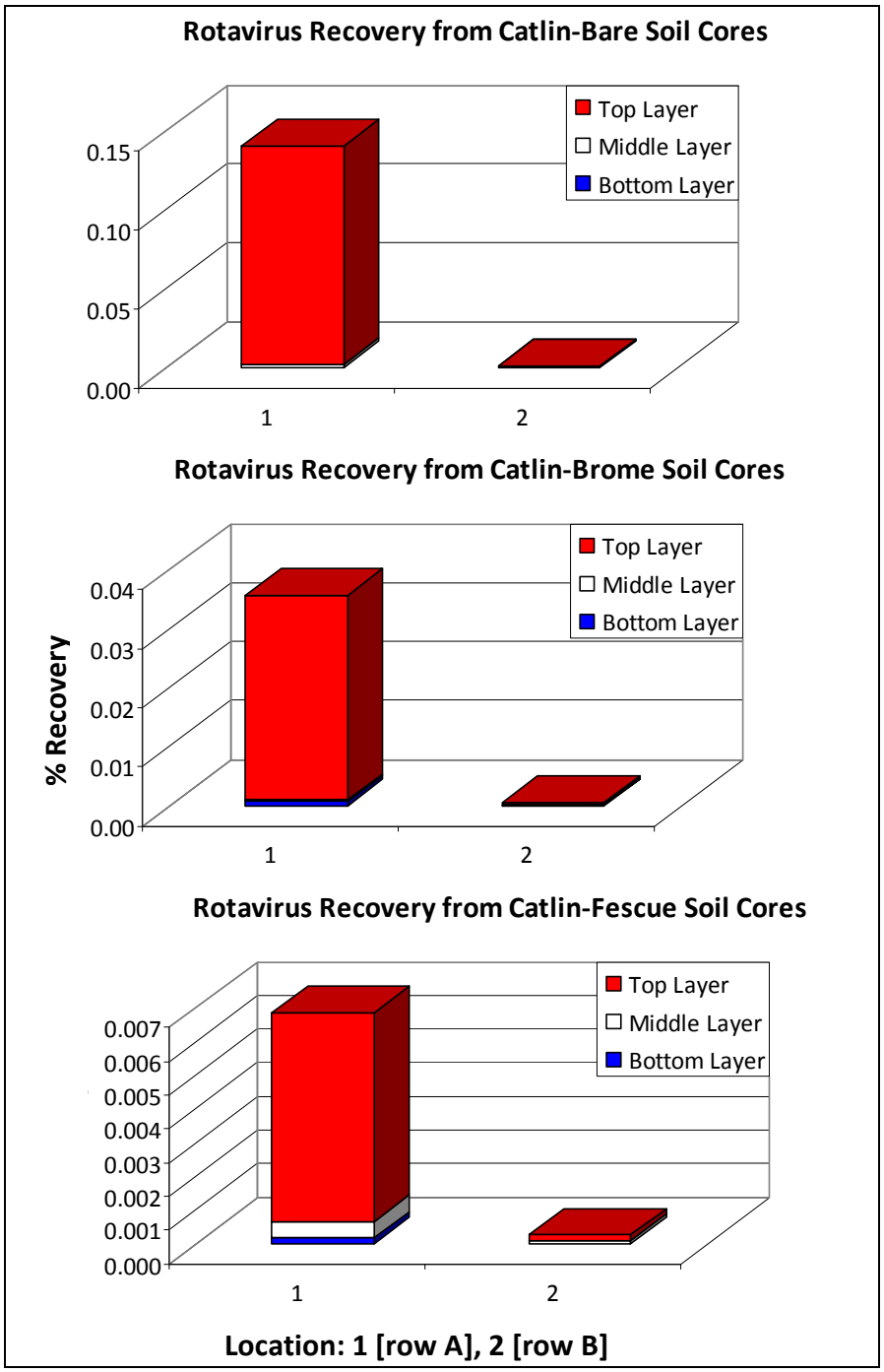


Figure 5.7. The percent recovery of infective rotavirus particles from Catlin soil conditions is shown as a bar graph. Location 1 is 5 cm (2 inches) and location 2 is 30.5 cm (12 inches) from the upslope edge of the soil bed. Each layer is 2.54 cm (1 inch) in depth, but displayed according to the percent recovered in each layer.

The reduced number of infective oocysts in the surface runoff appears to have increased the number entering and penetrating the soil profile. In all three conditions tested, infective

oocsyts penetrated the entire three-inch depth of soil, with the top one-inch layer being the highest. However, infective oocysts were found in near-surface flow only for the Fescue condition, showing that most infective oocysts are being adsorbed to the soil particles and not being allowed to escape with near-surface flow. The oocysts were recovered very near the point of application of the slurry, with only trace amounts being detected at the midpoint of the bed (12 inches from upslope end of bed).

The only infective rotavirus particles recovered in the soil cores of the bare soil were found in the top one-inch layer and near the point of application of the slurry. It appears that some rotavirus was transported just slightly beyond half the length of the bed, but probably quickly dissipated. For the vegetated conditions, infective rotavirus particles were detected throughout the soil profile, with a small amount detected in the near-surface flow (0.15% for Brome and 0.22% for Fescue). Only trace amounts (<0.001%) of infective rotavirus particles reached row B of the bed for vegetated conditions.

The Darwin soil (5% sand, 50% silt, 45% clay) was also used to investigate the transport of both *C. parvum* and rotavirus. The Darwin soil was collected from a site near Newton, IL, and is a common soil type in central Illinois.

Figure 5.8 shows the percent recovery of infective oocysts and infective rotavirus particles recovered in the surface runoff from the Darwin soil experiments along with the plot of runoff rate over time.

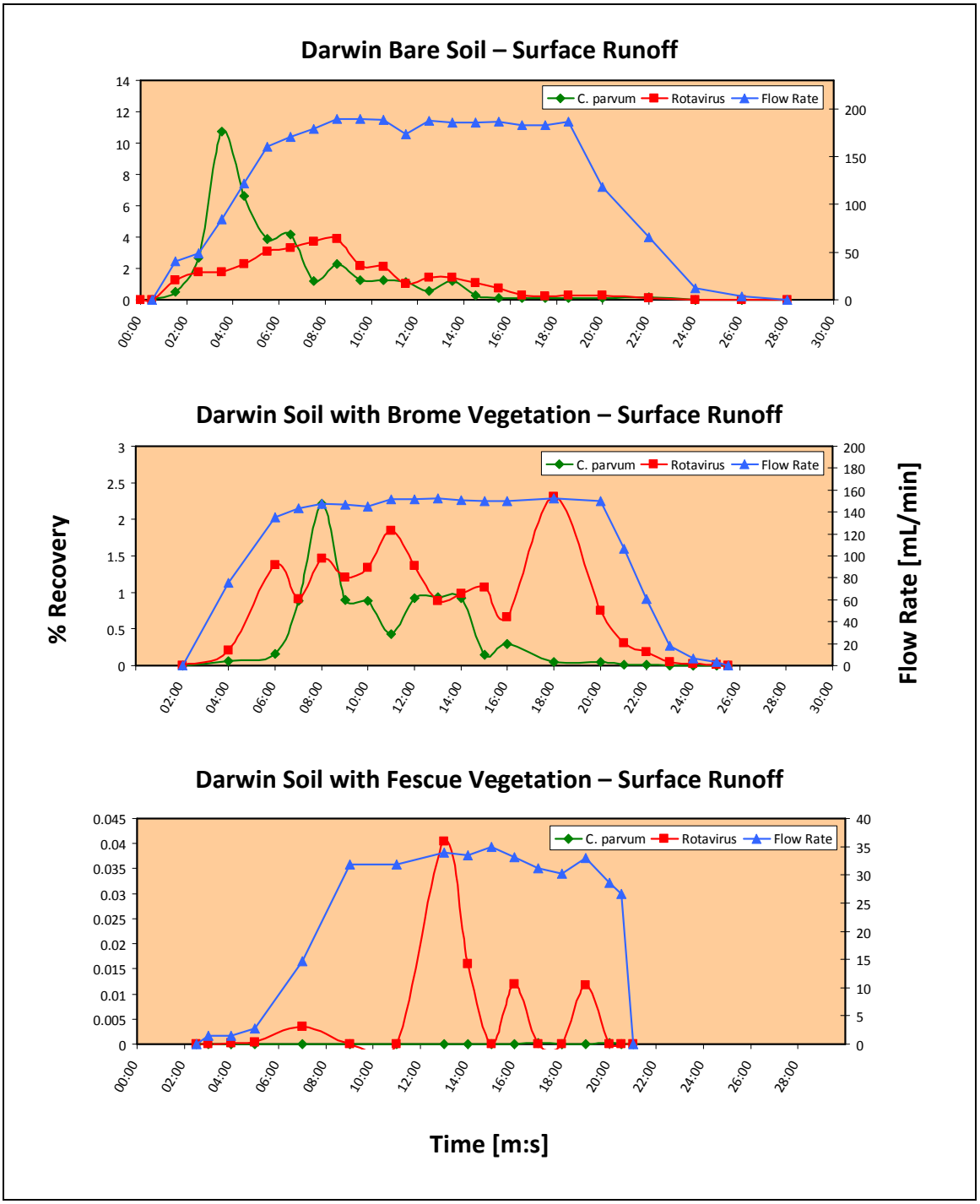


Figure 5.8. The percent recoveries (%) for infective *C. parvum* oocysts and infective rotavirus particles are plotted along with the flow rates (mL/min) of the surface runoff for Darwin soil conditions.

In bare Darwin soil, it appears that the transport of rotavirus is hindered more than the transport of *C. parvum* in bare soil. The recovery of infective *C. parvum* oocysts increased rapidly before reaching a peak of 11% after about four minutes. The recovery then quickly decreased before approaching zero after 14 minutes. The much higher content of clay likely prevented infiltration into the soil profile, causing a flush of oocysts very quickly. The recovery of infective rotavirus particles increased quickly after the start of rainfall, peaking at four percent after approximately eight minutes. The recovery then decreased to zero at approximately 16 minutes. Furthermore, no infective oocysts or infective rotavirus particles were recovered from the near-surface runoff of the bare Darwin condition, and only small amounts were recovered in the soil cores (0.92% of infective rotavirus particles and 0.19% of infective oocysts).

Vegetation causes a noticeable decrease in the recovery of both infective oocysts and infective rotavirus particles. The addition of Brome vegetation decreased the peak recovery of infective oocysts by 82% (11% recovery for bare soil conditions versus 2% for Brome vegetation) and the recovery of infective rotavirus particles by approximately 50% (4% recovery for bare soil conditions versus 2% for Brome vegetation). No infective oocysts were detected for the Fescue condition. Furthermore, Fescue vegetation reduced the peak recovery of infective rotavirus particles by 90% (4% recovery for bare soil versus 0.04% for Fescue vegetation). The significant reduction caused by the Fescue vegetation appears to be directly related to the much lower flow rates. The flow rate for the bare soil conditions is sustained at approximately 200 mL/min for most of the rainfall event, while only 160 mL/min for Brome vegetation and 35 mL/min for Fescue vegetation. The substantially reduced flow rate in the Fescue condition may have been caused by the lower bulk density (Table 5.2) which would have allowed water to more easily penetrate into the soil profile. The lower bulk density is most likely a product of the soil

bed not reaching a high enough compaction level, especially since Darwin soil has a very high clay content, which can make it difficult to compact after being collected from the field. Analysis of the near-surface flow (Table 5.1) shows that the reduced recovery in the surface runoff of the Fescue condition produced a higher recovery in the near-surface runoff for rotavirus (3.86%), but only produced a trace amount (0.28%) of infective oocysts in the near-surface runoff. This may be an indication that rotavirus, due to its much smaller size, is more easily transported through a soil profile. However, it is difficult to make any type of conclusion in regards to subsurface transport with only a 3-inch layer of soil. Figure 5.9 shows the recovery of infective oocysts from Darwin soil and Figure 5.10 shows the recovery of infective rotavirus particles from Darwin soil.

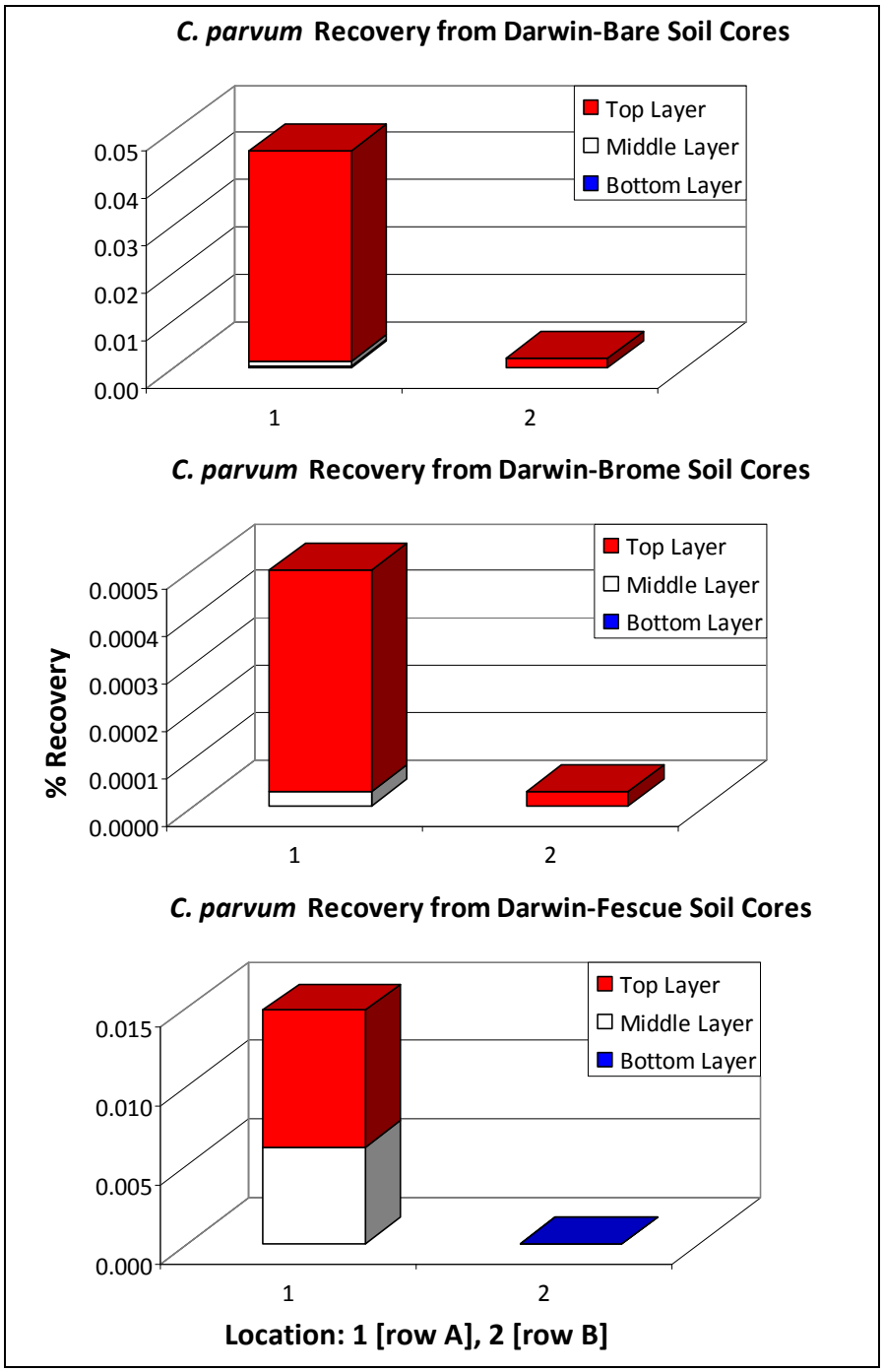


Figure 5.9. The percent recovery of infective *C. parvum* oocysts from Darwin soil cores is shown as a bar graph. Location 1 is 5 cm (2 inches) and location 2 is 30.5 cm (12 inches) from the upslope edge of the soil bed. Each layer is 2.54 cm (1 inch) in depth, but displayed according to the percent recovered in each layer.

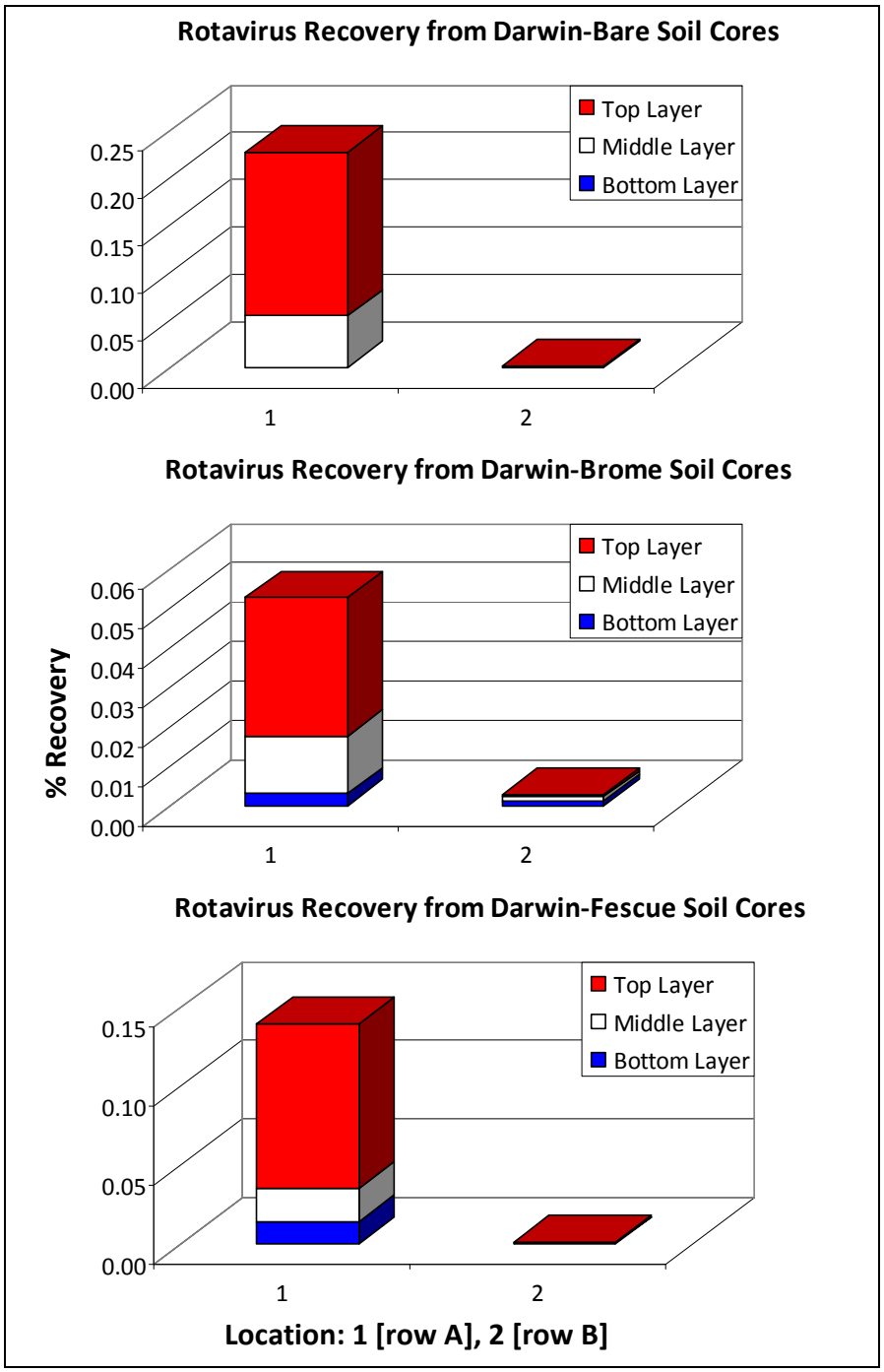


Figure 5.10. The percent recovery of infective rotavirus particles from Darwin soil conditions is shown as a bar graph. Location 1 is 5 cm (2 inches) and location 2 is 30.5 cm (12 inches) from the upslope edge of the soil bed. Each layer is 2.54 cm (1 inch) in depth, but displayed according to the percent recovered in each layer.

The reduced number of infective oocysts in the surface runoff does not appear to have increased the number entering and penetrating the soil profile. Of the three conditions tested, more infective oocysts were detected in the soil of the bare condition than either of the vegetative conditions. However, both Brome and Fescue vegetation reduced the amount of infective oocysts transported downslope, as expected. While more oocysts were detected in the soil for the bare condition, no infective oocysts were detected in the near-surface runoff. For the vegetated conditions, only small amounts of infective oocysts were detected in the soil (~0.0005% for Fescue and 0.06% for Brome), with only the Fescue condition showing a detectable amount of infective oocysts in the near-surface runoff (0.28%). Again, the majority of oocysts were recovered very near the point of application of the slurry, a point where the soil may have been overwhelmed and beyond its capacity to adsorb the oocysts.

Nearly all infective rotavirus particles recovered in the soil cores of the bare soil were found in the top two one-inch layers and near the point of application of the slurry. It appears that some rotavirus (~0.005% detected at midpoint of bed) was transported just slightly beyond half the length of the bed, but probably quickly dissipated. For the vegetated conditions, infective rotavirus particles were detected throughout the soil profile. Both vegetated conditions contained infective rotavirus particles in the near-surface runoff (2.8% for Brome and 3.86% for Fescue). Only trace amounts (<0.001%) of infective rotavirus particles were detected in row B soil cores for vegetated conditions. The majority of infective rotavirus particles are recovered near the point of application of the slurry, and in the top one-inch of soil. The rotavirus particles are transported to some extent down the length of the bed, but have nearly dissipated by the midpoint of the length of the bed.

The Alvin soil (60% sand, 25% silt, 15% clay) was used to investigate the transport of both *C. parvum* and rotavirus. The Alvin soil was collected from a site near Newton, IL, and is a common soil type in central and southern Illinois.

Figure 5.11 shows the percent recovery of infective oocysts and infective rotavirus particles recovered in the surface runoff from the Alvin soil experiments along with the plot of runoff rate over time.

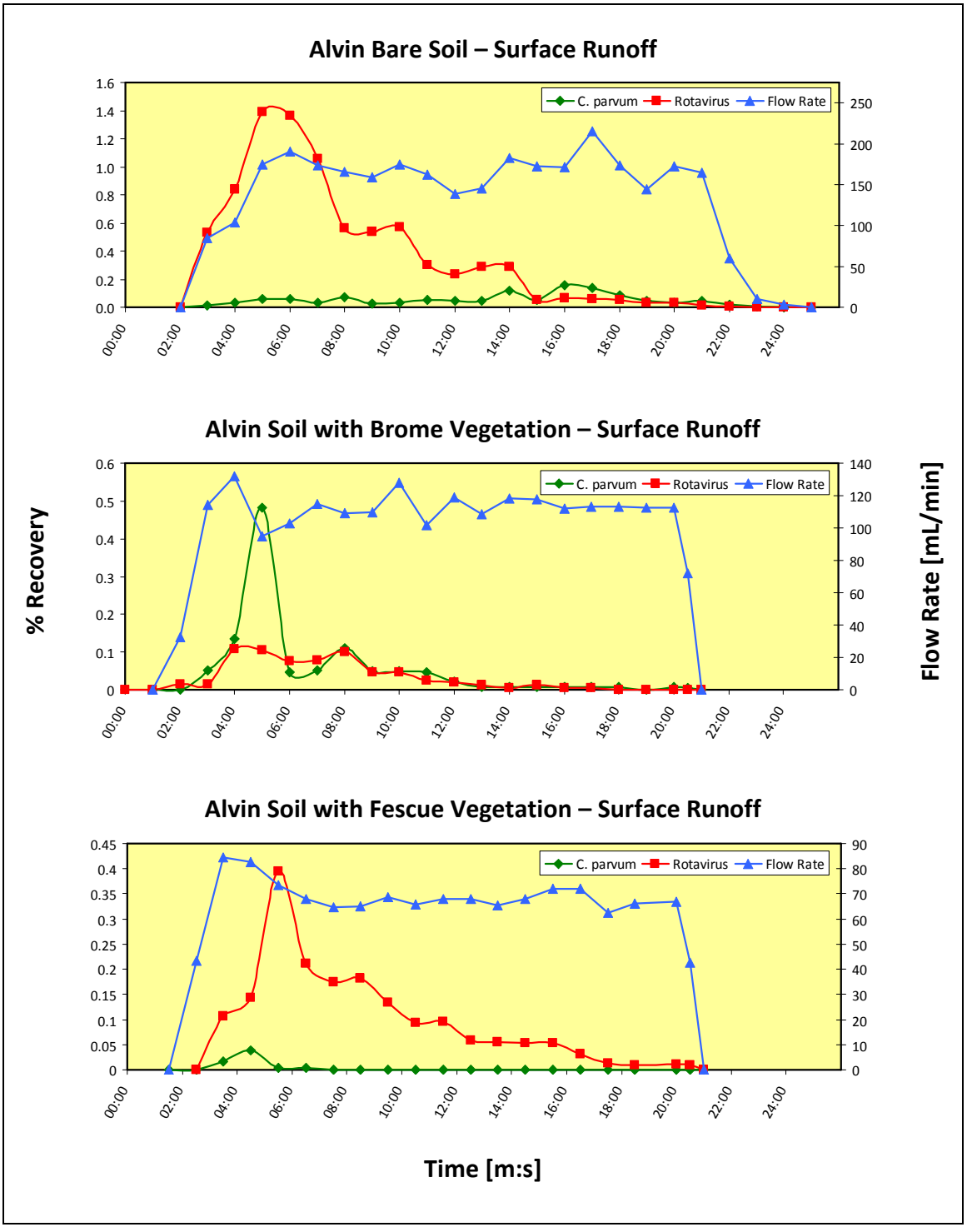


Figure 5.11. The percent recoveries (%) for infective *C. parvum* oocysts and infective rotavirus particles are plotted along with the flow rates (mL/min) of the surface runoff for Alvin soil conditions.

For bare soil conditions, *C. parvum* recovery begins with the surface runoff, with no distinct peak visible, but a maximum recovery of 0.15% occurring at 16 minutes after the start of rainfall. The recovery of infective rotavirus particles increases with the flow rate and reaches a peak of 1.4% at approximately 5 minutes after the start of rainfall. The recovery of rotavirus decreases to less than 0.1% at 15 minutes until completely diminishing by the end of the rainfall event.

With the addition of Brome vegetation, there is a higher peak recovery of infective *C. parvum* than with bare soil. This increase is the product of only one point, so it is possible that a flush of oocysts occurred during that time point. Even with this increased peak recovery, the total amount of infective oocysts recovered in the Brome vegetation condition is less than that from the bare condition (1.10% for Brome vegetation compared to 1.17% for bare soil). Brome vegetation is responsible for a 93% reduction in peak recovery for rotavirus and a 92% reduction in total recovery from surface runoff for the entire rainfall event. The addition of Fescue vegetation reduced the recovery of infective oocysts to nearly zero, with only 0.06% being recovered in the surface runoff for the entire rainfall event. A peak recovery for rotavirus of only 0.4% is seen in the presence of Fescue vegetation, a 71% reduction over bare soil conditions. In addition, a 72% reduction in rotavirus recovery from surface runoff was achieved with Fescue vegetation, reducing the total recovery of infective rotavirus particles in surface runoff to 1.83% for the entire rainfall event.

There is more variation in the flow rates for the Alvin experiments than for the Catlin or Darwin experiments. The variations are likely due to the rough surface produced by the high sand content of the Alvin soil. Small hills or depressions could cause small pulses in the surface runoff, accounting for the fluctuations in the hydrograph. The flow rate for the bare soil

condition fluctuates between 150 and 200 mL/min, while a flow rate of 110-130 mL/min is seen with Brome vegetation and 70-85 mL/min is seen with Fescue vegetation. Since the soil bulk densities for the Brome and Fescue conditions are similar, it is likely that the difference in flow rates is caused by the difference in surface vegetative cover (85% coverage of Fescue versus 54% coverage of Brome).

Figure 5.12 displays the percent recovery of infective oocysts from the soil cores of the Alvin soil conditions and Figure 5.13 displays the percent recovery of infective rotavirus particles from the soil cores of the Alvin soil.

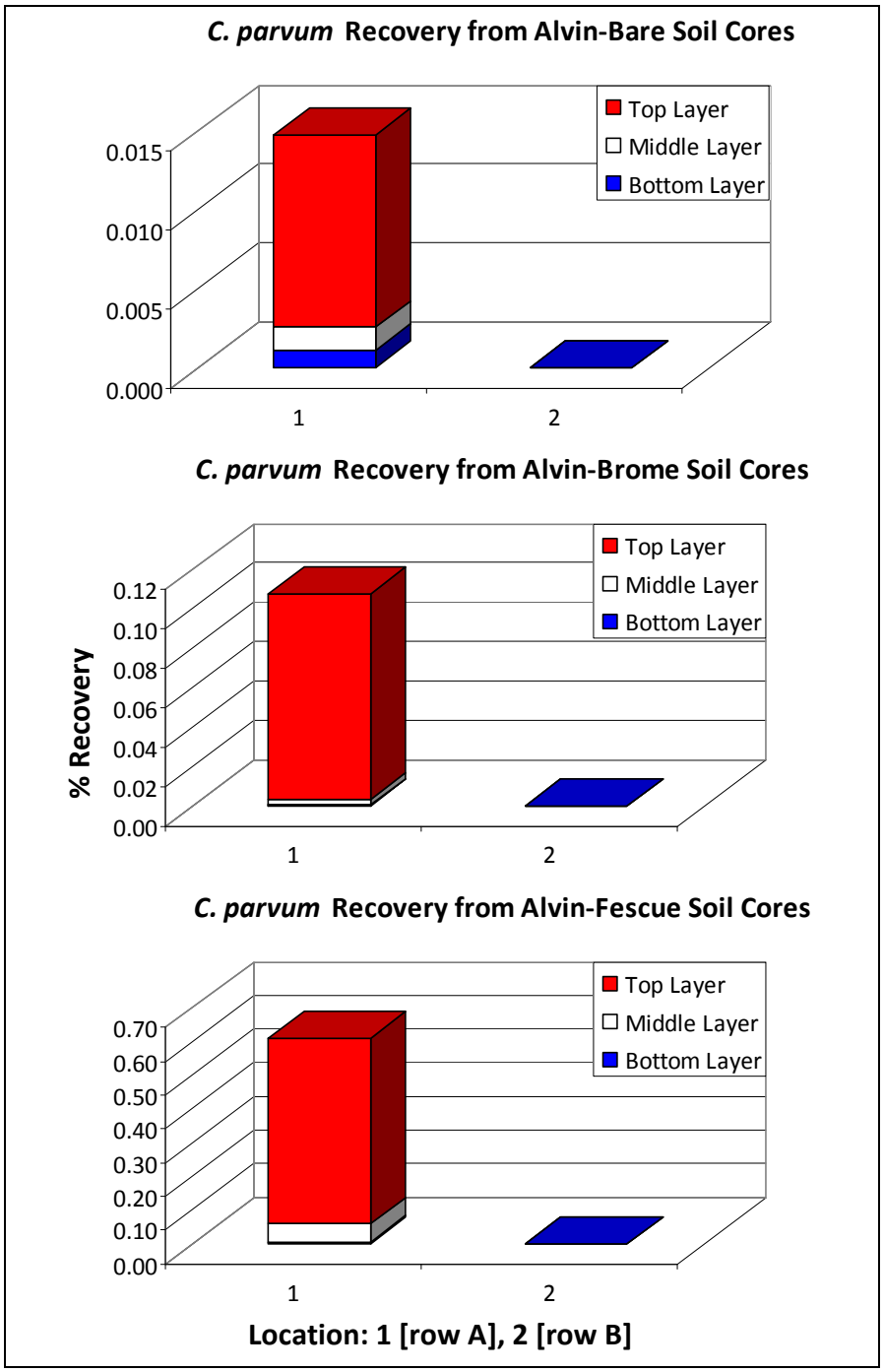


Figure 5.12. The percent recovery of infective *C. parvum* oocysts from Alvin soil conditions is shown as a bar graph. Location 1 is 5 cm (2 inches) and location 2 is 30.5 cm (12 inches) from the upslope edge of the soil bed. Each layer is 2.54 cm (1 inch) in depth, but displayed according to the percent recovered in each layer.

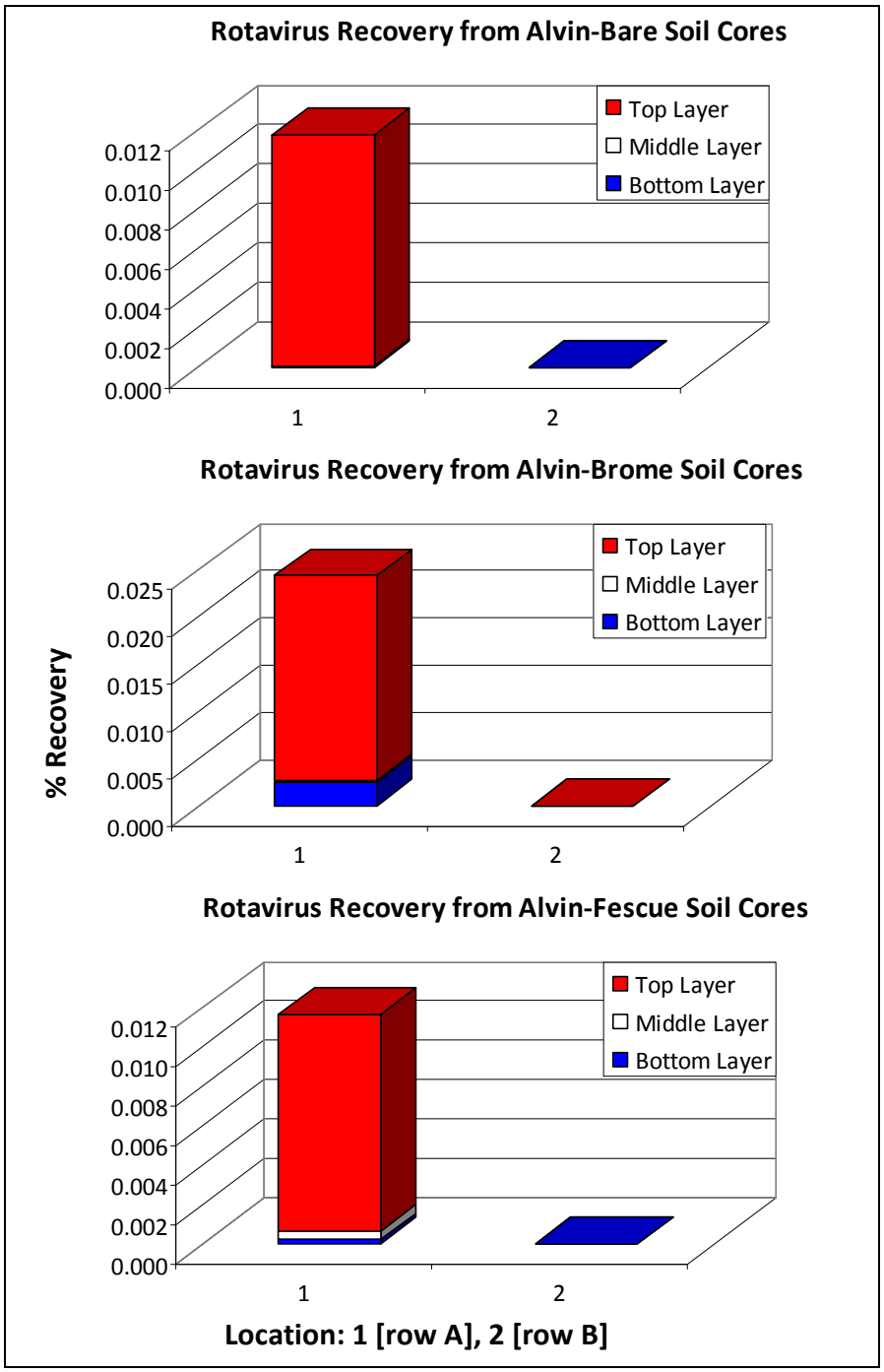


Figure 5.13. The percent recovery of infective rotavirus particles from Alvin soil conditions is shown as a bar graph. Location 1 is 5 cm (2 inches) and location 2 is 30.5 cm (12 inches) from the upslope edge of the soil bed. Each layer is 2.54 cm (1 inch) in depth, but displayed according to the percent recovered in each layer.

The reduced number of infective oocysts in the surface runoff appears to have increased the number entering and penetrating the soil profile. Of the three conditions tested, more

infective oocsyts were detected in the soil of the Brome (0.43%) and Fescue (2.44%) conditions than the bare condition. For the Alvin soil conditions, the recovery of infective *C. parvum* in the soil was restricted to the upslope half of the bed. The majority of the recovered oocysts were in the top one-inch layer of soil, but some oocysts were recovered in both of the other two one-inch layers as well. While some near-surface runoff did occur, there were no infective oocysts recovered in the near-surface runoff of the bare or Fescue condition and only a small amount (0.03%) in the near-surface of the Brome condition. There were no infective oocysts detected at location 2 (row B), at the midpoint of the length of the bed.

Nearly all infective rotavirus particles recovered in the soil cores of the bare soil were found in the top one-inch layer and near the point of application of the slurry. There were no infective rotavirus particles transported beyond half the length of the bed. Both vegetated conditions contained infective rotavirus particles in the near-surface runoff (0.08% for Brome and 0.07% for Fescue).

Table 5.1. Summary of partitioning of infective *C. parvum* oocysts and infective rotavirus particles between surface flow, near-surface flow, soil core extractions, and vegetation extractions. All values are percentages (%) of the amount recovered to the amount applied.

Catlin Soil								
Condition	% Recovery Rotavirus				% Recovery *C. parvum*			
	Surface	Near-Surface	Soil Cores	Vegetation	Surface	Near-Surface	Soil Cores	Vegetation
Bare	34.92	0.00	0.57	N/A	33.38	0.00	0.28	N/A
Brome	9.87	0.15	0.14	0.01	14.91	0.00	1.13	0.03
Fescue	14.23	0.22	0.03	0.01	22.07	0.02	0.90	0.02

Darwin Soil								
Condition	% Recovery Rotavirus				% Recovery *C. parvum*			
	Surface	Near-Surface	Soil Cores	Vegetation	Surface	Near-Surface	Soil Cores	Vegetation
Bare	26.56	0.00	0.92	N/A	38.58	0.00	0.19	N/A
Brome	16.91	2.80	0.22	0.03	8.89	0.00	0.00	0.00
Fescue	0.08	3.86	0.42	0.05	0.00	0.28	0.06	0.00

Alvin Soil								
Condition	% Recovery Rotavirus				% Recovery *C. parvum*			
	Surface	Near-Surface	Soil Cores	Vegetation	Surface	Near-Surface	Soil Cores	Vegetation
Bare	8.28	0.00	0.05	N/A	1.17	0.00	0.06	N/A
Brome	0.67	0.08	0.10	0.00	1.10	0.03	0.43	0.01
Fescue	1.83	0.07	0.05	0.03	0.06	0.00	2.44	0.01

Table 5.1 shows the surface recoveries as well as the recoveries in near-surface runoff, soil core extractions, and vegetation extractions. As discussed previously, surface vegetation not only decreases the amount of surface runoff, but also the amount of infective particles being discharged in surface runoff. The reduction of particles transported in the surface runoff, however, does tend to increase the amount being recovered in the near-surface. There is no apparent linear relationship between the recovery of infective oocysts or infective rotavirus particles and vegetative cover or vegetation type. Very small amounts of infective oocysts and infective rotavirus particles were from samples of surface vegetation. However, it appears that this finding could be misleading since small amounts of soil were attached to the grass when collected, and the soil may have contained reasonable amounts of either *C. parvum* or rotavirus, especially the grass samples collected along the point of application.

5.2 *C. parvum* overland transport kinetics

This section presents and discusses the overland transport of *C. parvum* oocysts and the effect of soil and vegetation type on the recovery of infective oocysts. The three soil types are first compared by surface condition to present the effect, if any, soil type has on recovery of infective *C. parvum* oocysts during overland transport. Then, the three surface conditions are compared according to soil type to present the effect, if any, vegetation type has on recovery of infective *C. parvum* oocysts during overland transport.

5.2.1 Effect of soil type on *C parvum* overland transport kinetics

The three soil types are first compared in this section according to the surface condition in order to examine the effect of soil type on the recovery of infective *C. parvum* oocysts during overland transport. Figure 5.14 shows the three soil types separated by surface condition.

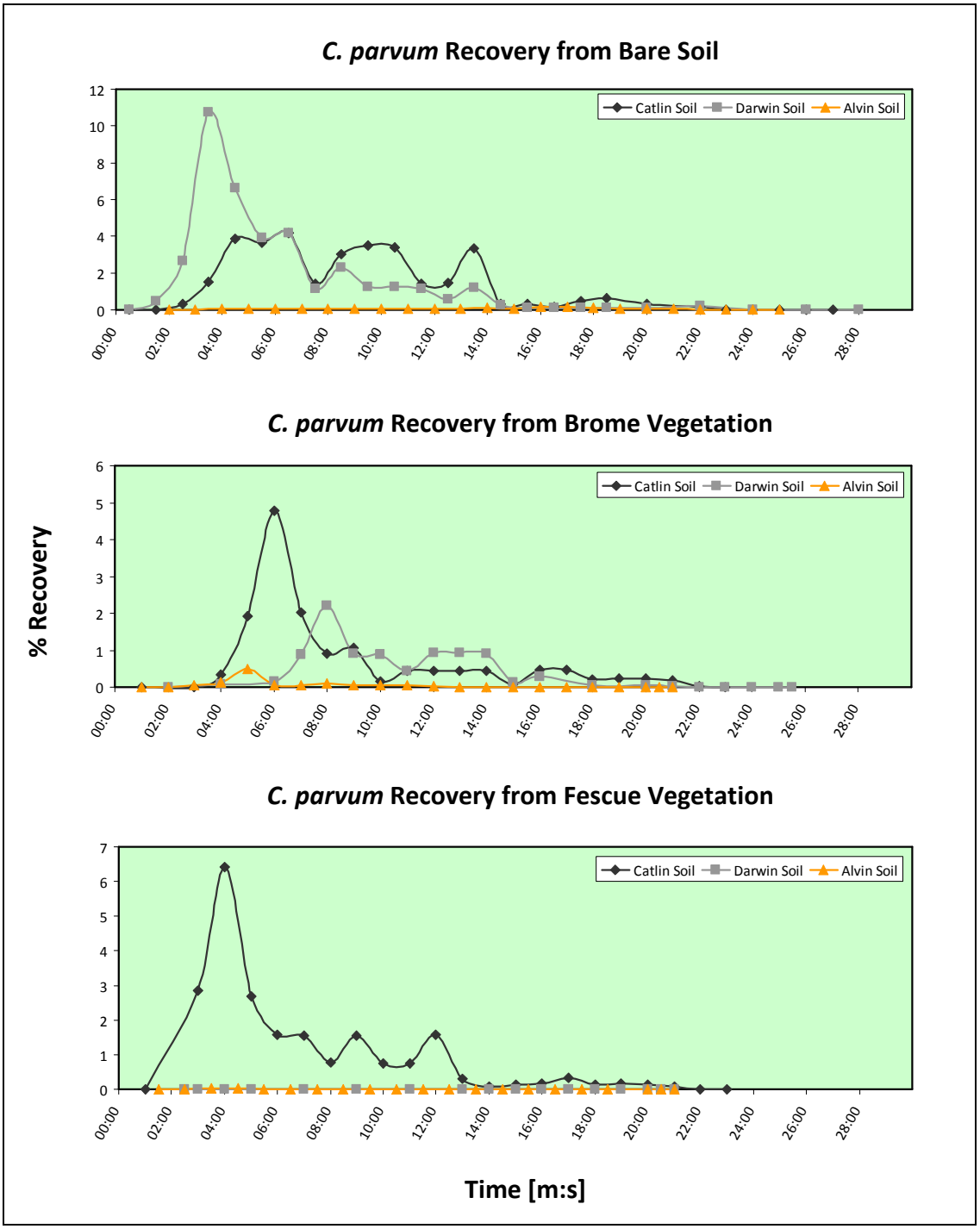


Figure 5.14. The recovery of infective C. parvum oocysts in surface runoff is plotted for all three soil types according to surface condition to compare the effect of soil.

At first glance, it appears Darwin (high clay) soil increases the amount of infective *C. parvum* oocysts recovered in surface runoff, in contrast to our previous findings that *C. parvum*

oocysts preferentially bind to clay particles. While this remains true, it is likely due to the reduced infiltration rate of soils with high clay contents. The oocysts may never come into contact with the clay soil particles: suspended in surface water and being directly transported in surface runoff. An inability for water to initially infiltrate the soil would cause this initial flush of oocysts. Over time, the recovery from Darwin soil decreases below that of Catlin soil. The total recovery for the entire rainfall event is similar for the Catlin (33.38%) and Darwin (38.58%) soils. The Catlin soil is approximately 50% silt and 26% clay while the Darwin soil is 50% silt and 45% clay. The silt and clay soil fractions compact more tightly, especially during rainfall events, sealing the surface soil structure and hindering soil infiltration. With a much reduced infiltration rate, several minutes are required for the oocysts to penetrate the soil surface. The recovery from Alvin soil, however, is much lower than that from both the Catlin and Darwin soils. The total recovery in surface runoff for Alvin soil is only 1.17%. As with the Darwin soil, this is likely due to the characteristics of the soil and the ability of water to better infiltrate a sandy soil such as Alvin. The Alvin soil is approximately 60% sand, with only 25% silt and 15% clay. The high sand content would allow for immediate soil penetration, at least into the thin mixing layer near the soil surface. After only a few minutes, the oocysts have the opportunity to thoroughly mix with the soil and soil water. Since sand particles are quite heavy, they are kept in place with some oocysts being transported in the overland runoff.

With the addition of Brome vegetation, recovery of infective oocysts is highest for Catlin soil. With the increased ability of water to infiltrate the soil, it is now apparent that the clay particles may be interacting with the oocysts. The Alvin soil has a much lower recovery than Catlin or Darwin soil. This reduced recovery from Alvin soil is controlled by flow

characteristics, with water more easily penetrating a sandy soil. With Brome vegetative cover, Alvin is the only soil with infective oocysts in the near-surface runoff.

With Fescue vegetative cover, no significant amount of infective oocysts is recovered from Darwin or Alvin soils. Again, this could be due to flow characteristics. The Catlin soil had the lowest percent of vegetative cover, and therefore, did not have as much obstruction to surface flow. This would account for the initial pulse of oocysts, followed by a quick decrease to less than two percent recovery. The total surface runoff volume from Catlin soil was substantially higher than that from either Darwin or Alvin soils (Table 5.2).

Table 5.2. Summary of *C. parvum* recoveries from surface runoff tabulated in conjuction with the seven experimental parameters collected for each combination of soil and vegetation type.

Bare Soil Conditions								
Soil	**% sand**	**% silt**	**% clay**	**% veg. cover**	**% OM**	**Bulk Density (g/cc)**	**Runoff Volume (mL)**	**% Recovery *C. parvum***
Catlin	24	50	26	0	7.18	1.05	4384	33.38
Darwin	5	50	45	0	5.45	1.08	3189	38.58
Alvin	60	25	15	0	2.10	1.28	3140	1.17

Brome Vegetation								
Soil	**% sand**	**% silt**	**% clay**	**% veg. cover**	**% OM**	**Bulk Density (g/cc)**	**Runoff Volume (mL)**	**% Recovery *C. parvum***
Catlin	24	50	26	49	7.18	1.05	2387	14.91
Darwin	5	50	45	72	5.45	0.95	2711	8.89
Alvin	60	25	15	54	2.10	1.25	2139	1.10

Fescue Vegetation								
Soil	**% sand**	**% silt**	**% clay**	**% veg. cover**	**% OM**	**Bulk Density (g/cc)**	**Runoff Volume (mL)**	**% Recovery *C. parvum***
Catlin	24	50	26	67	7.18	1.00	2894	22.07
Darwin	5	50	45	68	5.45	0.91	467	0.00
Alvin	60	25	15	85	2.10	1.28	1301	0.06

The data in Table 5.2 were used to analyze trends between individual characteristics of each condition and the actual percent recovery of infective *C. parvum* oocysts detected in the surface runoff (Figure 5.15). Since it is now believed that oocysts may never interact with the

soil in surface runoff (Darwin bare soil condition), trends were also plotted for soil core extractions (Figure 5.16), to examine the effect when interactions do occur between soil particles and oocysts.

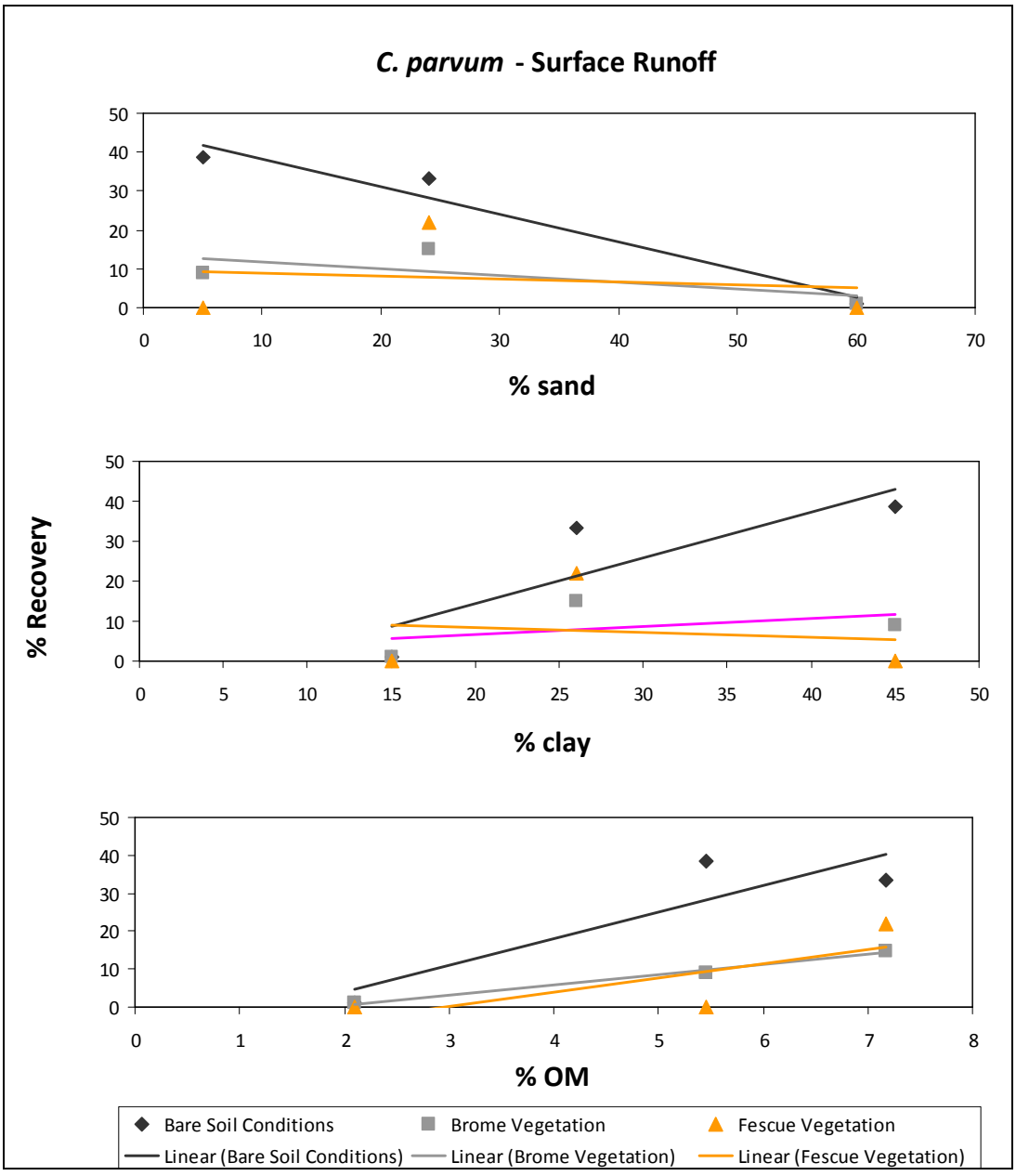


Figure 5.15. The recovery of infective C. parvum oocysts in surface runoff is plotted versus % sand, % clay, and % organic matter.

From these trends, it appears that sand decreases the recovery of oocysts in surface runoff, while clay increases recovery in surface runoff, both of which are in contrast to preliminary findings that oocysts primarily interact with clay particles. However, these trends also appear to be directly related to overland flow characteristics. The higher clay content would decrease soil infiltration rates and allow more runoff. Sand on the other hand, would provide a high soil infiltration rate and allow much more water, and oocysts, to infiltrate the soil profile.

The third plot shows that oocyst recovery increases with increasing organic matter content, which confirms findings by Blanc and Nasser (1996), who found that organic matter provides negative charge attachment sites that inhibit attachment of negatively charged microbial organisms.

If a portion of the infective oocysts are never interacting with the soil surface, it is not possible to determine if one particular fraction of soil is having a greater impact than another on oocyst survival. However, analysis of the soil core extractions could help explain this. The soil core extractions contain the fraction of infective oocysts that successfully entered the soil and were then extracted. The percent of oocysts recovered in the soil core extractions, then, is the fraction of oocysts that either does not interact with soil particles, or is not affected by the interaction with the soil. The soil core extractions are plotted against percent sand, percent clay, and percent organic matter in Figure 5.16.

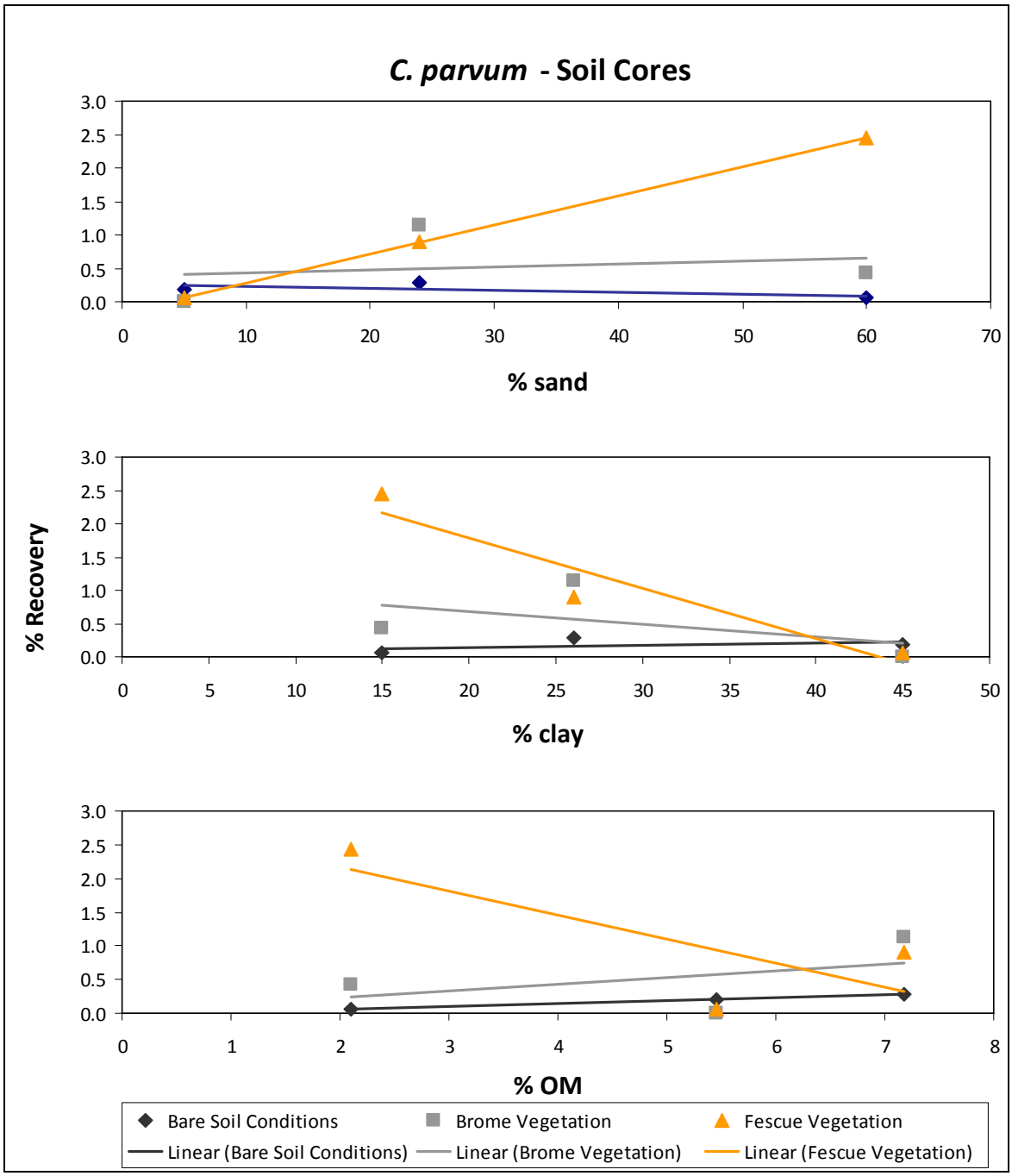


Figure 5.16. The recovery of infective C. parvum oocysts recovered from soil core extractions is plotted versus % sand, % clay, and % organic matter.

The soil core extraction trends are quite different than those for surface runoff. It appears that the oocysts which successfully enter the soil are more affected by the clay content, as was observed from preliminary results. While the relationships for percent sand and percent clay are

not conclusive, they show a strong trend confirming that soils high in clay content are better at reducing the recovery of infective oocysts. Organic matter slightly increases recovery with increasing organic matter content for the bare soil and Brome vegetation conditions, but decreases recovery with increasing organic matter content for the Fescue vegetation condition. Alvin soil with Fescue vegetation showed a recovery of 2.44% in the soil core extraction. This higher recovery could be due to a higher surface cover of Fescue vegetation (85% cover). The higher surface cover would correspond to a higher density of roots penetrating the soil, and in turn provide more macropores for storage of water. If the roots did not penetrate the entire depth of soil, the water would not enter the near-surface runoff. Instead, both the water and oocysts would be allowed to remain unaffected until extracted from the soil cores.

5.2.2 Effect of vegetation on *C. parvum* overland transport kinetics

The three surface conditions are compared in this section according to the soil type in order to examine the effect of vegetation on the recovery of infective *C. parvum* oocysts during overland transport. Figure 5.17 shows the three surface conditions separated by soil type.

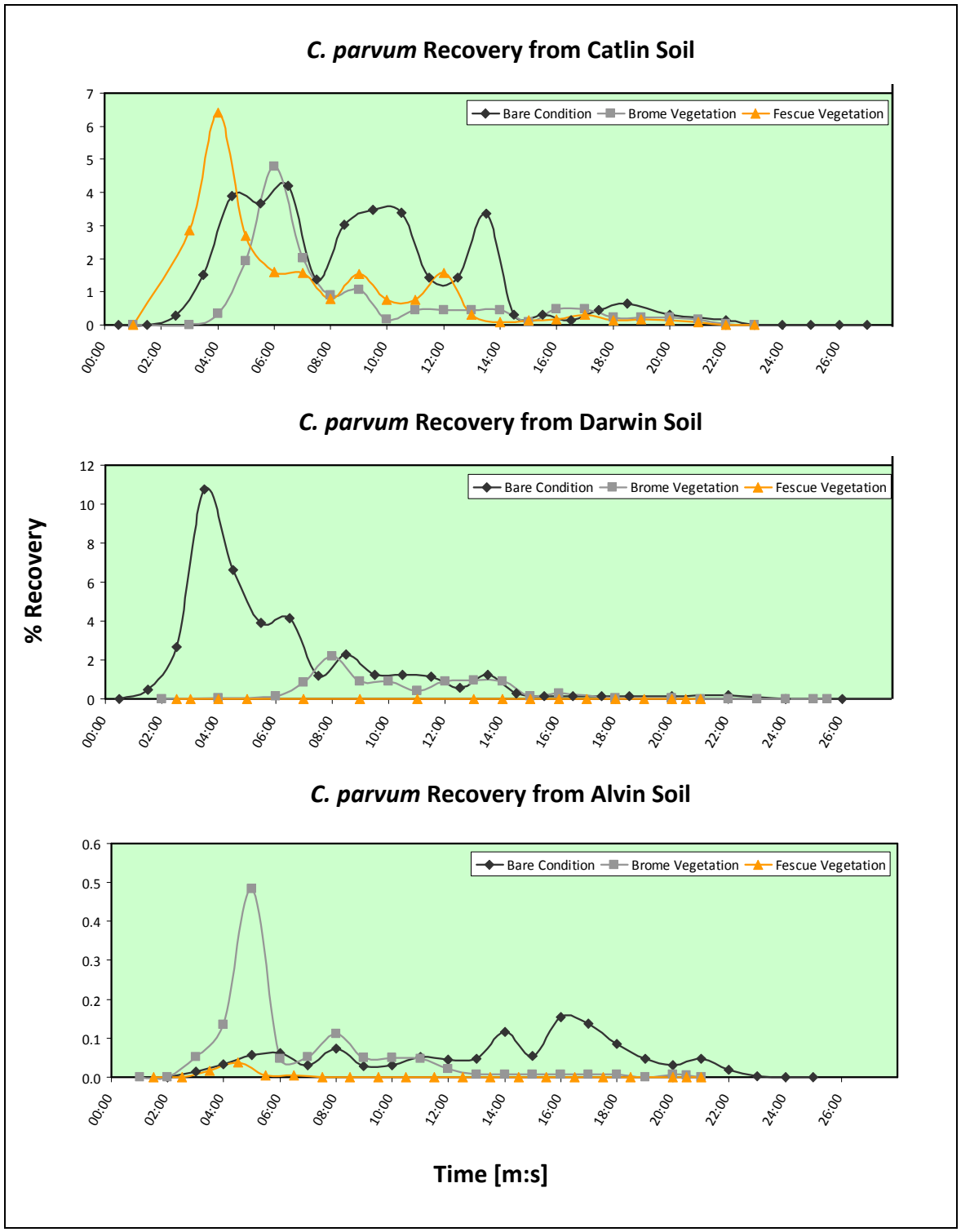


Figure 5.17. The recovery of infective C. parvum oocysts in surface runoff is plotted for all three surface conditions according to soil type to compare the effect of vegetation.

When all three surface conditions are plotted together according to soil type, the effect of surface condition is more easily noticed. For Catlin soil, both vegetated conditions show a higher peak recovery than that from the bare soil condition. However, it can be seen that the bare soil condition has a total of three peaks, while the Brome and Fescue vegetation conditions each only have one. Therefore, the grass appears to be responsible for limiting possible pulses of oocysts from being discharged in surface runoff. Vegetation serves a couple of purposes in this situation. The canopy cover that is provided by the vegetation is a source of interception, absorbing much of the impact from the rain droplets before colliding with the soil surface. With a lower state of energy upon collision with the soil surface, there is a reduced chance of surface soil pores being sealed upon impact. Surface sealing would reduce penetration into the soil profile and increase surface transport. In this case, however, the vegetation has reduced the consequences of the rain colliding with the soil surface and has also increased the potential for infiltration into the soil matrix. As an added bonus, the vegetation inhibits the flow of water over the soil surface. Instead of freely flowing over the soil surface, the stems of grass block direct flow down the slope of the bed and redirect the flow around each stem. Over the entire length of slope, these factors greatly delay the peak flow, and in turn the peak recovery of oocysts.

For Darwin soil, the bare soil condition has a much higher peak recovery than either of the vegetated conditions. As discussed previously, this is probably due to a low soil infiltration rate of the high clay soil. The roots of the vegetation create macropores that serve as small channels for water to enter the soil, reducing the volume of surface runoff as well as the number of infective oocysts in the surface runoff.

For the Alvin soil, the Brome vegetation condition has the highest peak recovery of 0.5%. While it is still a rather low recovery, it is much higher than the recoveries from bare soil and Fescue vegetation conditions. The high peak recovery of the Brome vegetation condition could be due to a preferential flow path in the soil surface that would have bypassed obstructions caused by the vegetation.

While vegetation, in general, appears to decrease the recovery of infective oocysts in surface runoff, there does not appear to be a clear advantage of any one vegetation type. Perhaps more important is the surface vegetative cover and the extent of the root system in the soil profile.

5.3 Rotavirus overland transport kinetics

This section presents and discusses the overland transport of infective rotavirus particles and the effect of soil and vegetation type on the recovery of infective particles. The three soil types are first compared by surface condition to present the effect, if any, soil type has on recovery of infective rotavirus particles during overland transport. Then, the three surface conditions are compared according to soil type to present the effect, if any, vegetation type has on recovery of infective rotavirus particles during overland transport.

5.3.1 Effect of soil type on rotavirus overland transport kinetics

The three soil types are first compared in this section according to the surface condition in order to examine the effect of soil type on the recovery of infective rotavirus particles during overland transport. Figure 5.18 shows the three soil types separated by surface condition.

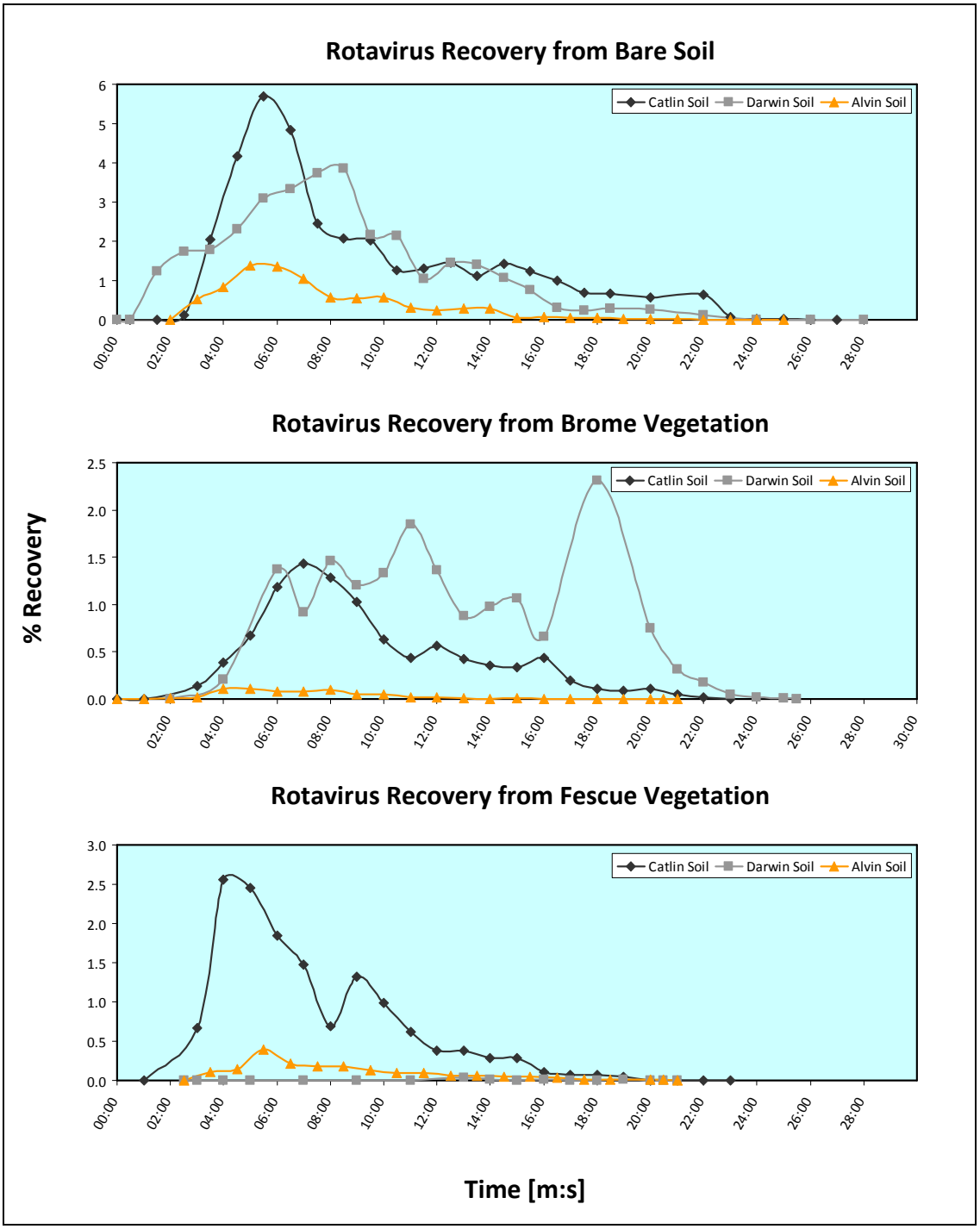


Figure 5.18. The recovery of infective rotavirus particles in surface runoff is plotted for all three soil types according to surface condition to compare the effect of soil.

As with *C. parvum* recovery, it appears here that soils with considerable amounts of clay (Catlin and Darwin) increase the amount of infective rotavirus particles recovered in surface

runoff, which are in agreement with our previous findings that rotavirus particles preferentially interact with sand particles. However, this trend with surface runoff is likely due to the reduced infiltration rate of soils with high clay contents. The rotavirus particles may never be coming into contact with the clay soil particles: suspended in surface water and being directly transported in surface runoff. An inability for water to initially infiltrate the soil would cause this initial flush of rotavirus particles. Over time, the recovery from Catlin soil decreases and follows that of Darwin soil very closely. As with *C. parvum* recovery, the recovery of infective rotavirus particles in surface runoff from Alvin soil is much lower than that from both the Catlin and Darwin soils. The total recovery in surface runoff for Alvin soil is only 8.28%, compared to 34.92% for Catlin soil and 26.56% for Darwin soil. Again, this is likely due to characteristics of the soil and the ability of water to better infiltrate a sandy soil such as Alvin. With more water infiltrating, there will also be more rotavirus particles entering the soil. Even with the increased infiltration rate, no infective rotavirus particles were detected in the near-surface runoff and only trace amounts in the soil core extractions.

With the addition of Brome vegetation, recovery of infective rotavirus particles is highest for Darwin soil. With the increased ability of water to infiltrate the soil, it is now apparent that the clay particles may be interacting with the rotavirus particles. An increased ability of rotavirus particles to interact or attach to clay soil particles could partly explain the higher recovery from Darwin soil. The Alvin soil has a much lower recovery than Catlin or Darwin soil. This reduced recovery from Alvin soil is probably mostly controlled by flow characteristics, with water more easily penetrating a sandy soil. However, the effect of interactions between sand particles and rotavirus particles can not be eliminated here.

With Fescue vegetative cover, no significant amount of infective rotavirus particles is recovered from Darwin or Alvin soils. The Catlin soil had the lowest percentage of vegetative cover and therefore did not have as much obstruction to surface flow. This would account for the initial pulse of rotavirus particles, followed by a quick decrease to less than two percent recovery. The total surface runoff volume from Catlin soil was substantially higher than that from either Darwin or Alvin soils (Table 5.3).

Table 5.3. Summary of rotavirus recoveries from surface runoff tabulated in conjunction with the seven experimental parameters collected for each combination of soil and vegetation type.

Bare Soil Conditions								
Soil	**% sand**	**% silt**	**% clay**	**% veg. cover**	**% OM**	**Bulk Density (g/cc)**	**Runoff Volume (mL)**	**% Recovery Rotavirus**
Catlin	24	50	26	0	7.18	1.05	4384	34.92
Darwin	5	50	45	0	5.45	1.08	3189	26.56
Alvin	60	25	15	0	2.10	1.28	3140	8.28

Brome Vegetation								
Soil	**% sand**	**% silt**	**% clay**	**% veg. cover**	**% OM**	**Bulk Density (g/cc)**	**Runoff Volume (mL)**	**% Recovery Rotavirus**
Catlin	24	50	26	49	7.18	1.05	2387	9.87
Darwin	5	50	45	72	5.45	0.95	2711	16.91
Alvin	60	25	15	54	2.10	1.25	2139	0.67

Fescue Vegetation								
Soil	**% sand**	**% silt**	**% clay**	**% veg. cover**	**% OM**	**Bulk Density (g/cc)**	**Runoff Volume (mL)**	**% Recovery Rotavirus**
Catlin	24	50	26	67	7.18	1.00	2894	14.23
Darwin	5	50	45	68	5.45	0.91	467	0.08
Alvin	60	25	15	85	2.10	1.28	1301	1.83

The data in Table 5.3 were used to analyze trends between individual characteristics of each condition and the actual percent recovery of infective rotavirus particles detected in the surface runoff (Figure 5.19). Since it is reasonable that rotavirus particles may never interact with the soil in surface runoff (Darwin bare soil condition) due to very low initial soil infiltration

rates, trends were also plotted for individual characteristics of each condition versus the percent recovery of infective rotavirus particles detected in the soil core extractions (Figure 5.20).

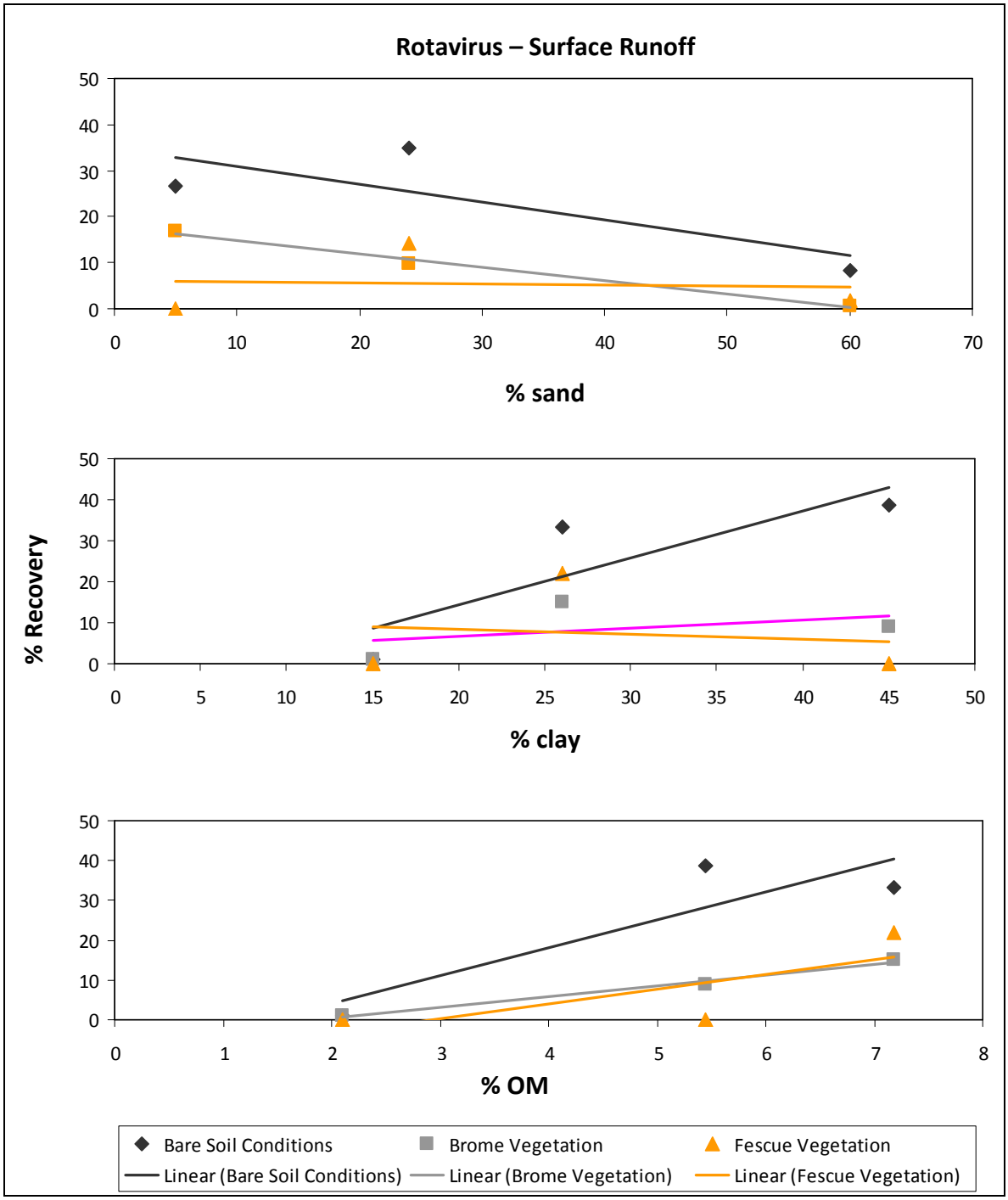


Figure 5.19. The recovery of infective rotavirus particles in surface runoff is plotted versus % sand, % clay, and % organic matter.

From these trends, it appears that sand decreases the recovery of rotavirus particles in surface runoff, while clay increases recovery in surface runoff, both of which are in agreement with preliminary findings that rotavirus particles primarily interact with sand particles. However, these trends also appear to be directly related to overland flow characteristics. The higher clay content would decrease soil infiltration rates and allow more runoff. Sand on the other hand, would provide a high soil infiltration rate and allow much more water, and rotavirus particles, to infiltrate the soil profile.

The third plot shows that the recovery of rotavirus particles increases with increasing organic matter content, which confirms findings by Blanc and Nasser (1996), who found that organic matter provides negative charge attachment sites that inhibit attachment of negatively charged microbial organisms. Clay particles are often associated with a higher organic matter content, and organic matter sorbed on soil particles can provide additional negative charges that repulse viruses or cover positively charged sites, decreasing the electrostatic interactions between viruses and soil particles.

If a portion of the infective rotavirus particles are never interacting with the soil surface, it is not possible to determine if one particular fraction of soil is having a greater impact than another on rotavirus recovery. However, analysis of the soil core extractions could help explain this. The soil core extractions contain the fraction of infective rotavirus particles that successfully entered the soil and were then able to be extracted. The percent of rotavirus particles recovered in the soil core extraction, then, is the fraction of particles that either does not interact with soil particles, or is not affected by the interaction with the soil. The soil core extractions are plotted against percent sand, percent clay, and percent organic matter in Figure 5.20.

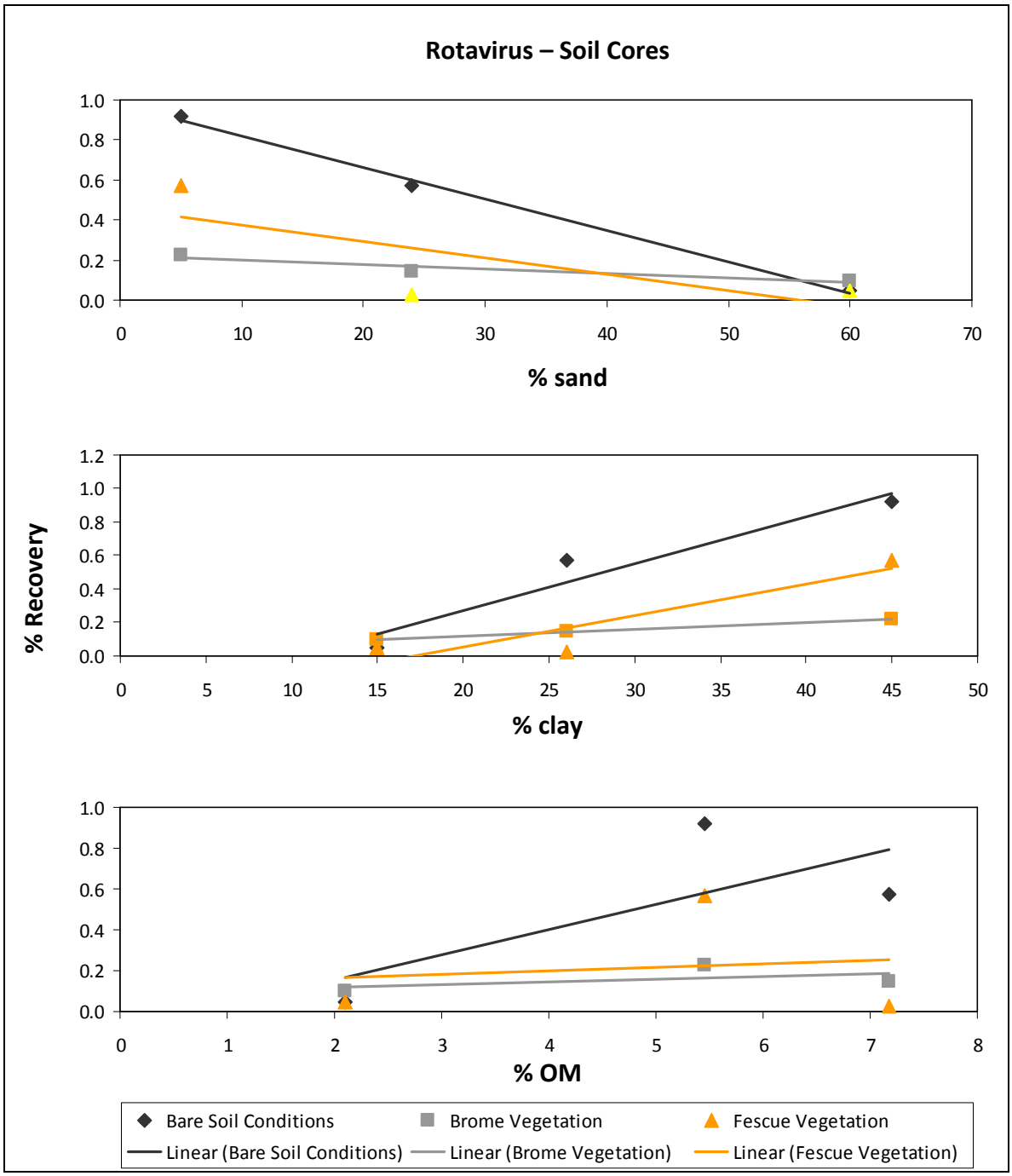


Figure 5.20. The recovery of infective rotavirus particles recovered from soil core extractions is plotted versus % sand, % clay, and % organic matter.

The soil core extraction trends are very similar to those for surface runoff. It appears the rotavirus particles that successfully enter the soil are more affected by the sand content, as was believed from preliminary results. While the relationships for percent sand and percent clay are

not conclusive, they show a strong trend confirming that soils high in sand content are better at reducing the recovery of infective rotavirus particles. Organic matter increases recovery with increasing organic matter content for all three surface conditions. This also is in agreement with previous findings.

5.3.2 Effect of vegetation on rotavirus overland transport kinetics

The three surface conditions are compared in this section according to the soil type in order to examine the effect of vegetation on the recovery of rotavirus particles during overland transport. Figure 5.21 shows the three surface conditions separated by soil type.

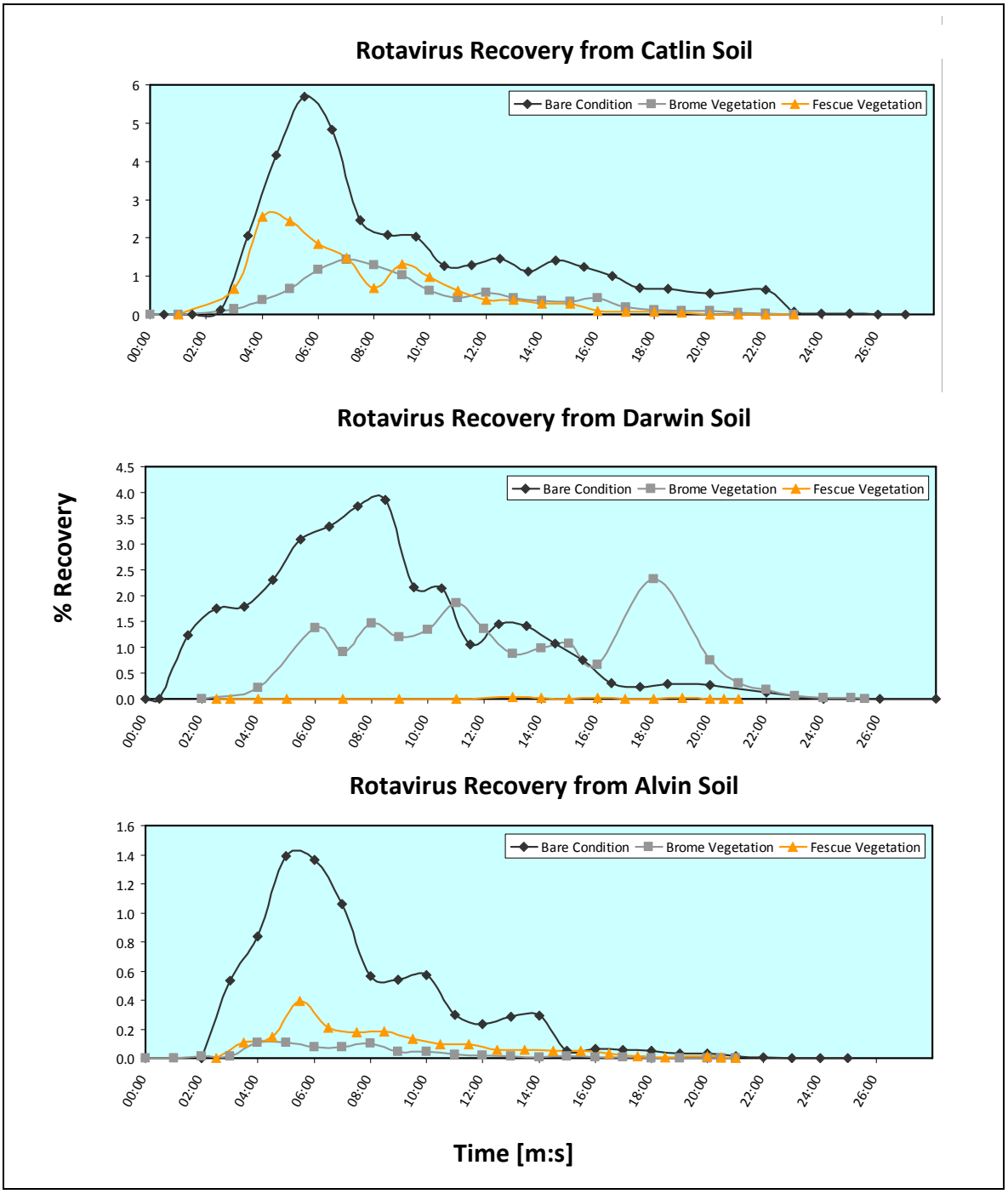


Figure 5.21. The recovery of infective rotavirus particles in surface runoff is plotted for all three surface conditions according to soil type to compare the effect of vegetation.

When all three surface conditions are plotted together according to soil type, the effect of surface condition is more easily understood. For Catlin soil, both vegetated conditions show a

lower peak recovery than the bare soil condition. This can be explained by the reduced surface flow rate and increased ability of water and rotavirus particles to enter the soil.

For Darwin soil, the bare soil condition has a much higher peak recovery than either of the vegetated conditions. As discussed previously, this is probably due to a low soil infiltration rate of the high clay soil. The roots of the vegetation create macropores that serve as small channels for water to enter the soil, reducing the volume of surface runoff as well as the number of infective rotavirus particles in the surface runoff. For the Alvin soil, the bare soil condition has the highest peak recovery.

Again, while vegetation, in general, appears to decrease the recovery of infective rotavirus particles in surface runoff, there does not appear to be a clear advantage of any one vegetation type. Perhaps more important is the surface vegetative cover and the extent of the root system in the soil profile. The percent vegetative cover has been used as one of seven parameters for developing models for *C. parvum* and rotavirus transport in surface runoff.

5.4 Overland transport kinetics modeling

The plots of overland kinetics served as an excellent way of studying the transport of infective *C. parvum* and infective rotavirus over the duration of a 20-minute rainfall event. While the plots of soil types separated by surface conditions helped explain the effect of soil type and the plots of surface condition separated by soil type helped explain the effect of vegetation, it remains difficult to quantify the relative importance of each factor. In addition, factors such as organic matter content, bulk density, and runoff volume may affect recovery of each pathogen. The analysis of a model assists in quantifying each of these parameters and the relative degree of effect each has on the recovery of either *C. parvum* or rotavirus.

An examination of the seven parameters that were collected during the transport studies individually makes the assumption that recovery from surface runoff is a linear relationship and that the parameters are independent of one another, with no interactions occurring. However, knowledge of the entire process shows that some parameters may be codependent, and the recovery from surface runoff may not be a linear relationship. For instance, the composition of soil is made up of sand, silt, and clay fractions. If one of these fractions is a dominant factor, it is only reasonable to assume that at least one of the three will have little or no effect. Therefore, separate models were developed for *C. parvum* and rotavirus. For each pathogen, two models were developed: one assuming a linear relationship and another assuming a nonlinear one. Table 5.4 defines the independent (X) and dependent (Y) variables that have been referred throughout the modeling section.

Nine separate overland transport studies were conducted to examine all combinations of soil and vegetation conditions. Pathogen recovery rates from these nine studies were used to develop regression models. In addition, the optimum linear and nonlinear models for each pathogen were used to predict pathogen recovery data not used for the development of the models. For the *C. parvum* models, one study was conducted in addition to the nine used for the model, but the bulk density (X_7) was not measured during that study. Therefore, bulk density was allowed to vary within a reasonable range until the model predicted a value very close to the observed result. As expected, different bulk densities were required for each model type, showing that both the linear and nonlinear models were capable of accurately predicting the observed value, given appropriate bulk density values. However, one bulk density is more typical of the conditions being tested.

Table 5.4. Dependent and independent variables considered in regression models.

Variable	Parameter
X_1	% Sand
X_2	% Silt
X_3	% Clay
X_4	% Vegetative Cover
X_5	% Organic Matter
X_6	Bulk Density (g/cc)
X_7	Runoff Volume (mL)
Y_1	% Recovery Rotavirus
Y_2	% Recovery *C. parvum*

5.4.1 *C. parvum* overland transport kinetics model

A linear model was first developed for the recovery of infective *C. parvum* oocysts in surface runoff (Equation 2). The linear model assumes each independent variable (X) has no interaction with another independent variable, and that the recovery of *C. parvum* in surface runoff is a linear relationship. Table 5.5 shows the number of parameters used in the model and a few statistical properties of the linear model.

$$\mathbf{Y_2} = -0.207962\,\mathbf{X_1} - 1.80789\,\mathbf{X_3} + 0.00903\,\mathbf{X_4} + 154.47607\,\mathbf{X_6} + 0.00421\,\mathbf{X_7} - 53.16255 \qquad (2)$$

Table 5.5. Statistical measures for the *C. parvum* linear model.

Parameters	R-squared	Adjusted R-squared	Mean Squared Error	Sum of Squares Error
5	0.9070	0.7525	55.36	166.09

Next, a nonlinear model was developed for infective *C. parvum* oocysts in surface runoff (Equation 3). The nonlinear model takes into account the possibility of an interaction between parameters. Table 5.6 shows the number of parameters used and the statistical parameters describing the accuracy of the nonlinear model.

$$\mathbf{Y_2} = 0.15831\ \mathbf{X_4} + 0.00038941\ \mathbf{X_2}*\mathbf{X_7} - 0.02196\ \mathbf{X_3}*\mathbf{X_4} + 0.00309\ \mathbf{X_4}^2 - 0.00232\ \mathbf{X_5}*\mathbf{X_7} - 65.41095\ \mathbf{X_6}^2 + 93.07463 \quad (3)$$

Table 5.6. Statistical measures for the *C. parvum* nonlinear model.

Parameters	R-squared	Adjusted R-squared	Mean Squared Error	Sum of Squares Error
6	1.0000	1.0000	0.00147	0.00293

Using equations 2 and 3, the predicted results are presented in Table 5.7 along with the values of all seven parameters. The same table (5.7) also provides observed *C. parvum* recovery rates for comparison purposes.

Table 5.7. Values of all seven parameters for each of the nine original and one additional study along with the observed *C. parvum* recoveries and predicted recoveries for the linear and nonlinear models. The additional study is independent of the nine used to develop the models, but was conducted under the same experimental procedure.

X_1	X_2	X_3	X_4	X_5	X_6	X_7	**Observed**	**Predicted**	
								Linear	**Nonlinear**
24	50	26	0	7.18	1.05	4384	**33.38**	**30.58**	**33.29**
5	50	45	0	5.45	1.08	3189	**38.58**	**35.35**	**38.55**
60	25	15	0	2.10	1.28	3140	**1.17**	**5.89**	**1.18**
24	50	26	49	7.18	1.05	2387	**14.91**	**22.61**	**14.87**
5	50	45	72	5.45	0.95	2711	**8.89**	**13.90**	**8.82**
60	25	15	54	2.10	1.25	2139	**1.10**	**-2.47**	**1.05**
24	50	26	67	7.18	1.00	2894	**22.07**	**17.18**	**22.03**
5	50	45	68	5.45	0.91	467	**0.00**	**-1.76**	**-0.05**
60	25	15	85	2.10	1.28	1301	**0.06**	**-1.08**	**0.02**
5	50	45	0	5.45	*1.28*	3750	**72.00**	**68.60**	*
					0.84			*	**72.52**

**model not capable of accurately predicting a recovery value for given conditions*

It is apparent that both models are reasonably good at predicting the recovery of infective oocysts in surface runoff. However, the nonlinear model is capable of predicting recoveries nearly identical to those observed. Both models show some difficulty predicting recoveries of nearly zero. Both models were capable of predicting a recovery close to the observed for the additional study, given appropriate values for bulk density. However, bulk densities for this soil

(Darwin) from the original nine studies were relatively low, due to the high clay content and inability to compact the soil in a small soil bed. Bulk densities for this soil ranged from 0.91 g/cc to 1.08 g/cc. Therefore, it can be assumed that the bulk density of the additional study was closer to the 0.84 g/cc used in the nonlinear model. The nonlinear model appears to be the more accurate model and probably more robust since it accounts for interactions between parameters.

Both the linear and nonlinear models were expected to predict the recoveries of the nine original studies with some accuracy since these same studies were used to create the model. However, the ability of the nonlinear model to perform well using data from a study of the same setup, but one that was not used in the creation of the model is very promising. The models were taken one step further, and used to predict recoveries from studies that were entirely independent of those used to develop the models. Table 5.8 shows data from Trask et al. (2003) as well as data from Atwill et al. (2002). Trask et al. (2003) used the same Catlin soil used in this study, but used slightly different combinations of rainfall intensity and slope. Therefore, a ratio of runoff volume is used to compensate for any differences. Atwill et al. (2002) did not include specific parameters of the setup used, but provided ranges. For instance, vegetation was grown to ≥85% surface cover, so 85% cover was used in these models. A ratio of runoff volumes was again used here to compensate for differences in rainfall intensity, slope, and surface area. In addition, bulk density measurements were not given by Trask et al. or Atwill et al. Therefore, bulk density values (shown in italics) were varied until predicted values very nearly matched the observed values.

Table 5.8. Values of all seven parameters and recoveries for five transport experiments conducted by Trask et al. (2003) and three transport experiments conducted by Atwill et al. (2002).

Trask et al. (2003)

X_1	X_2	X_3	X_4	X_5	X_6	X_7	**Observed**	**Predicted**	
								Linear	**Nonlinear**
2.54 cm/hr, 1.5% slope									
24	50	26	0	7.18	*0.97*	2067	**13.77**	**8.47**	*
					1.14			*	**13.88**
2.54 cm/hr, 3% slope									
24	50	26	0	7.18	*0.96*	2613	**9.53**	**9.22**	*
					1.18			*	**9.35**
2.54 cm/hr, 4.5% slope									
24	50	26	0	7.18	*0.92*	2293	**3.35**	**1.69**	*
					1.21			*	**3.76**
6.35 cm/hr, 1.5% slope									
24	50	26	0	7.18	*0.78*	7713	**2.53**	**2.88**	*
					1.31			*	**2.52**
6.35 cm/hr, 3% slope									
24	50	26	0	7.18	*0.81*	7267	**5.32**	**5.64**	*
					1.29			*	**4.67**

Atwill et al. (2002)

1.5 -4.0 cm/hr, 5-10 % slope									
X_1	X_2	X_3	X_4	X_5	X_6	X_7	**Observed**	**Predicted**	
								Linear	**Nonlinear**
19	47	34	85	4.00	*1.03*	13653	**63.00**	**63.21**	*
					1.39			*	**62.19**
45	37	18	85	1.40	*1.28*	13653	**75.00**	**76.69**	*
					1.63			*	**75.36**
70	25	5	85	1.70	*1.68*	6826	**81.00**	**81.25**	*
					1.15			*	**81.85**

**model not capable of accurately predicting a recovery value for given conditions*

Again, given appropriate bulk density values, both the linear and nonlinear models are reasonably good at predicting the observed values, with the nonlinear model predicting somewhat more accurate results. Since Trask et al. used the same Catlin soil used in this study, it was reasonable that a bulk density of 1.0-1.05 g/cc was used. However, Trask et al. used a soil bed much larger than the soil bed used in this study, so greater compaction may have been achieved without the risk of damaging the soil bed container. Therefore, the bulk density of the soil beds in the study by Trask et al. may have approached that of a natural field (1.25-1.45 g/cc).

If the bulk densities were indeed approaching levels of a natural field, it is likely that the nonlinear model may be describing the studies from Trask et al. quite well.

Both the linear and nonlinear models predicted the recoveries from the studies by Atwill et al. quite well. However, they did not provide the exact values of percent vegetative cover, bulk density, and runoff volume were not given. Therefore, without knowing more about the properties of the experimental setup, it is difficult to have confidence in either model at this point. It is important, though, that a model based on nine small-scale overland transport studies has the potential to accurately predict recoveries of *C. parvum* for entirely independent studies, given the appropriate experimental values.

It is also worth noting that the studies by Trask et al. and Atwill et al. did not use an infectivity assay for analysis of water samples. Therefore, it is likely that values predicted by the models in this study would underestimate the percent recovery of the independent studies: a higher recovery would be expected if the detection method was only concerned with the percent of *C. parvum* oocysts in the sample, rather than the fraction that remains infectious.

5.4.2 Rotavirus overland transport kinetics model

Similar to the method for developing the regression model for *C. parvum*, a linear model was first developed for the recovery of infective rotavirus particles in surface runoff (Equation 4). The linear model assumes each independent variable (X) has no interaction with another independent variable, and that the recovery of rotavirus in surface runoff is a linear relationship. Table 5.9 shows the number of parameters used in the model and a few statistical values describing the accuracy of the linear model.

$$\mathbf{Y_1} = -0.31559\ \mathbf{X_1} + 0.30359\ \mathbf{X_2} + 0.01382\ \mathbf{X_4} + 46.41102\ \mathbf{X_6} + 0.008\ \mathbf{X_7} - 62.19195 \quad (4)$$

Table 5.9. Statistical measures for the rotavirus linear model.

Parameters	R-squared	Adjusted R-squared	Mean Squared Error	Sum of Squares Error
5	0.9257	0.8018	28.628	85.88

Next, a nonlinear model was developed for infective rotavirus particles in surface runoff (Equation 5). The nonlinear model takes into account the possibility of an interaction between parameters. Table 5.10 shows the number of parameters used and the statistical values describing the accuracy of the nonlinear model.

$$\mathbf{Y_1} = -0.27259\ \mathbf{X_4} + 0.03496\ \mathbf{X_7} - 0.00382\ \mathbf{X_2}*\mathbf{X_4} + 0.003\ \mathbf{X_4^2} - 0.02866\ \mathbf{X_6}*\mathbf{X_7} + 13.67434 \quad (5)$$

Table 5.10. Statistical measures for the rotavirus nonlinear model.

Parameters	R-squared	Adjusted R-squared	Mean Squared Error	Sum of Squares Error
5	1.0000	0.9999	0.017	0.052

Table 5.11 presents the values of the seven parameters for all nine studies used to develop the models, as well as the three additional studies used here to validate both the linear and nonlinear models.

Table 5.11. Values of all seven parameters for each of the nine original and two additional studies along with the observed rotavirus recoveries and predicted recoveries for the linear and nonlinear models. The additional studies are independent of the nine used to develop the models, but was conducted under the same experimental procedure.

X_1	X_2	X_3	X_4	X_5	X_6	X_7	**Observed**	**Predicted**	
								Linear	**Nonlinear**
24	50	26	0	7.18	1.05	4384	**34.92**	**29.22**	**35.01**
5	50	45	0	5.45	1.08	3189	**26.56**	**27.05**	**26.45**
60	25	15	0	2.10	1.28	3140	**8.28**	**10.99**	**8.26**
24	50	26	49	7.18	1.05	2387	**9.87**	**13.92**	**9.78**
5	50	45	72	5.45	0.95	2711	**16.91**	**18.18**	**16.81**
60	25	15	54	2.10	1.25	2139	**0.67**	**2.34**	**0.69**
24	50	26	67	7.18	1.00	2894	**14.23**	**15.90**	**14.31**
5	50	45	68	5.45	0.91	467	**0.08**	**-1.68**	**0.17**
60	25	15	85	2.10	1.28	1301	**1.83**	**-2.55**	**1.82**
24	50	26	63	7.18	*1.07*	1550	**8.35**	**8.34**	*
					0.95			*	**8.36**
24	50	26	0	7.18	*1.56*	2906	**41.22**	**41.06**	*
					0.89			*	**41.14**
5	50	45	0	5.45	*1.7*	3750	**60.00**	**60.31**	*
					0.78			*	**60.94**

**model not capable of accurately predicting a recovery value for given conditions*

It is apparent that both models are reasonably good at predicting the recovery of infective rotavirus particles in surface runoff. However, the nonlinear model is capable of predicting recoveries very close to the observed values. The linear model shows some difficulty predicting recoveries near zero. Both models were capable of predicting a recovery close to the observed for the additional studies, given appropriate values for bulk density. However, bulk densities for these soils from the original nine studies were relatively low, due to the high clay content of both Catlin and Darwin soils and an inability to compact the soil in a small soil bed. From the original nine studies, bulk densities for Catlin soil ranged from 1.00 g/cc to 1.05 g/cc and those for Darwin soil ranged from 0.91 g/cc to 1.08 g/cc. Therefore, it may be argued that the bulk densities of the additional studies were closer to the 0.95 g/cc, 0.89 g/cc, and 0.78 g/cc as used in the nonlinear model. The nonlinear model appears to be the more accurate model and probably more robust since it accounts for interactions between parameters.

Both the linear and nonlinear models were expected to predict the recoveries of the nine original studies with some accuracy since these same studies were used to create the model. However, the ability of the nonlinear model to perform well using data from a study of the same setup, but one that was not used in the creation of the model is very promising. Due to the lack of overland transport studies investigating rotavirus, it was not possible to use an independent data set from a published journal to validate the rotavirus models. However, the ability of the *C. parvum* models to accurately predict recoveries of independent data give promise to the ability of the rotavirus models to do the same.

CHAPTER 6: CONCLUSION

Based on previous research (McLaughlin, 2003; Davidson, 2007), rotavirus particles are much more likely to interact with sand particles, with some reaction with clay particles. *C. parvum*, on the other hand, has been shown to interact almost exclusively with clay particles. Therefore, the Alvin soil was expected to show a greater reduction in recovery of infective rotavirus particles and the Darwin soil a greater reduction in recovery of infective oocysts. However, results from this study show a different trend. Recovery of infective rotavirus particles from surface runoff of bare Alvin soil is seven times higher than that of infective oocysts. Recovery from surface runoff of the bare Darwin soil is nearly one and a half times more for *C. parvum* than for rotavirus, with the recovery of *C. parvum* oocysts approaching 40 %. Recovery from the soil cores was slightly higher for *C. parvum* (0.06 %) than for rotavirus (0.05 %) in the case of the bare Alvin soil, with rotavirus percolating through all three one-inch layers and *C. parvum* only the top layer. Only 0.19% of infective oocysts were recovered from the cores of the bare Darwin soil, while 0.92 % of the infective rotavirus particles were recovered. Therefore, while the overland transport is in contrast to studies investigating soil interactions with *C. parvum* and rotavirus, it is quite possible that the higher recovery in surface runoff observed in this study is an issue of the pathogens penetrating the soil matrix. It could be especially significant that rotavirus particles are much smaller and therefore, more easily suspended in the surface runoff and less likely to settle from the water being transported. When *C. parvum* oocysts and rotavirus particles entered the soil profile, the trends observed in this study are in agreement with previous soil-microbe interaction studies.

Models for *C. parvum* and rotavirus are not only capable of accurately predicting the recoveries for the nine studies used to develop the models, but also independent data sets. Linear

and nonlinear models both have potential for predicting recoveries, but the nonlinear models appear to be much more robust in predicting a wider range of recoveries than the linear models. This is likely because the data used to create the models contained recoveries no greater than 40%. Therefore, it is encouraging that the nonlinear models were able to predict recoveries as high as 81% for *C. parvum* and 60% for rotavirus. Slight modification of these models could allow for recommendations of VFS based on the desired reduction in pathogen load.

The current research could have significant implications for design recommendations of best management practices, especially vegetative filter strips. While soil types vary considerably from one geographic region to another, it appears that soil types of a wide range of textural composition may be adequate for the removal of infective *C. parvum* oocysts and infective rotavirus particles. The critical factor to consider for each soil type may be the rate of overland flow. Given adequate time to penetrate the soil matrix and interact with soil particles, a significant reduction in pathogen loads can be achieved. This is evident with the Darwin and Alvin soils, with Darwin soil having a higher surface runoff rate and also a higher recovery of oocyss and rotavirus while Alvin soil had a much lower surface runoff rate and a lower percent recovery of oocysts and rotavirus. While there was no direct correlation between vegetation type and pathogen recovery, it appears that the percent ground cover of vegetation could be an important factor for pathogen removal.

RECOMMENDATIONS FOR FUTURE WORK

- Analyze the extraction method for each *C. parvum* and rotavirus to determine what percent is able to be extracted. This would give a more complete indication of the fraction that is remaining in the soil after an overland transport study.

- Examine other slope and rainfall intensity combinations to increase the ability of the pathogen models to predict surface runoff recoveries for conditions not tested in this study.

- Conduct field-scale overland transport studies for *C. parvum*, rotavirus, and potentially other pathogens of interest. Field-scale studies would be very beneficial for confirming results from the small-scale experiments in this study. In addition, field-scale studies would serve as valuable validation for the models developed in this study since one objective of this study was to analyze trends that can be applied to VFS.

- Develop more time-efficient detection methods. For instance, more than two weeks is necessary to complete the detection and analysis methods for one set of experimental conditions (when both *C. parvum* and rotavirus are being studying together). The ability to reduce the time needed for detection and analysis would increase the ability to test additional experimental conditions (soil type, vegetation type, slope, rainfall intensity, etc.).

- Develop a more efficient method of growing vegetation for the vegetated conditions. The time needed to prepare the vegetated beds was reduced by several weeks during this study, but approximately eight weeks is still needed to establish the necessary vegetative cover. Perhaps having the vegetation grown in a greenhouse and than transported to the lab for experiments would be an option.

- Use soil directly from the field to pack the soil beds. In this study, the soil was collected from the field and oven-dried to eliminate any previous *C. parvum* or rotavirus contamination. However, the drying process also made it more difficult to pack the soil to a natural compaction level (as evaluated by bulk density measurements). Perhaps the soil could be used straight from the field and a sample of spare soil analyzed for background activity. This background activity could then be taken into account when calculating the recovery of each pathogen from the soil cores of an overland transport study.

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