Effect of Tillage and Rainfall on Transport of Manure-Applied Cryptosporidium parvum Oocysts Through Soil

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Cryptosporidiosis is a debilitating gastrointestinal disease in humans and animals characterized by severe diarrhea. It is a serious opportunistic infection in immuno-compromised individuals. Cryptosporidium oocysts are spherical with a diameter of 3 to 5 μm and can be transmitted by direct human-animal contact or through contaminated food and water (Duffy and Moriarty, 2003; Slikto et al., 2000; Okhuysen et al., 1999; Weltman et al., 1997). Numerous outbreaks of cryptosporidiosis have been documented worldwide, demonstrating that Cryptosporidium is a significant waterborne pathogen, particularly in industrialized countries (Slikto et al., 2000; Solo-Gabrielle and Neumeister, 1996). The main public health concerns are that Cryptosporidium oocysts are resistant to disinfectants commonly used in water treatment and may survive for months in water environments (Robertson et al., 1992; Tamburrini and Pozio, 1999). Furthermore, a low number of oocysts are sufficient to establish infection and cause disease in susceptible hosts. The infective dose has been estimated as low as 10 oocysts (DuPont et al., 1995; USFDA, 1992), but may be as low as one oocyst for immunocompromised hosts (Medema and Schijven, 2001; Pereira et al., 2002).

Many studies have documented high concentrations of Cryptosporidium in untreated and treated water sources (Messner and Wolpert, 2000; Moulton-Hancock et al., 2000; De Carvalho et al., 2000; LeChevallier and Norton, 1995; Roach et al., 1993) and have implicated agricultural practices as an important source of contamination. Environmental contamination (water, soil, and food crops) has been casually linked to manure spreading and surface runoff from farmland due to the high incidence of the parasite in cattle (Bos taurus) (Graczyk et al., 2000; Tate et al., 2000; Tyrrel and Quinton, 2003). It has been suggested that rainfall and...
snowmelt may increase Cryptosporidium loads in water sources due to surface runoff. Rainfall intensity especially appears to influence the rapid and effective transport of oocysts (Ather-Holt et al., 1998, Curriero et al., 2001). In the United States, 68% of waterborne outbreaks reported from 1948 through 1994 were strongly associated with precipitation events (Curriero et al., 2001). In Germany, Kistemann et al. (2002) noted that Cryptosporidium oocysts were frequently detected in tributaries to reservoirs used for drinking water and that parasite load increased during heavy rainfall.

Most of the research designed to evaluate environmental contamination by Cryptosporidium spp. has focused on direct contamination of water sources. Although Cryptosporidium's potential to contaminate and survive in the environment is well established, few studies have considered the role of soil as a reservoir of Cryptosporidium oocysts. Similar to water environments, oocysts can remain infective for months in the soil (Jenkins et al., 2002, Kato et al., 2004). Large amounts of organic wastes are applied to land (USEPA, 2002). These wastes include sewage sludge and animal manure, which are potentially contaminated by Cryptosporidium oocysts that can survive conventional sewage treatment (Whitmore and Robertson, 1995). The extent of soil contamination due to the presence of the parasite is not widely documented, but it could be extensive in the vicinity of cattle facilities (Barwick et al., 2003a). Fruits and vegetables that contact soil may become contaminated with Cryptosporidium oocysts if untreated manure or wastewater has been used as fertilizer or for irrigation (Armon et al., 2002).

Rainfall has a significant effect on the movement of pathogens through the soil and could result in contaminated water (Auckenthaler et al., 2002). The movement of Cryptosporidium oocysts through different types of soil following rainfall has been demonstrated under several experimental and natural conditions (Barwick et al., 2003a, 2003b; Davies et al., 2004; Tate et al., 2000). Water can spread and carry pathogens into and through the soil, reaching surface water sources and possibly groundwater. Indeed, Cryptosporidium oocysts can migrate to a depth of 90 cm in soil and probably deeper in fractured soils (Armon et al., 2002). No-till soil often transports water faster and in larger amounts than tilled soil due to increased macropore continuity, suggesting that pathogen transport and dispersion can be increased by no-till management practices (Gagliardi and Karns, 2000). Earthworms (Lumbricus terrestris) can create burrows up to 2.5 m deep and these macropores have been shown to rapidly transmit up to 10% of natural rainfall through the soil profile. Furthermore, earthworm populations and burrow numbers frequently increase with no-till (Shipitalo, 2002; Shipitalo et al., 2000). Nevertheless no-till also provides numerous benefits to soil and crops, including greatly reducing soil erosion and improving soil productivity by increasing soil organic matter content, plant-available water, and plant root growth. Moreover, no-till is considered one of the most cost effective crop production systems because it lowers labor and machinery costs (Conservation Technology Information Center, 2009; Medema and Schijven, 2001). No-tillage practices are increasing annually in the United States and no-till was used on 26.3 million hectares in 2008 (Conservation Technology Information Center, 2009).

In this study, our objective was to assess the effect of soil tillage and rainfall on the transport of Cryptosporidium oocysts through no-till and tilled soils using simulated rainfall following the application of liquid dairy manure. The results will be useful for the development of better no-till and manure management strategies that maintain the many benefits of no-till while reducing pathogen loads to surface and subsurface waters.

### Materials and Methods

#### Soil Blocks

Twelve 30 by 30 by 30 cm blocks of intact Wooster silt loam soil (fine-loamy, mixed, active, mesic Oxyaquic Fragiu-Podzols) were collected during the first week of November from a hay field at The Ohio Agricultural Research and Development Center’s Wagner Farm. The history of the field before when the blocks were obtained was at least 3 yr of no-till corn followed by 2 yr of hay. Grass and weeds on the plots at the time of sampling were cut to about a 5 cm height. The soil blocks were isolated and removed from the field, as described previously (Shipitalo et al., 1990), and then stored in a glasshouse for several weeks to several months. In the laboratory, before treatments, the bottoms of the soil blocks were trimmed flat to achieve 30 cm depth and the bottoms vacuumed. The location of all pores ≥ 2 mm in diameter were recorded on tracing paper held to the base of each block.

To simulate tillage, the surface 10 cm of soil from six randomly selected blocks were removed, mixed, and then replaced on the top of the blocks. The other six blocks were left undisturbed as collected in the field.

#### Inoculum Preparation and Manure Application

Cryptosporidium parvum oocysts (OH strain), maintained by passages in neonatal calves, were used to spike liquid dairy manure. Oocysts were separated from the calf feces by sodium chloride and cesium chloride density gradient centrifugation (Current, 1990) and enumerated with a hemacytometer. Liquid manure (no straw and 3% solids) from a liquid manure storage facility on the campus of The Ohio Agricultural Research and Development Center, Wooster, OH was spiked to obtain a final concentration of approximately 10⁵ oocysts mL⁻¹. One liter of the spiked liquid manure was slowly and evenly poured over the entire surface of each soil block. This amount is equivalent to 11.1 mm of water and is within the range normally recommended for field applications in Ohio (Hoorman and Shipitalo, 2006). If any liquid manure immediately passed through the blocks following application the volume was recorded. Blocks were then kept undisturbed for 2, 4, 24, and 48 h before being subjected to the rainfall treatments (Table 1).

#### Rainfall Treatments and Leachate Collection

Rainfall was applied to the blocks using the rainfall simulator described by Shipitalo et al. (1990). The blocks were placed beneath an array of 105, 60-mL plastic syringes filled with tap wa-
ter and manipulated by motor driven plates that control the application rates while creating a random drop pattern. The blocks were supported by a 64-cell grid lysimeter (cell size 3.75 by 3.75 cm). Transparent plastic tubing connected to each cell led to a collection rack consisting of 64 50-mL tubes. These tubes were individually changed each time approximately 35 mL of leachate was collected for an individual cell and the time recorded. Simulated rainfall was applied as either a low intensity, light rainfall (i.e., 5 mm in 30 min—total 450 mL) or as a high intensity, heavy rainfall (i.e., 30 mm in 30 min—total 2700 mL). Six different rainfall treatments (T1–T6) were designed to simulate typical rainfall patterns observed in the state of Ohio (Table 1). Each treatment was applied to one no-till and one tilled block. Leachate was collected during and approximately 30 min after application of the rain had ceased. The total amount of collected leachate was recorded for each cell.

The blocks were weighed before and after each rainfall to determine soil water content. After the last rainfall, four of the no-till blocks and four of the tilled blocks were sliced into eight 3.75-cm-thick horizontal sections. Each soil section was carefully collected from top to bottom to minimize cross-contamination, placed in a plastic bag and mixed well before being subsampled for determination of oocyst content.

**Table 1. Description of experimental treatments.**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>T1 (24L/48H)†</th>
<th>T2 (48H)</th>
<th>T3 (48H/120H)</th>
<th>T4 (2L/4H)</th>
<th>T5 (4H)</th>
<th>T6 (4H/76H)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apply manure</td>
<td>Apply manure</td>
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<tr>
<td>Wait 24 h</td>
<td>Wait 48 h</td>
<td>Wait 48 h</td>
<td>Wait 2 h</td>
<td>Wait 4 h</td>
<td>Wait 4 h</td>
<td>Wait 4 h</td>
</tr>
<tr>
<td>Apply 5 mm/30 min</td>
<td>Apply 30 mm/30 min rain (2700 mL)</td>
<td>Apply 30 mm/30 min rain (2700 mL)</td>
<td>Apply 5 mm/30 min rain (450 mL)</td>
<td>Apply 30 mm/30 min rain (2700 mL)</td>
<td>Apply 30 mm/30 min rain (2700 mL)</td>
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<tr>
<td>rain (450 mL)</td>
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<tr>
<td>Collect leachate</td>
<td>Collect leachate</td>
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</tr>
<tr>
<td>Wait 24 h</td>
<td>Slice soil block</td>
<td>Wait 72 h</td>
<td>Wait 2 h</td>
<td>Slice soil block</td>
<td>Wait 72 h</td>
<td>Slice soil block</td>
</tr>
<tr>
<td>Apply 30 mm/30 min</td>
<td>Apply 30 mm/30 min rain (2700 mL)</td>
<td>Apply 30 mm/30 min rain (2700 mL)</td>
<td>Apply 30 mm/30 min rain (2700 mL)</td>
<td>Apply 30 mm/30 min rain (2700 mL)</td>
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<td>rain (2700 mL)</td>
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<tr>
<td>Collect leachate</td>
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<tr>
<td>Slice soil block</td>
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<td>Slice soil block</td>
<td>Slice soil block</td>
<td>Slice soil block</td>
<td></td>
</tr>
</tbody>
</table>

† The treatment abbreviations given in parentheses indicate both the number of hours since manure application and the rain intensity. The numbers in the treatment abbreviations represent the time between manure application to the soil block and the rain event. The letters represent the rain intensity with “L” being a low intensity rain of 5 mm applied in 30 min and the letter “H” a high intensity rain of 30 mm applied in 30 min.

**Cryptosporidium Detection in Water Samples**

Shortly after collection, 15-mL aliquots of the leachate samples were centrifuged at 1000 × g for 20 min at 4°C. The pellet was resuspended in 500 μL of sterile distilled water and stored at 4°C until further processing. Two-hundred microliters were used for DNA extraction with the QIAmp DNA stool Mini Kit (QIAGEN, Inc., Valencia, CA). Cryptosporidium DNA was amplified by PCR with the primers published by Laxer et al. (1991). PCR reaction of 20 μL final volume contained 1X PCR Buffer, 2.5 mmol L⁻¹ MgCl₂, 200 μmol L⁻¹ (each) dNTPs, 0.7 μg bovine serum albumin, 0.5 μmol L⁻¹ (each) sense and antisense primers, 1 U HotStart Taq DNA Polymerase (QIAGEN, Inc., Valencia, CA) and 2 μL of DNA. Both sense and antisense primers were biotinylated to produce a labeled PCR product, which was subjected to a solid-phase hybridization in 96-well microtiter plates described elsewhere (Sreevatsan et al., 2000). Briefly, each well of the microtiter plates was coated with a Cryptosporidium specific oligonucleotide (5’- TAACT-TCACGTGTGTGTTGCAATGCAATGAA-3’) designed to capture both strands of the labeled PCR product. Denatured PCR products were loaded into the precoated wells containing neutralization-hybridization buffer (1 mol L⁻¹ NaSCN, pH 4.5) and incubated at 37°C. After a series of washing and incubation steps Cryptosporidium detection was achieved using the enzyme neviravudin-peroxidase and its substrate tetramethylbenzidine (TMB). The optical density of each well was determined at 450 nm with an automated ELISA Reader (Emax precision microplate reader, Molecular Devices, Sunnyvale, CA). Positive and negative controls were included in each plate. The negative OD cutoff (<0.2 OD) was resolved previously.

**Cryptosporidium Detection in Soil Samples**

Oocysts were separated from soil by a modified sucrose flotation protocol described by Kato and Bowman (2002). In brief, 5 g of soil from each of the eight sliced sections of blocks receiving treatments T2, T3, T5, and T6 were weighed and added to 50-mL tubes. A small amount of zirconia/silica beads (0.5 mm diam.) and 20 mL of 0.5% 7X detergent in PBS were added to the sample and vortexed for 1 min. The mix was underlain with 20 mL of cold (4°C) sugar solution (specific gravity = 1.2) and centrifuged at 1000 × g for 30 min at 4°C. The interface was removed, transferred to a second 50 mL tube and distilled water was added to a final volume of 45 mL. The sample was vortexed and centrifuged. Supernatant was removed...
and the pellet was resuspended in 500 μL of sterile distilled water. DNA extraction, PCR, and hybridization were performed as described above.

**Cryptosporidium Oocysts Quantification**

For those leachate and soil samples that were positive by *Cryptosporidium*-specific PCR-hybridization, the remaining 300 μL of supernatant not used for DNA extraction, PCR and hybridization (see above sections) were subjected to immunomagnetic separation using Dynabeads anti-*Cryptosporidium* Kit (Dynal Biotech ASA, Oslo, Norway) according to manufacturer instructions. Oocysts were enumerated using a hemacytometer on aliquots that were obtained after thorough mixing of each sample.

**Data Analysis**

By combining data for individual blocks, we were able to test several treatment effects. For example, we compared tilled and no-till effects by combining the six rainfall treatments applied to the tilled soil blocks into a single value and by combining the six no-till soil blocks into a single value. Similarly, we combined soil blocks that had similar rainfall intensities applied or similar times between application of liquid manure and application of soil blocks that had similar rainfall intensities applied or similar times between application of liquid manure and application of first and second rainfall events. Recovery data for oocysts were log transformed prior to statistical analyses. A generalized linear modeling (GLM) procedure was used to analyze data and to test for treatment effects (SAS Institute, 2001).

**Results**

**Tillage Effect on Water Transport**

Visual inspection did not reveal the presence of cracks that may have formed, during sampling and treatment of the soil blocks, and that would permit water to rapidly infiltrate and percolate downward. In addition, the distribution of water collected from edge vs. nonedge cells was in agreement with the percentage of cells that were either edge or nonedge. The foam inserted between the soil and the wooden box that enclosed the soil blocks apparently created a good seal forcing the water through the soil blocks.

The number of ≥ 2 mm diam. macropores mapped at the bottom of the soil blocks (21) was the same for the tilled and no-till soil blocks (Table 2). This was not unexpected as all the soil blocks came from the same no-till field.

Sixty-four receiving cells, each 3.75 by 3.75 cm (i.e., 14.1 cm²) were placed under each soil block before application of manure and the rainfall treatments. No prerainfall leaching was observed in the tilled blocks after the liquid manure was applied. For the no-till soil blocks, an average of about 300 mL (30%) of the water associated with the application of the liquid manure was transported through the 30 cm depth even before rainfall application. This water seemed to have been little affected by its transport through the no-till soil blocks as it still retained the dark color associated with the liquid manure sample applied to the blocks.

An average total of 4750 mL of water was applied to each of the six tilled soil blocks and the same amount was applied to the six no-till soil blocks (Tables 1 and 2). Of this amount, 957 mL (20.1%) was recovered as leachate from the tilled soil blocks and 2360 mL (49.7%) from the no-till blocks, with 300 mL of this recovered before any rain was applied (see paragraph above). Several cells produced more than a single leachate sample of 35 mL. These cells contributed a high amount of the total leachate collected with the top three cells producing, on average, 12.7% (tilled) and 22.4% (no-till) of the total water applied to the soil blocks.

There was a difference in the total number of cells that produced leachate with the tilled blocks having an average of 12.7 leachate-producing cells compared to 25.7 for the no-till blocks (Table 2). Although some cells produced leachate that was clearly associated with macropores, other cells had substantial amounts of leachate that could not easily be attributed to a macropore directly above the collection bottle.

The breakthrough of water was rapid for both tillage treatments, probably because of continuous macropores that extended to 30 cm depth. The average breakthrough time, however, was about 2.6 times faster for the no-till soil blocks (3:06 min) than for the soil blocks that were surface tilled (8:08 min). Thus the simulated tillage reduced rapid infiltration and transport through the soil and more of the water applied as liquid manure or rainfall remained in the tilled than in the no-till soil blocks. Most of this water was retained in the surface layers of the tilled plots due to disruption of the water-transporting macropores to the deeper soil layers. There was a clear difference in water content in the top 15 cm of the soil blocks with significantly more water remaining in the tilled than in the NT blocks after the treatments were applied (Fig. 1).

**Tillage and Rainfall Effects on Oocyst Transport**

Tests indicated that recovery of *Cryptosporidium* oocysts was quantitative from water samples. Developing an accurate budget of oocysts recovered was difficult, however, because of the variation that was observed in the recovery of oocysts from soil. However, we believe the following observations are of interest. It is very clear that, by far, the greatest amount of *Cryptosporidium* oocysts was recovered from soil compared to the water samples with only about 0.04 and 0.16% of the total number of oocysts applied recovered in the water samples from the tilled and no-till soil blocks, respectively. Unfortunately, the leachate collected from the no-till blocks before any rain was applied was not analyzed for oocysts. Thus we do not have numbers of *Cryptosporidium* oocysts in the water that was rapidly transported through the soil and, presumably, came primarily from the applied liquid manure. However, based on the recovery values noted above for the different tillage treatments, it seems that a substantial number of oocysts were probably in these initial water samples from the no-till blocks. A crude estimate of recoveries indicates that 43% of total oocysts applied could not be accounted for as being present in the no-till soil blocks or the subsequent leachate samples collected. This suggests that the concentration of oocysts was little reduced by the rapid passage of water through the no-till soil from the applied liquid manure.
Significant difference between the tilled and no-till soil blocks at \( P < 0.05 \).

** Significant difference between the tilled and no-till soil blocks at \( P < 0.01 \).

*** Significant difference between the tilled and no-till soil blocks at \( P < 0.001 \).

† This includes both 1000 mL of the liquid manure plus 3750 mL rain that were applied to each of the soil blocks.

‡ Of the total leachate recovered, 300 mL were from the liquid manure before adding any rain. There was no leachate recovered from the liquid manure application to the tilled soil blocks.

Table 2. Recovery of water applied to individual tilled and no-till soil blocks.

<table>
<thead>
<tr>
<th>Parameter measured</th>
<th>Tilled</th>
<th>No-Till</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water applied/block, mL†</td>
<td>4750</td>
<td>4750</td>
</tr>
<tr>
<td>Leachate recovered/block, mL</td>
<td>957</td>
<td>2360‡</td>
</tr>
<tr>
<td>Leachate recovered/block, % of applied</td>
<td>20.1</td>
<td>49.7</td>
</tr>
<tr>
<td>Number of mapped macropores ≥ 2 mm/block</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>Number of collection cells/block</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>Number of cells/block from which leachate was recovered</td>
<td>17.1</td>
<td>25.7</td>
</tr>
<tr>
<td>Leachate recovered in top three producing cells/block – mean of total rainfall applied to individual soil blocks, %</td>
<td>12.7***</td>
<td>22.4</td>
</tr>
<tr>
<td>Time to collection of first leachate sample – mean of six blocks for each tillage treatment (min:sec)</td>
<td>8.08***</td>
<td>3.06</td>
</tr>
<tr>
<td>Number of cells/block that produced more than a single sample of leachate‡</td>
<td>4.8**</td>
<td>10.3</td>
</tr>
<tr>
<td>Number of leachate samples/block†</td>
<td>37.5</td>
<td>77.7</td>
</tr>
</tbody>
</table>

From a total of 691 leachate samples collected from the 12 soil blocks, 225 samples (32.6%) were collected from the tilled and 466 samples (67.4%) from the no-till soil blocks. This averaged to 37.5 leachate samples collected from each of the tilled blocks and 77.7 samples from the no-till blocks (Table 2). A greater number of samples tested positive for *Cryptosporidium* oocysts from no-till soil blocks (average, 49.0/block) compared with the samples collected from tilled soil blocks (average, 17.2/block) (Table 3). This represented 63.1% and 45.8% of the total samples collected from the no-till and tilled samples, respectively. Several cells produced more than a single 35-mL leachate sample and this was more commonly observed for the no-till than the tilled soil blocks. Of the cells that produced greater than a single sample, the no-till soil blocks had a greater number (average, 6.5/block) and a greater percentage (63%) of first leachate samples that tested positive for *Cryptosporidium* oocysts in the very first sample than did the tilled blocks. This indicates that the rain on the no-till soil blocks picked up oocysts from the soil surface and transported them to 30 cm depth. However, the oocysts in these samples accounted for only a small percentage of the total applied to the soil surface. In the tilled soil blocks, where macropores were disrupted in the top 10 cm, 15 first leachate samples (average, 2.5/block or 52%) out of the total number of cells that produced more than a single leachate sample tested positive for *Cryptosporidium* oocysts (Table 3).

Quantitative measurements of *Cryptosporidium* oocysts in leachate water samples collected from the soil blocks were made to compare the effect of various tillage and rainfall treatments. A low intensity rainfall after manure application (i.e., 5 mm in 30 min) significantly \( P \leq 0.05 \) decreased the amount of leachate and *Cryptosporidium* oocysts recovered compared to when the first rainfall after manure application was a high intensity event (30 mm in 30 min) (Comparison A, Table 4). The high connectivity of macropores that extended from the surface of the soil blocks to 30 cm depth contributed to leachate collection from the bottom of the no-till soil blocks, even when the amount of rain applied was only 5 mm in a 30 min period.

There was a complete lack of recovery of leachate from the tilled soil blocks, but not from the no-till blocks, when the low intensity rain was applied and differences in oocysts recovered after a delay from 2 to 24 h to onset of the first low intensity rain (Comparison B, Table 4) were not statistically different. For the NT blocks, there was an average of log 2.01 oocysts recovered in the leachate if the first low intensity rain was applied only 2 h after manure application. If 24 h was allowed to elapse before onset of the first low intensity rainfall, an average of log 1.22 oocysts was recovered. However, this observation is based on only a single replicate for each treatment applied to the no-till soil blocks. Substituting a high intensity rainfall event 4 h after manure application yielded an average of log 3.98 oocysts in the leachate water. Increasing the time to the first intense rainfall from 4 to 48 h decreased the recovery of *Cryptosporidium* oocysts slightly to an average of log 3.79 (Comparison C, Table 4).

The number of *Cryptosporidium* oocysts recovered after a first intense rainfall (log 3.83) was not much greater than the number recovered after a second intense rainfall (log 3.74) (Comparison D, Table 4). The difference, however, in the these two treatments...
Values in a leachate or oocysts column, for each comparison, that are recovered was not that different between the two treatments and rainfall treatment (T3). The highest average recoveries of Cryptosporidium oocysts across all the different rainfall treatments were from the no-till soil block (log 4.29 oocysts) compared to only log 3.64 oocysts from the tilled blocks.

Tillage and Rainfall Effects on Oocysts Remaining in Soil

Oocysts recovered from the soil block sections indicated that the tilled soil retained higher number of oocysts compared to the no-till soil (Fig. 2). The greatest number of oocysts was recovered from the fifth soil layer (i.e., 15.0–18.8 cm) of the tilled soil blocks although the high value for this layer seems to be an outlier because it resulted in a recovery >100% of the total number of Cryptosporidium oocysts applied to the soil blocks. Because of the great variability in the number of recovered oocysts, the differences between the tilled and no-till soil blocks were not statistically different at the P ≤ 0.05 level. The data for soil reflect, however, what was observed for the leachate samples where more oocysts were recovered in leachates from the no-till blocks. Also, large amounts of oocysts were also assumed to be in the 300 mL of leachate recovered from the applied liquid manure before rainfall was even applied. This would result in fewer oocysts remaining in the no-till soil blocks compared to the tilled blocks.

Discussion

Tillage had a greater effect on transport of Cryptosporidium oocysts through the soil compared to the other variables investigated in this study. All the soil blocks came from the same area of a single no-till field before applying simulated tillage to the top 10 cm of six of the soil blocks. This single treatment greatly affected oocyst transport because it disrupted macro pores that were continuous from the bottom of the soil blocks, at 30 cm depth, to near the soil surface.

The large size of Cryptosporidium oocysts, compared to other microorganisms in soil, affects how they are filtered and adsorbed to soil particles and influence their movement through soil (Mawdsley et al., 1996). In addition, soil properties such as type of soil, size and number of micropores, organic matter, and charge may also have considerable effects. Major differences between the intact and disturbed soil are the lack of natural soil

Table 3. Summary of Cryptosporidium parvum recovery data in individual collection cells of treated tilled and no-till soil blocks.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tilled</th>
<th>No-Till</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cells/block that produced leachate that tested positive for Cryptosporidium parvum†</td>
<td>10.5</td>
<td>*** 22.2</td>
</tr>
<tr>
<td>Number of samples/block that tested positive for Cryptosporidium parvum†</td>
<td>17.2</td>
<td>*** 49.0</td>
</tr>
<tr>
<td>Samples testing positive for Cryptosporidium parvum, †</td>
<td>45.8</td>
<td>* 63.1</td>
</tr>
<tr>
<td>Number of first leachate samples/block that tested positive for Cryptosporidium parvum‡</td>
<td>2.5</td>
<td>** 6.5</td>
</tr>
<tr>
<td>First leachate samples that tested positive for Cryptosporidium parvum, †‡</td>
<td>51.7</td>
<td>* 62.9</td>
</tr>
</tbody>
</table>

* Significant difference between the tilled and no-till soil blocks at P < 0.05.
** Significant difference between the tilled and no-till soil blocks at P < 0.01.
*** Significant difference between the tilled and no-till soil blocks at P < 0.001.
† Sum of the six tilled or the six no-till soil blocks.
‡ Several cells produced more than a single leachate sample and this row reports data for only the first sample, or breakthrough sample.

Table 4. Comparisons of mean recovery leachate (ml) and of Cryptosporidium parvum oocysts in leachate (number) from soil blocks after application of different tillage and rainfall treatments†.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Leachate (mL/block)</th>
<th>Oocysts (number recovered/block)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Intensity and amount of first rain event</td>
<td>log10</td>
<td></td>
</tr>
<tr>
<td>Low intensity rain (T1, T4– first rain only)</td>
<td>54.7 a‡</td>
<td>1.61 a</td>
</tr>
<tr>
<td>High intensity rain (T2, T3, T5– first rain only)</td>
<td>1110 b</td>
<td>3.89 b</td>
</tr>
<tr>
<td>B. Time to onset of low intensity rain</td>
<td>2 h (T4– first rain only)</td>
<td>69.3 a</td>
</tr>
<tr>
<td></td>
<td>24 h (T1– first rain only)</td>
<td>40.1 a</td>
</tr>
<tr>
<td>C. Time to onset of high intensity rain</td>
<td>4 h (T5, T6– first rain only)</td>
<td>882 a</td>
</tr>
<tr>
<td></td>
<td>48 h (T2, T3– first rain only)</td>
<td>1110 b</td>
</tr>
<tr>
<td>D. Transport in first or second high intensity rain</td>
<td>First storm (T3, T6)</td>
<td>782 a</td>
</tr>
<tr>
<td></td>
<td>Second storm (T3, T6)</td>
<td>1220 a</td>
</tr>
<tr>
<td>E. Time after low intensity rain to high intensity rain</td>
<td>2 h (T4– second rain only)</td>
<td>1034 a</td>
</tr>
<tr>
<td></td>
<td>24 h (T1– second rain only)</td>
<td>1170 a</td>
</tr>
<tr>
<td>F. Effect of tillage</td>
<td>No-till (T1, T2, T3, T4, T5, T6)</td>
<td>2080 a</td>
</tr>
<tr>
<td></td>
<td>Tilled (T1, T2, T3, T4, T5, T6)</td>
<td>957 b</td>
</tr>
</tbody>
</table>

† Treatments designated as T1 through T6 are described in Table 1.
‡ Values in a leachate or oocysts column, for each comparison, that are followed by different letters are significantly different (P < 0.05).

was that more of the Cryptosporidium oocysts were recovered in the first rainfall after only a 4 h time between manure application and rainfall treatment (Treatment 3) compared to the second rainfall 72 h after the first rainfall. If the first rainfall occurred 48 h after manure application, a lower number of oocysts were recovered in the first intense rainfall compared to the second intense rainfall 72 h later. Thus, even though the total of oocysts recovered was not that different between the two treatments describe in Comparison D of Table 4, the distribution of the oocysts in the two intense rainfalls was quite different.

Comparison E of Table 4 extends the study of what happens to the oocysts that were washed into the soil by a low intensity rainfall (i.e., Comparison B) but not recovered in a leachate water sample. Applying a high intensity rainfall 2 h after a low intensity rainfall resulted in log 3.94 Cryptosporidium oocysts recovered in leachate. Extending the time between the low intensity and high intensity rainfalls to 24 h, decreased oocysts recovery to log 3.70.

Of all the comparison in Table 4, the greatest significant difference in number of oocysts transported was observed for that of tilled vs. no-till (Comparison F). The highest average recoveries of Cryptosporidium oocysts across all the different rainfall treatments were from the no-till soil block (log 4.29 oocysts) compared to only log 3.64 oocysts from the tilled blocks.
structure and absence of macropores (e.g., wormholes) in the 10 cm soil surface layer due to the simulated tillage treatment. Macropores were clearly visible at the bottom of the soil blocks and if they extend to or near the soil surface, they can act as effective conduits for transport of Cryptosporidium oocysts. Our results suggest that high numbers of oocysts were transported through the no-till soil, even before application of rain. When the surface 10 cm of soil was tilled, the oocysts could not readily bypass the adsorptive and filtering effects of the soil and the volume of leachate and oocysts transport were reduced. Retention of oocysts by the surface soil layer has been reported by other investigators (Darnault et al., 2003, Mawdsley et al., 1996) and it was also thought to be due to a filtering effect by the surface soil.

Vertical transport of Cryptosporidium oocysts was also a function of rainfall treatment. When rainfall occurred 2 to 4 h after the manure application, more oocysts were transported through the soil than when the rain was delayed 48 h. This was especially evident for the no-till soil. Rainfall intensity can also affect oocysts movement through soil. A greatly reduced number of oocysts were transported through the soil after a low intensity rain compared to a high intensity rain, even if this first rainfall occurred only hours after liquid manure application. This suggests that, as much as possible, application of liquid manure to fields should be avoided before when an intense rainstorm is forecasted.

No attempt was made to determine spatial distribution of oocysts within the eight layers of the soil blocks. We assumed, however, that oocysts were more evenly distributed throughout the soil volume of the tilled plots, especially in the surface 10 cm of the soil blocks. In contrast, the oocysts in the no-till soil blocks may have been concentrated more in randomly distributed large macropores similar to what was found for atrazine in no-till soils which was greatly concentrated in the linings of earthworm burrows (Stehouwer et al., 1993).

It has been reported that storing animal wastes in piles before spreading may reduce the number of infective oocysts in the soil environment (Jenkins et al., 1999). Our results suggest that spreading the manure during dry seasons could be an effective management practice for reducing the oocysts movement as it allows time for reduction, via biological and environmental factors, in viable oocysts number before they can be vertically transported down into the soil. The negative side of this management practice is that, if applied to a no-till field without incorporation, the potential for odor and ammonia losses would be increased. Therefore, the combination of these practices (storing animal wastes and spreading it during dry periods) may significantly reduce number of infective oocysts to reach susceptible hosts and may help farmers to beneficially spread manure to fields without abandoning the use of no-till soil for crop production.

Hoorman and Shipitalo (2006) recently reported results from a survey they conducted that investigated 98 incidents, over a 4-yr period (2000–2003), where agricultural wastes in subsurface drainage waters contaminated streams in Ohio. Violations occurred most frequently with liquid swine or dairy wastes with all methods of application—irrigation, surface spreading, and subsurface injection. In most instances multiple factors contributed to each incident. The factor most commonly cited (41 cases) was application to saturated soils or heavy rainfall shortly after application. Avoiding these conditions when applying liquid manure to soil should reduce the number and severity of incidents. Some method of disrupting the macropores in a no-till soil also seems to be required for minimizing transport of manure to tile drain as only 17% of the incidents occurred on soils that were tilled or where wastes were incorporated. Thus the challenge is to develop liquid manure application technologies that can be used on no-till fields to minimize water contamination while maintaining the many benefits that are associated with no-till.

Possible solutions have been proposed by Hoorman and Shipitalo (2006) and include avoiding application of liquid manure directly over tile lines as transport in no-till soil is primarily in the vertical direction and making sure application is conducted when soils are not saturated and with at least 48 h between time of application and rainfall. This study also suggests the best combination of management practices that would lead to reduced Cryptosporidium oocysts transport through no-till soil profiles would be (i) applying some sort of tillage treatment directly over tile drains, (ii) spreading the liquid manure at least 2 d prior to rain, especially if a heavy rain is expected, and (iii) avoiding spreading the liquid manure directly over the tile lines. Practices that reduce the load of infective oocysts and environmental contamination should also be examined including manure composting and storing of the animal wastes before spreading.

References


