Extent and Magnitude of Agricultural Sources of *Cryptosporidium* in Surface Water

Project # 40
National Soil and Water Conservation Program

by:
Ron Fleming, Doug Hocking, Heather Fraser, and David Alves

submitted to:
David Armitage, Ontario Farm Environmental Coalition
c/o Ontario Federation of Agriculture
on behalf of
Agricultural Adaptation Council
90 Woodlawn Rd, West
Guelph, ON

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Final Report

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Executive Summary

In 1998, a study was begun at Ridgetown College, University of Guelph, to investigate levels of *Cryptosporidium* in livestock manure storages, tile drain water and surface water in southern Ontario. The objectives were:

1) to assess the viability of *Cryptosporidium* in liquid swine manure storages,
2) to determine the potential for a relationship between *Cryptosporidium* occurrence in storages and tile drains,
3) to quantify contributions from various sources in different watersheds, and
4) to investigate the relationship between the occurrence of *Cryptosporidium* and other water quality (and manure) indicators such as *Giardia*, *E. coli* and turbidity.

Ten swine farms were chosen and manure samples were collected over roughly a one-year period. Twenty tile drains were monitored (four samples from each during the period of flow in 1998/99). Half of these represented watersheds containing livestock. All of the swine farms and tile drain sites were also part of a study carried out in 1997, so much of the 1997 data could be used in the current study. In addition, eight surface water sites were chosen, and sampled between November, 1998 and May, 1999. These sites were chosen to represent a variety of typical land uses in an agricultural watershed.

Cryptosporidium was found in liquid swine manure, surface drain water, and subsurface tile drainage water. Both viable and non-viable oocysts were present in each of these. In total, 78 tile drain water samples were collected and analyzed. Each site was sampled four times. Each of the eight surface water sites was sampled four times. A total of six liquid manure samples were collected per farm from the 10 swine farms. The main findings of the study are as follows:

1. Conditions in a typical swine liquid manure storage are not such that there is a complete die-off of *Cryptosporidium* oocysts. Oocysts (non-viable and viable) were found in 22 of the 60 samples (37%). Viable oocysts were found in 19 of these 22 samples (86%). All manure storages tested positive at least once, of six sampling dates.

2. Concentrations of *Cryptosporidium* and *Giardia* in subsurface tile drains were significantly higher when the drainage area contained livestock barns than when no barns were present. *Cryptosporidium* concentrations averaged 771 and 323 oocysts per 100 L, respectively, when data from the 1997 and 1998/99 studies were combined.

3. Of 32 surface water samples, 14 (44%) tested positive for *Cryptosporidium* and the average concentration of all samples was 279 oocysts per 100 L (SD=564). No drainage area characteristics or combination of characteristics proved to be creating a significantly higher loading of oocysts than any other. This included presence of camping, field crops, houses, livestock, sewage treatment plant outfall, combined sewer outfall, migratory waterfowl or the date of sampling.

4. There was no significant relationship between *E. coli* concentrations or turbidity levels and concentrations of *Cryptosporidium* in the water samples.

5. There was no strong relationship between the presence or absence of *Giardia* and

Cryptosporidium in the manure samples, though statistical modeling showed a trend for the presence of these variables to be associated.

6. The average viability of oocysts in surface water samples, expressed as a percentage of the total oocysts detected, was 71%. The corresponding value for tile drainage water was 72%. Of all the samples collected, 44% of surface water samples and 32% of tile water samples contained viable oocysts.

7. In the drainage areas with no barns and no (obvious) manure spreading, *Cryptosporidium* was detected at least once (of four sample dates) in the tile water at eight of ten sites.

There are several issues that this study raises where further investigation may be needed:
 a) In the tile drainage study, the evidence points to some contribution of *Cryptosporidium* that is related to livestock in those drainage areas having livestock barns. However, in the "non-barn" watersheds, what are the sources? This represents a significant background level.

b) Tests are currently available to determine the source of *Cryptosporidium* organisms by examining the oocyst DNA. It may be necessary to use this approach on a watershed scale in order to accurately determine the source of contamination. Field-testing of this technique (or others) would be useful. It would allow for targeting of resources (e.g. where a cleanup is needed, or for identifying sources of recurring impairment).

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Ron Fleming[1], Doug Hocking[1], Heather Fraser[1], and David Alves[2]

1.0 Introduction

Cryptosporidium is a protozoan parasite that reproduces within the intestinal and respiratory tracts of many vertebrates (Garber 1993). A study by Fleming et al. (1997) found that 90 percent of swine farms tested positive for *Cryptosporidium* at least once, compared to 65 % of dairy (solid manure systems) and 50 % of dairy farms (liquid manure systems). Sixty farms were tested and about nine samples collected per farm over three visits. Some 26 % of all swine manure samples tested positive for *Cryptosporidium*, compared to 8.1 % solid dairy and 7.3 % for liquid dairy manure. Sixty tile water samples were taken from 20 tile drains. Half these tiles had manure spread in their catchment areas, half did not. Nine samples (15 percent) tested positive. *Cryptosporidium* oocysts were found in four of 10 tiles where livestock were present, and again in two of the remaining 10 where livestock were not present. No statistical significance could be determined from the tile samples, and consequently it was not possible to determine which half was the predominate source.

Since the parasite appeared to be widespread, more exhaustive examination was required to identify sources having the greatest potential to contaminate water. There was also a need to determine the viability of organisms in manure storages - to determine if there was any remaining potential to cause infection in a host.

2.0 Literature Review

Cryptosporidiosis (the disease) was first identified in humans in 1976. There are six recognized species of *Cryptosporidium,* however only *Cryptosporidium parvum* is thought to be infectious to humans and other mammals (Butler and Mayfield 1996).

Infections can be spread by "animal-to-human", or by "human-to-human" pathways (Kehl 1995). When a host is infected by this protozoa, it excretes infective oocysts into the environment. Oocysts are microscopic "eggs" 4-6 *u*m in diameter that can establish an infection in another host if they are ingested. An infected calf can excrete as many as $1x10^{10}$ oocysts per day for as long as 14 days (Carrington 1995). Oocysts can survive under a variety of conditions, including common drinking water disinfection processes like chlorination and filtration procedures. Contaminated water supplies have commonly been the cause of the self-limiting gastrointestinal illness,

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cryptosporidiosis, among immunologically healthy people. Health problems may be more serious, even fatal, in people suffering from AIDS (Acquired Immune Deficiency Syndrome) or undergoing chemotherapy, or having any other immunocompromising condition (Garber 1993). As few as 10 oocysts can establish an infection (Carrington 1995), and the minimum infective dose is generally less than or equal to 30 oocysts with a median dose of 132 oocysts (Dupont, 1995).

Recent evidence suggests that there are different genotypes of *Cryptosporidium parvum*. A report by Peng et al. (1997) reported identifying two distinct *Cryptosporidium parvum* genotypes. Genotype I was observed only in human-derived isolates and genotype II was found in calf isolates or isolates from humans reporting recent exposure to cattle feces. If developed further, this technology could help to identify the source of an outbreak. Further work on the heterogeneity of *C. parvum* us ongoing (Carraway et al. 1997; 1996).

2.1 Identification Techniques and Viability Testing

Due to their small size, *Cryptosporidium* oocysts are invisible to the naked human eye. Techniques with varying detection limits (the minimum detectable amount) and recovery rates (efficiency of the method to accurately screen samples) have been developed in order to identify an oocyst in a sample. The first method to be developed was a modified acid-fast stain which differentiates *Cryptosporidium* oocysts from other similar-sized organisms, like yeast. When viewed under a microscope, the yeasts and the oocysts are different colours (Garber 1993). The accuracy of this test depends on the examination by a qualified parasitologist. It is used in both clinical (fecally derived) and waterborne sample studies.

The enzyme immunosorbent assay (EIA) also uses colour to identify oocysts. It is a non-microscopic assay which detects an antigen-antibody reaction, using an enzyme and substrate reaction, which in turn causes a colour change (Murray et al. 1994). This method employs a standard-curve of known oocyst concentrations which have a light-absorbency to the enzyme amplification system (at wavelength of 450 nm). False positives can result if there are other antigens present in the sample that are not associated with an oocyst. Highly turbid samples may inhibit the light absorbance, thus limiting the analytical sensitivity of the test. At present, the EIA method is not widely used in environmental testing (Jakubowski et al. 1996).

The most accepted method for the diagnosis of parasite presence is the immunofluorescence assay (IFA). This technique uses a fluorescent second antibody, which recognizes the primary antiviral antibody and locates the viral antigen. A monoclonal antibody is used to recognize individual epitopes - a specific part of an antigen molecule which elicits immune reactivity (Murray et al. 1994). In a study comparing detection methods of *Cryptosporidium* and *Giardia*, direct immunofluorescent-monoclonal antibody stain had significantly higher detection rates than conventional staining methods (like the acid-fast method) (Bonnin et al. 1996). The recovery rates for this type of analysis have been reported at between 23 and 35 percent (Jakubowski et al. 1996).

In addition to staining and immuno-diagnostic methods, *Cryptosporidium* detection has been recently enhanced with the application of molecular diagnostics. The polymerase chain reaction (PCR) technique is based on enzymatic amplification of target nucleic acid sequences,

until a detectable level is reached. This method is most successful where there are large numbers of oocysts present, such as in feces from naturally infected animals (Leng et al. 1996). The method is developmental, and is not widely used in either clinical or environmental testing (Jakubowski et al. 1996).

There are two principal methods used to determine viability and infectivity: 1) animal infectivity (which is expensive, laborious, requires special accommodations and takes a long time) and 2) *in vitro* excystation alone or in combination with dye techniques. The latter estimates viability but can not determine infectivity (Fayer, 1997). With *in vitro* excystation conditions are re-created to imitate the gut; the sporozoite will exit the oocyst and thereby be able to cause infection if there is a suitable host (Unger, personal communication,1999).

2.2 Prevalence of *Cryptosporidium*

2.2.1 Prevalence In Surface Water and Groundwater - A common pathway for the oocysts to travel between hosts is through waterways. Because of this, its presence in water (especially surface water used as drinking water) has been closely scrutinized. LeChevallier (1991a) took raw water samples from 66 surface water treatment plants and found *Cryptosporidium* in 87% of the samples. Levels ranged from 0.07 to 484 oocysts per litre. It is important to note that only 32% of the oocysts observed were potentially viable. Sites from this study receiving urban sources of pollution had approximately ten times the oocysts that protected sites had. Rose (1988) tested *Cryptosporidium* levels at a lake outlet receiving water from mountain streams (33.5%), surface inflow (29.5%) and effluent from seven different sewage treatment plants (3.5%). A river diversion site passing through an area of intense animal husbandry was also tested. Twice the amount of oocysts were found at the river diversion compared to the lake outlet, showing livestock to be a potential source of elevated oocyst levels.

Following an outbreak in Kitchener-Waterloo, Ontario in 1993 in which over 200 people contracted cryptosporidiosis, testing of water from the Grand River revealed the presence of *Cryptosporidium*. Concentrations ranged from zero or near zero (i.e. not-detected) to 2075 oocysts per 100 L, with a geometric mean of less than 100 (Welker et al. 1994).

The degree to which livestock manure contaminates surface water can vary with management practices. In a study of two adjacent watersheds in British Columbia, with similar topography and geography, Ong et al. (1996) confirmed through comparison that livestock management practices restricting cattle contact with surface waters could significantly reduce the amount of parasite contamination. Concentrations of *Cryptosporidium* oocysts peaked during calving seasons. In a study conducted by Ongerth and Stibbs (1987), samples were taken downstream from agricultural, livestock and urban areas in six rivers in the Western USA. Each of the eleven samples tested positive for *Cryptosporidium*. Levels ranged from two to 112 oocysts per litre indicating all three types of areas as potential sources for oocysts. Todd et al. (1991) found four of the seven surface water sites around Manhattan, Kansas, tested positive for *Cryptosporidium* oocysts with concentrations ranging from 12 to17 oocysts per litre. Hansen and Ongerth (1991) concluded that continuous, as opposed to intermittent, concentrations of oocysts could be detected in a watershed of appreciable size. They also stated that wetter seasons can

result in a ten-fold increase in oocyst concentrations in river water compared to drier seasons where surface waters are not affected by runoff and land drainage.

Municipal water utilities are responsible for providing municipalities with safe drinking water and treating sewage so that effluents are clean enough to empty back into the water system. Studies have been conducted to test oocyst prevalence within these systems and their effectiveness at removing them. Wallis et al. (1996) tested water and sewage samples from 72 Canadian communities relying on surface water as their domestic water supply. *Cryptosporidium* oocysts were measured in 6.1% of the raw sewage samples, 4.5% of raw drinking water, and 3.6% of treated drinking water. Similar results were found by LeChevallier et al. (1991 b) in a study of 66 surface water treatment plants in 14 states and one province. They found that 27% of tested drinking water samples tested positive for *Cryptosporidium* oocysts. Finished drinking water supplies were more likely to have oocysts detected if there were high levels detected in corresponding raw water supplies, demonstrating the inefficiency of recommended water disinfection treatments in removing *Cryptosporidium*.

A source of these oocysts could be sewage effluents that are dumped into surface waters. A study by Madore et al. (1987) found levels of oocysts as high as 13,700/L in raw sewage, 3,960/L in treated sewage, and 5,800/L in surface water downstream of the effluent. Villacorta-Martinez de Maturana et al. (1992) investigated the viability of oocysts in sewage effluent after activated-sludge treatment. While the activated sludge procedure resulted in a removal rate of 80 to 84% of *C. parvum* oocysts, the remaining oocysts were still able to cause infection in mice (ie were still viable).

2.2.2 Prevalence In Livestock - A number of studies have been carried out to establish the prevalence of *Cryptosporidium* on livestock farms. Dairy and beef calves have been popular subjects, though numbers may also be found for swine farms. In most cases, the studies have concentrated on fresh fecal samples, in an effort to establish prevalence in a herd. Table 1 is an attempt to summarize the results of several of these studies. Ruest et al. (1998) conducted a study that found 88.7% of 505 dairy farms in Quebec tested positive for *Cryptosporidium*. Garber et al. (1994) found 59.1% of the 1103 dairy farms from 28 American states tested positive for *Cryptosporidium*. In the same study, 22.4% of the 1648 calves tested positive for oocysts. Oocysts were most prevalent in calves aged one to three weeks old with 48% of the calves in this age range testing positive. The rate of detection decreased to 22% for the three to five week old calves and was less than 15% in calves five weeks or older. The age of calf where oocysts turned up most frequently was 12 days. This same study also determined that incidence of *Cryptosporidium*-infected calves was higher in larger herds than smaller herds.

The USDA conducted a study in 1993 to determine the prevalence of *Cryptosporidium* in beef calves. 141 farming operations participated in the study. They determined that *Cryptosporidium* is a common protozoa in beef operations. 40% of the operations that submitted samples from diarrhetic calves tested positive at least once for *Cryptosporidium*. 42% of the operations that submitted samples from non-diarrhetic calves tested positive at least once. Of all the samples taken, 20% of the diarrhetic calves and 11% of the non-diarrhetic calves had positive test results (Atwill 1995). Olson et al. (ca1996) detected *Cryptosporidium* oocysts in 20% of the tested dairy fecal samples and in 11% of the tested swine fecal samples.

Table 1 – Summary of the prevalence of *Cryptosporidium* in livestock and manure

Farm Type (source of *Cryptosporidium*)	% of farms testing positive at least once	% of animals testing positive at least once	total % of samples testing positive	Source
Dairy - Feces	88%			Ruest et al. (1988)
Dairy - Feces	59%	all calves - 22.4% 1-3 wk - 48% 3-5 wk - 22% <5 wks - <15%		Garber et al. (1994)
Dairy - Feces		20%		Olson et al. (ca.1996)
Dairy - Solid manure	65%		8.1%	Fleming et al. (1997)
Dairy - Liquid manure	50%		7.3%	Fleming et al. (1997)
Beef calves - feces		Diarrhetic - 40% Non-diarrhetic - 42%	Diarr. 20% Non-D 11%	USDA (1993)
Swine - feces		11%		Olson et al. (ca.1996)
Swine - feces - with slotted floors		nursing pigs - 0% piglets - 0% sows - 0% weanlings - 27%		Xiao (1994)
Swine - feces - with concrete floors		nursing pigs - 29% piglets - 7% sows - 0% weanlings - 19%		Xiao (1994)
Swine - liquid manure	90%		26%	Fleming et al. (1997)

The effect of differing living conditions for young pigs was tested in a study by Xiao (1994). The first farm used slotted and wire floors. Only weanlings were shedding oocysts at a prevalence of 27%. The second farm utilized porous concrete floors. 29% of nursing pigs, 7% of piglets, 0% of sows and 19% of the weanlings showed evidence of oocysts in their feces. This study shows that reducing the exposure of young pigs to feces effectively reduces the incidence of oocyst shedding. A recent study of 60 farms in Ontario looked at oocyst levels in fresh manure as

well as stored manure. 90% of the swine farms, 65% of the dairy farms with solid manure storage, and 50% of the dairy farms with liquid manure tested positive for *Cryptosporidium* at least once during the study. Of the 552 samples taken, 26% of all swine manure samples tested positive for *Cryptosporidium*, compared to 8.1% for dairy with solid manure, and 7.3% for dairy with liquid manure (Fleming et al. 1997). There were fewer manure storage "positives" for the dairy farms, compared to swine. The most likely reason is that the calf manure was handled separately.

2.3 Water Quality Parameters as Indicators of *Cryptosporidium*

In order to minimize costs, efforts to establish relationships between oocyst levels and water quality indicators that are less expensive to test (such as turbidity, total coliform levels and fecal coliform concentrations) have been examined. Akin and Jakubouski (1986) and Rose et al. (1988) found no correlations in their studies between *Cryptosporidium* levels and water quality indicators such as turbidity, total coliform levels and fecal coliform. Conversely, in a study monitoring *Cryptosporidium* levels in surface water treatment plants, LeChevallier et al. (1991a) found significant correlations between elevated oocyst levels and water quality parameters such as turbidity, total coliform and fecal coliform. They concluded that elevated levels of total and fecal coliform and high turbidity readings should only be regarded as indicators of elevated pollution levels and therefore could indicate an increased probability of finding *Cryptosporidium* or *Giardia* in higher densities. Atherholt et al. (1998) found that rainfall appears to increase concentrations of *Cryptosporidium* in surface water through its influence on turbidity.

Standard turbidity levels have been established for safe drinking water with the belief that higher turbidity indicates contaminated water. Despite this regulation, 27% of the effluents from 66 water treatment plants in 14 American states and one Canadian province tested positive for *Cryptosporidium* oocysts. 78% of these sites would have been able to meet American turbidity standards - the standard is 0.5 NTU or less and the average turbidity of tested samples was 0.19 NTU (LeChevallier 1991b).

2.4 Viability and Survival

Cryptosporidium oocysts are not always able to establish an infection in a host. Various conditions can cause an oocyst to be rendered non-viable.
a) Temperature - Temperature plays a major role in the survival/viability of oocysts. Higher temperatures are more effective than cooler temperatures at rendering oocysts non-viable (Fayer 1994; Olson 1999; Carrington 1995; Chauret 1995). Olson (1999) tested oocyst viability at various temperatures in various conditions. The most favourable conditions for oocyst survival were at temperatures of 4° and -4°C in water and feces. The least favourable conditions for oocyst survival were at 25°C in water and feces. Similar results were found in a study by Carrington (1994) finding temperature to be the major factor influencing viability.
b) Dessication - Dessication is also effective at killing oocysts. After two hours of air drying on a slide at room temperature, 97% of the oocysts became non-viable. After approximately four hours, 100% of the oocysts were non-viable (Robertson 1992).

c) Ammonia - Ammonia is a strong base that is created by the hydrolysis of urea from urine by microorganisms in feces (Ruxton 1995). Normal ammonia levels in a manure slurry can range from 150 to 3000 mg/L. The results of a study testing the viability of oocysts under various conditions projected that it would take 8.2 days in a 0.06 M solution (1020 mg/L) to render 99.999% of the oocysts non-viable (Jenkins et al 1998). Volatilization, or the evaporation of ammonia, is a factor which decreases the amount of ammonia in manure slurries. In the top five centimeters of manure slurries, mathematical models indicate enough volatilization can occur so that ammonia levels are no longer strong enough to kill the oocysts. By stirring the slurry, levels of ammonia are more evenly distributed throughout the pit at levels that are strong enough to kill oocysts (Ruxton 1995).

d) Time - After 33 days in a river, 34 to 40% of recovered oocysts were non-viable. After 176 days, 89 to 99% of recovered oocysts were non-viable (Atwill 1995).

e) pH - It has not yet been determined what role pH plays in affecting oocyst viability. It can be said that extremes in pH make oocysts more susceptible to attack by other factors such as temperature (Merry 1997).

f) Other - Medema et al. (1997) found that biological and biochemical activity in water affected the survival of oocysts in water. Autoclaved water of comparable temperatures had larger numbers of oocyst survive than did natural river water. The rate of degradation increased as temperature of the water increased. This is in agreement with the fact that competing microorganisms, such as bacteria, thrive in warmer temperatures.

2.5 Transport

After conducting a review in 1995, Mawdsley et al. found that soil type and soil water content and flow are most frequently quoted as the main factors influencing movement of pathogens through the soil. However, very little research had been done on the survival and transport of pathogens in agricultural systems – specifically on the fate of those present in livestock manure following its application to the land. Mawdsley (1996a), conducted a study under laboratory conditions to determine to what degree soil type affects oocyst travel through the soil profile. She found that significantly more oocysts were recovered from the silty loam cores in the form of leachate than from the loamy sand or clay loam cores. Oocysts remaining within the soils were fairly evenly distributed in all of the soil types. In an experiment designed to simulate land applied manure conditions, Mawdsley et al. (1996b) observed oocyst movement through poorly drained silty loam soil on a tilting table. When water was sprayed over the tilting tables, the oocysts went into an aqueous stage and were not as available to adsorption by soil particles. In the aqueous stage, oocysts are more easily transported over the soil surface and not subjected to the filtering effects of passing through the soil profile. The breaking of macropores in the soil helps to slow water transport through the soil's profile, and hence transport of any organism contained within the water. This increases the soil's opportunity to adsorb organisms. Another way to encourage adsorption of organisms that might be present in runoff is with vegetated filter strips. Larsen et al. (1994) showed that filter strips as narrow as 0.61 m can reduce the risk of stream contamination due to runoff by 83%.

2.6 Sources in the Environment

Kemp et al. (1995) studied the fate of *Cryptosporidium* in manure after land application. Drainage from fields where manure was spread yielded low levels of oocysts fairly uniformly throughout the year. Levels were highest shortly after liquid manure application to the field, peaking at 3.2 oocysts/L. It appeared that the majority of oocysts entering the surface water originated from freshly deposited manure or leachate. Kemp examined a farm with a known case of cryptosporidiosis to see how oocysts would be disseminated into nearby surface waters. More than 50% of the examined cows were shedding oocysts. All of the farm drainage waters tested positive for oocysts, and specific events like calving activity and manure application to land seemed to coincide with peak oocyst concentrations.

Human waste has been identified as a source of oocysts in the environment (Madore et al. 1987). Though municipal sewage treatment plants have been studied extensively, there appears to be a lack of studies looking at rural septic systems and their potential to contribute oocysts in the environment.

Oocysts believed to be *Cryptosporidium parvum* have been identified in the feces of 79 species of mammals (Fayer, 1996). Some of these animals can have a *Cryptosporidium parvum* infection without showing any symptoms. Through defecation, these animals act as vectors for *Cryptosporidium* oocysts to contaminate other animals and water systems. A study conducted in England tested 510 barn rats for *Cryptosporidium parvum*. Over the period of a year, an average of 63% of the rats tested positive, with the highest count (95%) being during the spring season (Webster and MacDonald, 1996). White tailed deer (Fayer, 1996), and feral pigs (Atwill, 1997) have also been shown to shed oocysts in the environment. *C. parvum* oocysts can also be transported by animals that are not susceptible to infection. The Peking duck, a duck which is related to most waterfowl, is an example of this. It can ingest a viable oocyst, have it pass through its digestive system, and excrete it (still viable back into the environment (Graczyk 1996).

2.7 *Giardia*

Giardia, like *Cryptosporidium*, is a protozoan parasite that infects the intestinal tract. Though it is commonly a waterborne pathogen, it may be food-borne or transmitted by body contact (Smith 1993). It is recognized as the most common intestinal parasite world-wide. An infection due to the *Giardia* parasite is called Giardiasis. It can be asymptomatic or cause chronic diarrhea. Cysts, 7 to 14 *u*m in diameter, are shed intermittently in the feces of an infected organism (Olson et al. ca 1996). An infective dose could be fewer than 10 cysts (Smith, 1992).

There have been three species of *Giardia* identified. The species of major focus has been *Giardia lamblia* (also known as *G. intestinalis* or *G. duodenalis*) because it is the species infectious to mammals (including humans), birds and reptiles (Olson et al. ca 1996).

2.7.1 *Giardia* Identification Techniques - The same techniques used to identify *Cryptosporidium* oocysts and viability are used to identify *Giardia* cysts and viability.

2.7.2 *Giardia* **Survival and Viability** - Rice and Hoff (1981) tested the effect of UV irradiation and ozone on *Giardia* cysts. They found that levels that were quite suitable for eliminating coliform in drinking water did not result in *Giardia*-free drinking water. Similarly, West (1991) determined chlorine and UV irradiation disinfection techniques unable to destroy cysts. This immunity is due to the cyst's tough outer layer.

Bingham et al. (1979) conducted a series of tests to determine what effect temperature had on the viability of *Giardia* cysts. The first test compared survival rates of cysts in various water temperatures. They found that the storage of cysts in 8ºC distilled water permitted survival for up to 77 days. At 21ºC, cysts remained viable for five to 24 days, and at 37ºC, cysts remained viable for only four days. Their next test determined that although the freezing and thawing of cysts resulted in an almost complete loss of viability, less than one percent of the cysts survived for at least 14 days. This indicates that winter-time freezing cannot be relied upon to rid water of cysts. A study by Deng and Cliver (1992) determined that swine manure slurry played a role in the degradation of cysts. They observed cyst viability within swine manure slurry, human septic waste and a combination of both. Under field conditions, the time it took for a 90% reduction in total and viable cysts was 18.3 and 15.5 days in the swine slurry, compared to 41.6 and 26.8 days in the control solution of Dulbecco's phosphate-buffered saline (PBS). Rates of degradation similar to the control were found in septic tank effluent.

2.7.3 *Giardia* **Prevalence in Surface Water, Drinking Water, and Livestock** - A rise in cases of giardiasis was noted by Todd et al. (1991) in the Manhattan, Kansas region. Between 1965 and 1969, there were only two *Giardia* outbreaks reported in this region, while 56 outbreaks of giardiasis were reported between 1980 and 1985. The increase in outbreaks could have been due to an increased frequency of cysts in the environment, or to an increase in the diagnostic recognition of this intestinal parasite. Further to this study, Todd et al. (1991) examined surface water in the Manhattan area over a 16 month period. They found that *Giardia* and/or *Cryptosporidium* were found in 86% of the locations surveyed. 57% of the sites were found to have *Giardia* in concentrations ranging from 12 to 317 cysts per litre. Similar results were found by the American Water System. They collected 347 surface water samples from across the United States and tested them for *Giardia* cysts. Their results showed 54% of the samples tested positive for *Giardia* cysts (LeChevallier and Norton 1995).

Since *Giardia* is a waterborne parasite, drinking water facilities are often monitored for its presence. Isaac-Renton et al. (1996) tested 86 drinking water sites in British Columbia. 153 raw-water and 91 drinking water samples were taken. They determined cysts to be present in 64% of the raw water samples and 69% of the sites. They noted that cyst concentrations were lower, but not undetectable, in chlorinated water samples compared to raw samples. In another Canadian study, 72 drinking water facilities were tested for *Giardia*. It was detected in 73% of the raw sewage, 21% of the raw water and 18% of the treated water. This study reveals a few things. The relatively high amount of cysts detected in treated water exemplifies the inefficiency of drinking water disinfection processes to remove cysts from drinking water. Also, since a large proportion of raw sewage is human derived, humans could be considered a major contributor of cysts to drinking water (Wallis et al. 1996).

Giardia can be found in areas that have had relatively little human contact. In the Yukon, samples were taken from remote, pristine surface waters. *Giardia* was detected in 32% of the samples. In the same study, 21% of the tested scats were positive for cysts (Roach et al. 1993). Ongerth et al. (1995) determined from a study of pristine waters in Washington, USA, that a concentration of one cyst per 20 litres can be expected in relatively pristine rivers.

Levels of *Giardia* found in pristine surface waters are sometimes used as a basis for comparison. LeChevallier et al. (1991) compared *Giardia* levels in pristine surface waters to water receiving industrial (urban) pollution. They found ten times more *Giardia* in the water receiving industrial pollution. Todd et al. (1991) determined that water for agricultural and social use had a 270% increase in average cyst concentration when compared to pristine water.

Olson et al. (ca 1996) examined fecal samples from 104 cattle, 89 sheep, 236 pigs and 35 horses from up to six different locations in Canada – these were animals that showed no diarrhea symptoms. The overall prevalence of *Giardia* for cattle, sheep, swine and horses was 29%, 38%, 9%, and 20% respectively. There is evidence that management factors influence the incidence of *Giardia* infection on swine farms (Xiao et al. 1994) and on dairy farms (Garber et al. 1994). Management factors typically include practices relating to hygiene and sanitation, herd size, animal density, medication and biosecurity.

Good management practices don't always guarantee a herd's freedom from a *Giardia* infection. Olson et al. (1996) studied 20 farms operated under good management practices located in British Columbia. They found *Giardia* in all 20 farms with prevalence varying from 50% to 100% with an over all prevalence of 73%.

2.7.4 *Giardia* Sources in the environment - As mentioned earlier, giardiasis is the most common waterborne intestinal parasite in the world. Some cottage-goers call it "beaver fever" because they draw their water straight from nearby surface waters which may have beavers living in them. However, beavers are not the only wild animals that have been found to shed cysts in their feces. Others include muskrats, feral pigs, birds, cats, and dogs (de Regnier et al. 1989; Atwill et al. 1997). Erlandsen et al. (1990) attempted to determine the prevalence of *Giardia sp.* in beaver and muskrat populations in the northeastern American states and Minnesota by examining their fecal samples and their intestines for *Giardia* cysts. They found that 9.2% and 36.6% of beaver and muskrat fecal samples tested positive for cysts and 13.7% and 95.9% of the intestines of live-trapped beaver and muskrat tested positive for cysts.

However, wild animals are not the only source of cysts in the environment. Ong et al. (1996) took samples both upstream and downstream of a cattle ranch in British Columbia. They found downstream levels had significantly higher levels (0.6 to 42.9 cysts per 100L) than levels upstream of the ranch (0.5 to 34.4 cysts per 100L). Water samples were taken from two rivers near Pittsburgh over a two year period by States et al. (1997) to determine *Giardia* and *Cryptosporidium* levels. Possible sources of the protozoa included a dairy farm stream, effluent from a sewage treatment plant and several combined sewer overflows (CSOs). *Giardia* cysts and *Cryptosporidium* oocysts were detected in more than 50% of the river samples. The study concluded that the main source of *Giardia* could be treated and untreated (from the CSOs) sewage. A study by Ongerth et al. (1995) compared *Giardia* levels of two adjacent watersheds

with different amounts of human use in Washington, USA. They determined that the watershed with higher human use had higher rates of water contamination than the watershed with less human use. The U.S. Environmental Protection Agency suggested that elevated *Giardia* levels are due to sewage effluents while elevated *Cryptosporidium* levels may be due to non-point sources (1990). Agricultural effluent has also been shown to be a source of elevated *Giardia* levels in surface waters (Olson et al. ca1996).

3.0 Objectives

The objectives of the current study are to improve the understanding of the extent and magnitude of sources of *Cryptosporidium* in tile and surface drains in southern Ontario. Specifically:

1) assess viability of *Cryptosporidium* in liquid swine manure storage,
2) determine the potential for a relationship between *Cryptosporidium* occurrence in storages and tile drains,
3) quantify contributions from various sources in different watersheds, and
4) investigate the relationship between the occurrence of *Cryptosporidium* and other water quality (and manure) indicators such as *Giardia*, *E. coli* and turbidity.

4.0 Experimental Procedures

4.1 Site Selection

In order to achieve the greatest probable recovery of *Cryptosporidium* oocysts, swine farms were targeted for manure samples. Ten of the farms testing positive in the 1997 study by Fleming et al. (1997) were selected, with the farmers' permission. This was done in order to maximize the probability of capturing oocysts. These farms are located in southwestern Ontario, in the municipalities of Chatham-Kent, Lambton, Middlesex and Oxford.

Similarly all 20 tile drains from the same study were re-used. All receive agricultural drainage, and are not subject to urban runoff. They are all contained in the Thames River watershed. Half (10) have manure spread in the drainage area. Area drained varied from 16 to 121 ha (40 to 300 acres) and included more than one farm. Sampling points were made as close to public access roads as possible, to minimize time on site. There was no opportunity to examine tile drains on the swine farms.

Eight sample sites in surface drains were selected in order to illustrate impacts of urban runoff, municipal sewage treatment discharges, field crops, livestock access, wildlife and recreational uses. Expertise was solicited from three local Conservation Authorities (Kettle Creek, Upper and Lower Thames River) in selecting the best example of each. Table 2 summarizes the characteristics of the eight sites chosen for the surface water component of the study. Even with the attempt to identify areas having only one or two predominant land uses, it was very difficult to

achieve that level of selection. For reasons discussed later, Site #7 was dropped from the study.

Table 2 : Surface water drain major features

Surface Drain #	1	2	3	4	5	6	8	9
approximate watershed area (ha)	700	100	NA	700	5,900	5,300	1,500	1,000
livestock					U	U	U	U
livestock access					U			
field crops		U			U	U	U	U
seasonal campground	U							U
hamlet								U
migratory waterfowl	U							U
sewage treatment discharge			U					
urban runoff				U				
large remnant woodlot		U						U

NA - not applicable

4.2 Manure Sample Collection

All manure storages were sampled six times between mid-October 1998 and the end of July 1999, roughly every other month. Composite samples were collected from storage pits, and representative 5 mL aliquots were aseptically deposited into stool sample bottles (Para-Pak, SAF Fixative, Meridian Diagnostics, Inc.) containing 15 mL sodium acetate/acetic acid/formaldehyde (formalin) fixative. These were placed into a cooler immediately after collection, and delivered to the laboratory, or refrigerated until delivery in the next day or two.

4.2.1 Manure Sample Collection Procedure - Three one-litre samples were collected from each pit using a clean plastic bottle and stainless steel sampling bucket and pole. Liquid manure filled the bottle as it was plunged up and down through the standing manure. The pits were not regularly agitated by the farmer. Each sample was deposited into a clean four-litre pail lined with a

new disposable plastic bag. The contents were thoroughly mixed using a disposable plastic spoon, which was used to fill the sample bottle to the indicated mark on the label. The remaining contents of the pail were returned to the pit. The sample was then placed in the cooler. All non-disposable equipment was cleaned using commercially available detergent in a rinse-soap-rinse cycle, and placed directly into the vehicle. Municipally-supplied potable water was used for washing and rinsing. All disposable elements were placed into dedicated garbage bags. Waterproof footwear was washed with a 30 % ammonia solution and disposable outer "booties" (if used) were also placed in the dedicated garbage bag. Single-piece, single-use coveralls were removed and placed in separate plastic-lined storage "totes" for delivery to a commercial washing facility.

       For safety reasons, access for sampling storages was limited. Usually a small inspection plug or "cracked open" cover provided access to the stored manure. Open pits were typically fenced off, and/or had some type of berm or earthen access ramp for unloading. Snow drifts and ice also contributed to effectively limit the number of accessible sample collection points around the circumference of the open manure tanks. Ice and snow on the surface of outdoor pits never prevented collection of a specimen.

       A manure sampling "run" was usually completed over a three day period in the course of one week. The exception to this was over a period of inclement weather in the winter of 1999, which extended the third and fourth runs.

4.3 Water Sample Collection

       All surface water drains were sampled four times between early November 1998 and mid May 1999. Tile drains were sampled four times, except for two that produced only intermittent flow (due to low rainfall) and these were sampled three times each. Rainfall data and stream flow data for the Thames River at Dutton and Thamesville were collected and provided by the Lower Thames Valley Conservation Authority.

4.3.1 Water Sample Collection Procedure - *Cryptosporidium* and *Giardia* were filtered from tiles and surface drains using membrane dissolution filters charged by a small two-cycle Shindawa gas engine driven pump. The membrane dissolution filter method was developed by Aldom and Chagla at the Ontario Ministry of Health laboratory in London, Ontario. The sampling "kit" consisted of Millipore Corporation filters, a flow meter (with total volume readout), pressure gauge, plastic connector tubing, stopwatch, pocket knife, alcohol and a Hanna Instruments digital thermometer, rope, plus five plastic pails from four to 20 litres volume. Flow rate was adjusted to be less than four litres per minute and filter pressure was not allowed to exceed 138 kPa (20 psi). If pressure reached this upper limit, the filter was removed and replaced with a new filter. As much as possible, it was attempted to filter equivalent volumes of water for each sample.

       At most tile drain sites, discharge was collected in the four or 20 litre pail suspended from the protruding end. During high flow periods or at bed level outlets where the outlet was fully or partially submerged, the pump inlet pipe was inserted into the tile as far as possible in order to avoid backwash. Most often, tiles were not carrying volumes anywhere near full capacity. When flow was too slow for the pump to keep its prime, a clean pail was used to collect several litres

before the pump was run. At bed-level outlets a temporary dam was constructed using sand, gravel or earth in a clean disposable bag wadded into the tile outlet. Tile water was then allowed to build up behind the dam and periodically drawn down by the pump. Several times snow drifts had to be cleared to allow access to the tiles, but these delays never caused the pump, samples or filters to be in danger of freezing.

The surface drains were handled somewhat differently. To prevent streambed sediments from clogging the filter prematurely, the inlet pipe was placed on stones or in a submerged plastic bucket. As far as possible, samples were collected in mid-channel or where movement could be observed.

In order to eliminate the possibility of contamination from site to site with the sampling apparatus, the entire pumping system was purged with source water, using a minimum of 50 litres of water from the tile or the surface drain before the sample was run through the collection filter.

The filters containing the samples were placed in zip-lock plastic bags and placed on artificial ice in portable coolers for delivery to the laboratory. If samples were to be delivered next day, samples were refrigerated overnight.

Resources also permitted the collection of 70 grab samples from study tiles and surface drains for *E. coli* analysis and 68 turbidity measurements.

4.4 Laboratory Analysis Procedure

All microbiological analyses were carried out by GAP EnviroMicrobial Services Inc. at their London, Ontario facility. Turbidity measurements were made with a DRT 100B HF Instruments turbidimeter at Ridgetown College (University of Guelph).

4.4.1 Manure Sample Analysis - Manure samples were processed using a direct immuno-fluorescent assay kit purchased through Meridian, supplied by Oxoid. The analysis was conducted by mixing the sample to create a uniform suspension. Once the sample was well mixed, a loopful of manure was distributed on a well slide (supplied in the kit). Three replicates of the manure sample were transferred to the slides. The slides were then air dried and the fluorescent antibody for *Cryptosporidium* and *Giardia* were applied to the dried well. The wells were almost flooded with antibody. At the same time, two vital dyes were added to the antibody flooding the dried sample. They were 4',6-Diamadino-2-phenyl-indole (DAPI) and Propium Iodide (PI). The DAPI was used to determine potential viability of *Cryptosporidium* and PI for the non-viable *Cryptosporidium*. An oocyst that is determined to include DAPI is considered to be potentially viable. An oocyst observed to include PI is considered to be dead or non-viable. The three immuno-fluorescent stains were added to the wells and were incubated at 37^0 C for one hour to enhance the fluorescent signal. The samples were then examined using immuno-fluorescent microscopy. This assay does not lend itself to quantification because analysis is initiated with an unknown volume (from the loop) to start with. It is possible to make a semi-quantitative measurement by scoring the number of *Cryptosporidium parvum* oocysts and *Giardia lamblia* cysts observed per field. Positive and negative controls were processed with every sample.

One set of manure samples for nutrient analysis was taken from each farm in July 1999,

for reference.

4.4.2 Water Sample Analysis - The large volume water sample filtrations were processed using the membrane dissolution method (Aldom and Chagla 1995). Once concentrated, the samples were set up to perform an immuno-fluorescent assay as was conducted for the manure samples. A set aliquot volume of the sample was inoculated onto a well slide, where it was air dried. Once the sample was dried, an immuno-fluorescent antibody obtained by Waterborne Inc. was applied to the wells with the vital dyes (DAPI and PI). The fluorogenic stains were incubated at 37ºC for one hour. Then the samples were observed using immuno-fluorescent microscopy. Results were tabulated as potentially viable and non-viable, based on the inclusion/exclusion of DAPI and PI.

5.0 Results and Discussion

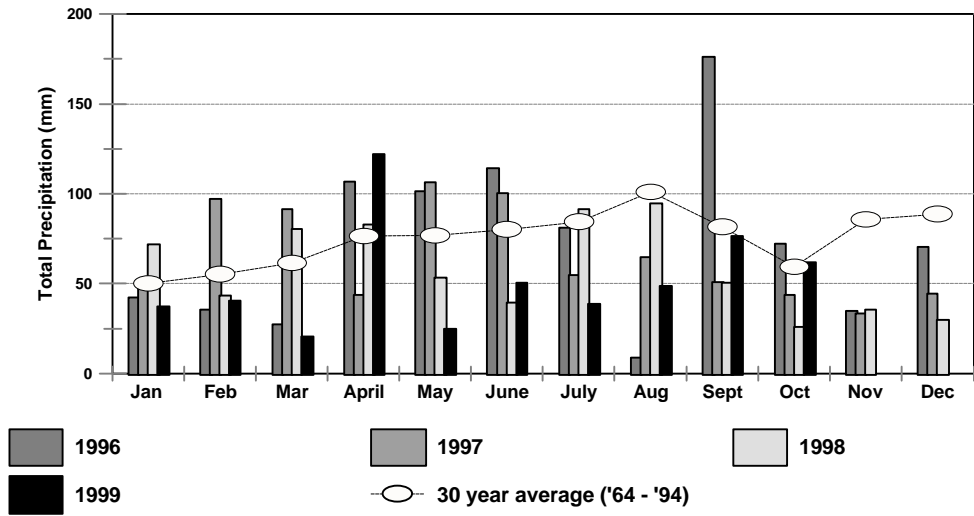## 5.1 Sample Collection

The predominately dry conditions in southwestern Ontario in 1998 seriously affected flow in surface and subsurface tile drains. This delayed the start and shortened the time frame for sample collection, generally disrupting orderly collection and presenting scheduling problems for the laboratory. For a time in late winter/early spring, sampling had to be delayed as the seasonal supply of filter cartridges had been exhausted. There was also a concern that if the dry conditions persisted, the tiles would cease flowing before the surface drain and tile water samples could be gathered. As it was, two tiles flowed only from the first week of February until the first week of April, and did not flow again. Lack of flow in tiles delayed first sample collection in seven tiles until the first week of February. All four visits to each tile and surface drain were completed, except for two tiles where only three samples were collected. Sample collection for tile drains started on November 9, 1998 and ended June 3, 1999. Surface drains were sampled from November 4, 1998 to May 13, 1999. One agricultural surface drain (originally labeled as Site # 7 and not listed in Table 2) was dropped when it had not started flowing by mid-January, 1999.

Precipitation monthly averages for Ridgetown are shown in Figure 1. This covers the past four years and compares the monthly amounts with the 30-year average for the Ridgetown College weather station. It is easy to see the high number of months during the study period where precipitation was less than normal. Figure 2 shows river flow volumes for two locations in the Thames River. This graph is included to show the variability of flow rates that are possible in the local river system. Long-term average flow rates were not readily available for comparison, though the graph does show the contrast in flow rates from the period leading up to and the period after February, 1999. This corresponds to the water sampling period for the current study.

Turbidity testing was started partway through the study. It was not included in the original proposal but was deemed to have value as a potential indicator of water contamination. Samples were collected from the tiles and surface drains from December 22, 1998 until the end of the sampling period.

Manure storage *Cryptosporidium* samples were collected between October 14, 1998 and July 28, 1999. The sampling interval was roughly once every two months.

Figure 1 Ridgetown Total Precipitation 1996 to 1999 versus 30-Year Average (1964 to 1994)
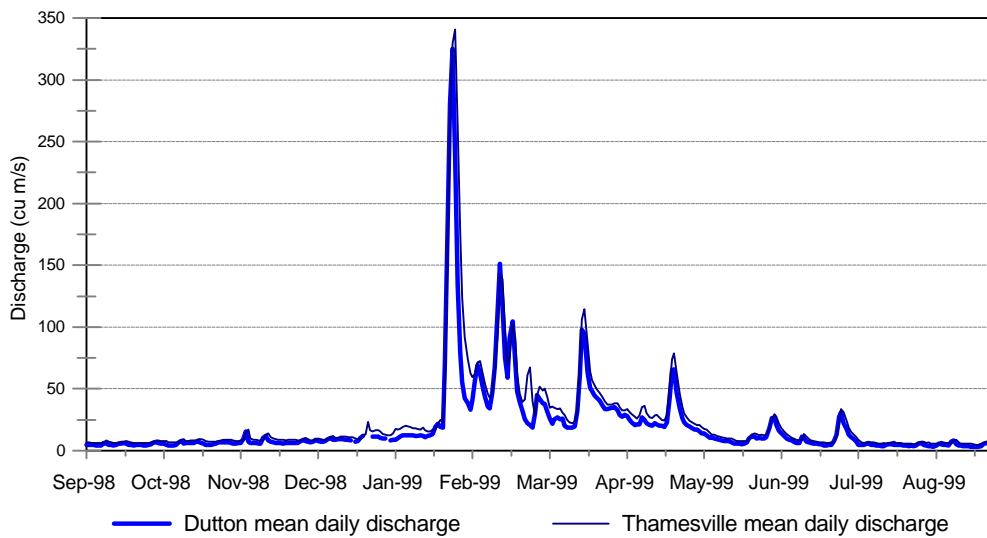
Figure 2 Lower Thames River flow rates from September, 1998 to August, 1999

It was the intention with this study to spread sample collection over a one-year period for the manure and the water samples. Given the seasonal nature of water flow in the tile drains and streams in the study, the sampling gives a fair representation of water flow conditions.

5.2 Comparison of Studies

As mentioned earlier, there was an attempt to achieve continuity between this study and the one carried out in 1997 (Fleming et al. 1997). The tile drain sites were identical and the 10 swine manure storages were selected based on having tested positive for *Cryptosporidium* in the previous study (i.e. from the 20 original sites). For purposes of analyzing results, data for tile water and manure from the 1997 study have been included, in order to give more precision to the statistical analysis. This does not apply to all tests done in the current study, but where appropriate, the results from both studies are combined.

5.3 *Cryptosporidium* - General

Table 3 summarizes the detection of *Cryptosporidium* in tile, surface drains and swine liquid manure sites in 1998-99. Materials and analysis methods for the manure storage samples are different and results are not directly comparable.

Viable *Cryptosporidium* oocysts were detected in 32% of all tile water, 44% of all surface water and 37% of all manure storage samples analyzed. The water from 15 of 20 (75%) tile drain sites contained *Cryptosporidium* oocysts at least once, as did seven of the eight (88%) surface drain sites. There have been multiple detections of viable *Cryptosporidium* in seven tiles and four surface drains. The highest frequency of detection (three out of four times) were found at a tile drain serving a drainage area containing both houses and barns, and at the discharge pipe of the urban sewage treatment plant.

5.4 *Giardia* - General

Table 4 gives corresponding summary statistics for *Giardia*. As before, laboratory analysis techniques were different for the manure samples, so concentrations are not reported. Tile water, surface water plus two-thirds of the manure storage samples were examined for *Giardia*. It has been detected 12 times in the 78 tile water samples. It appeared in the same sample as *Cryptosporidium* eight times. Only once was it found in a tile that did not have *Cryptosporidium* detected during the study. In surface drains, it was found only four times, once without *Cryptosporidium* on the same sample day. In manure storage samples, it was found five times at four sites, all in the last and second-last samples taken (May and July). In four of these instances, it was found with viable *Cryptosporidium*.

Table 3: Summary of *Cryptosporidium* sampling results 1998 - 1999

	TILE WATER	SURFACE WATER	MANURE STORAGE
sites	20	8	10
total number of samples	78	32	60
# samples with *Cryptosporidium* detected (% total)	25 (32%)	14 (44%)	22 (37%)
# times viable *Cryptosporidium* detected (% total)	22 (28%)	11 (34%)	19[1]
% viable (of all detections)	88 %	79 %	100 %
# of times non-viable *Cryptosporidium* reported	13	5	5
# of sites viable and non-viable *Cryptosporidium* detected (% of total)	15 (75 %)	7 (88 %)	10 (100 %)
# of sites with repeat occurrences (viable and non-viable)	8	5	7
average concentration - all sites (Standard Deviation)	142 (350)	279 (564)	NA
average viable concentration per 100 L [3]	349	471	Low [2]
range of viable concentrations per 100 L [3]	61 to 1,308	91 to 1,132	Low to High [2]
average non-viable concentration per 100 L [4]	259	748	Low [2]
range of non-viable concentrations per 100 L [4]	7 to 1,244	73 to 2,615	Low to High [2]

Notes: 1. Viability testing not performed on first three samples of *Cryptosporidium* detected in liquid swine manure storages.
2. No enumeration done for manure samples - ranges of concentrations only.
3. of all samples testing positive and viable
4. of all samples testing positive and non-viable

Table 4: Summary of *Giardia* sampling results 1998 - 1999

	TILE WATER	SURFACE WATER	MANURE STORAGE
# samples analyzed	78	32	39 [1]
# of times *Giardia* detected (%)	12 (15 %)	4 (13 %)	5 (13 %)
# of sites where detected	10	3	4
# of sites with repeat occurrences	2	1	1
average concentration per 100 L	658	144	Low [2]
range of concentrations	7 to 2,711	67 to 273	NA [2]
# sites detected with *Cryptosporidium*	9	3	4
# times detected with *Cryptosporidium* in same sample	9 of 25	3 of 14	4 of 5

Notes: 1. *Giardia* testing not performed on first 21 manure storage samples.
          2. No enumeration done for manure samples - ranges of concentrations only.

5.5 Manure Storages

All manure storages had *Cryptosporidium* detected at some point during the sampling period (six samples per storage). This was predicted, as all the storages had tested positive in the previous study. Since a key objective was to assess viability, it was considered an advantage to re-visit sites where *Cryptosporidium* had been found before. One manure storage tested positive for *Cryptosporidium* four times. In total, 37% (of the 60 samples) tested positive for *Cryptosporidium.* This compares with 26% (fresh and stored manure) from the 1997 study (Fleming et al. 1997), summarized in Table 1. Seven storages tested positive for *Cryptosporidium* more than once in 1998-99. Of 60 liquid swine manure samples, 19 yielded **viable** *Cryptosporidium* (of 22 times detected). This indicates that there is not a complete die-off of oocysts in manure storages - some may be killed over time but viable oocysts remain. Unlike the water samples, it was not possible to do an accurate enumeration of viable or total oocysts. Unfortunately, therefore, a viability percentage could not be established.

There was no direct correlation between the presence of *Cryptosporidium* and *Giardia* in the manure samples. A frequency table of all manure sample results from both studies (i.e. 1997 and 98/98 studies) is given in Table 5. This shows the results only for those samples where both organisms were tested. Both organisms were detected in only 10 of the 69 samples where both measurements were made.

Table 5: Frequency table showing incidence of positive and negative tests for *Cryptosporidium* and *Giardia* in manure samples

	No *Cryptosporidium* present	*Cryptosporidium* present	Row Totals
No *Giardia* present	33 (48%)	21 (30%)	54 (78%)
***Giardia* present**	5 (7%)	10 (15%)	15 (22%)
Column Totals	38 (55%)	31 (45%)	69 (100%)

Manure nutrient analyses were performed and are reported in Table 6. From a nutrient standpoint, the samples all were within normal concentration ranges for liquid swine manure. Concentrations of NH_4-N ranged from 932 to 4911 mg/kg which, according to some reports, should be enough to kill oocysts. As mentioned earlier, this complete die-off was not happening.

Table 6: Manure Storage Nutrient Analysis

Farm #	N %	P %	K %	NH_4- N mg/kg	NO_3-N mg/kg	pH	Dry Matter %
1	0.27	0.16	0.22	2553	0.50	7.6	3.48
2	0.21	0.02	0.14	1633	0.32	7.6	0.82
3	0.40	0.08	0.19	2675	0.15	7.6	2.25
4	0.82	0.26	0.38	4911	0.57	7.2	8.61
5	0.26	0.04	0.11	2215	0.21	7.3	1.23
6	0.24	0.05	0.15	1792	0.59	7.4	1.43
7	0.14	0.02	0.07	980	0.27	7.3	0.91
8	0.14	0.01	0.16	932	0.47	7.4	1.06
9	0.28	0.02	0.22	2251	0.23	7.1	1.58
10	0.34	0.04	0.26	2166	0.31	7.7	1.36

5.6 Surface Water

Most surface drain waters contained oocysts. Table 3 shows that seven of the eight sites tested positive on at least one of the four samples taken over the sampling period. *Cryptosporidium* concentrations were compared in order to determine if any sites were significantly different from the others. In most cases, there was no significant difference between the sites. However, Site #2 (natural area) and Site #3 (sewage treatment plant discharge) did

have significantly higher levels than Site #8 (the agricultural surface drain upstream of the livestock access site). No drainage area characteristics or combination of characteristics proved to be creating a significantly higher loading of oocysts than any other. This included presence of camping, field crops, houses, livestock, sewage treatment plant outfall, combined sewer discharge, migratory waterfowl or the date of sampling. It is possible that a significant relationship exists, but a greater number of samples would be needed, given the high level of variability of results.

Sites #5, #6 and #8 are progressively upstream on the same watercourse. *Cryptosporidium* was detected in the two downstream sites, both above and below a location where livestock have access to the stream for watering. The fact that oocysts were detected upstream of the watering site suggests that downstream spot sampling in large drains is not sufficient to characterize the source of *Cryptosporidium*.

As noted in Table 3, 11 of the 14 times that *Cryptosporidium* was detected, viable oocysts were present. The percentage of viable oocysts in a sample (as a percentage of the total oocysts) ranged from 0% to 100% and the average viability was 71%.

A summary of surface water statistics is given in Table 6. It brings together selected values from previous tables and includes selected comparisons to *E. coli* and turbidity.

Table 6: Surface water summary statistics

Statistic	Total *Cryptosporidium*	Viable *Cryptosporidium*	Total *Giardia*	*E. coli*	Turbidity
Total samples	32	14	32	23	23
# detected	14	11	4	23	----
Average of all	279 per 100 L	162 per 100 L	18 per 100 L	2,622 per 100 mL	11.55 NTU
Standard deviation	564	317	55	8,057	18.51
Minimum	0	0	0	4	0.38
Maximum	2,615	1,132	273	32,000	86.6

An *E. coli* count of 25,000 per 100 mL was measured in a sample from the sewage treatment plant discharge with viable *Cryptosporidium* detected. The next sample taken 24 days later had a concentration of 5 per 100 mL. The reduction is likely due to the fact that the discharge is chlorinated during the spring and summer in order to reduce the potential for contamination of downstream beaches. As expected, viability of the *Cryptosporidium* detected in the corresponding sample was not affected by chlorination. All were viable in this sample.

Turbidity readings were made for 23 surface drain samples. There was no correlation between turbidity levels and presence of *Cryptosporidium* or *Giardia*. Viable *Cryptosporidium*

were found in the highest (86.6 NTU) and second lowest (0.71 NTU) turbidity samples. .
Approximately half the detections in surface drains were near 1.0 NTU.

5.7 Tile Water

The previous tile water study (Fleming et al. 1997) divided the same 20 tile drains into two groups of ten, with and without barns in the catchment areas. It was assumed that livestock barns in a watershed corresponded to areas where livestock were present, therefore manure was spread on the land. Likewise, if no barns were present, it was assumed no manure was spread in that particular drainage basin. There was one exception to this. One of the drainage areas had no barns, but manure was spread onto the land. From this site, of the six water samples analyzed in the two study periods, there were no detections of oocysts. Even though the assumptions about barns and manure were not verified with a rigorous survey of the areas, every effort was made to visually verify the accuracy. Other than the exception noted, no observations were made that cast doubt on the validity of the assumptions.

Eighty percent (eight of ten) of the drainage areas without barns have had *Cryptosporidium* detected at least once. The source of these oocysts is not obvious. The tile drains represent a closed system with few opportunities for surface water to enter. In some cases, surface inlets along the drains allow surface water entry. This overland flow could become contaminated with oocysts shed by wildlife or pets. No study was carried out to determine numbers of these inlets, but it is likely that very few existed. Another possible source of oocysts is household septic systems. Six of the ten sites in this group contained rural residences. Anecdotal evidence strongly suggests that in certain areas of the province, especially in areas of heavy clay soils, septic systems are occasionally illegally connected to subsurface tile drain systems. No attempt was made to determine if this was happening in the test watersheds. Only one drainage area had no houses or barns. Normally, rodents (e.g. muskrats) in tile drains would not be a likely source of oocysts, as all of the outlet tiles contained a rodent guard designed to keep these animals out of the tiles. In one of the tiles, however, dead raccoons were removed from inside the rodent gate. How they gained access to the tile is unknown.

In contrast to the 80% above, 70% (seven of ten) with barns had *Cryptosporidium* detected. When concentrations are considered, however, the levels of total *Cryptosporidium* in tile water samples from drainage areas having barns is significantly higher ($p<0.01$) than sites with no barns or manure spreading. The transport mechanism to tiles is not clear, but it does appear that the risk of *Cryptosporidium* gaining access to tile drain systems is significantly higher in areas where livestock are present, and, by extension, where manure is spread on the land.

Figure 3 shows the number of positive tests for *Cryptosporidium*, expressed as a percentage of total samples, using data from both studies. It shows that the drainage areas with barns yielded drainage water containing oocysts more often (36% of samples) than drain water from areas with no barns (19% of samples). Figure 4 shows the average concentration of *Cryptosporidium* and *Giardia* in the drainage samples for the two land uses (both studies).

As with the manure and surface water samples, viability testing was performed on all samples testing positive for *Cryptosporidium*. The average viability of oocysts in the tile drain samples was 72%, almost identical to the average viability found in surface water.

Turbidity readings were made for 68 tile samples. Both the highest (38.4 NTU) and lowest (0.05 NTU) contained viable *Cryptosporidium*. No significant relationship was found between the presence of *Cryptosporidium* and turbidity levels of the water. There is, however, a significant relationship between total *Cryptosporidium* and *Giardia* ($p < 0.05$) when 1997 and
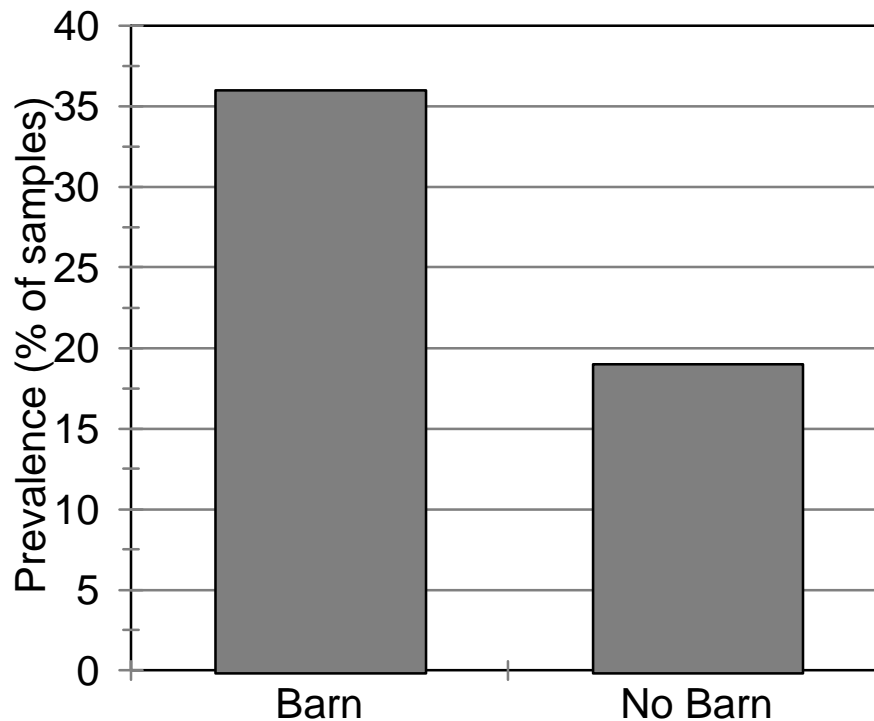
Figure 3 *Cryptosporidium* detected in tile drains in watersheds with barns and without barns - combined data from both studies - number of samples testing positive expressed as a percentage of total samples (n=118)

1999 data are combined. There were no significant differences between the months sampled for *Cryptosporidium*.

Tile water concentrations of total *Cryptosporidium* compared to *E. coli* yielded no significant relationship. Of 70 samples collected from tile drains, *E. coli* was not detected (0 per 100 mL) in eight samples. The highest reading (35,000 per 100 mL) was found at a drainage area having houses and beef cattle barns. No *Cryptosporidium* or *Giardia* were detected in the corresponding sample, although both *Cryptosporidium* and *E. coli* were later detected at this site. There were 22 tile samples (31 percent) that exceeded the Ontario Provincial Water Quality
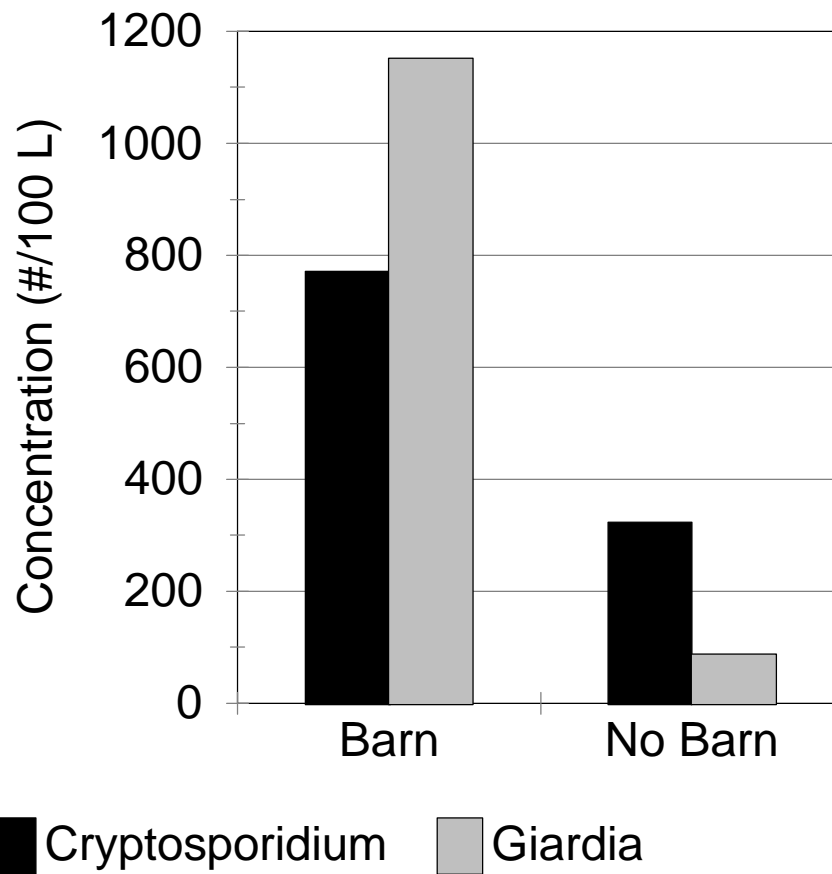
Figure 4 Average concentrations of *Cryptosporidium* and *Giardia* in tile drains from watersheds with and without livestock - for both study periods

Objective (100 *E. coli* per 100 mL) for swimming.

Discharge measurements could be taken from 15 of 20 tile drains. *Cryptosporidium* and *Giardia* were not detected when flow rates exceeded 10 L per second in any of the tiles. The maximum measured flow during sample collection was 20 L per second. The tiles ranged in diameter from 200 to 1100 mm (8 to 44 inches).

5.8 Statistical Modeling

A mixed model and fixed effect model approach was used to fit the outcome of *Cryptosporidium* presence or absence with key variables in a logistic regression model (using Egret - Cytel Software MA; Statistix - Analytical Software CA). The presence of *Cryptosporidium* or *Giardia* organisms in manure storages was not associated with the month or

the depth of the sample taken. In this model, however, there was a trend for the presence of these pathogens to be associated (p=0.056).

Statistical modeling of surface water data found that the factors: dwelling, wildlife and livestock were too closely correlated to all be included in the full models. The presence of *Cryptosporidium* or *Giardia* was not statistically associated with any of the factors in these statistical models.

For tile drain water, statistical modeling examined the impact of key variables on the presence or absence of *Cryptosporidium*. The random effect of "site" was found to adjust for the potential influence of clustering of the outcome across time, by location and through other unknown correlations.

There was little difference between results, indicating that the clustering is not likely having a major influence in these data. In a full model (all independent variables considered using backwards selection), the presence of *Cryptosporidium* was associated with the presence of *Giardia* ($p<0.05$) Similarly, with the presence of Giardia as the outcome of interest, the only significant factor is the presence of *Cryptosporidium*. This indicates that the two organisms are highly correlated and were more likely to be found together in the samples.

A reduced model was considered to examine other variables since these two outcomes are not causally associated (they should not both appear in the model). In the reduced model, the presence of a barn is highly significant ($p<0.01$) - the presence of a barn increases the risk of recovering *Cryptosporidium* oocysts from the water samples. A relationship between turbidity, flow rate and barn was also observed in the step-wise selection. Turbidity reduced the effect of the barn variable and the effect of turbidity was reduced by flow rate. Turbidity was not directly associated with flow rate, presence of a barn, or the outcome variables. The exact nature of these associations could not be determined, other than the initial moderately strong association of barn and presence of *Cryptosporidium*.

Total concentration of *Cryptosporidium* (transformed as a log-total concentration of oocysts in the sample) was extremely variable within site over time and across sites at a given time. There was no significant association between total concentration of *Cryptosporidium* and flow rate, volume filtered or turbidity ($p>0.05$). The presence of a barn had a significant association with the total concentration of *Cryptosporidium* ($p<0.05$). This association was not significant with flow rate, volume filtered and turbidity in the model. The concentration of *Cryptosporidium* was associated with the *Giardia* concentration ($p<0.05$) - high levels of one were generally found with high levels of the other.

Overall, there was evidence that the presence of a barn (as a proxy for livestock presence) was associated with the presence of *Cryptosporidium* or *Giardia* in the tile water samples. The exact nature of other factors (such as turbidity) could not be determined in this study.

6.0 Summary

Cryptosporidium was found in liquid swine manure, surface drain water, and subsurface tile drainage water. Both viable and non-viable oocysts were present in each. In total, 78 tile drain water samples were collected and analyzed. This represented 20 drainage areas, half of which contained livestock barns. Eight surface water sites were sampled, representing a variety of land uses or potential contributions within a typical southern Ontario watershed. Each site was sampled four times. Samples of liquid manure were collected from 10 swine farms over roughly a one-year period. A total of six samples were collected per farm. The main findings of the study are as follows:

1. Conditions in a typical swine liquid manure storage are not such that there is a complete die-off of *Cryptosporidium* oocysts. Oocysts (non-viable and viable) were found in 22 of the 60 samples (37%). Viable oocysts were found in 19 of these 22 samples (86%). All manure storages tested positive at least once, of six sampling dates.
2. Concentrations of *Cryptosporidium* and *Giardia* in subsurface tile drains were significantly higher when the drainage area contained livestock barns than when no barns were present. *Cryptosporidium* concentrations averaged 771 and 323 oocysts per 100 L, respectively, when data from the 1997 and 1998/99 studies were combined.
3. Of 32 surface water samples, 14 (44%) tested positive for *Cryptosporidium* and the average concentration of all samples was 279 oocysts per 100 L (SD=564). No drainage area characteristics or combination of characteristics proved to be creating a significantly higher loading of oocysts than any other. This included presence of camping, field crops, houses, livestock, sewage treatment plant outfall, combined sewer outfall, migratory waterfowl or the date of sampling.
4. There was no significant relationship between *E. coli* concentrations or turbidity levels and concentrations of *Cryptosporidium* in the water samples.
5. There was no strong relationship between the presence or absence of *Giardia* and *Cryptosporidium* in the manure samples, though statistical modeling showed a trend for the presence of these variables to be associated.
6. The average viability of oocysts in surface water samples, expressed as a percentage of the total oocysts detected, was 71%. The corresponding value for tile drainage water was 72%. Of all the samples collected, 44% of surface water samples and 32% of tile water samples contained viable oocysts.
7. In the drainage areas with no barns and no (obvious) manure spreading, *Cryptosporidium* was detected at least once (of four sample dates) in the tile water at eight of ten sites.

There are several issues that this study raises where further investigation may be needed:
a) In the tile drainage study, the evidence points to some contribution of *Cryptosporidium* that is related to livestock in those drainage areas having livestock barns. However, in the "non-barn" watersheds, what are the sources? This represents a significant background level.

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b) Tests are currently available to determine the source of *Cryptosporidium* by examining the oocyst DNA. It may be necessary to use this approach on a watershed scale in order to accurately determine the source of contamination. Field-testing of this technique (or others) would be useful. It would allow for targeting of resources (e.g. where a cleanup is needed, or for recurring impairment).

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8.0 References

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