Cryptosporidium in Livestock, Manure Storages, and Surface Waters in Ontario

prepared by:

Ron Fleming, Jennifer McLellan, David Alves,
Don Hilborn, Katarina Pintar, Malcolm MacAlpine

prepared for:
Ontario Federation of Agriculture
(representing the Ontario Farm Environmental Coalition)
40 Eglinton Ave. E., 5th Floor
Toronto, Ontario
M4P 3B1

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FINAL REPORT

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Executive Summary:

Cryptosporidium (krip-toe-spor-id-ee-um) *spp.* is a protozoan parasite that reproduces in vertebrates. It is most commonly known as a cause of gastroenteritis in people and can cause relatively large outbreaks of human illness. In the past, agricultural sources have been implicated as a contributing factor in major outbreaks of the disease. This study was initiated in light of the scarcity of information about levels of *Cryptosporidium* in manure storages and tile drainage water, and in an attempt to put together information and recommendations for farmers.

Manure study - 60 farms in southwestern Ontario were chosen and a total of 552 fecal samples were collected and submitted for analysis during three farm visits between November, 1996 and March, 1997. There were 20 each of: swine farrowing operations with liquid manure; dairy with solid manure and runoff storages, and dairy with liquid manure. Numbers of *Cryptosporidium* oocysts from fresh manure from calves or young pigs were compared to levels in manure from the storages. No measurements of viability of the organisms were made. Information on farm management (relating to herd health) practices was collected.

For the 60 farms in the study, 90% of the swine farms, 65% of the dairy farms with solid manure systems, and 50% of dairy farms with liquid manure tested positive for *Cryptosporidium* at least once during the study. In total, 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. Swine farms had significantly more samples test-positive than dairy farms over all visits ($p<0.0001$). For each of the three farm types, 50 to 55% of the farms tested positive for *Cryptosporidium* at least once for the fresh manure samples (i.e. from young pigs or calves). In contrast, 75% of the swine farms tested positive at least once for a storage sample; 20% for dairy farms with solid manure storages (plus runoff tanks); 0% for liquid dairy manure storages. This represents a marked difference between levels of *Cryptosporidium* in swine versus dairy farm manure storages. Positive manure storage test results tended to cluster (occur more than once) within swine farms significantly more than dairy farms.

Tile water study - A total of 60 water samples were analyzed to compare the prevalence of *Cryptosporidium* in tile drainage discharge water from 2 different areas - those having a high concentration of livestock in the drainage basin (i.e. livestock manure was spread on the land), and those having no livestock in the drainage basin. Two samples were collected at each of 10 subsurface drain outlets representing each of the two types of watersheds (i.e 20 drain outlets, in total). In addition, one sample was collected from the receiving stream (or ditch). *Cryptosporidium* oocysts were detected in tile drainage water samples from four of the 10 "livestock" watersheds and from two of the 10 "no-livestock" sites. The numbers of samples were too low to establish the significance of these numbers.

Information Packages - Following a review of previous studies, summary information and recommendations were compiled in three different formats (appended). These will form the basis of paper factsheets on *Cryptosporidium* and information that will be distributed via the Internet.

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Ron Fleming[1], Jennifer McLellan[2], David Alves[3], Don Hilborn[4],
Katarina Pintar[2], Malcolm MacAlpine[1]

1.0 Introduction

Cryptosporidium parvum is a protozoan parasite that reproduces within the intestinal and respiratory tract of many vertebrates (Garber 1993). It was first identified early in the 20th century, and cryptosporidiosis (the disease) was first identified in humans in 1976. It is most commonly known as a cause of gastroenteritis in humans. There are six recognized species of *Cryptosporidium,* however only *Cryptosporidium parvum* is thought to be infectious to humans (Butler and Mayfield 1996). The protozoa is transmitted via a fecal-oral route by various pathways, including drinking water. Infections can be spread by "animal-to-human", or by "human-to-human" pathways (Kehl 1995). The presence of *Cryptosporidium* in water supplies has commonly been related to a self-limiting gastrointestinal illness among immunologically healthy people, and may result in more serious health problems for those who are immunocompromised (Garber 1993).

Due to the widespread impact of this protozoa, extensive research has been devoted to the study of the organism's life-cycle, virulence, viability, detection, as well as the treatment of cryptosporidiosis. However, there is a lack of understanding of the pathways by which the protozoa enter the environment. One commonly assumed source is agricultural runoff (Garber et al. 1994; Bridgeman et al. 1995; and Butler and Mayfield 1996).

Prevalence studies have shown the incidence of the disease in farm animals in various regions of North America. However, there has been no indication of the prevalence among farms in Ontario. A variety of manure management practices are used throughout North America. In certain regions (e.g., Alberta), it is common for livestock to pasture or be housed adjacent to surface water. In Ontario, however, there has been an effort to fence cattle out of streams. Farmers typically store manure for periods of up to a year before land application. Few studies have looked at the levels of these protozoa in manure storages or in the runoff from solid manure storages.

This report details a study carried out on southern Ontario farms and drains. The primary interest of the study was ultimately to further develop Best Management

1 Ridgetown College (University of Guelph), Ridgetown
2 Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA), Guelph
3 Health Management - OMAFRA, Fergus
4 OMAFRA, Woodstock

Practices for farmers that reduce the risk of infections from *Cryptosporidium* spp. in the environment. This research compared the levels of *Cryptosporidium* spp. in manure storages, livestock, and water samples. The water samples were taken from tile outlets of agricultural watersheds - "livestock" and "non-livestock" areas.

2.0 Background

2.1 General - *Cryptosporidium parvum* has been recognized as a human pathogen since 1976. Cryptosporidiosis, the disease caused by the organism, was rarely reported from 1976 to 1982. During that period, it was reported mainly in persons with impaired immune systems. Numbers of reported cases rose sharply in 1982, largely because of the AIDS epidemic. As diagnostic tests improved, it became evident that immunocompetent persons were also contracting the disease. In immunocompetent people, cryptosporidiosis is an acute, self-limiting diarrheal illness that lasts about 7 to 14 days. It is often accompanied by nausea, abdominal cramps and low-grade fever. In immunocompromised people, the disease is usually chronic and more severe. Infections in animals occur predominantly in young animals (under six months of age), while humans may be infected at any time in their lives.

The life cycle of *Cryptosporidium* is completed within one host. Each generation can develop and mature within 12 to 14 hours. Mature oocysts are shed in the feces of the infected animal or human. These oocysts can survive under a variety of environmental conditions - even chlorination is not effective at killing oocysts, hence the concern with municipal water supply systems.

Many of the studies done to detect *Cryptosporidium* have also measured levels of *Giardia*. This water-borne parasite can cause giardiasis (sometimes referred to as "beaver fever"), an illness contracted by animals and humans. The life cycle stage found in the environment is the "cyst". Because of the similarities of the two organisms and the fact that the information is reported along with *Cryptosporidium* numbers, selected references to numbers of *Giardia* cysts are included in the review.

Juranek (1995) in a literature review, summarized the known sources of infection associated with *Cryptosporidium*:

a) transmission from person to person - believed to be one of the most common - e.g., children wearing diapers who attend day care centres;

b) transmission through ingestion of fecally contaminated water or food - water includes not only surface water used as a drinking water supply, but also well water, springs, swimming pools, and amusement park water slides or wave pools;

c) from animal to person - the strongest evidence deals with transmission from dairy calves to humans; and

d) by contact with contaminated environmental surfaces.

Recent evidence suggests that there are strains or subgroups of *Cryptosporidium parvum* that tend to infect animals. Genotyping of some isolates from human outbreaks suggests the *C. parvum* may be from human sources rather than other animal sources. Further work on the heterogeneity of *C. parvum* is ongoing (Carraway et al. 1997; 1996).

2.2 Sources of Contamination

- *Cryptosporidium* is usually associated with surface water (as opposed to groundwater). The relative importance of the variety of sources of contamination is not well understood. Possible sources include:

a) sewage treatment plant discharge - either treated discharge or accidental overflows of untreated waste
b) overland runoff from manure storages and feedlots
c) illegal connections of septic systems to subsurface drains emptying into surface water
d) wildlife - defecation in or near streams
e) runoff from fields receiving livestock manure
f) runoff from fields receiving sewage sludge
g) livestock manure entering streams as a result of defecation in or near streams
h) other sources of sewage (e.g., interception of septic plume by surface water, marine discharge)

Several researchers have measured background levels of *Cryptosporidium* in surface waters. Ongerth and Stibbs (1987) found *Cryptosporidium* oocysts in each of the 11 samples of water from six rivers in the State of Washington. Concentrations ranged from 2 to 112 oocysts/L. Hansen and Ongerth (1991), found that *Cryptosporidium* oocysts were present in river water of both inhabited and uninhabited areas, and that the concentrations were continuous (over a 3 month period) as opposed to intermittent. Ongerth et al. (1995) found that concentrations of *Giardia* cysts of 1 cyst per 20 L can be expected in relatively pristine rivers. A rise in concentration corresponded to an increase in the level of human activity on the river.

Rose et al. (1988) found no association between levels of coliform bacteria, turbidity, *Cryptosporidium* oocysts and *Giardia* cysts in a surface water study in a western USA watershed. *Cryptosporidium* was detected in 20 of 39 samples (51%) collected over a one year period. *Giardia* was detected in 12 of 39 samples (31%). Todd et al. (1991) sampled surface water in Kansas and found four of seven sites (57%) had *Cryptosporidium* oocysts, with concentrations ranging from 45 to 66 oocysts/gal. Four sites also had *Giardia* cysts, with concentrations ranging from 45 to 1200 cysts/gal. Estimated recovery rates were 2.0% for *Giardia* and 5.6% for *Cryptosporidium*.

Wallis et al (1996) tested water and sewage samples from 72 Canadian communities that relied on surface water as their domestic water supply. Of 1760 samples tested,

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. Most of these samples contained less than 0.5 oocysts per 100 L. Further, the viability of oocysts recovered from water and sewage was much less than 100%. *Giardia* cysts were found in 72.6% of raw sewage samples, 20.9% of raw drinking water, and 18.2% of treated drinking water. Most of the water samples contained less than 2 *Giardia* cysts per 100 L. The sewage samples most frequently contained less than 1000 cysts per litre.

In a study by LeChevalier and Norton (1995) samples were collected from 72 North American surface water treatment plants between March 1991 and January 1993. *Cryptosporidium* oocysts were detected in 51.5% of the samples, with a geometric mean of 2.4 oocysts/L (ranging from 0.065 to 65.1 oocysts/L). *Giardia* cysts were detected in 45.0% of the 262 raw water samples. The geometric mean of detectable *Giardia* was 2.0 cysts/L, with levels ranging from 0.02 to 43.8 cysts/L. This study concluded that if enough samples were analysed, it would be highly likely to eventually detect *Giardia* and/or *Cryptosporidium*. An earlier study at many of the same water treatment plants (LeChevalier et al. 1991) found *Cryptosporidium* oocysts in 27% of the drinking water samples and *Giardia* cysts in 17% of the 83 filtered water samples.

Goatcher et al. (1996) studied levels of *Giardia* and *Cryptosporidium* in raw water at treatment plants serving Edmonton, Alberta. *Cryptosporidium* was detected in about 1/2 of the water samples while *Giardia* was detected in about 85% of all samples. At the Rossdale water treatment plant, after 3 1/2 years of sampling, *Cryptosporidium* levels ranged from 3 to 480 oocysts/100 L (geometric mean = 27). Levels of *Cryptosporidium* were substantially higher during spring, corresponding to spring runoff. Levels of *Giardia* were in the range of 5 to 780 cysts/100 L (geometric mean = 79).

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. The sewage treatment plants using sand filtration along with activated sludge had significantly lower levels of oocysts present in 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 (i.e. were still viable).

Roach et al. (1993) studied waterborne *Giardia* and *Cryptosporidium* in the Yukon, Canada. No cattle were present in the Whitehorse watershed during this study. Raw water occasionally tested positive for *Giardia* cysts and *Cryptosporidium* oocysts. *Giardia* was found in all raw sewage samples. The highest levels of *Cryptosporidium* were found in a treated sewage sample (333 oocysts/L). *Giardia* cysts were found in 21% of the wildlife fecal samples, but no oocysts were detected. The authors suggested that in the Yukon, humans are the most important reservoir of *Cryptosporidium*.

These studies point out that *Cryptosporidium* is a very common environmental pathogen in North American surface water.

2.3 Major Outbreaks - While there are many ways that cryptosporidiosis can be spread, the most dramatic cases are those associated with infection of municipal water systems. These have done the most to raise people=s awareness of the issue. Most outbreaks have been attributed to poor water treatment, or rather, inadequate removal of the infective oocyst stage of the protozoa (Garber 1993).

A major outbreak occurred in Milwaukee, Wisconsin in the spring of 1993. An estimated 403,000 people became infected (MacKenzie et al. 1994). *Cryptosporidium* oocysts entered the city=s drinking water through one of the two water treatment plants drawing water from Lake Michigan. Because of a change in chemical used to coagulate particulates prior to sand filtration, and lack of employee experience with this chemical, the turbidity levels in the treated water from the southern plant rose dramatically. This corresponded with high levels of oocysts in the drinking water (Edwards 1993). The initial reaction of health officials (as reported in an Associated Press release in the Toronto Globe and Mail, April 10, 1993) was that the source of contamination was most likely farm or slaughterhouse runoff. MacKenzie et al. (1994), however, maintained that the source was speculative. Possible sources included cattle along two rivers that flow into Milwaukee harbour, slaughterhouses, and human sewage.

In January and February, 1987, an estimated 13,000 people in Carroll County, Georgia became infected (Hayes et al. 1989). Once again, the presence of *Cryptosporidium* oocysts in the drinking water was linked to a failure in the treatment system. No source of contamination could be traced - a sewage overflow was found, and a few cattle in a nearby watershed tested positive for *Cryptosporidium*.

In July 1984, Braun Station, Texas, experienced an outbreak of cryptosporidiosis. Fecally contaminated water entered the water supply, an artesian well. Attempts to identify the exact site of surface water or sewage contamination were unsuccessful. No association was evident between pet ownership, exposure to farm animals, or swimming in the community pool (D=Antonio et al. 1985).

From January to June, 1992, a large outbreak occurred in Jackson County, Oregon**.** It was linked to the water supply (surface water sources), and with problems in the filtration system. There were no concentrated livestock operations such as feedlots or dairies in the watershed. No evidence established the source of oocysts in the water. The most likely factors were: a) reduced stream flows resulting in a higher-than-normal fraction of municipal wastewater in the stream (as high as 30% of stream flow) and b) runoff caused by several small rainstorms, from agricultural and livestock grazing areas (Leland et al. 1993).

2.4 Livestock Agriculture - A number of studies have been carried out to establish the prevalence of *Cryptosporidium* on livestock farms. Calves have been popular subjects, both on dairy and beef farms, 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.

In a study in Manitoba, Mann et al. (1986) examined fecal samples from 3593 people having diarrhea, and 182 calves from 148 herds having a diarrhea problem. Oocysts were found in 1% of humans and 25% of the calves. Children under 5 had a higher infection rate than older people. Rates in Northern communities were higher than in southern areas (both rural and city). Infection in beef calves was highest in winter and spring while human infection was most common in late summer and fall. The source of human infection was not established but there was evidence of person-to-person transmission and also animal-to-person (e.g., pets, calves).

Olson et al. (1996?) examined fecal samples from 104 cattle, 89 sheep, 236 pigs and 35 horses from up to 6 different locations in Canada - these were animals that showed no diarrhea symptoms. Overall prevalence of *Cryptosporidium* for cattle, sheep, swine and horses was 20%, 23%, 11%, and 17%, respectively. For *Giardia,* the overall prevalence for cattle, sheep, swine and horses was 29%, 38%, 9%, and 20% respectively.

Kemp et al. (1995), in a study in Scotland, found that 60 to 94% of the dairy calves on several farms developed clinical cryptosporidiosis. All calves on the farm were sampled - no selection criteria were used. Calves shed oocysts at age 5 to 23 days old. Numbers shed ranged from less than one oocysts/g to in excess of 10^8 oocysts/g feces. Each calf has the potential to shed 10^{10} oocysts over a period of about 7 to 10 days. In adult cattle, there appeared to be a protracted low-grade infection, with shedding of oocysts happening over a two to five month period. On farms known to have cryptosporidial infection in the calves, up to 34% of mature calves were found to be shedding oocysts. Ongerth and Stibbs (1989) measured *Cryptosporidium* levels in 445 Holstein calves at 10 dairy farms, using an acid-fast stain technique. Forty-one percent of the samples contained *Cryptosporidium*. The prevalence varied with calf age - 51% for calves between 7 and 21 days old, 60% in the 8-14 day old group, and 0% in the >21 day old group (of 30 calves). Prevalence did not appear to be related to season.

In contrast, Myers et al. (1984) examined fecal specimens from 136 healthy beef calves (1 day to 12 weeks of age) and found no evidence of *Cryptosporidium*, even though there were often other calves in the herds with diarrhea.

Many of the prevalence studies have looked at conditions at one point in time. Others, however, have followed a herd for a longer period and have found that given a long enough sampling period, nearly all the animals in a herd may shed oocysts. Quigley et

al. (1994) analysed fecal samples biweekly for 90 dairy calves from birth to about 4 weeks of age. *Cryptosporidium* was found in 28% of all samples and was shed by 96% of the calves during the study. Samples tested positive only during weeks 1 to 4 of the study. Most shedding occurred during the first 3 weeks of life. *Giardia* was found in 27% of all samples (79% of the calves). Fecal excretion of *Giardia* generally occurred after 2 weeks of age.

Tacal et al. (1987) found 10 of 200 (5%) market swine at a livestock auction were infected with *Cryptosporidium*.

There is evidence that management factors influence the incidence of *Cryptosporidium* and *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, biosecurity, etc.

2.5 Measurement Techniques - Due to the widespread impact of this protozoa, extensive research has been devoted to the study of the organism=s life-cycle, virulence, viability, detection, as well as the treatment of cryptosporidiosis. Of particular interest are the developments in the detection of the oocyst in both waterborne and clinical (fecal) samples. However, both the detection limit and the recovery rate for all detection methods are rather unsatisfactory, and there is still a great need for continuing research in this field.

The **detection limit** refers to the minimum amount detectable by the diagnostic tests. In some cases these tests can only detect a concentration of 10^3 oocysts per gram of feces. Thus, any sample with a lower concentration of oocysts will result in a false negative for the presence of *Cryptosporidium*.

Some methods return false positives, due to interfering fluorescing algae, or inhibitory substances in the sample which disrupt the detection test. False positives are more commonly seen with the immunofluorescence assay and the enzyme-linked immunosorbent assay.

There are large discrepancies between laboratories on reported **recovery rates** (LeChevallier et al. 1995). Poor recovery rate is an ongoing challenge in *Cryptosporidium* detection, and the importance of developing a more efficient method is integral to accurately screening water samples - issues include entrapment, filtration, purification, separation, and concentration.

The diagnosis of cryptosporidiosis, or the detection of the oocyst stage, is most often made by microscopic detection in both clinical and waterborne samples. However, before any method of observation is used, samples are commonly preserved. The

clinical samples are exposed to either 2.5% potassium dichromate, 10% formalin, or sodium acetate-acetic acid-formaldehyde (SAF), immediately after retrieval to maintain the morphology of the oocyst. This is usually followed by filtration, elution, centrifugation and a sucrose density gradient.

Sample filtration is required as an initial step to remove the larger particles in both water and clinical samples. Some losses of oocysts are inevitable in this step, combined with the elution of the sample (LeChevallier et al. 1995). Previously the most commonly used filter for waterborne samples was a polypropylene-wound fibre filter. However, this method presented many problems, including a low recovery rate. A more recent approach involves filtering the sample water and dissolving the filter. The *Cryptosporidium* oocysts are retrieved from this mixture of filter and filtrate. This procedure is more time-efficient and results in a recovery rate of up to 60%, compared with a two to ten percent recovery rate for the polypropylene-wound filter (Palmateer 1997).

Once the oocysts have been eluted, centrifugation follows, and this step may result in oocyst loss. In many cases, a large number of oocysts remain in the supernatant after centrifugation, which is then discarded. If the sample is highly turbid the debris aids in pulling the oocysts down into the useable pellet, reducing oocyst loss (LeChevallier et al. 1995). The sucrose density gradient enables separation of the oocysts from the debris in the sample. The lighter oocysts will float on top of the gradient, and the heavier debris will sink to the bottom, due to the specific gravity of sucrose (LeChevallier et al. 1995).

The first method developed for the detection of *Cryptosporidium* oocysts was a modified **acid-fast stain**. *Cryptosporidium* is a small and transparent protozoa which is often mistaken for yeast. However, differential stains such as the acid-fast stain are able to stain yeast and the oocysts different colours. *Cryptosporidium* are acid-fast and yeast are not (Garber 1993). The benefits of this method include time efficiency, ease in processing large volumes, and moderate cost. However, the accuracy of this test depends on the examination by a qualified parasitologist. Often, there is difficulty in viewing the internal makeup of the oocyst, which then requires an additional stain to confirm the result. As well, there is the additional cost of a fluorescence microscope (MacPherson and McQueen 1993). This test is used in both clinical and waterborne sample studies.

The **immunofluorescence assay** (IFA) is the most accepted method for the diagnosis of parasite presence. There are several test kits on the market specifically designed for the detection of *Cryptosporidium* by immunofluorescence. The indirect immunofluorescence assay (IFA) uses a fluorescent second antibody, which recognizes the primary antiviral antibody and locates the viral antigen. In the IFA kits, a monoclonal antibody is used, which recognizes individual epitopes (a specific part of an

antigen molecule which elicits immune reactivity) (Murray et al. 1994). Monoclonal antibodies are also able to detect viral mutants and strains which differ in these proteins.

There is also a direct immunofluorescence assay available, which uses a fluorescent primary antiviral antibody. Of all the methods available to monitor for the presence of *Cryptosporidium*, this is one of the most expensive and time-consuming. Special equipment is necessary, including microdilutors, reciprocal shakers, and a fluorescence microscope (MacPherson and McQueen 1993). For detection purposes, the largest drawback is the tendency for background fluorescing interference, usually caused by certain algal species. These algae are of similar shape and size to both *Giardia* and *Cryptosporidium*, and in turn increase the frequency of false-positive IFA results. The recovery rates for this type of analysis have been reported at between 23 and 35 percent (Jakubowski et al. 1996).

One type of assay which has become increasingly more common is the **enzyme immunosorbent assay (EIA)** test, also referred to as the enzyme-linked immunosorbent assay (ELISA). This method is considerably less labour-intensive. However, there is a loss of parasitological expertise (Murray et al. 1994). 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. This method employs a standard-curve of known oocyst concentrations which have a light-absorbance to the enzyme amplification system (at a wavelength of 450 nm). This procedure typically requires only two hours (Jakubowski et al. 1996).

False-positives can occur if free antigens which aren't associated with an oocyst are present in the sample, causing a positive reaction. Highly turbid samples may inhibit the light absorbance, thus limiting the analytical sensitivity of the test (Jakubowski et al. 1996). There are no published recovery rates available. Currently, commercial ELISA kits are used for the analysis of clinical samples, but this method can also be used to screen water samples. At present, the EIA method is not widely used for environmental testing.

In addition to staining and immunodiagnostic methods, *Cryptosporidium* detection has been recently enhanced with the application of molecular diagnostics. In this field, the **polymerase chain reaction (PCR)** has perhaps the most potential. PCR is based on enzymatic amplification of target nucleic acid sequences, until a detectable level is reached. The purification step for this method is critical, after which purified oocysts remain, ready for PCR amplification. The oocysts must also undergo a freeze-thaw procedure to break open the shell, and liberate the *Cryptosporidium* DNA. This method is most successful where there are large numbers of oocysts present, such in feces from naturally infected animals. However, detection in asymptomatic cases and waterborne samples is more difficult due to the lower numbers of oocysts (Leng et al. 1996).

Once the samples are centrifuged and purified, the amplification reaction is an automated series of cycles, including template denaturation, primer annealing, and extension of the annealed primers by DNA polymerase I. The PCR products are then electrophoresed on an agarose gel, and can be visualized with ethidium bromide staining. There are certain limits to this method and they include the presence of humic substances which can interfere with the activity of the enzymes used in PCR. The presence of formalin or potassium dichromate (two preservatives often used in clinical samples) inhibits the PCR. Recently it has been found that nested PCR is useful for detection, and often PCR is used in conjunction with other optimizing methods such as flow cytometry and magnetic separation. This method is developmental, and is not widely used in either clinical or environmental testing (Jakubowski et al. 1996).

Magnetic separation involves the use of small paramagnetic beads coated with antibodies against surface antigens of cells. The beads are magnetic in a magnetic field, but once the magnetic field is removed, they are nonmagnetic. The sample is first exposed to a primary antibody, which reacts with the target. This complex then reacts with the antibody-tagged beads, and they are pulled towards a magnetic plate by magnetic forces. This enables separation of the oocysts from other particulate matter in the sample. Although this protocol is still being developed, it is proving to be effective in the purification step of the detection method for environmental samples (Jakubowski et al. 1996).

2.6 Viability in the Environment - In order to develop measures that farmers may take to reduce potential environmental impacts of spreading manure containing *Cryptosporidium* oocysts, an understanding of the factors that lead to a reduction in viability is important. Robertson et al. (1992) looked at the survival of oocysts under a variety of environmental conditions. Small proportions of oocysts survived for long periods of being frozen (775 hours at -22 ^{o}C). Dessication (drying) was 100% effective at killing the oocysts in less than 4 hours. Oocysts were able to survive for long periods (several months) in tap water, river water, and in liquid cattle manure.

Kemp et al. (1995), found that up to 8% of the total oocysts shed by calves may leach from the calf pen bedding. However only 30% of the oocysts recovered from the straw bedding and 10% of those recovered from the leachate were viable. Composting was effective at rendering oocysts non-viable. They showed that the viability of oocysts in solid manure declines over time, and the decline is even more rapid in liquid manure storages. Storage of liquid manure for 13 weeks at 4 ^{o}C resulted in a low residual viability, while storage at 15 ^{o}C was enough to kill all of the oocysts.

Cool, moist, stable conditions are important to the survival of oocysts (Kemp et al. 1995). These conditions are not found in manure storages, but they are found in surface water. Once in soil, oocysts are capable of surviving for long periods (e.g., in excess of

100 days following application to land). Composting or other treatment of manure, where temperatures of 30 to 50 ^{o}C can be achieved, can effectively reduce oocyst viabilities to negligible levels in a matter of hours (Kemp et al. 1995).

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 but was 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.

Mawdsley et al. (1996) applied *Cryptosporidium parvum* oocysts to the surface of undisturbed soil columns and irrigated water onto the columns over a 21 day period. They were attempting to establish the potential for leaching of oocysts through the soil to subsurface drains following application of livestock manure to the soil. Consistently, more than 70% of the oocysts were recovered in the surface two cm of soil, with numbers decreasing with increasing depth. Low numbers of oocysts were found in the leachate from the clay loam and silty loam soils, but not in that from the loamy sand soil. The soil columns were 30 cm deep.

In a discussion of viability, it is important to be aware of the concept of "infectious dose". This is the actual number of oocysts that must be ingested before a person or an animal becomes infected. The range in humans is from a low value of only 10 oocysts to a high of about 1000 (Barta 1997). The fact that some humans can become infected after ingesting only 10 oocysts suggests that even at low survival rates for the organism (in the environment), the risk of human infection persists.

2.7 Current Awareness Programs

- In recent years, several communities have established programs aimed at education, monitoring, risk reduction, etc. Some examples are described:

New York City - Watershed Agricultural Program - The New York City Watershed covers nearly 2000 square miles and supplies approximately 9 million people with high quality, unfiltered drinking water. It is the intention of the Watershed Agricultural Program to reduce the risk of diseases caused by *Giardia* and *Cryptosporidium* in the unfiltered water. Farms within this watershed have adopted operational and management techniques to protect water quality. Multiple barriers (e.g. at the farmstead, farm fields and margins of watercourses) were developed so the levels of pollutants could be contained and controlled. This is an example of a partnership between New York City and the agricultural community (Anon, 1994).

Edmonton, Alberta - In the spring of 1983, Edmonton experienced a *Giardia* outbreak

with over 500 cases. City officials then decided to monitor the North Saskatchewan River for *Giardia* and viruses, and later for *Cryptosporidium.* Studies showed that *Cryptosporidium* oocysts in surface waters are commonly associated with agricultural wastes as there are several livestock and cattle operations along nearby creeks which belong to the watershed of the North Saskatchewan River. The high numbers appeared to be a reflection of the major snow melt and spring runoff conditions that occurred after a winter of very heavy snow accumulation. Although there were fluctuations in the number of parasites, levels of *Cryptosporidium* appeared to be the highest in samples taken during the spring runoff months.

Waterloo Region, Ontario - Grand River Watershed - There are periodic samples taken at the intake of the water treatment plant at Kitchener to monitor *Cryptosporidium* levels. Sampling has been ongoing since 1993 and has shown that the Grand River has detectable levels of *Cryptosporidium* all year - not just during the spring runoff. A study is taking place to investigate the correlation between levels of *Cryptosporidium* and other organisms (e.g., viruses). If a relationship is established, perhaps a cost-effective, fast, indirect technique in predicting the presence of *Cryptosporidium* can be developed (Pett 1997).

3.0 Objectives

In light of the scarcity of information about levels of *Cryptosporidium* in manure storages and tile drainage water, and in an attempt to put together information of direct use by farmers, the following objectives were set:

1. To investigate the prevalence of *Cryptosporidium* in typical livestock manure storages and in sewage sludge in southwestern Ontario;
2. To compare the prevalence of *Cryptosporidium* in tile drainage discharge water from 2 different areas - those having a high concentration of livestock in the drainage basin, and those having no livestock in the drainage basin.
3. To prepare an information series designed to educate rural residents, livestock farmers, and the general public on this issue, using the results of this study and a literature review.
4. To develop a sampling protocol that could be used in future studies involving livestock manure storages.

4.0 Experimental Procedures

4.1 Site Selection - Many prevalence studies have focused on cattle. There is a lack of information about the prevalence on swine farms, yet this sector represents a large proportion of the manure produced in southern Ontario. Our study included forty

dairy farms (20 with a liquid manure system and 20 with a solid manure system) and twenty swine farms. The farms were selected based on prior knowledge of their manure system and the willingness of the farmers to participate in the study. There was an attempt to cover a fairly wide geographic area, representing a significant livestock area of the province. All farms were located in southwestern Ontario. Information was gathered at each farm to help determine how representative the farms were of "typical" Ontario dairy and swine farms.

Dairy farms were chosen over beef farms due to the ease in locating farms with adult and young calves together, considering the winter time line of the study. As well, dairy farms are more likely to have solid manure storage with a runoff storage system, and the runoff was of particular interest.

For the water study, 20 drainage basins were chosen, all located within the Thames River watershed. Ten of those had a high concentration of livestock, and ten had no livestock. The criteria for selection included: a) the presence of a subsurface drainage system handling the drain water from more than one farm, b) the subsurface drain outlet located close to the road (for easy access), and c) draining agricultural areas, rather than residential areas.

Sewage sludge is commonly spread on agricultural crop land. In an effort to compare *Cryptosporidium* concentrations in manure to those in sludge, we planned to get 20 sludge samples from 10 different sewage treatment plants located in southwestern Ontario. These would represent the most commonly used systems in the region. Unfortunately, we were unable to get the necessary approvals at the plants contacted and only were able to get 10 samples, all from the same plant. With the exception of the sole cooperator, the plant managers appeared to be suspicious of the study's motives and fearful of reprisals if their plant tested positive for *Cryptosporidium*. Due to a lack of control over this part of the study, it was not followed through (the 10 samples all subsequently tested negative for *Cryptosporidium*, but we had no information on their background with which to draw any conclusions).

4.2 Manure Sample Collection

- All farms were visited three times between November, 1996 and March, 1997. Qualitative data was retrieved from surveys distributed at a farm-level. A survey form is included in the Appendix. The questions related to sanitation practices, herd size, management practices, and *Cryptosporidium* infection history. This information was then used in the statistical analysis of the study and to examine the representativeness of the farms.

Stored manure, runoff from manure storages, and fresh feces were collected in stool sample bottles (Para-Pak, SAF Fixative, Meridian Diagnostics, Inc.). These contained 15 mL of sodium acetate-acetic acid-formaldehyde (formalin) fixative and were

designed for adding a 5 mL sample. They were then placed into a cooler during the day of sampling, and stored in a refrigerator until delivery to the Ontario Ministry of Health lab (within one month).

4.2.1 Liquid Manure: Using a clean plastic one litre bottle inserted into a sample holder, five representative samples were collected from each liquid manure tank. The sample holder was dipped into the manure storage and filled - at various depths in the storage. Each of the five samples was emptied into a plastic pail lined with a disposable plastic bag. The combined sample was mixed thoroughly using a disposable spoon. A representative sample (5 mL) was then removed, and poured into the sample bottle. This procedure was also used for the liquid runoff tank. On the uncovered storages, it was possible to move around the tank and retrieve the five initial litres of liquid from various locations. The covered storages usually only had one access opening and every attempt was made to get a representative sample from this one location.

Due to the onset of freezing temperatures early into the study, some refinements were made in the sample collection protocol. Outside manure storages were frozen or partially frozen by the end of December. In total, six swine farms, 15 dairy farms with liquid manure storages, and all 20 dairy farms with runoff storages were affected. In these cases, samples were collected using one of the following methods:

a) Liquid swine manure was normally collected from storages under the barn if outside storages were frozen. Typically, this manure would be fresher than that found in outside storages.

b) On a few of the swine farms, outside storages were filled from the top (as opposed to pumping manure into the bottom of the storage). In the cold weather, these tended to be partially frozen. Samples were collected from the outdoor pit, but were comprised of the unfrozen, freshest, manure from the top of the storage.

c) Liquid dairy manure stored in earthen pits that were frozen was sampled by cutting through the ice with an axe and sampling through the hole.

d) On dairy farms with liquid manure in open tanks, when the tank was frozen, manure samples were drawn from covered holding pits - either outside or in the barn. Typically, the manure in these tanks was up to one week old.

e) On dairy farms with solid manure, when the liquid runoff storage was frozen, the liquid sample was collected in the solid storages where liquids were trapped. This tended to be liquid that had only recently run off the pile, otherwise, it also would be frozen.

4.2.2 Solid Manure: A pitch fork was used to dig into the pile to a depth of about 0.5 m at 5 different locations. Using a disposable spoon, a small sample of manure was retrieved (with the least amount of straw possible). These manure samples were then

combined in a 2 L pail lined with a disposable bag. If the surface was frozen, samples were taken from deeper in the pile. When all the samples were mixed thoroughly in the pail, a representative sample was removed and put into the sample bottle.

4.2.3 Swine fecal samples: Due to the biosecurity programs in place at the swine farms, most sampling (on 17 farms) was conducted by farm workers using a standard protocol (we collected the samples on the remaining three farms) . Five samples of fresh manure from baby pigs in farrowing crates were collected. They were placed into a disposable cup. The five samples were then mixed and a representative sample was taken and put into the sample bottle. Five samples of fresh manure from weaner pigs (older, housed in a separate location) were collected in a similar fashion.

4.2.4 Calf fecal samples: A clean disposable spoon was used to collect five samples of fresh calf manure from calves less than two months old (i.e., from five different calves). These were then placed into a disposable cup, mixed, and a representative sample was removed and put into the sample bottle. For those farms with hutches (individual calf housing, usually located outside), where the calf manure was not stored in the manure tank or in the manure pile, a sample was still taken from the calves in hutches. An additional composite sample was then taken from about five young animals inside the barn whose manure was stored in the tank or pile.

4.3 Water Sampling - At each of the 20 sites, a pump was submerged into the water. For the drain outlets, a pail or tub was placed under the outlet to hold the pump and to prevent entry of water from the receiving stream. The filtration kit was connected to the pump, and flow was adjusted to approximately four L/min. Up to 200 L of water were then filtered, although this value decreased later on in the study, following findings that concluded only 50 L of water needed to be filtered. Once the volume was filtered, the filter was disconnected and placed into a plastic bag provided with the kit. It was then placed in a cooler with an ice pack, ready for transport. To decontaminate the unit for the next filtration, approximately 200 L of surface water was run through before filtration began.

The membrane dissolution filter method used was developed in London, at the GAP EnviroMicrobial Services Inc. It was developed by John Aldom and Abdul Chagla at the Ministry of Health lab in London, Ontario (Palmateer, 1997). The filter in the kit was designed by Millipore Corporation, specifically for the recovery of *Cryptosporidium* and *Giardia* from water. The kit contained a pressure tubing, a back pressure gauge, a water volume totalizer, and the filter. Once the water was filtered through the apparatus, the membrane was dissolved and from the residue, the oocysts were stained with a monoclonal antibody stain (Palmateer, 1997).

Water samples were collected during February and March, 1997. For each of the subsurface drain outlets, two samples were collected, on two separate visits. As well as

at the tile outlets, one sample per site was collected from the receiving stream or ditch, just upstream of the outlet.

4.4 Sample Analysis -

4.4.1 Sample Preparation: For all analysis methods, a sample preparation step was needed, and it varied between each method.

4.4.2 Staining: The auramine/rhodamine acid-fast staining procedure was performed on the first few samples which were recovered, as an initial screening test. However, the results were inconclusive, and the step was removed from the analysis of the remaining samples.

4.4.3 Immunofluorescence assay (IFA): *Cryptosporidium* oocysts were detected in fecal samples by immunofluorescent assays (Merifluor kits—Meridian Diagnostics), for both the fecal and water samples, and the assay was performed as described by the manufacturer.

4.4.4 Enzyme linked immunosorbent assay (EIA): The fecal and water samples were analyzed by an enzyme immunosorbent assay at the Ontario Ministry of Health lab in London, Ontario (fecal samples) and GAP EnviroMicrobial Services Inc, London (water) . The assay was performed as described by the manufacturer (IVD Research, Inc.).

4.4.5 Nested PCR: The nested PCR was performed by the Laboratory Service Division of the University of Guelph, Ontario. The original intention was to analyze all of the first approximately 1/3 of the samples, then only the samples testing positive using the IFA method (300 samples total). The PCR method was to be used for both fecal and water samples. In total, 103 fecal samples and all 60 water samples were analyzed using PCR. Three different fecal sample types were analyzed, including fresh/frozen, formalin preserved, and Potassium dichromate preserved.

4.5 Analysis of Data -

Bivariate associations were examined between the herd factors from the farm questionnaire and the *Cryptosporidium* test status of the farm. A farm was considered to be positive for *Cryptosporidium spp.* when a positive test result in the manure storage or livestock feces among the three sample periods was found. For categorical herd factors, a Fischer's exact test was conducted and for continuous factors a one-way analysis of variance was used. McNemar's test for symmetry was used to test the association between test results in livestock and in the manure storages within the same farm (Statistix ver. 6.0 for Windows). These analyses were stratified by dairy and swine farms.

An analysis of variance with “farm visit number” as a random effect was used as a method of detecting a significant effect of time across the three sample dates while adjusting for type of farm and type of manure sample (storage vs. livestock) (SAS for Windows).

Intraclass correlation coefficients were calculated to examine the degree of clustering of test results within farms across the study farms stratified by type of farms and type of manure sample (Donald 1988; McDermott et al. 1994).

The test results from the tile outlet samples among “livestock” and “no livestock” areas were compared using a Fischer exact test.

5.0 Results and Discussion

5.1 Site Descriptions - An attempt was made to choose sites (both for livestock farms and for drainage water sampling) from as wide a geographical area as practical and representing intensive livestock areas of southwestern Ontario. Figure 1 shows the geographical distribution of livestock farms, by county.
Similarly, Figure 2 shows the approximate locations of the water sampling sites. These were all located in the Thames River watershed.

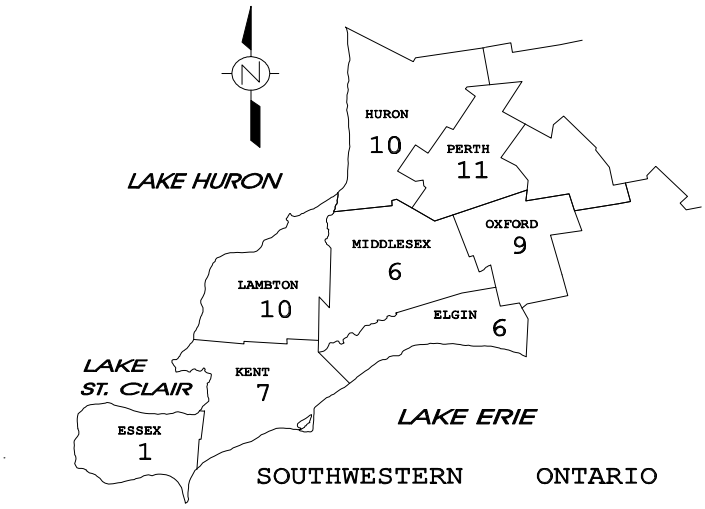

Figure 1 Numbers of participant farms in the various counties in the study - 60 total

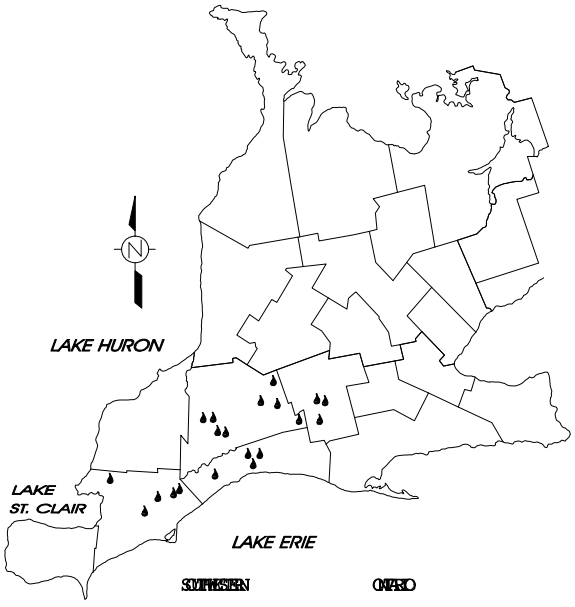

Figure 2 Locations of water sampling sites - 20 total

5.2 Livestock Manure -

5.2.1 Dairy and Swine Farms: The farmers who were contacted were, for the most part, quite interested in the study and willing to help. For those farmers who were contacted, the main reason for not ending up in the study was failure to meet the selection criteria (e.g., different type of manure system than what we wanted).

The dairy farms in the study represented a range of herd sizes. The breakdown of housing types is as follows: 25 tie stall barns, 14 free stall, and 1 bedded pack barn. The average number of milking cows per farm was 58 (SD=23, does not include dry cows - average 10 per farm), and the distribution of numbers of milking cows per farm is shown in Figure 3. Similarly, for the swine operations, the average number of sows per farm was 240 (SD=210). The distribution of numbers of sows is shown in Figure 4.

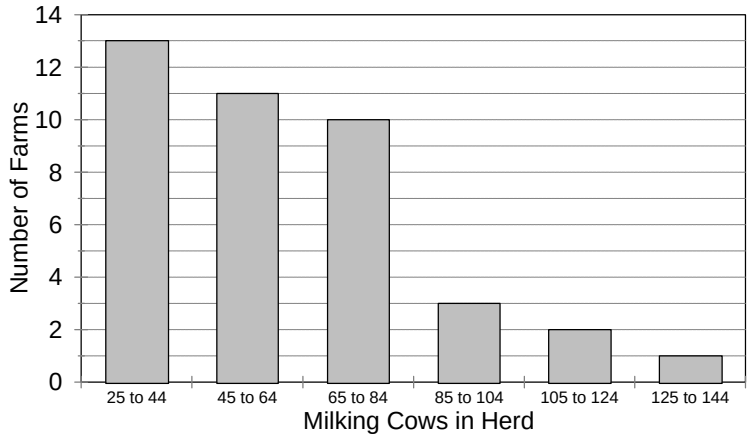

Figure 3 Number of milking cows per herd - 40 dairy farms

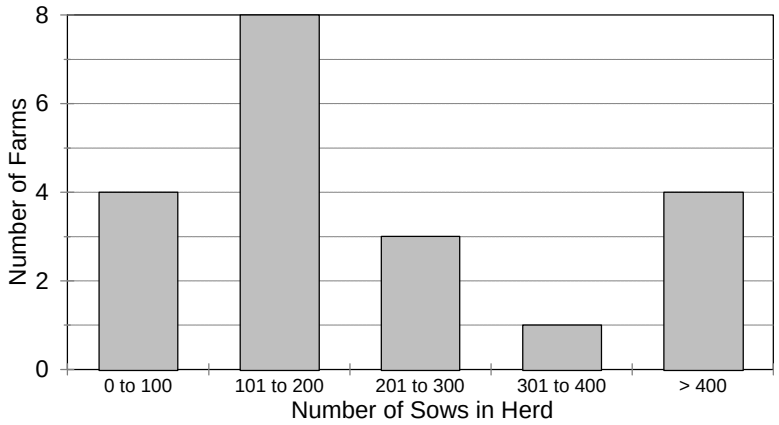

Figure 5 Number of sows per herd - 20 swine farms

The confidence limits on the estimates of herd sizes of the farrowing units and the dairy farms were within what would be expected for these types of farms in Ontario (OMAFRA 1995).

The farmers were asked to record the number of cases of scours in the herd in the previous month - for calves up to three months old, or for piglets on the sow. Results for the calves are shown in Figure 5. Of the 40 dairy farms, 27 reported having 2 or fewer cases of scours in the previous month. There was a higher incidence of scouring on the swine farms, as

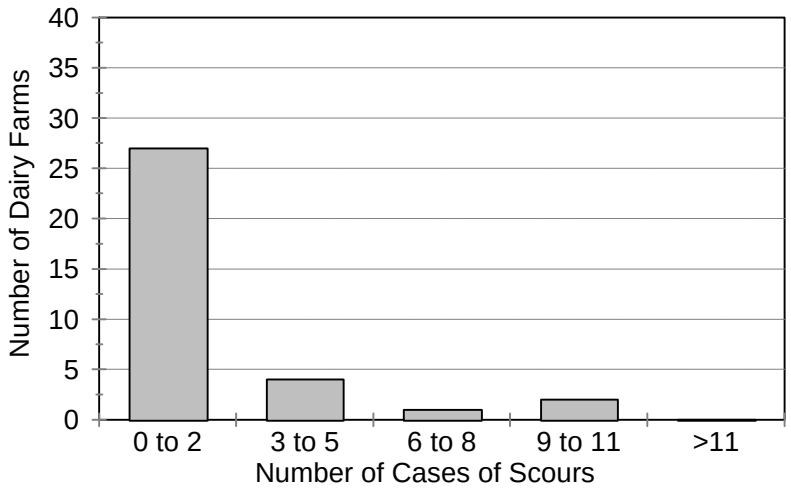

Figure 5 Numbers of reported cases of scours among dairy calves under 3 months old during the month prior to the start of this study

shown in Figure 6. This is likely due to the higher number of young animals at risk of scouring on swine farms.

For the dairy farms, bulk tank somatic cell counts (measured monthly in shipped milk) were recorded. Averages of the results for the previous three months were calculated. Half of the 40 farms had average counts in the range 100 to 200; 13 were in the range 201 to 300; 5 were in the range 300 to 400; and one was in the range 401 to 500. These averages and the dispersion of these milk quality values were not significantly different from other estimates of the Ontario average among dairy farms (OMAFRA 1995).

Sanitation practices were recorded along with other biosecurity measures included in the management of the farms. Of the 40 dairy farms, only one did not use bedding for the calves. Only on eight of the remaining farms was the bedding changed more frequently than once a month. All of the farrowing crates were pressure-washed between farrowings. This washing occurred at least once a month on 15 of the 20 farms. The difference in levels of biosecurity on the farms, between low levels on dairy and higher levels on swine farms, is thought to be representative of these farm populations, based on studies by the USDA

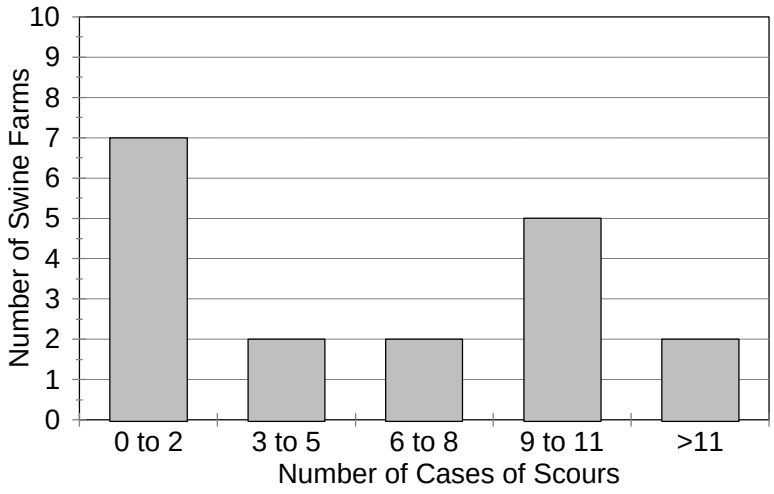

Figure 6 Number of reported cases of scours among piglets on the sow during the month prior to the start of this study

(USDA:APHIS:VS 1995; USDA:APHIS:VS 1996). However, there are no population-based studies on biosecurity in Ontario from which to make a valid comparison.

Overall, these results suggest that these groups of sample farms were representative of the population of herds of these commodities in Ontario. However, demographic data on a random sample or census of farms stratified by the type of manure storage does not currently exist, making accurate comparisons to the population of farms in Ontario difficult.

5.2.2 IFA Test Results: While the EIA and PCR test procedures were conducted, for the purposes of data analysis the IFA results will be used. This is mainly because IFA has been the most commonly used test in other similar studies. Experiences with EIA and PCR are experimental and will be discussed later.

The IFA test yielded a result of either positive (*Cryptosporidium* present) or negative (no *Cryptosporidium* detected). The IFA test did not distinguish the species *C. parvum*, but for the manure samples, we felt that very few, if any, of the other species of *Cryptosporidium* should be present. Also, the test was not able to distinguish between living (viable) and dead (structure intact but no longer viable) oocysts. While a precise enumeration was not performed, the lab staff were able to estimate concentrations of oocysts - the designations were as follows:

0	- negative - no oocysts detected
Few	- approx. 200 to 1000 oocysts per gram of manure
+	- 1001 to 5000 oocysts/g
++	- 5001 to 20000 oocysts/g
+++	- >20000 oocysts/g
++++	- dense mass

Of the total of 552 manure samples tested using the IFA method, 71 tested positive (12.9%). The breakdown by estimated concentration was:

Few	33
+	17
++	9
+++ or ++++	12

The breakdown by farm type is shown in Figure 7. This represents the percentage of each farm type that tested positive at least once during the study. Each farm was visited 3 times and several samples were collected each time. The average total number of samples collected, by farm type, was: eight for swine, nine for dairy with liquid manure, and 11 for dairy with solid manure. Figure 7 shows us that of the eight samples (on average) submitted from each swine farm, at least one tested positive on 18 of the 20 farms.

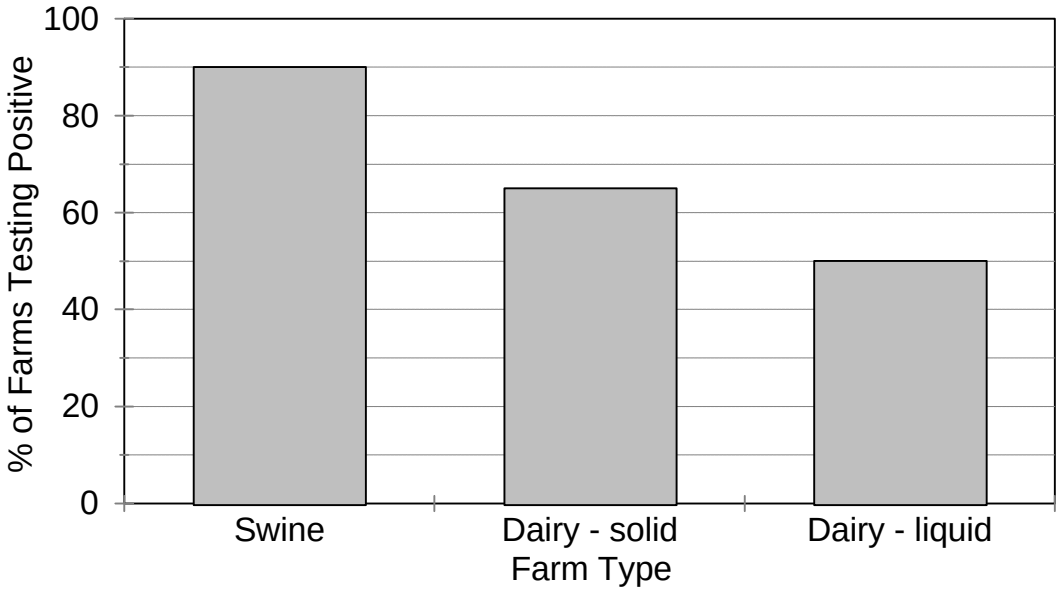

Figure 7 Percentage of farms testing positive at least once during the study

Figure 8 gives the number of test-positives by farm type, expressed as a percentage of the total number of samples taken for that farm type. This shows that 26.5% of all swine

samples tested positive, 8.1% of all dairy farms with solid manure, and 7.3% of all dairy-liquid systems.

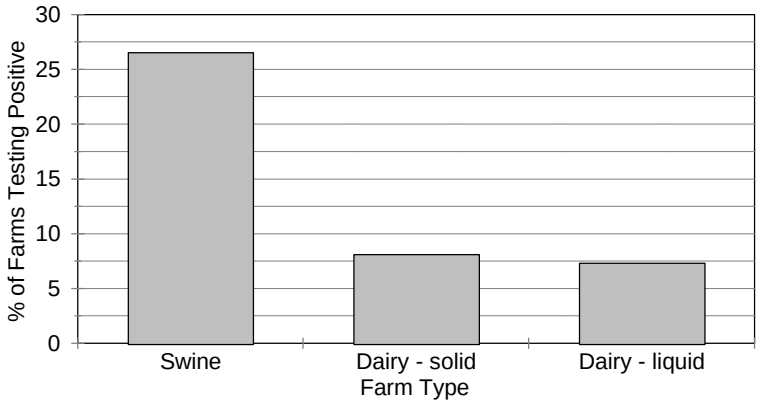

Figure 8 Percentage of total numbers of samples, by farm type, testing positive for *Cryptosporidium*

One of the main reasons for carrying out this study was to examine the difference in levels of oocysts between "fresh" and "stored" manure. Figure 9 shows where, by farm type, the concentrations of oocysts were found. Over the course of the study (3 visits), oocysts were found on 50 to 55% of the farms in the samples of fresh manure from the calves or young pigs. However, no oocysts were found in any of the dairy liquid manure storage samples. Oocysts were found in the samples from only a small number (20%) of storages on farms with solid manure systems. These were mostly in the actual solid manure pile - only one of the runoff storage samples tested positive. In contrast, 75% of liquid swine manure storages

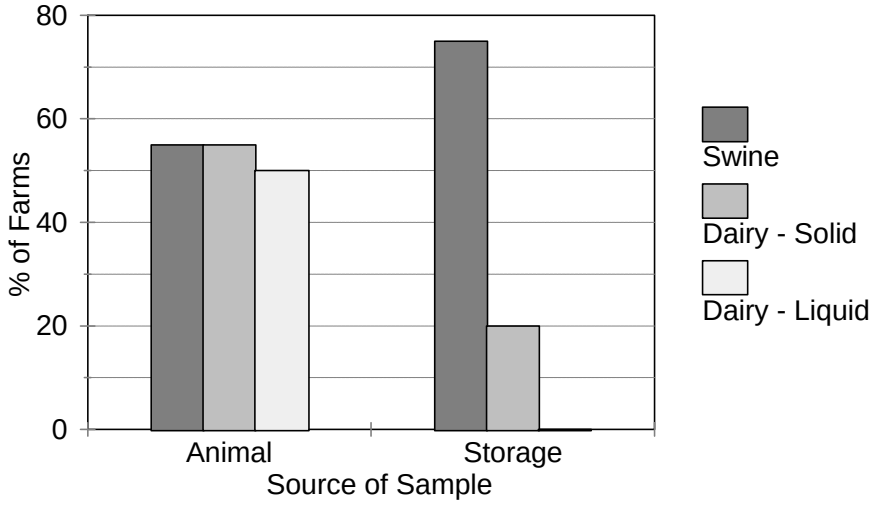

Figure 9 Percentage of farms, by source of sample, testing positive at least once during the study

tested positive for oocysts at least once in the three visits.

Part of the reason for no detections in the liquid storages on the dairy farms may have been due to the manner of handling the calf manure. Typically, the calves were housed in hutches and their manure was either stored separately or spread onto the land at clean-out. If the main source of oocysts was the calves, keeping their manure separate would prevent contamination of the main storage tank. This explanation, however does not take into account the fact that occasionally, the manure from older calves (manure enters the large tank) also tested positive for oocysts. Also, it is not clear whether the problems caused by the freezing of many of the liquid storages (i.e. manure samples were more fresh than if they had all come from the long-term storage) caused any difference in oocyst levels in the storage samples (increase or decrease).

The period of the study and the sampling method used were biased towards a "best case scenario". We feel that the levels and comparisons of levels estimated were likely on the conservative side of the true population levels.

No attempt was made in this study to measure the viability of oocysts. It is possible, as others have suggested, that even where oocysts were detected in the manure storages, the percentage of viable organisms may be low. However, even low levels **may** represent a significant level of infectious dose.

From a detailed statistical analysis of the data, the following emerged:

a) Swine farms were more likely than dairy to be classed as test-positive when all test results were pooled together ($p=0.04$). This association (odds ratio=3.3; 95% CL= 1.1-10.6) was strengthened when only liquid dairy and swine manure storages were compared (odds ratio=4.5; 95% CL= 1.2-17).

b) Swine farms had significantly more samples test-positive than dairy farms over all visits ($p<0.0001$). This effect was mainly due to the number of positive manure storage samples on swine farms ($p=0.01$). The relative risk of test-positive swine manure storages was 3.5 times that of dairy.

c) There was no significant association between the farm types and the test results of the livestock fecal samples ($p>0.05$).

d) Within swine farms there was a significant association between the test results of the livestock fecal samples and the manure storage samples ($p<0.03$). On dairy farms there was no association between the manure storage and livestock fecal samples ($p=0.58$).

e) Positive manure storage test results tended to cluster within swine farms significantly more than dairy farms (the intraclass correlation coefficients were 0.44 and 0.24, respectively).

f) There were no significant differences of test results among visits ($p=0.38$). This was relatively consistent among the farm types and sample source types.

g) There were a few herd factors that were associated with a farm having a positive test status - not disinfecting footwear, using a stacker style of manure handling,

infrequent removal of manure, infrequent addition of bedding and routine medication of piglets ($p<0.10$).

The resources available for this study did not permit the selection of a large random sample of herds needed for a precise estimate of the prevalence of *Cryptosporidium sp.* in livestock and manure storages. The focus of this study was to estimate the relative importance of this organism in manure storages and to examine the association with the test-status of the livestock on the farm. This highlights the importance of our research finding of significantly more positive swine herd samples and positive swine herds than dairy herds.

The clustering of livestock sample results with similar manure storage test results establishes a link between the young animals testing positive and contaminating the manure storages in swine. There was not a similar connection on the dairy farms studied in this project. The mixing of manure in liquid storages on swine farms, from all swine on the premises, may be a significant risk factor for ongoing contamination of the manure storage on farms with infected pigs.

The clustering of test-positive results within farms, especially on swine farms, is evidence for herd factors that explain the persistence of the infection on some farms and not others. If the disease was simply randomly distributed among farms, preventive strategies may be futile. However, our results indicate that further study may uncover critical control points that will reduce the risk of this pathogen contaminating manure storages and possibly the environment.

This study uncovered some herd factors that may lead to control measures or at least be proxies for other control points. Proper disinfection of footwear may highlight the importance of adequate farm biosecurity. Infrequent removal of manure and infrequent addition of fresh bedding may be related to the infection pressure in the farm environment for young calves and piglets. Routine medication of piglets may indicate farms that have poor colostral immunity, or be a proxy for farms with a recurrent history of scour problems.

There was no strong evidence for a seasonal pattern of the test results in these herds. The sampling was conducted through fluctuating but mostly cold temperatures that may have lowered the number of organisms in the farm environment.

5.3 Water Quality

5.3.1 Drain Sites: Table 1 gives a profile of the drain sites selected for the study. The average size of drainage basin was 176 acres (72 ha), and usually more than one landowner was represented. Many subsurface drainage systems in Ontario contain surface inlets. These could be catch-basins or inspection ports where only a minimum of surface water generally enters the system. However, they could include actual inlets designed to divert surface water through subsurface drain systems, usually in order to minimize soil erosion. The numbers of surface inlets reported in Table 1 are estimates based on what could be observed

from the roadway. These numbers are important in that they represent a pathway for surface water to enter the subsurface drainage system. No attempt was made to determine the presence of surface inlets near the building sites. Also, no attempt was made to determine if any of the household septic systems (which would be the typical system to handle domestic wastewater) were illegally connected to the subsurface drainage systems.

Table 1 Summary information for 20 tile drain sites

	Average	Range (Min. - Max.)
Size (acres)	176	100 - 300
Outlet pipe diam. (inches)	16.9	8 - 44
Water Temperature (^{o}C)	3.3	1.0 - 7.0
Tile flow rate (L/s)	11.7	0.3 - 20.0
Number of houses in watershed	2.7	0 - 13
Number of barns (livestock watersheds)	2.6	1 - 5
Estimated % of land receiving manure (livestock watersheds)	48	25 - 75
Number of surface inlets in system	3.5	0 - 11

A total of 60 water samples were collected and analyzed. The samples from the open ditch were, in most cases, collected at the second visit to each site. The second visit corresponded to spring snowmelt conditions. It has been under these spring runoff conditions that the reported "outbreaks" have typically occurred.

5.3.2 IFA Test Results: Of the 60 samples, nine tested positive for *Cryptosporidium*, using the IFA test. Two of these were from surface water sources and two were from tiles draining non-livestock watersheds. The remaining five were from tiles draining livestock watersheds. One of the latter group was a repeat - i.e. the sample tested positive for each of the two samples. In total then, *Cryptosporidium* was detected at two of the 20 surface water sites, four of the 10 tiles draining livestock watersheds and two of the 10 tiles draining watersheds where no livestock were present. The average recovery rate for the test procedure was calculated to be 54.4%.

During the water testing, oocysts were counted and the oocyst concentration was calculated. Concentrations ranged from a low of 7.7 oocysts/L to a high of 333 oocysts/L. The concentrations are in the range reported by others (e.g., Ongerth and Stibbs 1987; Hansen and Ongerth 1991). However, the prevalence in the surface water was lower than that found

by others (e.g., Ongerth and Stibbs 1987). While it is possible that livestock manure contributed to the positive readings in the livestock watershed, it is unclear what caused the positives in the watershed where no livestock were present.

This part of the study was intended to get a feel for the potential of tile drain water to be contaminated. The number of samples tested from tile drains was very low and the resultant statistical power of this part of the study was low. We needed to see a marked difference in the proportion of positive samples between the livestock and no-livestock areas to identify a significant result. The trend towards higher levels of positive water samples from livestock dense areas may or may not be a statistical aberration. The perception of this trend merits further study. There was no significant difference between the proportion of test-positive results in tile drain outlets from livestock versus no-livestock areas ($p=0.17$).

5.3.3 Lab Procedures

Initially, the acid-fast stain technique was to be used as a simple screening method. Unfortunately, it soon became apparent that the manure contained a large amount of acid-fast artifacts (such as yeasts, fungal spores, and pollen grains), making it technically challenging to use a straight acid-fast stain.

The EIA test did not prove to be satisfactory for the analysis of manure samples. For the first two runs of 90 samples each, the positive rate was extremely high. All controls were as expected. More vigorous washing improved the correlation with the results of the IFA test somewhat, but not to an acceptable level. The problems appeared to be related to the large amounts of mucous and viscous substances that were difficult to remove from the microwells of the test kit. Another possible explanation is that the antibodies used in the EIA kit are not directed exclusively at the antigens of the oocyst wall, but detect antigens produced during the asexual stage of the *Cryptosporidium* life cycle. This would help explain the higher incidence of positives.

The polymerase chain reaction (PCR) was used for two reasons:

a) as a test to confirm the presence of the species *C. parvum* in samples that had previously been identified as positive for *Cryptosporidium* using the Meridian immunofluorescence assay (IFA); and

b) to verify false negatives identified using the IFA method - reflecting a concern that we were operating at or near the detection limit of the IFA method.

Fourteen DNA isolation procedures were screened for their applicability in the detection of *C. parvum* in fecal and environmental water samples. One procedure was chosen that allowed the detection of approximately 10^3 oocysts/gm of feces. Magnetic capture of the oocysts prior to DNA extraction was required when processing environmental water samples; the detection level was 10^3 oocysts/mL of water pellet.

A total of 103 animal fecal samples were tested using PCR. Excellent correlation was observed between the PCR and IFA data when oocysts numbers were large (>1000 oocysts/ 10 µL) but correlation decreased as the oocyst number decreased. In total, 44% of the samples that were presumptive-positive by IFA were positive by PCR. All thirty-two fecal samples testing negative by IFA (chosen randomly) were negative using the PCR assay. The detection level was approximately 10^3 oocysts/gm of feces, making the PCR method an inappropriate choice for finding false negatives.

A total of 60 environmental water samples were tested by PCR. Forty-four percent of samples that were presumptive-positive by IFA were confirmed positive using PCR amplification. No correlation between the EIA presumptive-positive data and the PCR data was observed.

Further optimization of the PCR technology is required to improve the detection limit and accuracy of the assay. The use of magnetic capture of the oocysts should be investigated further as it allows the concentration of the sample at least 10-fold.

6.0 Conclusions

1. For the 60 farms in the study (20 of each type) and over livestock and manure storage samples, 90% of the swine farms, 65% of the dairy farms with solid manure systems, and 50% of dairy farms with liquid manure tested positive for *Cryptosporidium* at least once during the study.

2. In total, 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. Swine farms had significantly more samples test-positive than dairy farms over all visits ($p<0.0001$). There are marked differences between swine and dairy farms that could influence the levels of *Cryptosporidium* on the farms.

3. For each of the three farm types, 50 to 55% of the farms tested positive for *Cryptosporidium* at least once for the fresh manure samples (i.e. from young pigs or calves). In contrast, 75% of the swine farms tested positive at least once for a storage sample; 20% for dairy farms with solid manure storages (plus runoff tanks); 0% for liquid dairy manure storages. This represents a marked difference between levels of *Cryptosporidium* in swine versus dairy farm manure storages.

4. Positive manure storage test results tended to cluster within swine farms significantly more than dairy farms (the intraclass correlation coefficients were 0.44 and 0.24, respectively). There are herd factors that will likely influence levels of *Cryptosporidium* on the farm.

5. Cryptosporidium oocysts were detected in tile drainage water samples from four of the 10 watersheds where livestock were present (i.e. livestock manure was spread on the land). Oocysts were also detected at two of the 10 sites where no livestock were present. The source of this latter contamination is not obvious. The numbers of samples were too low to establish the significance of these numbers.

7.0 Proposed Future *Cryptosporidium* Research

Following are future research needs identified during the course of this study:

< The spreading of formalin-killed *Cryptosporidium*- infected liquid manure over tile drains, to measure the amount of *Cryptosporidium* in the tile drainage water. This would provide some insight into the transmission of the organism through the soil.

< The spreading of liquid manure or solid manure over a frozen area of land would aid in understanding the impact of such a practice, and specifically the effect of low temperatures on Cryptosporidium viability.

< The spreading of manure under conditions that allow for a study of the effects of desiccation and UV light exposure on the viability of oocysts.

< A groundwater study , to determine if oocysts are capable of migrating through the soil and entering the groundwater. Shallow piezometers placed near the top of the water table would allow surveillance of the oocyst migration through the soil. Application of a mixture of formalin-killed *Cryptosporidium* oocysts and liquid manure, along with a bromide tracer would then be followed by periodic sampling of the water, until the bromide tracer was detected.

< A continuous surveillance of a few tile outlets, in non-livestock and livestock watersheds, for the presence of *Cryptosporidium* oocysts would be helpful in obtaining a larger data set for the water study. This project would have a one to two year time line.

< A study of the viability of oocysts in manure storages, especially on swine farms, to determine if the oocysts that may be spread on the land pose any real environmental danger.

< A comprehensive study of sources of oocysts to help put into perspective the relative contributions to surface water of livestock agriculture, faulty septic systems, municipal sewage treatment plants (treated and untreated discharge), land-applied sewage sludge, wildlife, and possible other sources. Related to this is a validation of heterogeneity and sub-grouping work with *C. parvum* to examine human versus livestock origins of oocysts in surface water and manure storages.

< A pamphlet to generate public awareness of *Cryptosporidium*, distributed on farms in Ontario, would be a first step in the awareness and prevention motto of this project group.

8.0 Acknowledgments

The authors would like to acknowledge the contribution of the 60 farmers involved in this study. They willingly participated in the hopes of helping to advance the knowledge base in the area of *Cryptosporidium*, an organism of which many had no prior knowledge. Funding for this project was provided by the Environmental Farm Plan (Canada-Ontario Green Plan Accord), through a program administered by the Ontario Farm Environmental Coalition and the Ontario Federation of Agriculture. EIA test kits were provided for evaluation purposes (at no cost) by IVD Research, Inc.

9.0 Glossary

Antibody: A glycoprotein produced in response to the introduction of an antigen into the system. The antibody complexes with the antigen.

Antigen: A foreign substance, such as a protein, which induces the immune system. The protozoa secretes these antigens, which then activate the host's immune system.

Antigen-Antibody reaction: For detection purposes, an antibody specific to Cryptosporidium oocyst antigens, is added to an unknown sample, and the formation of an antibody-antigen complex indicates the presence of Cryptosporidium.

Association: Variables which are highly correlated. Additional tests must be applied to establish causation.

Asymptomatic: No symptoms of infection or disease are evident.

Composite sample: A mixture of a number of grab samples, to form a heterogeneous sample, representative of the original sampled material (ie: manure reservoir).

Concentration: A measure of the number of oocysts in a sample, usually in oocysts/gram of feces.

Cryptosporidiosis: The disease state from a *Cryptosporidium spp*. infection.

Cryptosporidium parvum: The genus and species names of the infective organism. This species is the only one known to infect humans.

Desiccation: The removal of moisture from an object. One of the environmental factors which *Cryptosporidium* oocysts are susceptible to.

Detection limit: The ability of the test method to determine an oocyst concentration. The larger the detection limit, the less accurate the test is.

(EIA) Enzyme Immunoassay: A test which detects the fecal *Cryptosporidium* antigen using special antibodies and other compounds. A change in colour indicates a positive test.

False negative: A false negative (negative outcome for a sample which has oocysts present) in this field is common in all detection methods, and usually is caused by low oocyst concentrations, which the tests are unable to detect.

False positive: A false positive (a positive outcome for a sample which is oocyst-free) in this field is common in both the IFA and EIA methods. Algal fluorescing interference is common in water samples tested with the IFA method. Free antigens not related to the oocyst shell can interfere in the fecal samples tested with the EIA method and also result in mis-diagnosis.

Fecal-Oral pathway: The infection pathway of *Cryptosporidium*, referring to the transmission of the oocyst from the feces and ingested either directly or through food or water.

Gastroenteritis: Illness related to the gastrointestinal portion of the digestive system, with symptoms including nausea, vomiting, and diarrhea.

Grab sample: A single sample taken at one instance.

Immunocompromised: The lack of a properly functioning immune system, leading to a higher susceptibility to infection, and a slower and weaker response to invasion. This state is common among patients undergoing chemotherapy and organ transplants, HIV positive patients, and very young or elderly people.

(IFA) Immunofluorescence: A technique used to identify particular antigens microscopically in a sample, by the binding of a fluorescent antibody conjugate, which forms an antibody-antigen complex.

Independent data: Each observation is not systematically correlated to other observations.

Infectivity: The capability of an organism of infecting a host with a disease.

Inhibition: The hindering of detection of oocysts by other materials present in the sample, masking or preventing accurate diagnosis.

Masking: Disguising or concealing the presence of the oocysts, hindering detection.

Monoclonal antibody: An antibody of a single type that is produced by a population of genetically identical plasma cells.

Occurrence: The presence of the protozoa in the test material.

Oocyst: A cyst formed around a protozoa, utilized like a protective covering from harsh environmental factors. The infective stage in the life cycle of *Cryptosporidium spp.* .

Pathogen: A parasite with the ability to cause disease.

Parasite: An organism that lives on or within a host (another organism) and benefits from the association with the host, while harming the host.

(PCR) Polymerase chain reaction: A technique which is used to make a large quantity of a specific DNA sequence, from small amounts of DNA. This large quantity of DNA is then bound to a known fragment, representative of the genetic sequence of Cryptosporidium parvum. If the two fragments bind, a fluorescing band is produced on an electrophoresis gel, and results in a positive sample for the presence of C. parvum.

Precision: The level of variability of test results when repeatedly examining an identical sample.

Prevalence: The proportion of positive samples among those at risk at one period in time.

Protozoa: Microorganisms classified in the Protozoa subkingdom. A unicellular eukaryotic protist.

P-value: A proportion that indicates the chance of error in the result of a statistical test.

Random sample: A sample taken in a formal manner to minimize unknown biases.

Recovery rate: Refers to the ability of the detection methods to retrieve the oocysts from test material with a known level of contamination. Larger recovery rates are desired for more accurate detection methods.

Repeatability: A measure that indicates how well the test performs on a series of identical samples.

Sample heterogeneity: Manure samples were diverse in content, varied throughout by consistent sub-sampling from different areas of the reservoirs.

Scours: A common term for diarrhea in livestock.

Self-limiting: Refers to the disease process, and the ability of the patient to overcome the infection without chemical intervention.

Sensitivity: The ability of the test to detect the presence of oocysts in a population of positive field samples. The greater the sensitivity of the method, the more accurate the results.

Shedding: The release of oocysts from the intestinal wall of the host organism, into the deposited fecal material. This fecal material is then considered infectious.

Sporozoite: A motile, infective stage of a protozoan life cycle.

t-test: A statistical test for comparing samples of continuous data.

UV light: Light with a wavelength just beyond the visible spectrum. Cryptosporidium oocysts are susceptible to UV light, and remain non-viable through exposure to UV light for a short period of time.

Viability: The capability of an organism of living and causing disease.

Virulence: The degree of pathogenicity of an organism, indicated by fatality rates or ability to invade new hosts and cause disease.

Watershed: A line of separation between waters flowing to different source waters.

10.0 References

Anon. 1994. First progress report of the New York City watershed agricultural program. New York City Watershed Agricultural Council, Walton, NY

Barta, J. 1997. Personal communication - September, 1997. Pathobiology Department, University of Guelph, Guelph, Ontario

Bridgeman, S. A., Robertson, R. M. P., Syed, Q., Speed, N., Andrews, N., Hunter, P. R. 1995. Outbreak of cryptosporidiosis associated with a disinfected groundwater supply. Epidemiology and Infection. 115; pp.555-566

Butler, B. J., Mayfield, C. I. 1996. *Cryptosporidium spp*. - A review of the organism, the disease, and implications for managing water resources. Report prepared for Waterloo Centre for Groundwater Research, Waterloo, Ontario

Carraway, M., Tzipori, S., and Widmer, G. 1997. A new restriction fragment length polymorphism from *Cryptosporidium parvum* identifies genetically heterogeneous parasite populations and genotypic changes following transmission from bovine to human hosts. Infect Immun. Sep; 65(9):3958-3960

Carraway, M., Tzipori, S., and Widmer, G. 1996. Identification of genetic heterogeneity in the *Cryptosporidium parvum* ribosomal repeat. Appl Environ Microbiol. Feb; 62(2):712-716

D'Antonio, R. G., Winn, R. E., Taylor, J. P., Gustafson, T. L., Current, W. L., Rhodes, M. M., Gary, W. Jr., Zajac, R. A., Lackland Air Force Base. 1985. A waterborne outbreak of cryptosporidiosis in normal hosts. Annuals of Internal Medicine, 103(6 pt 1):p. 886-888.

Donald, A. 1988. Statistical analysis of binary data from matched designs that require multiple observations on the same herd, litter or animal. Acta Vet Scan 1988, supplement 84.

Edwards, D. D. 1993. Troubled waters in Milwaukee. American Society of Microbiology News. Vol 59, No. 7, pp. 342-345

Garber, L. P., Salman, M. D., Hurd, H. S., Keefe, T., Schlater, J. L. 1994. Potential risk factors for *Cryptosporidium* infection in dairy calves. JAVMA., 205(1):p.86-91

Garber, L. 1993. *Cryptosporidium parvum* literature review. Animal Health Insight, Fall 1993. p. 3-11.

Goatcher, L. J., Poon, P., Gammie, L. 1996. Monitoring for *Giardia* and *Cryptosporidium* in the North Saskatchewan River at water treatment plants in Edmonton, Alberta.

Paper presented at the 7th National Conference on Drinking water, Charlottetown, P.E.I., August, 1996

Hansen, J. S., and Ongerth, J. E. 1991. Effects of time and watershed characteristics on the concentration of *Cryptosporidium* oocysts in river water. Applied and Environmental Microbiology, 57(10):p. 2790-2795.

Hayes, E. B., Matte, T. D., O'Brien, T. R., McKinley, T. W., Logsdon, G. S., Rose, J. B., Ungar, B. L. P., Word, D. M., Pinskey, P. F., Cummings, M. L., Wilson, M. A., Long, E. G., Hurwitz, E. S., Juranek, D. D. 1989. Large community outbreak of cryptosporidiosis due to contamination of a filtered public water supply. New England Journal of Medicine, 320(21):p. 1372-1376.

Jakubowski, W., Boutros , S., Faber, W., Fayer, R., Ghiorse, W., Lechevallier, M., Rose, J., Schaub, S., Singh, A., and Stewart, M. 1996. Environmental methods for *Cryptosporidium*. Journal of American Water Works Association, 88(9):p.107-121

Juranek, D. D. 1995. Cryptosporidiosis: sources of infection and guidelines for prevention. Clinical Infectious Diseases, 21:p. S57-61.

Kehl, K. S. C., Cicirello, H., Havens, P. L. 1995. Comparison of four different methods for detection of *Cryptosporidium* species. Journal of Clinical Microbiology. 33(2);pp.416-418

Kemp, J. S., Wright, S. E., Coop, R. L., Mawdsley, J. L., Merry, R. J., Pain, B. F., Theodorou, M. K., Read, L. A., Svoboda, I. F., Bukhari, Z., and Smith, H. V. 1995. Protozoan, bacterial and viral pathogens, farm wastes and water quality protection - final report.

LeChevallier, M. W., Norton, W. D., Lee, R. G. 1991. *Giardia* and *Cryptosporidium spp*. in filtered drinking water supplies. Applied and Environmental Microbiology, 57(9):p. 2617- 2621.

LeChevallier, M. W., and Norton, W. D. 1995. *Giardia* and *Cryptosporidium* in raw and finished water. American Water Works Association, :p. 54-68.

LeChevallier, M. W., Norton, D. W., Siegel, J. E., Abbaszadegan, M. 1995. Evaluation of the immunofluorescence procedure for detection of *Giardia* cysts and *Cryptosporidium* oocysts in water. Applied and Environmental Microbiology, 61(2).p.690-697

Leland, D., McAnulty, J., Keene, W., Stevens, G. 1993. A cryptosporidiosis outbreak in a filtered-water supply. Journal of American Water Works Association, 85(6):p. 34-42.

Leng, X., Mosier, D.A., Oberst, R.D. 1996. Simplified method for recovery and PCR detection of *Cryptosporidium* DNA from bovine feces. Applied and Environmental Microbiology, 62(2):p.643-647

MacKenzie, W. R., Hoxie, N. J., Proctor, M. E., Gradus, M. S., Blair, K. A., Peterson, D. E., Kazmierczak, J. J., Addiss, D. G., Fox, K. R., Rose, J. B., Davis, J. P. 1994. A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. New England Journal of Medicine, 331:p. 161-167.

MacPherson, D.W., McQueen, R. 1993. Cryptosporidiosis: Multi-attribute evaluation of six diagnostic methods. Journal of Clinical Microbiology, 31(2).p.198-202

Madore, M. S., Rose, J. B., Gerba, C. P., Arrowood, M. J., Sterling, C. R. 1987. Occurrence of *Cryptosporidium* oocysts in sewage effluents and selected surface waters. Journal of Parasitology, 73(4):p. 702-705.

Mann, E. D., Selka, L. H., Nayar, G. P. S., and Koschik, C. 1986. Infection with *Cryptosporidium spp*. in humans and cattle in Manitoba. Canadian Journal of Veterinary Res, 50:p. 174-178.

Mawdsley, J. L., Brooks, A. E., Merry, R. J. 1996. Movement of the protozoan pathogen *Cryptosporidium parvum* through three contrasting soil types. Biol Fertil Soils, 21:p. 30-36.

McDermott, J. J, Schukken, T. H., Shoukri, M. M. 1994. Study design and analytic methods for data collected from clusters of animals. Prev Vet Med, 1994; 18: 175-191.

Murray, P.R., Kobayashi, G.S., Pfaller, M.A., Rosenthal, K.S. 1994. Medical Microbiology - Second Edition Mosby-Year Book, Inc., p.546

Myers, L. L., Firehammer, B. D., Border, M. M., and Shoop, D. S. 1984. Prevalence of enteric pathogens in the feces of healthy beef calves. American Journal of Veterinary Resources, 45(8):p. 1544-1548.

Olson, M. E., Thorlakson, C. L., Deselliers, L., Morck, D. W., McAllister, T. A. 1996?. *Giardia* and *Cryptosporidium* in Canadian farm animals. research report - University of Calgary, Alberta

OMAFRA. 1995. Ontario Farm Management Analysis Project. Ontario Ministry of Agriculture Food and Rural Affairs.

Ongerth, J. E., Hunter, G. D., DeWalle, F. B. 1995. Watershed use and giardia cyst presence. Watershed Resources, 29(5):p. 1295- 1299.

Ongerth, J. E., and Stibbs, H. H. 1987. Identification of cryptosporidium oocysts in river water. Applied and Environmental Microbiology, 53(4):p. 672-676.

Ongerth, J. E., and Stibbs, H. H. 1989. Prevalence of cryptosporidium infection in dairy calves in western Washington. American Journal of Veterinary Resources, 50(7):p. 1069-1070.

Palmateer, G. 1997. New method to recover *Cryptosporidium* and *Giardia* from drinking water. Environmental Science and Engineering, p.88

Pett, W.B. 1997. Personal communication - June, 1997. Engineering Dept., Water Services, Regional Municipality of Waterloo, Kitchener, Ontario.

Quigley, J. D., Martin, K. R., Bemis, D. A., Potgieter, L. N. D., Reinemeyer, C. R., Rohrbach, B. W., Dowlen, H. H., Lamar, K. C. 1994. Effects of housing and colostrum feeding on the prevalence of selected infectious organisms in feces of jersey calves. Journal of Dairy Science, 77:p. 3124-3131.

Roach, P. D., Olson, M. E., Whitley, G., Wallis, P. M. 1993. Waterborne *Giardia* cysts and *Cryptosporidium* oocysts in the Yukon, Canada. Applied and Environmental Microbiology, 59(1):p. 67-73.

Robertson, L. J., Campbell, A. T., and Smith, H. V. 1992. Survival of *Cryptosporidium parvum* oocysts under various environmental pressures. Applied and Environmental Microbiology, 58(11):p. 3494-3500.

Rose, J. B., Darbin, H., Gerba, C. P. 1988. Correlations of the protozoa, *Cryptosporidium* and *giardia*, with water quality variables in a watershed. Water Science Technology, 20(11/12):p. 271-276.

Tacal, J. V. Jr., Sobieh, M., El-Ahraf, A. 1987. *Cryptosporidium* in market pigs in southern California, USA. Veterinary Record, 120:p. 915-917.

Todd, S. C., Phillips, M. S., Marchin, G. L., Upton, S. J. 1991. *Cryptosporidium* and *giardia* in surface waters in and around Manhattan, Kansas. Transactions of the Kansas Academy of Science, 94(3-4):p. 101-106.

USDA:APHIS:VS 1995. Part I: Reference of 1995 swine management practices. Centre for Epidemiology and Animal Health, Fort Collins, Colorado.

USDA:APHIS:VS 1996. Part III: Reference of 1996 dairy health and health management practices. Centre for Epidemiology and Animal Health, Fort Collins, Colorado.

Villacorta-Martinez de Maturana, I., Ares-Mazas, M. E., Duran- Oreiro, D., and Lorenzo-Lorenzo, M. J. 1992. Efficacy of activated sludge in removing

Cryptosporidium parvum oocysts from sewage. Applied and Environmental Microbiology, 58(11):p. 3514- 3516.

Wallis, P. M., Isaac-Renton, J. L., Olson, M. E., Robertson, W. J., Van Keulen, H. 1996. Prevalence of *giardia* cysts and *Cryptosporidium* oocysts and characterization of *giardia spp*. isolated from drinking water in Canada. Applied and Environmental Microbiology, 62(8):p. 2789-2797.

Xiao, L., Herd, R. P., and Bowman, G. L. 1994. Prevalence of *Cryptosporidium* and *giardia* infections on two Ohio pig farms with different management systems. Veterinary Parasitology, 52:p. 331- 336.

Appendix

Farm Survey Sheet

Field Study of Cryptosporidium and Manure Storage 1996-97

Name of Farmer: _____

Mailing Address: _____

_____

_____

Location: **(eg. lot, concession, township)**

_____

_____

Phone: **(519)**_____

Fax: **(519)**_____

Farm number assigned for this study: _____

Questionnaire

These questions will help a field study of Cryptosporidium sp. and manure storages on dairy and swine farms in Ontario. Cryptosporidiosis is an intestinal infection in animals and humans associated with outbreaks of diarrhea. Localized epidemics have occurred in communities in Ontario in the past five years.

The questions pertain to the farm operation and farm personnel/family at the location where the manure samples will be taken. All answers will be held in strict confidence.

Note: Y=yes, N=no, DK=don't know, NA=question doesn't apply

ref. 961202

1. General farm information

1-1 Farm manure storage system: liquid-swine **G** liquid-dairy **G** solid-dairy **G**

1-2 Number of animals - Include a note (under Additions) if any livestock were brought on to the farm from another source/location in the past year (do not include natural increases) .

Livestock-type	**Number**	**Additions**
Milking cows	_____	**G**
Dry cows	_____	**G**
Dairy Heifers (over 3 months)	_____	**G**
Dairy calves (up to 3 months)	_____	**G**
Sows	_____	**G**
Piglets (on the sow)	_____	**G**
Weaner pigs	_____	**G**
Grow-finisher pigs	_____	**G**
other livestock, specify_____	_____	**G**

2. Dairy Farms:

2-1 In the past month, indicate the number of cases of scours in calves (up to 3 months of age)
_____ DK **G** NA **G**

2-2 In the past month, indicate the number that died - calves (up to 3 months of age)
_____ DK **G** NA **G**

2-3 Are you on Dairy Herd Improvement (DHI)?
Y **G** N **G** DK **G** NA **G**

2-4 What was the bulk tank somatic cell count (SCC) for the past three months?

_____, _____, _____ DK **G** NA **G**

2-5 Housing for the milking cows:

tie stall **G**
free stall **G**
combination **G**
other, specify _____.

2-6 How many maternity pens do you have for calvings? _____ DK **G** NA **G**

2-7 Type of housing for calves (under 3 months):

housed among cows **G**
calf pens in the main cow barn **G**
separate calf barn **G**
hutches **G**

other _____

2-8 Type of bedding used in the area where baby calves are housed:

straw **G**
shavings **G**
sawdust **G**
sand **G**
concrete **G**

other, specify _____.

2-9 In the area where baby calves are housed, how often (number per month):

is used bedding removed? _____ DK **G** NA **G**
is the area pressure washed? _____ DK **G** NA **G**
is the area disinfected? _____ DK **G** NA **G**
is fresh bedding added? _____ DK **G** NA **G**

2-10 Estimate the amount (litres) of colostrum that calves are routinely fed in the first 12 hours of life.
_____ DK **G** NA **G**

2-11 Is the manure/bedding from clinically ill (diarrhea) animals kept separate from the rest of the herd manure/bedding?
Y **G** N **G** DK **G** NA **G**

2-12 Do you routinely medicate (for prevention of disease) baby calves with antibacterials?
Y **G** N **G** DK **G** NA **G**

2-13 At what age do you routinely wean (days) dairy calves?_____ DK **G** NA **G**

2-14 Do you require visitors to the main barn to:
disinfect footwear? **G**
change footwear? **G**
change clothing? **G**
other, specify _____

2-15 Is there any disinfectant routinely applied to the floor area where the cows are housed (eg. lime)? Y **G** N **G** DK **G** NA **G**

3. Swine Farms

3-1 In the past month, indicate the number of cases of scours in piglets (still on the sow) .
_____ DK **G** NA **G**

3-2 In the past month, indicate the number that died - piglets (still on the sow).
_____ DK **G** NA **G**

3-3 Estimate the number of pigs/sow/year _____ DK **G** NA **G**

3-4 Estimate the number of hogs marketed in the past 12 months
_____ DK **G** NA **G**

3-5 Type of housing for farrowing sows and piglets:

crates **G**
box stalls **G**

other, specify _____.

3-6 Type of floor covering used in the area where baby piglets are housed:

straw **G**
concrete **G**
plastic or plastic-coated slats **G**
stainless steel slats **G**

other, specify _____.

3-7 In the area where baby piglets are housed, how often (number per month):

is used bedding removed? **G**
is the area pressure washed? **G**
is the area disinfected? **G**
is fresh bedding added? **G**

3-8 Is the manure/bedding from clinically ill (diarrhea) animals kept separate from the rest of the herd manure/bedding?

Y **G** N **G** DK **G** NA **G**

3-9 Do you routinely medicate (for prevention of disease) baby piglets with antibacterials?

Y **G** N **G** DK **G** NA **G**

3-10 At what age do you routinely wean (days) piglets? _____ DK **G** NA **G**

3-11 Do you require visitors to the main barn to:

disinfect footwear? **G**
change footwear? **G**
change clothing? **G**

other, specify_____

3-12 Is there any disinfectant routinely applied to the floor area where the sows are housed (eg. lime)? Y **G** N **G** DK **G** NA **G**

4. Manure Storage Details

Solid manure storage pad with runoff storage

4-1 Dimensions of the solid manure storage pad? _____

4-2 How is manure transferred from the barn to the storage?

stacker **G**
tractor and front end loader **G**

underground transfer system **G**

other, specify _____

4-3 Runoff storage type:

open circular tank **G**
earthen pit **G**
covered circular tank **G**

other, specify

4-4 Runoff storage dimensions _____

4-5 Other sources of liquid into runoff storage:

yard	**G**	area involved	_____(square feet)
roof	**G**	area involved	_____
milkhouse	**G**	daily input	_____

Other, specify _____

Liquid manure storage

4-6 Type of storage:

open circular tank **G**
rectangular covered storage (incl. under barn) **G**
earthen pit **G**
covered circular tank **G**

other, specify _____

4-7 Storage dimensions _____

5. General Health Questions

5-1 During the past 30 days did any farm personnel/family member have diarrhea that lasted for two or more days?

Y **G** N **G** DK **G** NA **G**

5-2 Has Cryptosporidium infection been diagnosed in farm personnel in the past 12 months?

Y **G** N **G** DK **G** NA **G**

5-3 Has Cryptosporidium infection been diagnosed in livestock on the farm in the past 12 months?

Y **G** N **G** DK **G** NA **G**

Factsheet on *Cryptosporidium* - DRAFT - September, 1997

What is Cryptosporidium parvum*?*

Cryptosporidium (krip-toe-spor-id-ee-um) *spp.* is a protozoan parasite that reproduces in vertebrates and causes disease in humans and agricultural livestock. This parasite was associated with large outbreaks of human illness in the US and Canada. It can be found within the epithelial cells of the digestive organs and respiratory tract. It was first identified early in the 20th century, and cryptosporidiosis (the disease) was first identified in 1976. It is a cause of gastroenteritis in people and can cause relatively large outbreaks of human illness. There are six recognized species of *Cryptosporidium* including *C. parvum* and *C. muris* (which infect mammals), *C. baileyi*, and *C. meleagridis* (which infect birds), and *C. nasorum* and *C. crotalis* (which infect fish and reptiles.)

The life cycle of these protozoa occurs within one host, and consists of several stages. The oocyst stage is the infectious stage, and is released from the intestine into the feces of the affected animal. Subsequent infections are transmitted by fecal-oral routes. Transmission to new hosts can be through person-to-person or animal-to-person contact, or by the ingestion of contaminated water or food. This parasite has a rapid life cycle, and can reproduce in the intestinal wall within twelve hours. The oocyst stage of the life cycle is approximately 4 µm in width, but is able to fold over and travel through smaller pore sizes. This can be a problem in water filtration systems.

What is the prevalence of Cryptosporidium parvum *in livestock?*

Many studies have been done on the prevalence of *C. parvum* among farm animals world wide, although most studies focus on dairy or beef cattle operations. Many of the prevalence values which are published refer to the occurrence of at least one positive sample on a farm. A study by the USDA examined 210 operations and indicated that 22% of pre-weaned dairy calves, and 50% of dairy calves in a 1 to 3 week age group tested positive for *C. parvum.* In Manitoba , Canada a study of beef farms showed that 22 % of beef calves among 148 herds tested positive for *C. parvum*. Some of the factors which may affect the presence of *C. parvum* on farms include the herd sizes (the larger herds showing significantly larger numbers of infected calves), disinfection of footwear upon entering the barn facilities, frequency of addition of bedding, and routine medication of piglets on swine farms, and the type of manure storage (open tanks compared with closed tanks).

What is the prevalence of Cryptosporidium parvum *infection among wildlife?*

There is little known about the prevalence of shedding among wildlife species with access to surface water. Oocysts from one mammal, however, appear to be infectious to other mammals. In a survey of 100 wild raccoons, 13 tested positive for *C. parvum*. Studies demonstrated *C. parvum* in captive deer and a 30% prevalence among wild mice trapped at a dairy. These oocysts were shown to be infectious to calves, indicating a possible mouse-calf cycle.

How well do Cryptosporidium *oocysts survive in the environment?*

Although the oocyst shell is thick and resistant to many chemicals such as chlorine, it is susceptible to drying, freezing, and ultraviolet light. Drying appears to kill oocysts in a matter of hours. With 10 or more days of freezing, one study showed that over 90 % of the oocysts were found to be non-infective. However, at temperatures as high as 30ºC, oocysts are able to survive for up to two weeks, indicating that this protozoan remains infectious during warm weather. A viability study found that 34-40 % of oocysts in fecal matter deposited directly into the water supply were no longer infectious after 33 days. After 176 days, 89-99% of the oocysts were estimated to be non-infectious.

What is the prevalence of Cryptosporidium parvum *in surface waters?*

A study of western United States surface waters indicated a 77% prevalence of *C. parvum* oocysts. One study found no significant variability in oocyst concentration between protected surface water and surface water open to agriculture run-off. And, 68% of oocysts in the agricultural runoff were non-infectious. *C. parvum* in pristine surface waters has been reported (at a concentration of 0.005-18 oocysts/L). This may indicate that *C. parvum* occurs naturally in the environment, perhaps through wildlife populations. The presence of *C. parvum* oocysts appears to increase following heavy rain, due to an increase in the amount of runoff from both wild and domestic animals. The increase in oocysts could also be attributed to overflow of storm sewers into sanitary sewers, resulting in direct discharge of untreated raw sewage.

What are the symptoms of a Cryptosporidium parvum *infection among cattle?*

The symptoms of *C. parvum* in calves can begin in calves as early as five days after birth, though they are most commonly seen in calves over two weeks old. The clinical symptoms of an acute infection include watery, yellow diarrhea (sometimes containing flecks of blood), mild fever, dehydration, and sometimes lethargy. The clinical symptoms of a chronic infection include semi-formed stools, little to no fever, and weight loss. Severe infections are associated with younger animals, inadequate colostrum intake, larger herd size, and poor sanitation. The duration of infection can persist for months in a herd. The duration of the clinical infection is related to the strength of the animal's immunity. Most calves become infected under six weeks of age, and approximately 25% of calves with diarrhea between five days to one month old are infected with *C. parvum.*

What are the symptoms of a Cryptosporidium parvum *infection among humans?*

C. parvum in humans is typified by diarrhea, abdominal cramps, headaches, nausea, vomiting and a low-grade fever. The initial symptoms can persist and develop into weight loss and dehydration. Pulmonary and tracheal infections can also result, and are characterized by coughing and low grade fever, accompanied with gastrointestinal distress. Not everyone exposed to the protozoa will contract the disease. Some people will become infected by ingesting only 10 oocysts, while others may need to ingest 1000 oocysts to become infected. The infection is self-limiting, clearing up on its own, and usually lasting for one to two weeks. Asymptomatic infections can also occur in humans.

People with a compromised immune system are at risk of severe disease from this infection. The disease can last up to six months. Those most susceptible to this disease are patients undergoing chemotherapy, recent transplant recipients, AIDS patients, the elderly, and young children.

How is Cryptosporidium parvum *detected in both farm animal and human samples?*

C. parvum infection in humans is a disease that is reportable to the Ministry of Health in Ontario. A fecal sample is usually taken. Human fecal samples are easier to analyse for the presence of *C. parvum* due to the lack of inhibitory substances in the feces. Some tests include direct fecal smears, acid-fast staining, immunofluorescent assays, and monoclonal antibody tests (such as the ELISA test). Bovine and swine samples, for example, are harder to analyse, and the accuracy of the tests presently used in laboratories is variable, indicating a great need for more research into the methodology of *C. parvum* testing.

How is a Cryptosporidium parvum *infection treated in both farm animals and humans?*

To date, there is no effective treatment for *C. parvum* infections among humans or farm animals, and treatment is aimed at controlling the dehydration and diarrhea symptoms of the disease, as well as prevention.

How can a Cryptosporidium parvum *infection be prevented?*

Prevention centres around limiting the fecal-oral route of transmission. The best way to decrease the chance for an infection on the farm is through good hygiene, and farm workers should pay particular attention to personal hygiene, and hygiene between the barn and the house. Care should be taken when treating sick livestock, and contact with others should be limited. Children should be particularly careful, and their hand washing should be as thorough as possible. *C. parvum* and other pathogens are prevalent in animal manure. Preventing unreasonable exposure of young children playing in the barn or around the barnyard should be a part of farm safety. The washing of hands with soap and water should follow contact with toilets, diapers, animals or animal feces, after working in dirt or touching objects which may have been exposed to fecal matter, and before preparing or serving food. All fruit and vegetables should be thoroughly washed if eaten raw, due to possible contact with manure.

How can one prevent Cryptosporidium parvum *on farms?*

The best method of control is to limit the fecal-oral route of transmission of oocysts between young animals. Since the *C. parvum* oocysts are not susceptible to most disinfectants, emphasis should be put on maintaining regular removal of manure and bedding. The areas where young animals are held should be thoroughly cleaned with an ammonia solution and left to dry for a few days before new animals are brought in. Other recommendations include raising young animals in clean and dry environments, and in the case of dairy farms, raising the young calves in separate hutches or boxes. Healthy and sick animals should be separated, as should the manure of these animals. As well, as mentioned previously, mice and rat populations should be controlled as much as possible, since they may be a reservoir

for infective feces. Adequate colostrum intake and proper diagnosis in cases of persistent diarrhea outbreaks is critical.

How can farms control the release of Cryptosporidium parvum *to the watershed?*

Best farm management practices will help control the release of *C. parvum* oocysts into the environment, and there are certain procedures which should be followed to ensure this.

Purchasing Animals: The purchasing, addition, or boarding of *C. parvum* infected animals is a known source of spread of the infection to other healthy animals. Thus, it is important to ensure that the health history of the herds of origin do not include persistent diarrhea problems. All animals purchased should be quarantined for a period of 2 weeks, to observe for scouring, and there should be no contact between new animals and the original livestock. If the animal is scouring, isolation procedures should be maintained for at least one week after the diarrhea symptoms have ceased and proper veterinary consultation should be pursued. The contaminated manure and bedding should be disposed of according to recommendations for housing and bedding (see below).

Housing and Bedding: Once an animal is infected in a herd, there is a significant risk for spread to other animals in the herd, due to the large number of *C. parvum* oocysts which are shed. Calves should be housed separately from the herd, ideally in separate pens, and bedding should be removed and replaced routinely. The housing areas for both the adult animals and the young stock should be pressure washed with an ammonia-based disinfectant at least annually - more frequently, if possible.

Manure management: Oocysts are capable of remaining infectious for long periods of time in the environment, due to their hard outer shell. The proper management of manure on farms is of utmost importance to reduce the number of viable oocysts. The oocysts are resistant to most environmental pressures, but are sensitive to drying, freezing, UV light, and ammonia-based cleaners. The control of *C. parvum* infections is an important benefit from following the suggested best management practices for livestock waste management. Liquid manure should be collected in tanks or lagoons, as well as any runoff wastes from solid manure systems, barnyard runoff, and milkhouse runoff.

Treatment of manure prior to application: Including bedding with manure aids in absorbing liquid, reducing the moisture content and allowing more aeration, to encourage composting. Composting of solid manure is an effective control measure, provided aeration is achieved by turning of the manure heap. The heat thus generated reduces the number of viable oocysts. Methane digestion of liquid manure also generates heat.

Manure application to land: It is recommended that manure be applied prior to or early in the growth stage of any crop. Manure should be in storage for the months of November to March. Manure should not be spread on fields with a history of floods or runoff. Runoff can pollute surface water, increasing the risk of infection further along the waterway.

Liquid manure should not be spread on land within 10 metres of an open watercourse, and solid manure should not be applied within 5 metres of an open watercourse.

Following the application of manure, animals should not be allowed to graze, and cutting of forages or other crops should be postponed until all signs of the manure have disappeared. The application of manure to impermeable soils with significant gradients near water sources should be avoided. If the manure is applied to a field having a subsurface drainage system, due to the continuous macropores that may be present in the soil, the manure should be applied to dry soil. This will help reduce the rapid flow of manure to the tile drains. Performing light tillage prior to application is even more effective at reducing the potential for macropore flow.

References:

Angus, K. W. 1997. Cryptosporidiosis in domestic animals and humans. In Practice. 9,(2)., pp.47-49

Fayer, R. D., Trout, J., Nerad, T. 1996. Effects of a wide range of temperatures on infectivity of Cryptosporidium parvum oocysts. J. Euk. Microbiol. 43,(5)., p.64S

Garber, L. P., Salman, M. D., Hurd, S. H., Keefe, T., Schlater, J. L. 1994. Potential risk factors for *Cryptosporidium* infection in dairy calves. JAVMA. 205,(1).,pp.86-91

Garber, L. 1993. *Cryptosporidium parvum* Literature Review Animal Health Insight. pp.3-11

Kemp, J. S., Wright, S. E., Coop, R. L., Mawsley, J. L., Merry, J. L., Pain, B. F., Theodoru, M. K., Read, I. A., Svoboda, I. F., Bukhari, Z., Smith, H. V. 1995. Protozoan, bacterial and viral pathogens, farm wastes and water quality protection - final report. MAFF open contract csa 2064

Makuch, J. R. 1996. *Cryptosporidium*: A waterborne pathogen. <http://inform.umd.edu:86/EdRes/Topic/AgrEnv/Water/.cryptfac.html> Accessed July 10,1997

Makuch, J. R. *Cryptosporidium parvum* and cattle: Implications for public health and land use restrictions. <http://inform.umd.edu:86/EdRes/Topic/AgrEnv/Water/.cornell.html> Accessed July 10,1997

Moon, H.M., Woodmansee, D. B. 1986. Cryptosporidiosis. JAVMA. 189,(6)., pp.643-646

Rings, D. M., Rings, M. B. 1996. Managing Cryptosporidiosis and *Giardia* infections in domestic ruminants. Food-Animal Practice. pp.1125-1131

Upton, S. J. *Cryptosporidium* research. <http://www-personal.ksu.edu/~coccidia/> Accessed July 10,1997

Cryptosporidium Infosheet - Plain Language - DRAFT September, 1997

What is Cryptosporidium parvum*?*

Cryptosporidium (krip-toe-spor-id-ee-um) *parvum* is a very small parasite that can reproduce in animals. It is found in digestive organs and the respiratory tract. It was first identified early in the 20th century, and cryptosporidiosis (the disease) was first identified in 1976. It is most commonly known as a cause of gastroenteritis in people, and can cause relatively large outbreaks of human illness. There are six recognized species, including two which infect mammals, two which infect birds, and two which infect fish and reptiles. The life cycle of this parasite occurs within one animal, but consists of several stages.

How does Cryptosporidium parvum *infect people?*

The oocyst stage is the infectious stage. Oocysts are released from the intestinal wall into the fecal material of the infected animal or person. Other people become infected after oocysts somehow enter their mouths, after they come in contact with fecal material. Poor hygiene practices often lead to infection. Contact with toilets, diapers, animals or animal feces, and dirt are common sources of infection. The ingestion of contaminated food or water is perhaps the most publicized route of infection.

What are the symptoms of a Cryptosporidium parvum *infection among humans?*

Infection in humans may result in diarrhea, abdominal cramps, headaches, nausea, vomiting and a low-grade fever. The initial symptoms can persist and develop into weight loss and dehydration in severe cases. Not everyone exposed to the protozoa will get sick. The infection often clears up on its own in two weeks, without any treatment. This disease often produces no symptoms, but can still be spread to others.

People with a weak immune system are more severely affected. In these cases symptoms may persist for up to six months. Patients undergoing chemotherapy, recent transplant recipients, AIDS patients, the elderly, and young children are at greatest risk of severe disease.

How is a Cryptosporidium parvum *infection treated?*

To date, there is no effective treatment for *Cryptosporidium parvum* infections. The treatment is limited to addressing the symptoms of the disease, and making sure the patient doesn't become dehydrated.

How are Cryptosporidium parvum *infections prevented in people?*

Prevention centres around limiting contact with feces. Farm workers should pay close attention to personal hygiene and hygiene between the barn and house. Children should be especially careful, and their hand washing should be as thorough as possible. *Cryptosporidium parvum* and other pathogens are present in animal manure. Preventing unreasonable exposure of young children playing in the barn or around the barnyard should be a part of farm safety. The washing of hands with soap and water should follow contact

with toilets, diapers, animals or animal feces, after working in dirt or touching objects which may have come in contact with fecal matter, and before preparing or serving food. Everyone should avoid drinking untreated surface water or water from a poorly constructed or maintained well. All fruit and vegetables should be thoroughly washed if eaten raw, if there was any possible contact with manure.

Care should be taken when treating sick livestock, and contact with others should be limited. Proper medical care should be sought in persistent gastrointestinal problems in animals and humans. Drinking water in rural areas should be checked occasionally for the presence of pathogens. As well, proper well-head construction should be followed, to limit the possibility of contamination by manure or septic systems.

Cryptosporidium Infosheet - Technical Version - DRAFT - September, 1997

What is Cryptosporidium parvum*?*

Cryptosporidium (krip-toe-spor-id-ee-um)*spp.* is a protozoan parasite that reproduces in vertebrates. It can be found within the epithelial cells of the digestive organs and respiratory tract. It was first identified early in the 20th century, and cryptosporidiosis (the disease) was first identified in 1976. It is most commonly known as a cause of gastroenteritis in people and can cause relatively large outbreaks of human illness. There are six recognized species of *Cryptosporidium* including *C. parvum* and *C. muris* (which infect mammals), *C. baileyi*, and *C. meleagridis* (which infect avians), and *C. nasorum* and *C. crotalis* (which infect fish and reptiles.) The life cycle of these protozoa occurs within one host, and consists of several stages.

How is Cryptosporidium parvum *contracted by humans?*

The oocyst stage is the infectious stage, and is released from the intestinal wall into the fecal material of the affected animal. Subsequent re-infections are transmitted by oocysts through fecal-oral contact. Transmission is through person-to-person or animal-to-person contact, or by the ingestion of contaminated food or water. Poor hygiene practices are often associated with infection - by coming into contact with toilets, diapers, animals or animal feces, dirt, preparing or serving food, or touching objects which may have come in contact with fecal matter. The ingestion of contaminated food or water is perhaps the most publicized route of infection.

What are the symptoms of a Cryptosporidium parvum *infection among humans?*

The infection in humans may result in diarrhea, abdominal cramps, headaches, nausea, vomiting and a low-grade fever. The initial symptoms can persist and develop into weight loss and dehydration in severe cases. Pulmonary infections can also result, and are characterized by coughing and low grade fever, often accompanied with gastroenteritis. Not everyone exposed to the protozoa will show signs of the disease. The infection is self-limiting, often clearing up in one to two weeks.

People with a weak immune system are more severely affected. In these cases, intestinal symptoms may persist for up to six months. Patients undergoing chemotherapy, recent transplant recipients, AIDS patients, the elderly, and young children are at greatest risk of severe disease.

How is a Cryptosporidium parvum *infection treated?*

To date, there is no effective treatment for *Cryptosporidium parvum* infections. The treatment is limited to addressing the symptoms of the disease, and ensuring the dehydration of the patient is kept under control.

How are Cryptosporidium parvum *infections prevented among humans?*

Prevention centres around limiting the fecal-oral route of transmission by person-to-person or animal-to-person contact. Farm workers should pay particular attention to personal hygiene and hygiene between the barn and house. Children should be particularly careful, and their hand washing should be as thorough as possible. Preventing unreasonable exposure of young children playing in the barn or around the barnyard should be a part of farm safety. The washing of hands with soap and water should follow contact with toilets, diapers, animals or animal feces, after working in dirt or touching objects which may have come in contact with fecal matter, and before preparing or serving food. One should avoid drinking untreated surface water or water from a well subject to surface water entry. Any untreated drinking water should be brought to a rolling boil for a minimum of one minute. All fruit and vegetables should be thoroughly washed if eaten raw, if there was any possible contact with manure.