

# **Report on a Scoping study for an Agro-Ecosystem Indicator of the Risk of Water Contamination by Pathogens from Agricultural Operations**

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## **1. Introduction**

Sustainable use of Canada's natural resources by the agri-food sector requires both the benchmarking of the magnitude of those resources and continued monitoring of their quality. A further step required is the development of management practices and their implementation at locations of greatest vulnerability. Freshwater resources in agricultural regions are important for continued primary production, but also because the water is commonly used for recreational purposes, provides the basis for fisheries, is harvested from these regions for potable supplies used by municipalities, and provides a varied habitat for plants and animals. Establishing vulnerable locations and providing a minimum level of water quality monitoring is expensive, and identifying likely beneficial management practices across the country requires considerable effort. Remote sensing techniques coupled with simulation modelling offers the most effective means of achieving these objectives, but our knowledge of many processes that result in the contamination of surface and ground water resources at the watershed and regional level is still rudimentary in many cases (Goss, 1994). Another approach is to identify and develop indicators that combine elements of the processes that contribute to contamination with some simple measures of the potential for contamination (Girardin *et al.*, 1999).

This report considers the information requirements to develop an agroecosystem indicator that assesses the risk that pathogens from one or more agricultural operations may contaminate water resources. It consists of three major sections: the first is a review of the literature that identifies the potential sources of pathogens on agricultural operations, and the relative importance of those sources in leading to degradation of water resources; the second part deals with the activities underway in organizations other than agricultural departments in Canada, and in jurisdictions outside of Canada; the third section considers elements and critical control points from the literature review, and comments on existing or planned indicator frameworks for other AEIs that could contribute to a pathogen AEI.

## **2. Detailed Objectives**

The objectives of this study are:

1. Assess the available information appropriate to the development of an Agroecosystem Indicator, which is a protocol or framework that can be used to estimate from knowledge at each critical point the risks to water resources from the on-farm use of organic materials, including but not limited to livestock manure, human wastes (septic tank effluent discharge into ground water and sewage biosolids), paper biosolids, and food processing industry wastes.

The critical points include the locations of generation, storage, treatment, transportation and use. The framework needs to be sensitive to feasibility and the practicality of using treatment methods or BMPs to enable the safe use of the materials, so those aspects need to be assessed.

The framework could be or include computer models or decision support systems. It should be usable at the watershed, regional, provincial scale and be national in its applicability.

2. Identify current studies or works being undertaken to fulfill Objective 1 in other jurisdictions in Canada, North America, Europe or other regions facing issues of zoonosis from agricultural activity.
3. Report on information and consensus of any recent studies on what are the appropriate surrogate indicators of the presence of pathogens (bacteria, virus, protozoa, parasites) for identifying the sources of contamination within a watershed.
4. Identify components of any existing AEIs that may in turn contribute to an AEI for pathogens.

### **3. Literature Review**

#### **3.1 Background**

This review considers the sources and potential pathways of pathogen movement to water resources. Pathogens can be found on livestock farms associated with animal manure, domestic septic systems, milk house septic systems, pets and wild animals. Manure from housed animals, and waste from either domestic or processing activities that is treated using septic systems, can be considered as being held at some level of confinement or storage for a time before land application or release into the environment. Faeces dropped by grazing animals is under some level of control depending on the decisions of the farm operator. In contrast, the behaviour of pets and wild animals are not readily predictable or managed, and so such animals will not be regarded as sources for this study. The risk of contamination from family pets will not be discussed in this paper, and an assumption will be made that any contamination from, wild animals is not enhanced and is probably less from agricultural than non-agricultural land. That said, it may be the case that there are more wild animals concentrated near waterways because that can act as conduit for migration and hunting as well as being an area where there is more natural cover. Application to land of manure, sewage and other organic biosolids brought in from municipalities and industry for land application, and the faeces from grazing animals forms a diffuse unconfined source. Once materials leave the tile lines of the weeping bed of a septic system it also forms an unconfined source.

We will consider first a manure source subject to some confinement. The manure shed in barn stays on the floor or enters a gutter where it is held in temporary storage before moving to longterm storage. In some instances the manure may be treated. At each stage there is the possibility that pathogens can move to a surface or ground water resource. The risk of this happening needs to be considered. Risks associated with septic tanks are considered at the same time.

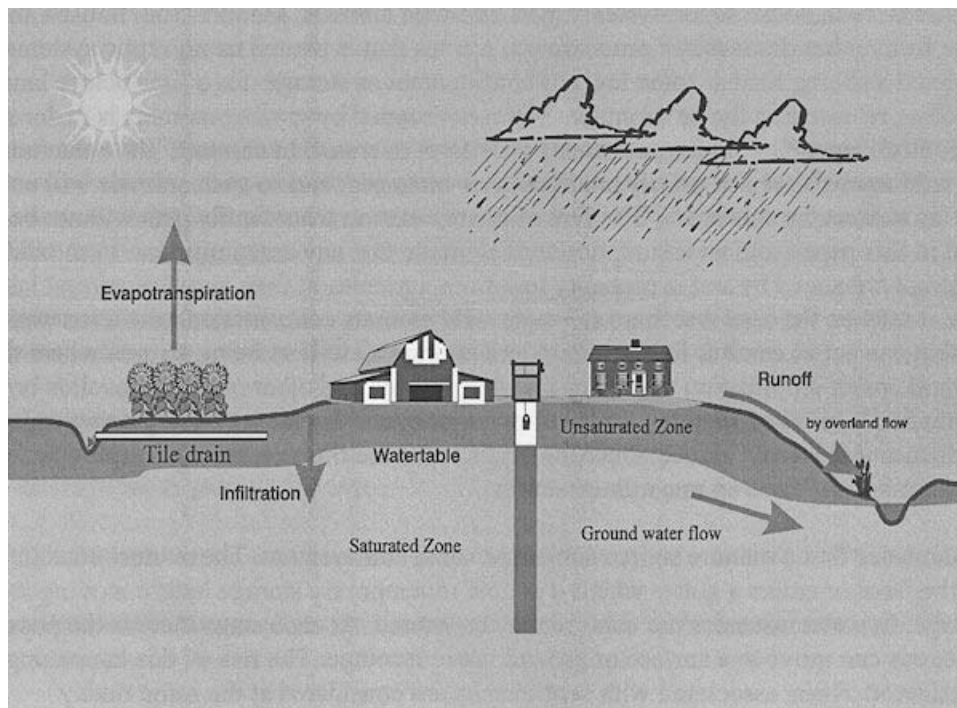
The risks associated with the next stage of manure management, the application of manure to the field, can be considered along with other unconfined sources including sewage biosolids, septic system effluent, and treatment waste from milk-house septic systems and waste water trenches.

Two major water resources are recognized as sources of drinking water:

- surface water, which includes streams, municipal drains, rivers and lakes; and
- groundwater, which consists of saturated zones in various strata of subsoil and rock

The water or hydrological cycle (Figure 1) is a major driver in determining the movement of contaminants. It affects the distribution of contaminants in the surface and groundwater as well as losses in gaseous form. The main components of the hydrological cycle are precipitation, evapotranspiration, drainage, and runoff.

Evapotranspiration, drainage, and runoff represent the fate of the precipitation component of the cycle. Averaged across four Ontario sites, evapotranspiration accounted for 64% of precipitation, drainage 23%, and runoff 12% (Parkin *et al.*, 1999). Some of the drainage water moves laterally in the near-surface layer of the soil (interflow) and then to streams. Where tile drains are present, some proportion of drainage enters the drains and is consequently emitted to surface water instead of recharging groundwater. Contaminants from manure can enter surface water bodies in the surface runoff from fields and yards, in



**Figure 1.** Schematic diagram of the hydrological (water) cycle.

interflow, in discharge from tile drains, and in groundwater. Contaminants enter groundwater by direct transport through rock and overlying soil or through man-made ducts such as abandoned, improperly sealed, or poorly maintained wells. In the process called leaching, materials are dissolved or displaced from the soil or rock as the water moves through. Runoff in this document is restricted to mean surface runoff. Both surface and groundwater can therefore be impacted by runoff that picks up contaminants on the surface of the soil or on yards, as well as from material that has been incorporated into shallow soil layers.

### 3.1.1 Manure

As shed from animals, manure contains up to  $10^{10}$  microorganisms per gramme. When present, pathogenic microorganisms may be  $10^5 \text{ g}^{-1}$ . The more animals on a farm, the greater the likelihood of pathogens in the manure. Identifying the risk of environmental contamination from manure is complex. It is impossible to predict precisely what will happen to manure under any given set of conditions. Many factors impact the fate of manure constituents:

- the nature of manure itself, which varies considerably depending on the type, diet, and age of the livestock;
- the type of livestock housing and use of bedding; type and length of manure storage;
- the method, timing, and rate of manure application on fields;
- the characteristics of the land receiving the manure, including soil texture, slope, depth to water table, proximity to water resources, and tillage;
- the weather (e.g., wind speed during application and rainfall intensity, frequency, and duration before and after manure application), which determines if potential contaminants move off- site in surface runoff, tile drainage flow, groundwater, or air or remain in the rooting zone where nutrients can be taken up by crops.

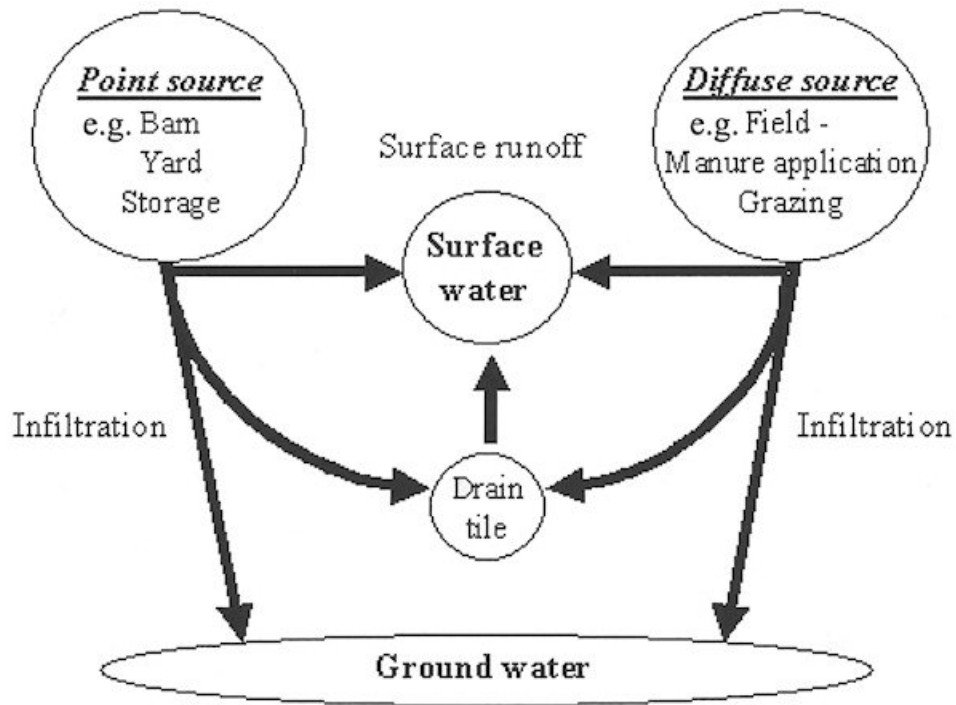
The ways in which the number of pathogens can decline or become immobilized greatly influence the likelihood that a given water resource may become affected. This is summarized in Figure 2.

The key groups of pathogens in farm animals are bacteria, viruses, and protozoa.

#### *Bacteria.*

Very large numbers of bacteria are present in manure and septage, and may total  $10^{10}$  organisms  $\text{mL}^{-1}$  in liquid manure. The greatest numbers are of faecal coliforms and streptococci (Table 1). Bacterial pathogens that are of high priority from a human health perspective are the enterohemorrhagic and related forms of *Escherichia. coli*, *Salmonella*, *Listeria monocytogenes*, and *Campylobacter*. Pathogens of medium priority are *Yersinia enterocolitica*, *Clostridium perfringens*, and other pathogenic forms of *E. coli*. While bacteria species from these two groups are always present in manure, *Salmonella* spp. (another important group of bacterial pathogens) are known to represent a risk to water supplies (Pell, 1997) and are mostly found in swine and poultry manure (Strauch, 1987). However, the

numbers may be similar across species when comparisons are made per unit of dry matter. Due to the greater mobility of bacteria in the liquid phase compared with the solid phase, liquid manure tends to be more uniformly contaminated than solid manure.



**Figure 2.** Schematic diagram of the sources and movement of contaminants from manure to water resources.

**Table 1.** Examples of bacterial numbers in some animal manure (CFU mL<sup>-1</sup> or g<sup>-1</sup> fresh manure).

Manure type	Faecal coliforms	Faecal Streptococci	Salmonella spp.	Protozoa & Others	Source
Liquid swine manure	4.3x10 <sup>3</sup> to 1.3x10 <sup>5</sup>				Unc, 1999
	2.4x10 <sup>3</sup>	9.3x10 <sup>3</sup>	0		Weigel, 1995
	9.5 x10 <sup>4</sup> to 1.1x10 <sup>6</sup> <i>E. coli</i>	7.2x10 <sup>4</sup> to 4.5x10 <sup>5</sup> Streptococci-D	0 to 1.5x10 <sup>3</sup> ( <i>S. infantis</i> )		Ruprich, 1994
Liquid cattle manure	2.4x10 <sup>3</sup>	9.3x10 <sup>3</sup>	0		Weigel, 1995
	4.5x10 <sup>2</sup> to 1.5x10 <sup>6</sup> <i>E. coli</i>	4.5x10 <sup>2</sup> to 9.5x10 <sup>5</sup> Streptococci-D	0		Ruprich, 1994
Dairy slurry	6.3x10 <sup>4</sup> to 1.0x10 <sup>7</sup> Enterobacteria	2.7x10 <sup>7</sup>			Ostling and Lindgren, 1991 Crane <i>et al.</i> , 1983
Solid beef manure	2.4x10 <sup>5</sup>	1.5x10 <sup>7</sup>	0		Weigel, 1995
	1.9x10 <sup>6</sup> to 6.8x10 <sup>6</sup>				Unc, 1999
Solid dairy manure	2.0x10 <sup>5</sup> to 1.0x10 <sup>7</sup> Enterobacteria				Ostling and Lindgren, 1991
Fresh cow manure	up to 1.0x10 <sup>9</sup>		up to 1.0x10 <sup>9</sup>	<i>Cryptosporidium parvum</i> From 25 to 1.8x10 <sup>4</sup> in healthy animals to 1x10 <sup>9</sup> in sick animals	Mawdsley <i>et al.</i> , 1995 Clinton <i>et al.</i> , 1979 Scott <i>et al.</i> , 1994 Smith, 1992
Sheep	6.0x10 <sup>6</sup>	6.6x10 <sup>5</sup>			Crane <i>et al.</i> , 1983
Horse	9.4x10 <sup>4</sup>	6.3x10 <sup>6</sup>			Crane <i>et al.</i> , 1983
Poultry	1.3x10 <sup>6</sup> to 1.4x10 <sup>8</sup>	6.2x10 <sup>5</sup> to 1.9.7x10 <sup>8</sup>			Crane <i>et al.</i> , 1983

As pathogenic species or strains are present in far fewer numbers than are the benign or beneficial ones, the non-pathogenic organisms have commonly been used as indicators of faecal contamination in water resources. Total coliform counts, numbers of faecal coliforms, and the presence of *Escherichia coli* (*E. coli*) are all used in this way. Some strains of *E. coli* can cause disease. These strains are recognized by the presence of particular proteins or polysaccharides on the surface of the bacteria. One sero group, the enteropathogenic *E. coli* (EPEC), have given rise to *E. coli* 0157:H7 which contains the genes for 'Shiga toxin' or 'Verotoxin.'

These genes are thought to have been introduced through infection with bacteriophage (a virus that attacks bacteria), which carried the genes together with a virulence plasmid. Other disease-causing strains, developed from the enteroaggregative serogroup of *E. coli*, have also acquired the same toxin-forming genes and virulence plasmids. The Verotoxin-forming *E. coli* (VTEC) may therefore conform to 0157 or non-0157 serogroups. Not all *E. coli* with the 0157 serotype actually give rise to disease in humans. However, cattle and other ruminants appear to carry those that do cause illness (Table 2). Concentrations of *E. coli* 0157:H7 in cattle faeces range from  $10^2$  to  $10^5$  cfu fresh weight.

The smaller amounts are common in younger animals. Infection in an individual animal is not continuous; rather, animals experience a series of reinfections, the frequency declining with age. Furthermore, the release of *E. coli* 0157:H7 in the faeces shows a strong seasonality, being greatest in July and August. Consequently, the concentration of colony-forming units in faeces is expected to be highly variable with time. Based on the surveillance of beef carcass contamination, the concentration of *E. coli* 0157:H7 in faeces may vary between years as well as between seasons. *E. coli* 0157:H7 has been detected infrequently in swine or poultry (Table 2), probably giving less risk of human infection from these sources.

*Salmonella* spp. are an important group of bacterial pathogens known to represent a risk to water supplies (Pell, 1997) and are mostly found in swine and poultry manure (Strauch, 1987).

**Table 2.** The frequency of detection of *E. coli* 0157 in animals from different groups (J. Van Donkersgoed, 2000).

Animal type	Range of reported frequency of detection (%)
Cattle	
Dairy	0-68
Beef feedlot	0.3-88
Cow-calf	0.7-20
Sheep	0-31
Pigs	0-1.4 *
Poultry	0-1.3
Deer	1.9-9.0
Birds	0.5
Rodents	0-40
Flies	3.3
Horses	1.1
Pet dogs	3.1

\* Not human pathogenic strain of *E. coli* 0157:H7, but note that *E. coli* 0157:H7 has now been reported in swine (Feder *et al.*, 2003)

*Leptospira*, a waterborne pathogen spread through urine, has been found in pigs. Survival is enhanced by warm temperatures (19-30°C) and alkaline media.

*Yersinia enterocolitica* in humans is thought to come mainly from infected pigs. In Canada, its prevalence appears to be about 20% in finisher pigs.

*Campylobacter* spp. are commonly found in swine (66% to 95%) and poultry manure, but are of lesser concern in cattle. However, others have concluded that cattle are the largest reservoir in the agricultural environment. For example, Jones *et al.* (2002) reported that cattle herd infection rates typically vary from 70 to 90% with infection exhibiting a distinct seasonality (bimodal) similar to the peaks reported for human infections (Koenraad *et al.*, 1997; Stanley *et al.*, 1998 a,b). Of the many recognised species of *Campylobacter*, *C. jejuni* is the most predominant cause of enteric disorders (90%) while *C. coli*, *C. lari*, *C. hyointestinalis* and *C. upsaliensis* have also been identified as causing disease in humans (USFDA, 1992; HMSO, 1993). The frequency of infection in sheep tends to be less than in other farm animals (Manser and Dalziel, 1985). *C. jejuni* has been isolated from chickens, pigs, and cattle in Ontario. Isolates from chickens and cattle were of the same serotypes that occur in humans. Most of the pigs that tested positive for *Campylobacter* carried a serotype of *C. coli* that was uncommon in humans (Munroe *et al.*, 1983). Using laboratory-based microcosms (long-term testing units), Thomas *et al.* (1999) identified that water systems

could act as a reservoir for *Campylobacter* infections. The primary infection of young animals is likely the consumption of faecally contaminated water and vegetation (Weijtens *et al.*, 1997; 1999). Grazing herds with access to rivers or streams have been shown to have a greater incidence of infection than those drinking chlorinated water supplies (Humphrey and Beckett, 1987). Contamination of pig and cattle faeces is common at rates of  $10^2$ - $10^6$  cfu g<sup>-1</sup>. To prevent the colonization of poultry chicks by *C. jejuni*, 'competitive exclusion' can be used (Phillips, 1995). In this technique, a specific mixture of other intestinal bacteria, taken from adult birds, is introduced into the cecum of one-day-old chicks. As human faeces are known to contain more than  $10^6$  cfu in infected cases, *Campylobacter* can be commonly found in sewage and untreated water (Fricker and Park, 1989; Jones *et al.*, 1990). Untreated abattoir waste which is also applied to land in many countries has also been shown to be heavily contaminated with *Campylobacter* ( $>10^3$  mL<sup>-1</sup>), and has been implicated in water course contamination (Jones *et al.*, 1990).

*Listeria monocytogenes* can be carried by healthy animals. *L. monocytogenes* is the dominant cause of human infection, however, other species including *L. innocua*, *L. ivanovi* and *L. seeligeri* may also cause illness. Shedding of the bacterium is greater in winter than summer, and it can grow over a wide range of temperatures from 3 to 42°C. It is pH tolerant in the range of pH 5.5-9.0 (Pell, 1997). Typically, 50 to 80% of farm environments are contaminated with *L. monocytogenes* with many of the isolated strains pathogenic. However, strains which are pathogenic to humans are commonly non-lethal to animals (Welshimer, 1968). As *L. monocytogenes* is present in the intestines of some farm animals, it is not surprising that it is also present in the faeces of these animals at rates  $>10^2$  cfu g<sup>-1</sup>. *L. monocytogenes* is frequently described as a facultative intracellular parasite that can be shed both within the milk of cows as well as in faeces. Studies looking at the incidence of *L. monocytogenes* in faeces of farm animals have typically shown that 2 to 50% of pig, cattle and sheep faeces to be contaminated with the pathogen (Skovgaard and Morgen, 1988; Skovgaard and Norrung, 1989; Van Renterghem *et al.*, 1991). *L. monocytogenes* can be frequently detected in soil, associated litter and vegetation, indicating that the pathogen can exist in the wider environment as a saprophyte on both soil organic matter and plants (Weiss and Seelinger, 1975). Typical concentrations in soil range from 1 to 500 cfu (MacGowan *et al.*, 1994; Dowe *et al.*, 1997). The persistence of *L. monocytogenes* in soil up to 10 years after incorporation of animal residues in soil has been reported (Welshimer, 1968). Other studies have indicated that *L. monocytogenes* is incapable of surviving in soil for longer than 2 months.

*Leptospira*, a waterborne pathogen spread through urine, has been found in pigs. Survival is enhanced by warm temperatures (19-30°C) and alkaline media.

*Clostridium perfringens* is a bacterium that forms spores, which are resistant to environmental stresses including disinfecting agents. It is excreted in the faeces of many animals, but is not present in samples of sludge taken from septic tanks. Antibiotic-resistant strains of *C. perfringens* can be used to distinguish the source of faecal contamination of domestic farm

wells; the presence of *C. perfringens* together with faecal coliforms indicated that animal manure was the source (Conboy and Goss, 2001).

### *Viruses*

Although viruses are common contaminants of manure, information about their occurrence and longevity in manure is very limited. Many animal viruses that are likely to be excreted in manure do not cause disease in humans (Pell, 1997). Viruses that are of high priority are the rotaviruses groups A & B (bovine, swine), Hepatitis E (swine & rats), and Myxovirus (swine & poultry). Rotaviruses cause diarrhoea in neonates of humans and a number of other animals (Estes and Cohen, 1989). The closeness of the human and swine forms of rotaviruses, together with the analysis of associated antigens and antibodies, suggest a crossover between the two hosts (Holland, 1990). Large numbers of these viruses can be excreted in faeces from infected pigs, with sows being an important source of contamination of young piglets (Benfield *et al*, 1982). Bovine rotaviruses may be isolated from cattle manure, but it is not thought to be common (Pell, 1997). Swine hepatitis E is closely allied to the human form of the virus. The virus is common in animals of three months or older throughout the mid-western U.S. states. The human form of the virus is known to be transmitted through contaminated water (Meng *et al*, 1997).

Medium priority viruses include Reovirus - type 3, Enterovirus (bovine, swine), Calicivirus (bovine, swine). Some viruses, which do give rise to diseases in humans, can be found in large numbers in manure (Addis *et al*, 1999). For example, coronaviruses, which cause diarrhoea in calves and pigs, are found in manure. Reoviruses excreted by cattle are found mainly in manure (Strauch, 1987).

Low priority viruses include *Herpes*, *Corona*, *Parvo*, *Paramyxa*, *Hepadna*, and *Retrovirus*.

Enteroviruses and adenoviruses in animals are not thought to represent a significant threat to humans (Stelma and McCabe, 1992). Swine vesicular disease does not appear to pose a threat to water supplies. Survival outside the host appears to be relatively short. Bovine parvoviruses do not appear to be related to those that affect humans.

Influenza virus is very widespread, and pigs may be a potential reservoir of human strains. The virus can survive outside the host for a prolonged period. For example, the infectious avian influenza virus can survive in water for 207 days at 17°C (Brown and Alexander, 1998). Other animal viruses that can cause disease in humans, such as cowpox and paravaccinia, are not likely to be found in manure.

### *Protozoa*

High priority protozoan parasites are *Cryptosporidium parvum* (bovine, swine, sheep) and *Giardia lamblia* (bovine & swine). *Cryptosporidium* is carried by a wide variety of animals (Graczyk *et al.*, 1998; de Graaf *et al.*, 1999) which contribute to the wide distribution of oocysts found in the environment. *C. parvum*, can also cause severe disease symptoms in

humans. In one-third of the diarrheal outbreaks in 1993 to 1994 for which the causal agent was positively identified, *C. parvum* and *Giardia* species were the pathogens involved. River-water samples in the Ottawa region contained significant numbers of *C. parvum* oocysts and *G. lamblia* cysts, but the origin was likely from sewage treatment plants (Chauret *et al*, 1995). *Cryptosporidium parvum* requires the ingestion of between 1 and 100 oocysts to cause disease in humans. It is considered a threat to surface water supplies, but recent evidence suggests that the oocysts can move through macropores in the soil and contaminate shallow groundwater (Endale *et al*, 2000). *C. parvum* cannot be controlled by chlorination at levels that are safe for use in domestic water supplies (Pell, 1997).

Oocysts of *C. parvum* have been found in manure from dairy farms and swine farms in Ontario, although more were present in liquid manure from the swine farms. In the UK, oocysts were found on 59% of dairy farms and 22.4% of heifers and a similar number of beef calves were infected (Pell, 1997). Fleming *et al*. examined the manure on 60 farms in southwestern Ontario on three occasions over one year (Fleming *et al*, 1997). *C. parvum* was found on 90% of the swine farms. Evidence suggests, however, that the strain associated with swine does not cause disease in humans (Olson, 2000). *Giardia lamblia* requires only about 10 cysts to cause disease in a human. It is the most commonly isolated intestinal parasite. In one study, 3% of young pigs and 19% of adult animals were infected (Olson *et al*, 1997).

*G. lamblia* has been found on 7-67% of swine operations (Xiao *et al*, 1994). There is evidence that *G. lamblia* from grazing cattle contributed to the contamination of a piped domestic water supply in British Columbia (Issac-Renton *et al*, 1995). A surface reservoir was the source of the water. *Worms Ascaris suum*, a helminthic worm, appears to be able to pass from pigs to humans, although evidence from China suggests that the strains that infect pigs are genetically different from those isolated from humans. Prevalence in swine may be as high as 50%. Up to 2 million eggs can be shed per day by infected animals. *Tenia solium*, the human tapeworm, is very uncommon in North America, and is not thought to pose a risk to water supplies.

Hutchison *et al*. (2002) investigated the presence of pathogens in manures in the United Kingdom. The percentage isolation and geometric mean level for each pathogen ranged from 3.4 % and 1.1 cysts per gramme for *Giardia* to 29.4 % and 5.98 CFU g<sup>-1</sup> for *Listeria* (Tables 3 and 4).

**Table 3.** Percentage of British livestock manures that contained zoonotic microorganisms.

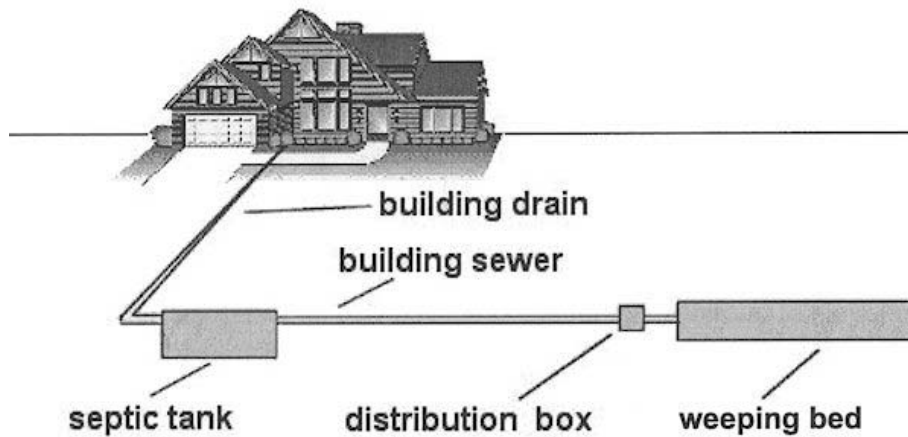
Manure	Animal species							
	Cattle		Pig		Poultry		Sheep	
	Fresh (n=522)	Stored (n=249)	Fresh (n=93)	Stored (n=35)	Fresh (n=48)	Stored (n=17)	Fresh (n=23)	Stored (n=6)
<i>E. coli</i> 0157	15.4%	13.2%	16.1%	26%	ND	ND	21.7%	16.7%
<i>Salmonella</i>	4.5%	4.8%	8.6%	2.8%	12.5%	5.8%	8.6%	0%
<i>Listeria</i>	28.8%	34.5%	20.4%	22.8%	18.75%	11.8%	30.4%	33.3%
<i>Campylobacter</i>	15.9%	15.6%	18.3%	17.1%	22.9%	11.8%	21.7%	16.7%
<i>C. parvum</i>	7.1%	4%	18.3%	5.7%	ND	ND	30.4%	0%
<i>G. intestinalis</i>	3.8%	1.6%	3.2%	2.9%	ND	ND	21.7%	0%

**Table 4.** Mean levels of zoonotic pathogens observed British livestock manures. Data are geometric means for positive isolations only. ND were not determined because it was not appropriate to test manures from these species for all pathogen types.

Manure	Levels of pathogens found in positive samples for each animal species (CFU g <sup>-1</sup> )							
	Cattle		Pig		Poultry		Sheep	
	Fresh	Stored	Fresh	Stored	Fresh	Stored	Fresh	Stored
<i>E. coli</i> 0157	(n=85) 467	(n=33) 306	(n=15) 3908	(n=9) 1296	ND	ND	(n=5) 782	(n=1) 5000
<i>Salmonella</i>	(n=25) 670	(n=12) 296	(n=8) 738	(n=1) 2000	(n=6) 12	(n=1) 1900	(n=2) 707	-
<i>Listeria</i>	(n=159) 213	(n=86) 554	(n=19) 113	(n=9) 414	(n=9) 420	(n=2) 110	(n=7) 198	(n=2) 159
<i>Campylobacter</i>	(n=88) 529	(n=38) 397	(n=17) 624	(n=6) 351	(n=11) 447	(n=2) 589	(n=5) 386	(n=1) 100
<i>C. parvum</i>	(n=39) 19	(n=10) 8.4	(n=17) 58	(n=3) 33	ND	ND	(n=5) 20	-
<i>G. intestinalis</i>	(n=12) 21	(n=4) 1	(n=3) 68	(n=1) 12	ND	ND	(n=7) 10	-

### 3.1.2 Septage

The key features of on-site waste treatment systems are the holding tank and the distribution system (Figure 3). For septic systems, the distribution of waste water is a series of perforated pipes, which form a weeping bed. The septic tank and the drain lines form the confined part of the system, but once the waste water enters the perforated pipe of the distribution system it is largely unconfined. Primary digestion of the solids in the waste takes place in the holding tank.



**Figure 3.** Key features of on-site waste treatment systems.

The basic septic system consists of a buried tank where waterborne wastes are collected and settleable solids are removed from the liquid by gravity separation; and a subsurface drain system where clarified effluent percolates into the soil. System performance is essentially a function of the design of the system components, construction techniques employed, characteristics of the wastes, rate of hydraulic loading, climate, areal geology and topography, physical and chemical composition of the soil mantle, and care given to periodic maintenance (Canter, 1988). One of the key concerns associated with the design and usage of septic tank systems is the potential risk of inadvertently polluting ground and surface water resources. Potential pollutants from septic tank systems are primarily those associated with domestic wastewater. The typical wastewater flow from a household unit is about 150-170 litres/day/person. Typical sources of household wastewater expressed on a percentage bases, are: toilets — 22-45 percent; laundry rooms — 4-26 percent; bath and shower rooms — 18-37 percent; kitchen — 6-13 percent and other (less common) — 0-14 percent (Canter, 1988).

The same groups of pathogens found in animal manure can also be present in human sewage, with very much the same species involved. In addition there can be a number of pathogenic yeasts and fungi (Table 5).

**Table 5.** Some pathogenic yeasts and fungi found in sewage (after Strauch, 1991).

Yeasts	Fungi
<i>Candida albicans</i>	<i>Aspergillus spp.</i>
<i>C. Krusei</i>	<i>Epidermophyton spp.</i>
<i>C. Tropicalis</i>	<i>Geotrichum candidum</i>
<i>C. Guillermondii</i>	<i>Phialophora richardsii</i>
<i>Cryptococcus neoformans</i>	<i>Trichophyton spp.</i>
<i>Trichosporon spp.</i>	

### **3.2 Critical Phases for the Protection of Water Resources from the Management of Manure and Other Biosolids Containing Pathogens**

#### **3.2.1 Confined systems**

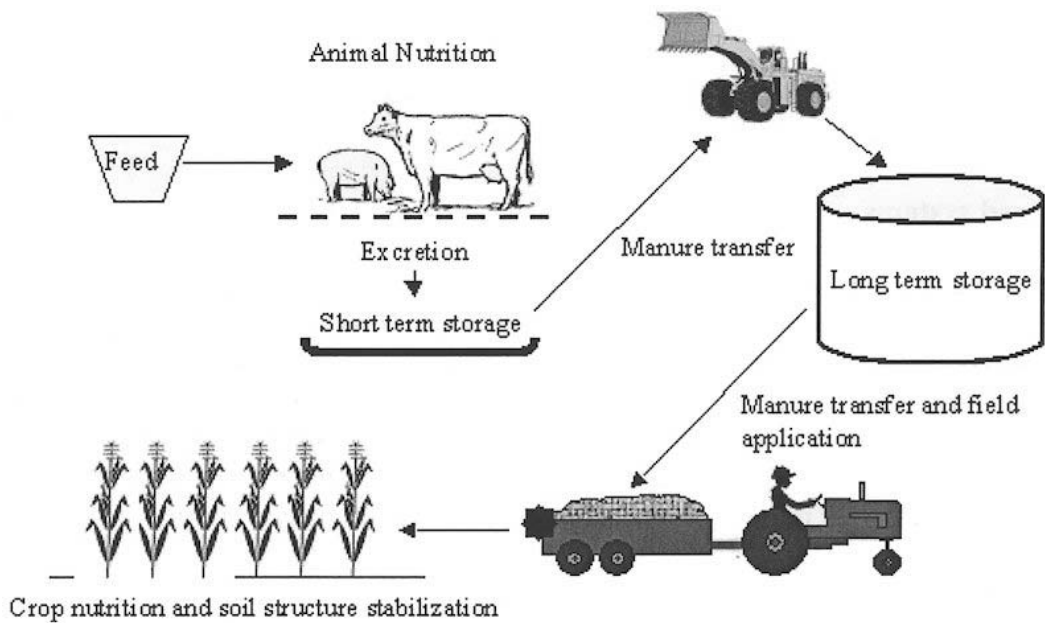
For housed animals as well as for on-site waste treatment facilities, faecal material is handled for some period under confinement, and any escape into the environment is largely because of a structural breakdown. The relative likelihood that pathogens are able to enter surface and ground water resources from these structures will be assessed. The focus will be on source locations associated with animal production facilities as well as on-site systems for milk house and domestic waste water. Sources following the land application of manure, sewage biosolids including septage and other organic waste material will be covered in Section 3.2.2.

##### **3.2.1.1 Manure**

The initial handling of the manure from confined animals is a major factor in determining the final consistency of the manure that will be spread on fields.

Feed and water for livestock are the sources for pathogenic bacteria and other microorganisms that are present in manure. If animals drink contaminated water, diarrhea can result, (Peer and Merritt, 1997) which modifies the concentration of materials within the manure. Water quality guidelines exist to protect animal health.(CCME, 1999). However, other than considering the transmission of pathogens between animals, there is little information on the impacts of animal manure on the quality of water for animal welfare. Manure management should, and increasingly does, start with the formulation of the animal diets (Figure 4). A properly designed diet provides all the nutrients and roughage required for growth, body maintenance, and reproductive capacity, while preventing unnecessary excess. Excess nutrients either pass through the alimentary tract and are excreted in the faeces or are absorbed and then removed from the body, together with metabolic breakdown products, via the kidneys. In mammals, materials removed from the bloodstream by the kidneys are excreted along with water as urine. Avian species conserve water, and waste products separated by the kidneys are voided through the same opening as undigested feed. The alimentary tracts of animals provide ideal environments for microbial growth, including species and strains that are parasitic or pathogenic in humans. Part of the microbial

population is voided along with the faeces. The fate of these microbes, as well as that of other components that can influence pathogens in the environment, is considered in this section. Most farm animals in Canada spend significant time in confinement or at least under cover, so that most manure (the mix of urine and faeces for mammals and the droppings of avian species) is deposited in barns or exercise yards. These locations provide an initial temporary store of the manure. In some cases, the manure is removed from the point of defecation and transferred into longer-term storage. Alternatively, it may be moved into short-term storage within the same area before being moved into longer-term storage. Sometimes, the manure undergoes treatment as part of long-term storage.



**Figure 4.** The main parts of a manure management system that are relevant to environmental contamination by manure constituents.

The fixed facilities, where manure is deposited or stored, therefore can be considered as distinct potential point sources for the contamination of the environment and of water resources in particular.

Feed and water for livestock are the sources of the mineral nutrients, metals, and pathogenic bacteria that are present in manure. Research aimed at improving feed utilization has, as one consequence, shown how the nutrient loading into manure can be modified by diet and how the form of N may change with the partitioning between urinary and faecal excretion. Diet can also affect the microbial activity in the hindgut of cattle, which could influence the survival of pathogens. The potential role of antibiotics in the diet (either at therapeutic or sub-therapeutic levels) in the release of pathogens into manure is of considerable importance.

### 3.2.1.2 Feed manipulation

The feed provided for farm animals largely determines the potential for the contamination of water resources with nutrients and biological oxygen demand (BOD), the latter being due to labile organic carbon compounds, in subsequent phases of manure management. Large variations in the nutrient content of manure can be related to differences in levels of animal performance, feed intake, type and quality of diet and feed management, and environment factors affecting water and food intake (AASE, 1998; Holter and Urban Jr., 1992; Van Horn *et al*, 1994). The amount of microbial production in the hindgut of animals depends on the availability of fermentable carbohydrates and protein (Orskov *et al*, 1970). Diets that have slower rumen degradability of carbohydrates or faster passage rates provide greater quantities of these materials. Pathogenic bacteria can infect animals through contaminated feed and water supplies (Herriott *et al*, 1998; Shere *et al*, 1998). Contamination can come from the manure of other herd members or from other animals such as rodents. Diet, or at least changes in diet, appear to influence the shedding of *E. coli* 0157:H7 (Russell *et al*, 2000).

Antibiotics are applied to treat disease, but may also be given prophylactically in feed, particularly for poultry, swine, and beef cattle, to improve the feed conversion to animal growth and production as well as to prevent and control disease. The antibiotics are used for both sub-therapeutic and for treating disease and include penicillin and tetracycline compounds. Ionophores, a type of antibiotic, depress or inhibit the growth of specific microorganisms in the rumen of cattle. This selective inhibition alters rumen processes, including changing the types of volatile fatty acids produced and decreasing the breakdown of feed protein. The improved animal performance associated with the use of ionophores results from the increased energy retention associated with the change from acetic acid to propionic acid production (Bergen and Bates, 1984).

These antibiotic compounds can also be excreted, but there is little information on how the loading of pathogens in manure or their later survival is affected or whether it affects their transfer to water resources. Strains of *Clostridium perfringens* in manure (both pig and cattle) were found to have a high resistance to antibiotics. Their spread through the environment has been related to land application of livestock waste (Van Stappen *et al*, 1990). Significant amounts of an ingested antibiotic can also be excreted in an active form (Gamal-El-Din, 1986). There is also evidence that some antibiotics can increase shedding of *E. coli* 0157:H7 (Gyles, 2000).

### 3.2.1.3 Excretion

Water resources become at risk from pathogens because they are released by animals in their excreta. The components of manure are important because of their possible impacts on survival and transport of pathogens released into the environment. Nitrogen is an important nutrient for living organisms. In freshly deposited manure, nitrogen is present as urea and

uric acid, which are very labile and are thought to change rapidly to ammoniacal nitrogen. Carbon compounds in feed are broken down during aerobic cell respiration to provide energy for the animals, but many are not readily metabolised, particularly by monogastric animals. The ability of ruminants to break down cellulose and complex starches in their alimentary tracts is associated with the likelihood that their manure will tend to contain more bacteria than that of non-ruminants. The risk of contamination of water resources depends, at least in part, on the level of containment where the manure is excreted.

#### **3.2.1.3.1 Excretion in confined or sheltered areas**

Once manure is excreted, any pathogens it contains become subject to environmental stresses that can affect their survival. Some can still reproduce even though they are outside the body of the host animal. Some, such as *Clostridium perfringens*, form spores while others enter a resistant phase in which they fail to form colonies when attempts are made to culture them (Davies *et al*, 1995). The concentration of nutrients and carbon substrates in manure are likely to change as the carbon compounds are used as an energy source by microbes.

Data on the amounts of nutrients in animal manure is well documented, but there is little information on the content of metals, pathogens, endocrine-disruptive compounds, and labile carbon compounds that can modify the survival and transport potential of pathogens to water resources. There is considerable seasonality in the release of pathogens, for example the largest numbers of *E. coli* 0157:H7 are shed into manure in July and August.

Up to 23% of ammonia released in a barn may come from animal urine (Pain *et al*, 1991). The release and volatilization of ammonia shortly after excretion reduces the final loading of N in the manure that is subsequently stored and eventually applied to the land. This can greatly alter the optimum loading of manure to fields and consequently change the number of pathogens applied to the land later in the manure management program.

Animal manure can be the source of pathogenic organisms such as bacteria (Tables 6 and 7), viruses, protozoa, and helminthic worms (Strauch, 1987). The microbial population in the animal alimentary tract comprises both long-term colonizers as well as more transitory strains. As a result it is not always easy to identify the source of a contamination event in the environment. Relatively few pathogenic organisms are found in manure compared with organisms that have no effect on human health. Furthermore, pathogens may be present in manure even if the animals present no symptoms, and a few infected animals can contaminate a whole source of manure (Strauch, 1988). Consequently the more animals on a farm, the greater the likelihood of pathogens being present in the manure.

**Table 6.** Pathogenic bacteria found in animal manure (after Strauch, 1988).

Manure type	Bacteria species
Cattle manure	<i>Brucella</i> sp., <i>Bacillus anthracis</i> (anthrax), <i>Leptospira</i> sp., <i>Mycobacterium</i> sp., <i>Escherichia coli</i> , <i>Clostridium perfringens</i>
Swine manure	<i>Brucella</i> sp., <i>Leptospira</i> sp., <i>Treponema</i> sp, <i>Clostridium tetani</i> , <i>Mycobacterium</i> spp., <i>Escherichia coli</i> , <i>Salmonella</i> sp...
Poultry manure	<i>Salmonella</i> sp., <i>Pasteurella</i> sp., <i>Clostridium</i> sp., <i>Listeria</i> sp., <i>Mycobacterium</i> sp.

**Table 7.** Frequency of detection of pathogenic organisms in cattle.

Source	Study description	Organism	Proportion of carriers
Pell A.N., 1997	US	<i>E. coli</i> 0157:H7	Faeces of mature cows: usually under 1% of the animals but as high as 5%
	Two national wide studies and two studies at state level (Wisconsin and Washington) of cow faeces		Calves under 24 months: 2.8%
Mafu <i>et al.</i> , 1988	Quebec, Canada	<i>Salmonella</i> sp.	18% of animals
	Faeces from slaughtered cows	<i>E. coli</i>	99% of animals
Atabai and Cory, 1998	UK	<i>Yersinia</i> sp.	18% of animals
		<i>Campylobacter</i>	37% to 81% of animals
Busato <i>et al.</i> , 1999	Switzerland	VTEC (verotoxin producing <i>E. coli</i> )	78% of the farms; 43% of the animals
	67 larger cow herds	<i>Campylobacter jejuni</i>	32% of the farms
		<i>Campylobacter coli</i>	19% of the farms; 3% of the animals
		<i>Yersinia</i> sp.	22% of the farms; 1.7% of the animals (infection limited to animals younger than 8 months)

#### 3.2.1.4 Initial handling of manure and its short-term storage

The current information available through the Canada Plan Service (CPS) shows the latest designs for slatted floors and short-term storage. The internal arrangements of pens, ventilation fans, animal walk alleys, feeders, and waterers are all aimed at keeping the defecation area as small as possible. The type, amount, and nutrient content of the bedding used and the amount of water added to manure from drinking water can modify the quality of the manure after defecation (Beauchamp, 1983). The consistency of manure depends on the type of animal as well as the feed mix, the water intake, and the amount of water and bedding combined with the urine and faeces. If the mixture contains less than 12% dry matter it can usually be handled as a liquid. Manure with a dry matter content of 10-16% may behave as a semi-solid material, making it difficult to handle. Above about 14% dry matter, manure generally behaves as a solid material and is more readily handled. Surface water courses need to be protected from any runoff that might carry manure from feedlots, exercise yards and solid manure storage pads. Many modern feedlots have lagoons to collect any runoff. Of the 229 manure spills recorded by the Ontario Ministry of the Environment (OMOE) that impacted surface water bodies in the Southwestern Region of Ontario between 1988 and 1999, 216 related to liquid manure systems and only three to solid manure systems (Blackie, 2000). Manure type was not recorded for the remaining spills.

Barn cleaning is aimed at improving animal welfare. However, alley flushing systems resulted in an 8-fold higher rate of contamination with *E.coli* 0157:H7 in dairy animals than was found for other cleaning systems (Garber *et al*, 1999). *Salmonella agona* was found in 84% of swine faecal samples in an open-flushgutter barn compared with only 9% from pigs on a partly slotted floor (Davies *et al*, 1997).

Once outside the host, viruses are unable to multiply. Their survival depends on the pH of their environment, temperature, and whether they are adsorbed onto suspended solids or embedded in them (Pell, 1997). Viruses tend to be inactivated more rapidly in summer than in winter.

#### 3.2.1.5 Long-term manure storage

Manure from confined animals has to be stored for at least part of the year. There are two basic storage methods:

- keep the manure as dry as possible and store as solid or semi-solid material;
- add cleaning water and produce a slurry that can be handled as a liquid

Implications for engineering and economics and the possibilities for treating the manure during storage differ markedly between solid and liquid systems (Barrington and Piche, 1992; Paul *et al*, 1992). Solid manure can be composted during storage or simply allowed to break down (Anon, 1991; Guidi and Poggio, 1987). Major changes in the composition and the form of the nutrient fractions can result (Baldwin, 1981; Ngoddy *et al*, 1971; Wild, 1988). Liquid manure can also undergo transformations, particularly resulting in the release of gaseous products (Webber and Lane, 1969). The consistency of liquid manure and the concentration

of nutrients may be further modified on transfer to long-term storage if wash water from barn or milk house cleaning is added.

There are various types of liquid storage. The majority are open top storage systems, considered to be the most economical construction. However, such storage facilities collect rain and snow while allowing free volatilization of ammonia, which again can have consequences for the loading of pathogens to fields during land application. Cracks in liquid manure tanks and earthen storages can lead to groundwater pollution, although this may be a relatively rare event (Roswell *et al*, 1985; Barrington *et al*, 1991). While clean water has been found to infiltrate through unsealed cracks into concrete storages from high water tables, the reverse flow was not as great (Barrington *et al*, 1987a; Barrington *et al*, 1987b). When manure with 10% solids was in the tank, the leakage was greatly reduced (by more than 10:1). Even though leakage was slow, the products remained in the soil through which they flowed (Barrington *et al*, 1991). Once all the soil surrounding a well became contaminated, it was not practicable to clean it up. Jofriet has developed new plans for the Ontario Ministry of Agriculture and Food (OMAF) which attempt to present best practices in the design and construction of concrete underground storage (Jofriet, 1992). Earthen storage, in areas with shallow bedrock, pervious soils, and shallow water tables, also endanger water supplies. Some townships require soils engineering to determine the depth of suitable soil; otherwise artificial liners are needed. Placing a storage tank above ground is not really a solution because of cost and the difficulty of filling and agitation. Particular concern for groundwater quality relates to clay-lined lagoon storage units located on sandy loam or loamy sand soils with shallow water tables (Ritter and Chirnside, 1990).

Unless properly constructed using impervious liners, manure liquids can leak into the subsoil (Barrington *et al*, 1991). If cracks develop in the walls of the liner after the lagoon has been emptied, newly added manure can seep out before solids can effect a reseal. Leaks can also develop if plant roots are allowed to penetrate the liner. Problems with liquid manure storage systems contributed 17% of the 229 listed manure spills mentioned above (Blackie, 2000). The failures of storages in terms of cracks or collapse, although small in number, are of concern. Cracks in storage walls have allowed manure to enter the soil. In the most prominent cases, manure entered a tile drain and flowed into a watercourse. Both earthen and concrete storages have been involved, but concrete storages were involved in the most prominent cases (Johnson and Hilborn, 1999). In 1999, Ontario Pork investigated 50 concrete storages for liquid manure. Eight of these warranted further detailed investigation to identify whether leakage or spills during transfer of manure to tankers was responsible for elevated nutrients in the soil close to the tanks. Further studies indicated that one of them was leaking, and either the tank or associated pipes were leaking in a second case. Spillage around storages during the transfer of manure to tankers being used in land application was identified as a more significant factor in ground water contamination (Rudolph, 2003). Fleming *et al*. reviewed the leakage of manure from storage facilities (Fleming *et al*, 1999). They concluded that as long as Ontario guidelines (OMAF, 1994b) were adhered to, significant leakage was unlikely from either concrete or earthen storage facilities because of the self-sealing properties of manure.

Engineering solutions are available to prevent problems associated with the transfer of manure from gutters in a barn to the long-term storage; this has been the cause of some spills. The size of storage has been an important issue in relation to water quality. Inadequate storage volume was involved in 34 of 38 manure spills associated with problems from stored manure in the Southwestern Region of Ontario between 1988 and 1999. Three times as many reports were related to concrete storage facilities as to earthen ones (Blackie, 2000). Too little long-term storage (e.g., storage capacity of less than 180 days) also requires the spreading of manure on partly frozen ground and risks endangering surface water supplies (see Timing of manure applications, 3.2.2.1.2).

Solid manure can be stored where it is produced and then transferred to the field. Such a system rarely allows storage for more than six months. Another possibility is to regularly transfer the manure to a concrete pad, which may also have sidewalls and a roof to keep out snow and rain. If the storage is not roofed, runoff might develop. This must be addressed, preferably by containment in a liquid storage. Some farmers still store solid manure in windrows directly on the soil. These piles can be leached by precipitation, and it is possible that pathogens could move to groundwater as a result, but the sealing properties of manure may filter out the relatively large particles that pathogens present.

Temperature also affects survival rate of pathogens, but not always in the same direction. Survival of many bacteria, including *E. coli* 0157:H7, is enhanced by cooler temperatures.

Liquid or slurry manure undergoes anaerobic decomposition unless it is artificially aerated. Solid manure undergoes mainly aerobic decomposition if loosely packed or anaerobic decomposition if tightly packed. Aerobic decomposition of manure organic matter results in the release of CO<sub>2</sub> and the formation of compounds that are more resistant to breakdown by microbes. When free oxygen is not present, organic matter is converted to low-molecular weight C-compounds, mainly volatile fatty acids (VFA). Methane gas (CH<sub>4</sub>) is also produced. VFA are a readily available carbon source for microorganisms under aerobic conditions.

The temperature, water content, and aeration status of manure are important for the survival of potential pathogens (Table 8). In solid manure stores, pathogens close to the outside of a pile may be subject to cooler temperatures than are those near the centre. Consequently, the former may survive, even if those at the centre do not, and form the source for contamination when spread on the land (Sutton, 1983).

The bacterial population in excreta undergoes considerable change during storage. Decomposition processes in manure are aerobic if free oxygen is present and anaerobic if free oxygen is not present. Aerobic microorganisms produce about 5.5 times more microbial biomass per unit of organic substrate than do anaerobic microorganisms. Both cattle slurry and poultry excreta contain a high density of microorganisms (Nodar *et al*, 1990). The concentration of microorganisms (number per unit volume) in these manures is about ten times greater than in pig slurry. At the beginning of slurry storage, the population of viable organisms in most microbial groups abruptly declined (Nodar *et al*, 1992). Denitrifying and sulphate-reducing microbes, together with algae, increased during this time. Thereafter, the

total population multiplied rapidly, becoming five-fold greater than the initial value after 14 weeks. The increase was mainly attributed to anaerobic bacteria (proteolytic, ammonific, amylolytic, anaerobic-cellulytic and anaerobic-nitrogen fixing species). Aerobic heterotrophic bacteria, actinomycetes, and fungi showed little change.

**Table 8.** Survival of potentially pathogenic organisms in manure (Wang *et al.*, 1996; M. Olson, 2000).

Organism	Survival under experimental conditions (days)					
	Frozen	5°C	30°C	Liquid manure	Compost	Dried
<i>E. coli</i>	>100	>100	10	100	7	1
<i>Salmonella</i>	>150	150	28	75	14	7
Campylobacter	50	21	7	100	7	1
Giardia	<1	7	7	300	14	1
<i>Cryptosporidium</i>	>300	50	28	>300	28	1
		5°C	22°C	37°C		
<i>E. coli</i> 0157:H7		70	56	49		

Rotaviruses are stable in faeces for up to nine months. The longevity of other viruses can be adversely affected by some bacteria present in manure. These bacteria have developed various strategies to inactivate viruses, including the formation of proteases (Pell, 1997).

*Cryptosporidium parvum* oocysts were found to survive in liquid manure storages, despite the high levels of ammonium (Fleming *et al.*, 1997). *Giardia* appears to be sensitive to freezing, whereas survival of other pathogens is enhanced. Temperatures above 30°C reduce survival times for these organisms, with the possible exception of *Giardia* (Table 8). None of the organisms appear to survive for long in dried manure.

### 3.2.1.6 Processing and treatment

There may be some risk to water resources from the processing or treatment of manure on farms. The microorganism content may also change during treatment (Tables 9, 10, and 11).

#### 3.2.1.6.1 Composting

Composting greatly reduces the bulk volume of the material, allowing economic transportation over greater distances than with untreated manure. While the basic requirements for composting are known, many on-farm operations do not achieve complete stabilization. Various recipes exist to mix the various carbon- and nitrogen-contributing materials. Applying raw (fresh or non-composted) manure to soil and allowing decomposition to occur in the soil adds more carbon, particularly in compounds that are readily assimilated by microorganisms.

This would be expected to stimulate the microbial population, thereby improving soil structural development and stability.

One benefit of properly controlled composting is that harmful bacteria and unwanted weed seeds can be killed (Table 9). However, it is important to ensure that all the material is subject to temperatures above 55°C, which is difficult in the absence of forced aeration (St. Jean, 1997).

Neither bovine enterovirus nor bovine parvovirus survived aerobic composting for 28 days. Temperature in the pile was maintained at 60°C from day 3 (Monteith *et al*, 1986).

It is difficult to ensure that all the manure reaches 55°C during the composting process, thereby killing all pathogens.

### **3.2.1.6.2 Other processing treatments**

Other processing treatments commonly address odour formation or gas production, including the volatilization of ammonia. Mechanical separation of coarse solids from slurry results in a material that can be stacked and composted. The liquid can also be treated more readily because crusts and solid settlement are less of a problem. Such liquids can be aerated in storage to reduce odour release during land application. Simply separating solids by passing a slurry through a mesh screen can have a significant effect on NH<sub>3</sub> volatilization. However, for cattle slurry, the solids need to be separated using a 0.1 mm mesh to reduce ammonia volatilization by 50% (Frost *et al*, 1990). Acidification of the same slurry to pH 5.5 decreased volatilization by about 85%. Read and Svoboda introduced *Cryptosporidium* oocysts to the liquid remaining after solids were separated from cattle slurry (Read and Svoboda, 1995). The material was kept at 15°C with minimal aeration. The dissolved oxygen in the liquid was 0%. The oocysts became non-viable after 4.1 days.

During biogas production (another option for processing manure), much of the manure-N is converted to the ammonium form and is still available in the residues from the process. The anaerobic digesters operate either at ambient temperatures, at which bacteria are not killed, or at elevated temperatures, at which pathogens do not survive if the minimum temperature is at least 55°C. However, the efficiency of digesters can be reduced if the manure contains levels of antibiotics concomitant with therapeutic doses of penicillin, ampicillin, tetracycline, oxytetracycline or chloramphenicol supplied in feed (Gamal-El-Din, 1986). Digestion at temperatures below 40°C may not control pathogens, and 10% of *E. coli* and *C. jejuni* may survive for periods in excess of 50 days (Kearney *et al*, 1993). However, bovine enterovirus and bovine parvovirus survived for only 30 minutes during thermal anaerobic digestion at 55°C, but at 35°C the enterovirus survived for 13 days (Monteith *et al*, 1986). Other treatments can ensure that manure reaches a sufficiently high temperature to kill pathogens, but in the past these approaches have been too expensive to establish on farms of the size typical in Canada.

The use of anaerobic digesters to produce methane for on-farm energy generation is more prevalent in Europe than in the United States. Germany alone has approximately 400 digesters, compared with 28 on U.S. farms. USEPA, USDA, and Dept. of Energy officials indicate that the relatively low cost of energy in the United States as compared to Europe make these options less attractive to U.S. farmers. Some European countries, such as Denmark, Germany, and the Netherlands, have quasi-government or commercial companies that operate centralized plants that accept organic waste material for anaerobic digestion. Some countries have imposed specific nutrient management regulations.

Denmark, Japan, the Netherlands, Sweden, and the UK regulate and limit application of animal wastes to agricultural lands. Denmark requires that farmers meet specific cropland acreage-to-animal ratios. USEPA officials have investigated municipal sewage treatment technologies for the treatment of wastewater and sewage from large dairy and hog operations. They concluded that the technologies would require significant modifications to handle the more concentrated wastes from farm operations. Also, the capital investment and operating and maintenance costs would be very high. The construction of such on-farm treatment plants may require financial assistance, as is often the case for municipal facilities.

Another process that has been employed is a lime treatment (addition of quick lime or slaked lime) to raise the pH to 12 for at least 2 hours (Tables 9 and 10).

**Table 9.** Effect of treatment on the survival of bacteria (Millner, 2003).

Treatment	Log Reduction	Stress
Lagoon	1-3	time
Constructed Wetland	2-3	time, filtration
Deep Stack	1-?	NH <sub>3</sub> , heat
Digestion -Mesophilic	1-2	time; heat
Digestion -Thermophilic	5	
Composting	1-5	heat, time
Air Drying	1-2	dessication
Heat Drying	4-5	heat, dessication
Pasteurization	5	time, heat
Alkaline Process	3-5	heat, NH <sub>3</sub>

**Table 10.** Effect of manure treatment on the reduction in viruses (Millner, 2003).

Treatment	Log Reduction	Animal Systems
Lagoon	1-3	beef, dairy, swine
Const. Wetland	1-2	beef, dairy, swine
Deep Stack	?	poultry, beef, dairy
Digestion - Mesophilic	1-2	beef, dairy, swine
Digestion -Thermophilic	3->4	
Composting	1-5	all
Thermal Process	3-4 @ 55-60°C, >4 @>60°C	all
Drying and Dry Storage	<1 at >3% Moisture, >3 at <1% Moisture	all
Alkaline process	1-2 pH<11, 3-4 pH>11	all

**Table 11.** Effect of treatment on the survival of parasites (Millner, 2003).

Treatment	Cryptosporidium	Giardia lamblia	Ascaris suum
Digestion - Aerobic	0	+++	+++
Digestion -Anaerobic	Unknown	+++	+++
Composting	Unknown	Unknown	0
Lagoon Storage	Unknown	Unknown	0
Air Drying	Unknown	Unknown	Unknown
Lime Stabilization	Unknown	+++	0

### 3.2.1.7 Other Sources of Pathogens Associated with Animal Agriculture

With the advent of charges for animal removal by rendering companies, alternate methods of disposal have had to be found.

No studies on movement of pathogens (or general bacteria, chemicals or salt) from deadstock burial were found in the literature. However, the British Geological Survey conducted a study in Wolverhampton at the Danescourt cemetery in 1999 and found faecal streptococci and *Staphylococcus aureus* in high concentrations in the groundwater under the cemetery (Trick *et al.*, 2001). Though deemed heavily contaminated by the WHO, the bacteria are not expected to survive long enough to travel far from the cemetery. A Portuguese study in Sao Paulo found that ground water under a cemetery was contaminated by bacteria (including *Clostridium perfringens*). The areas with the highest concentrations of bacteria were predominately recent (less than one year) burials, located in areas with a shallow water table. The bacteria did travel a few metres, but with increasing distance from the grave, the

numbers dropped. Viruses appeared to have a higher mobility than the bacteria (Pacheco and Matos, 2002).

Composting of dead animals has been a disposal method on poultry farms in the United States since the 1980's and was later adapted to swine (Keener *et al.*, 2000). Composting became legal in Canada in 1994 but the practice has not become common (Walker, 2001). Recently this method of disposal has been extended to include cattle and sheep (Keener *et al.*, 2000).

Once finished, compost is land applied like manure. Thus, potentially providing another path for the entry of pathogens to the environment. Though dependent on the type and size of the carcass, composting generally consists of a mix of animal manure (litter), carcasses and carbon rich source (corn stalks, wood shavings, straw) in a primary composter. There are two to three composters, generally, in this process. The carcasses are placed in the first bin and after three months are moved to the second bin, thus allowing the material to be turned. Again the carcass is composted for three months. If after this time there is still mortalities showing, the compost is transferred to the third bin.

The ratios of carcasses and carbon sources vary with animal type, size and regulating body. OMAF recommends a one-one ratio by weight of carcass to carbon source (Morris *et al.*, 1997). The carcass is the main source for nitrogen and moisture in the process. Temperature is key to the degradation of the carcass. The compost must be kept at 55°C (130°F) for three consecutive days for proper composting. Pathogens are assumed to be destroyed by the combination of time and temperature within the composter. However, the temperatures may not be effective along the edges of the primary bins. Without proper mixing, pathogens may survive (Collins, 1996). Additionally, if carcasses are loaded along the sidewalls of the bins, putrefaction will occur and composting will be poor. In a 6-month old compost pile, *E. coli* was found on the outer layers, but there was no recovery deeper into the pile than 20 cm (Murphy and Carr, 1991).

Once finished, compost is then spread on fields as an additional source of nutrients. The material can be stored in solid manure facilities until needed (Morris *et al.*, 1997).

Composting of deadstock may be a current source of pathogens transported to groundwater, especially as the methodologies have not been strictly defined and the method has no true form of regulation.

### **3.2.2 Unconfirmed Systems**

This section deals with the risks from pathogens associated with the management of manure and other biosolids once they are no longer contained in buildings, pipes or storage facilities. It includes risks associated with the application of organic waste to soil as well as to the risk from on-site waste treatment systems once the material enters the soil.

Models we have are common to both transport of pathogens from land application of sewage

and the land application of manure.

### **3.2.2.1 Direct deposition and application of manure to the land**

While some manure reaches the soil by direct deposition from grazing animals, the majority is applied as part of the fertilizer requirement for crops. The various stages in manure management to this point determine the likelihood that pathogens will be present. They also determine the form and concentration of carbon compounds and mineral nutrients that reach the land, thereby influencing the survival and transport of pathogens from the soil to water resources.

#### **3.2.2.1.1 Direct excretion into water resources**

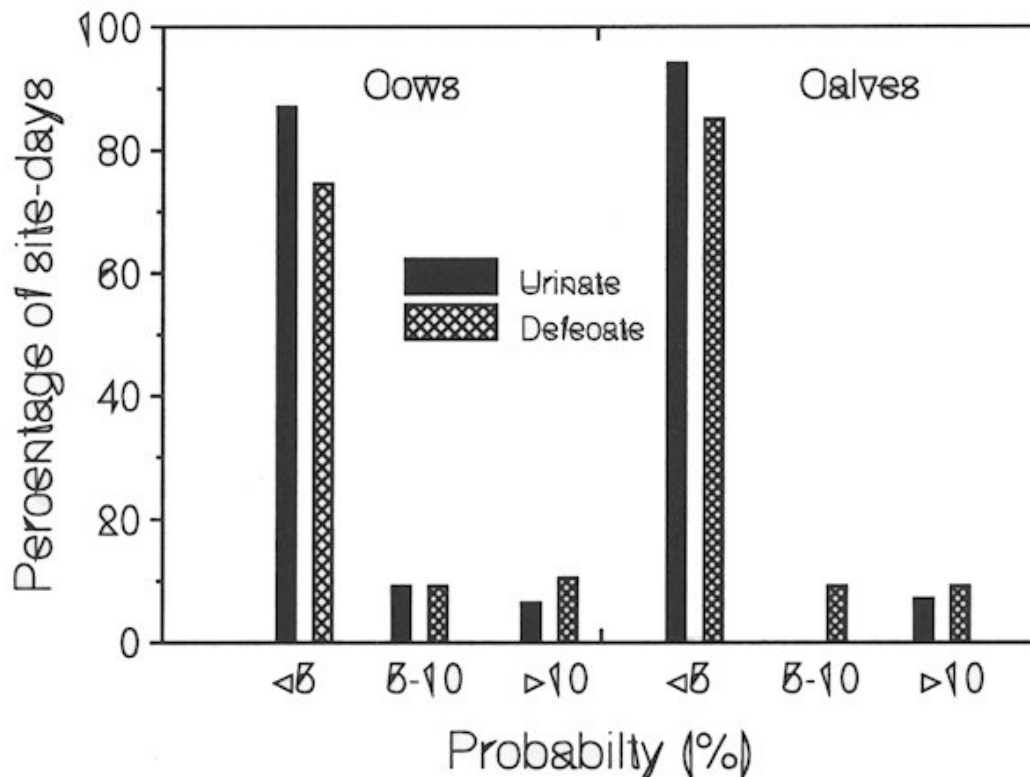
If animals have access to water and defecate directly into it, the resource will be contaminated. The risk can be lessened by reducing the opportunity for animals to access rivers, streams and other flowing water courses to drink. Seasonal behavioural studies (Duncan *et al*, 1998; Gary *et al*, 1983; Veira *et al.*, 2003) show that animals do not spend extended periods of time in the water and usually void little urine or faeces there (Figure 5) However, this normal pattern was not always followed, and on one day at each experimental site, considerably more direct defecation did take place (Duncan *et al*, 1998). Once voided, bacteria rapidly become attached to sediment on the stream bed, where they can survive for at least two months (Davies *et al*, 1995). Few are present in the water beyond 50 m from the point of entry (Whiteley, 1998).

However, bacteria have been found at a concentration of 10/mL almost 20 km downstream from a source of contamination (Feresu and Van Sickle, 1990). Access to streams also allows animals to disturb the sediment, causing the release of coliforms and other bacteria into the water. These coliforms likely originate from earlier direct defecation into the stream, in runoff and sediment from the adjacent fields, or from other sources such as waste treatment plants. Enhanced flow associated with major rainstorms also moves bacteria downstream (Whiteley, 1998). Alternative methods to keep cattle away from water courses have been investigated. Fencing is effective but expensive. Providing drinking water in the field discourages access, as does preventing the animals from forming trails along stream banks. Providing shade away from streams may also help (Duncan, 1996). Constructing low-level crossings at locations normally used by animals to enter water can prevent collapse of banks and the disturbance of sediment.

Once in surface water, the survival of *E. coli* (ETEC), *Campylobacter jejuni* and *Yersinia - enterocolitica* is such that this could be a persistent site of transmission between animals and humans (Terzieva and McFeters, 1991). If drinking water wells in shallow aquifers are poorly maintained or badly located, they can be impacted by surface runoff. There is evidence of a child being infected with *E. coli* 0157:H7 from dairy cattle through drinking water from such a well (Jackson *et al*, 1998).

### 3.2.2.1.2 Application to land

Most manure is applied to cropped land (Baldwin, 1981), though there is encouragement to apply dairy slurry to grassland. Solid manure lends itself only to surface spreading which then requires a second tillage operation for incorporation. Liquid manure can be spread from a tanker, applied by irrigation, or injected using hollow tines. After spreading, liquid manure may also be incorporated by tillage. Application of liquid manure adds solids plus water, thereby increasing soil water content. This effect may be sufficient to result in flow through tile drains. Applying solid manure causes little change in soil water content. Almost all of the incidents of water course contamination in the Southwestern Region of Ontario between 1988 and 1999 were related to land application of manure (Blackie, 2000). Crane *et al.* (1983) tabulated results from a number of studies reporting the concentrations of faecal bacteria found in surface runoff from land after manure applications. They concluded that land application of organic waste can significantly increase bacteria contamination of surface water from runoff, especially if farmers do not follow wise management options and safety precautions. Results from the *Ontario Farm Groundwater Quality Survey* indicated that farmstead drinking water wells were more likely to be contaminated where manure was spread (Goss *et al.*, 1998a; Rudolph *et al.*, 1998b).



**Figure 5.** Probability that cows and calves will void urine or faeces into a stream (Duncan, 1998)

Only a small fraction of sewage and other organic biosolids that are produced annually are applied to agricultural land as part of regular agronomic practices, but their characteristics have much in common with animal manure.

### *Transportation of manure to sites of application*

Liquid manure is transported via pipelines, tanker-trailers, or custom truck-spreaders. Liquid biosolids are transported by tanker trailers. Equipment manufacturers have increased tanker size to meet market demands. The mass of tanker and contents often exceeds the capacity of the tractor brakes to stop a fully loaded unit, which could lead to a spill. Semi-solid manure is not easily transported and can result in spillage in transit. Solid manure and biosolids are somewhat easier to transport. The cost of transportation is high because of the large volume-to-weight ratio and the relatively small concentration of nutrients. Poultry manure tends to be the exception, and poultry producers have greater opportunities to have the manure taken by other farmers. The cost of transportation has also resulted in manure being spread more regularly on fields close to the barn or storage than on more distant fields. The nutrient levels, particularly of nutrients such as phosphorus that are less mobile in the soil, can become excessive if such practices have continued over many years. The consequences for the survival and transport of pathogens have not been explored. Nutrient management strategies are designed to ensure that excess nutrients are not applied to the land, thereby reducing the risk to water resources, but the potential impact on the loading of pathogens has not been considered. Furthermore, implementing such strategies also means that manure needs to be transported farther from the storage sites, which may mean increased transportation from one farm to another. Transportation of manure to the field has been a factor in some manure spills, but it is the application, mainly of liquid manure, that is the most frequently reported cause of manure and associated contaminants entering surface water bodies.

### *Impacts of application techniques*

There are three main methods of application: broadcasting (solid, semi-solid, and liquid manure), irrigation, and injection (liquid manure). The mode of application has the greatest effect on the amount of ammonia that is lost by volatilization during this phase. Importantly, the more nitrogen lost through this route, the less that is potentially available to be lost to water resources, but the impacts on the environment are more extensive, including the movement of pathogens, and associated odour issues greater. Liquid manure is applied to the soil surface of arable land either from a tanker (broadcasting) or by using a sprayer linked to pipes that are connected to the storage system (irrigation).

Broadcasting has traditionally used a splash-plate to distribute the manure, but low-level or low-pressure nozzles on booms are increasingly in use. These give a more even distribution or can be used to apply the manure in bands between rows, thereby reducing odour release, but the impact on pathogen movement has not been investigated. Liquid manure can also be directly injected below the soil surface (injection), using hollow tines preceded by coulters to cut through crop residues. The injector system can be mounted directly behind a tanker or set on a tool bar connected to the three-point hitch of a tractor and linked to a stationary

tanker via a flexible hose.

Broadcast application of liquid manure from a tanker has resulted in fewer than a third of the problems encountered when using spray irrigation, a practice that is declining in popularity. Failure of equipment associated with the land application has been the cause of 27% of manure spills that resulted in contamination of water courses in the Southwestern Region of Ontario between 1988 and 1999 (Blackie, 2000).

The techniques for both irrigation and injection are well developed, and manufacturers continue to improve the equipment for surface-spreading liquid manure from tankers. They are improving the uniformity of application, which also helps to reduce odour. Uniform distribution is essential if farmers are to rely solely on the nutrients contained in manure for their crop's nutrient needs. Uneven application is likely to result in localized ponding and hence increased likelihood of preferential flow. This in turn will enhance the possibility that ground water will be impacted by pathogens (see Section 3.2.6). Flow metres enable operators to apply liquid manure more judiciously. Furthermore, the possibility of surface runoff immediately after application is greater with surface spreading than injection. Incorporating manure by tillage immediately after application dramatically reduces runoff losses.

Compared with broadcasting, injection requires greater tractor power and less manure can be applied per hour. Therefore, cost and the small window of time available to most farmers in the spring often limit the potential use of injection. Poor distribution patterns result from all types of manure spreaders due largely to the nature of the material. Using injection on rolling topography has also resulted in problems. The most frequently reported route by which liquid manure can contaminate surface water courses is in outflow from tile-drain systems.

Fleming and Bradshaw identified macropore flow of manure liquids into subsurface drains after spreading (Fleming and Bradshaw, 1991; Fleming and Bradshaw, 1992a; Fleming and Bradshaw, 1992b). Pre-tillage tines have been incorporated into injection machinery to limit macropore flow (J. Houle & Fils Inc., Drummondville, Quebec; Husky Farm Equipment Ltd., Alma, Ontario). Large tankers can cause problems with soil compaction in the field. Compaction is a significant concern because it can increase surface runoff as well as decrease crop yield. This problem is also being reduced by new machine design (e.g. tankers with tracks now made by Husky Mfg.). Solid manure is applied by spreader machines that propel the manure to the rear or to the side. Although new machines operate effectively, the spread tends to become less uniform with use. Hawkins (personal communication, November 2002) has developed an improved system that gives uniform spreading with well-used beaters. The more variable nature of solid manure also tends to reduce the uniformity of nutrient application.

In addition to the choice of application method, producers also have to make decisions on the timing of their land application. Factors to consider include the risks from soil compaction, likelihood of runoff, and nutrient loss through ammonia volatilization. The timing of manure applications is critical for the availability of nitrogen both to crops and on the potential for

environmental impacts. As manure storage on many farms is limited, the common periods for application are the fall, winter, and spring. In spring, applications may be as a pre-plant fertilization or as a side- or top-dressing. The experimental evidence shows that compared with spring applications, manuring land in fall or winter results in longer survival of bacteria. Although it is impossible to predict accurately the fate of manure constituents following land application of manure, the development and use of agricultural best management practices could be expected to minimize the risk of environmental contamination.

Evidence from the Ausable-Bayfield Conservation Authority indicates that bacteriological contamination from tile drains can be greater after injection than after surface spreading (Foran *et al*, 1993). Transport of bacteria in surface runoff was similar for surface spreading, incorporation, or injection of manure (King *et al*, 1994). When the system was modified by placing a cultivating tine ahead of the injector tine, there was a significant reduction in bacterial transport through runoff. Pre-tillage of soil before spreading liquid manure minimized the direct impact of manure on the quality of tile-drain effluent (Fleming and Bradshaw, 1992b). Bacteria from poultry manure were not detected in runoff when the manure was applied to bare soil, but was present when the manure was applied to grassland (Giddens and Barnett, 1980). In the first day after applying liquid manure, more bacteria may be lost in overland flow from no-till land than from ploughed land, but the rate of decline in the concentration of bacteria in the runoff water can also be greater (King *et al*, 1994).

#### *Timing of manure applications*

When determining when to apply manure, producers have to consider several factors including the risk of soil compaction, likelihood of runoff, and nutrient loss through NH<sub>3</sub> volatilization. The timing of manure applications is critical both for the availability of nitrogen to crops and for potential impacts on the environment.

As manure storage on many farms is limited, the common periods for application are the fall, winter, and spring. In spring, applications may be as a pre-plant fertilization or as a side- or top-dressing. The experimental evidence shows that compared with spring applications, manuring land in fall or winter results in lower recovery of applied nitrogen by the crops and greater risk of leaching or surface runoff and denitrification (Table 12) (Thompson *et al*, 1987; Goss *et al*, 1995a). Current guidelines indicate that manure should "not be spread on frozen or ice-covered soil." If the soil is unfrozen, then winter spreading should not occur on land with more than a slope of 3%. In an emergency, winter spreading is permitted, but only on land with residues or vegetation and only where there is no danger of runoff or flooding.

Fleming and Fraser (2000) have reviewed the literature on winter spreading of manure. In general, winter spreading of manure results in greater nutrient losses than at other times. As many soils are impervious when frozen, manure spread on the surface is likely to be carried off in runoff from snow-melt or rain. The likelihood of surface runoff does not appear to differ whether the manure is spread on frozen soil or snow or onto a cover crop. The effect of slope has received little critical attention. Losses to the environment depend on whether the first snow-melt or rainfall event results in runoff or infiltration, which is greatly influenced by

weather factors. However, solid manure may reduce the amount of runoff. Clearly, as current weather patterns are critical, it is difficult to predict whether there will be significant environmental contamination in any one winter. Local weather records might be used to identify locations where the risks are greatest, but Fleming and Fraser (2000) concluded that the evidence supports the discouragement of winter spreading.

**Table 12.** Sinks for N following application of slurry in three treatments to grassland in winter and spring in the UK - results corrected for the appropriate control plots. Values in parentheses are the amounts of N expressed as % of the total N applied. Data from Thompson *et al.* (1987).

Application	Nitrogen Sinks <sup>†</sup>			Σ Sinks
	Apparent Recovery in Herbage	NH <sub>3</sub> Volatilization Loss	Denitrification Loss	
<i>Winter Experiment</i>				
	kg N ha <sup>-1</sup> (%)			
Surface spread slurry	49.0 (19.8)	77.1 (30.8)	29.9 (12.1)	156.1 (62.9)
Injected slurry	82.7 (33.4)	2.1 (0.9)	52.7 (21.3)	137.5 (55.4)
Injected slurry <sup>†</sup> nitrapyrin	90.1 (36.3)	2.1 (0.9)	22.7 ( 9.2)	114.9 (46.3)
CV <sup>‡</sup>	17.0% -	25.3% -	98.2% (42.6%)	--
<i>Spring Experiment</i>				
Surface spread slurry	66.9 (25.5)	53.0 (20.2)	4.5 (1.7)	124.4 (47.5)
Injected slurry	93.9 (35.5)	2.4 (0.9)	17.7 (6.8)	114.0 (43.5)
Injected slurry <sup>†</sup> nitrapyrin	109.9 (42.0)	2.4 (0.9)	14.0 (5.3)	126.3 (48.2)
CV <sup>‡</sup>	13.8% -	21.1% -	182% (74.8%)	--

† In both experiments leaching losses from all treatments were negligible

‡ Coefficients of variation determined as follows:

Apparent recovery: from the total apparent recoveries for each of the four plots for the three treatments in each experiment.

NH<sub>3</sub> volatilization: from the total NH<sub>3</sub> loss determined for each of the three tunnels used for the surface application treatment.

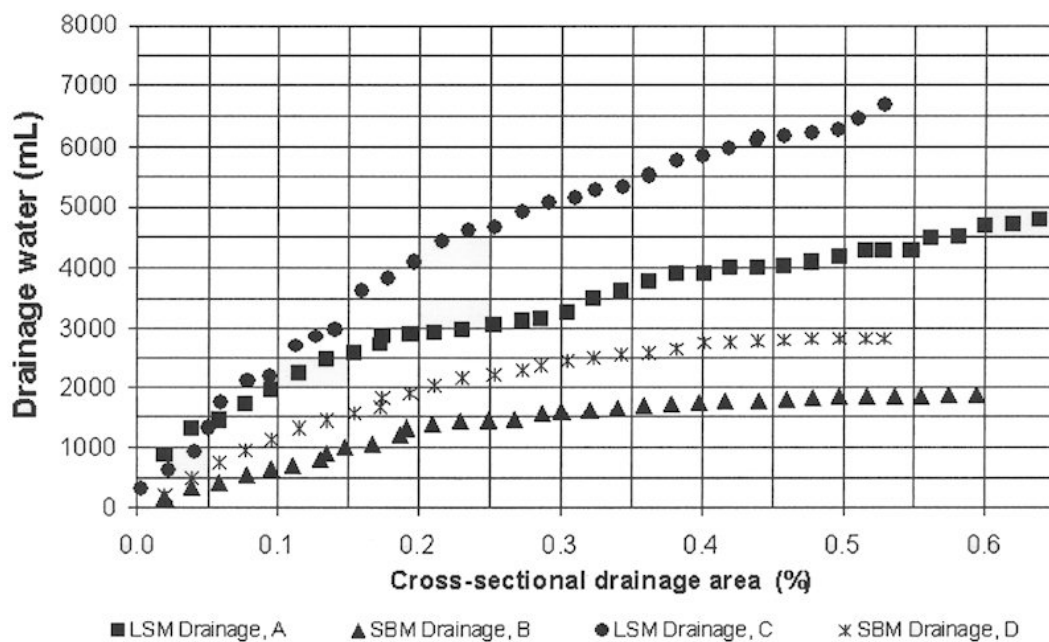
Denitrification: the average coefficient of variation for all denitrification measurements in each experiment. In parenthesis the average for values greater than 0.10 kg N ha<sup>-1</sup> d<sup>-1</sup>.

### 3.2.2.2 Retention of pathogens applied to the soil in manure and biosolids

While many pathogenic microorganisms can survive outside host environments, their potential for movement to, and eventual contamination of, water resources by microorganisms depends on their concentration in manure and biosolids at the time of application and their survival.

A number of aspects influence the survival of non- indigenous bacteria following land application of manure: properties of the soil, availability of nutrients, including carbon, and interactions with soil biota (Abu-Ashour *et al*, 1994b). These interactions include competition with and predation by indigenous soil micro- and meso-organisms (for example, earthworms can reduce bacteria populations). Populations of microorganisms are dynamic — they are influenced by factors that affect their survival. Many are also motile. The application medium also affects the survival of bacteria: Ostling and Lindgren (1991) found that 20-40 times more indigenous *Bacillus* spores were present on manured crops than on un-manured crops, and these numbers remained constant with time to harvest. However, bacteria originating in the manure itself, such as *Clostridium*, some coliforms, and *E. coli*, all declined with time after manure application. Thelin and Gifford showed that if a sample of freshly voided manure was subject to water from a rainfall simulator within 5 days, the concentration of faecal coliform bacteria in runoff was in the order of  $10^4$  mL<sup>-1</sup>, but this number declined to 400 after 30 days (Thelin and Gifford, 1983).

The distribution of faecal bacteria following land application and subsequent rainfall simulation was studied by Unc (2002) in four undisturbed clay loam cores (Figure 6). The majority of bacteria (up to 80%) applied with liquid swine manure was transported through the 50cm length of the cores and was recovered in the drainage water. On the other hand a significant proportion of the faecal bacteria applied with the solid beef manure was retained within the manure matrix.



**Figure 6.** Proportional partitioning of faecal coliforms following manure application and irrigation through four undisturbed clay-loam soil cores (45 cm in diameter and 50 cm in length).

The survival of pathogens in water and soil is very variable (Tables 13 and 14), because of both differences between species and the resilience of different individuals and groups. Among bacteria, many Gram positive organisms form resistant spores, whereas in Gram negative organisms physiological adaptation to environmental stress may involve the reduction in cell size and metabolic rate (Roszak and Colwell, 1987). Some pathogenic bacteria can survive in a dormant state in soils and water, and cannot be grown in conventional media, being viable but non-culturable (Byrd *et al.*, 1991). Sunlight reduces the longevity of bacteria in water, and factors, such as erosion, that decrease the transmission of light because of increased suspended solids tends to increase survival time. Hence, turbidity appears to be important for the survival of some pathogens in water supplies (Aramini *et al.*, 2000). *Campylobacter* survival rates in freshwater can be up to 4 months with survival greatest at 4°C (Rollins and Colwell, 1986; Thomas *et al.*, 1999). Survival time, however, is highly dependent upon strain type, previous growth conditions, water quality, and environmental conditions (Buswell *et al.*, 1998, 1999). Two studies on the survival of *E. coli* 0157:H7 in animal faeces found that the pathogen can persist for more than 30 days at 22-23°C and survival is extended at the lower temperature (4°C). In addition, *E. coli* 0157:H7 survived for 21 months in a manure pile stored outside under fluctuating environmental conditions (Kudva *et al.*, 1998). Survival of the pathogen was less in faecal slurries and was undetectable after 5 days of incubation at 23°C (Kudva *et al.*, 1998).

**Table 13.** Survival time of pathogens in water and sewage at 20-30°C  
(Based on Feachem *et al.*, 1983).

Pathogen Group	Example	Survival time in fresh water and sewage (days)
Bacteria	Faecal coliforms	< 60 but commonly <30
	Salmonella	< 60 but commonly <30
	Shigella	< 30 but commonly <10
Viruses	Enteroviruses	<120 but commonly <50
Protozoa	Cryptosporidium (oocysts)	>360
Helminths	Ascaris lumbricoides (eggs)	>180

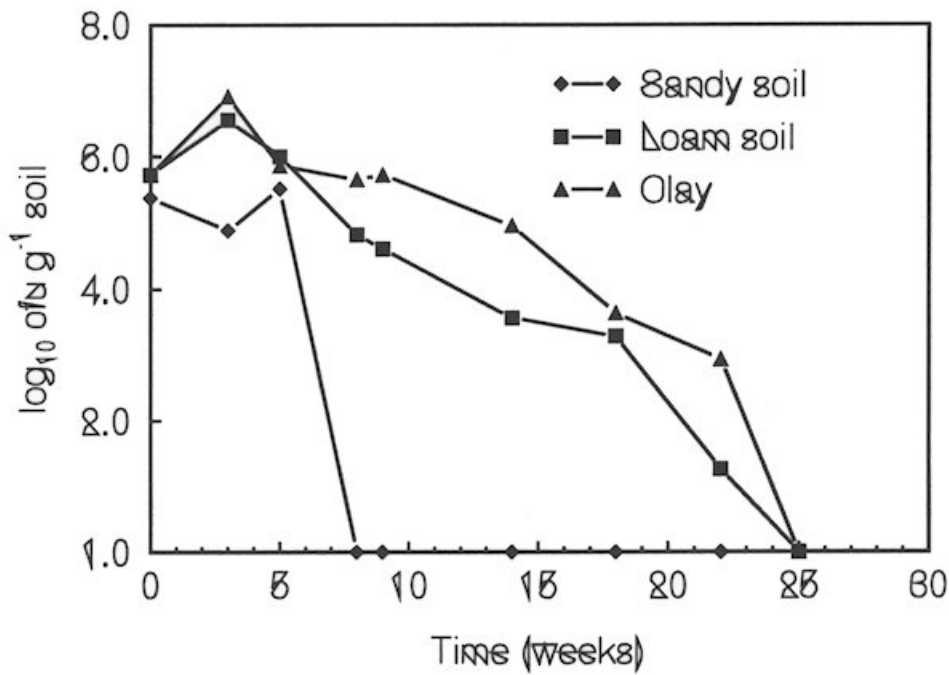
**Table 14.** Survival time of pathogens in soil at 20-30°C (Based on Feachem *et al.*, 1983).

Pathogen Group	Example	Survival time soil (days)
Bacteria	Faecal coliforms	< 70 but commonly <20
	Salmonella	< 70 but commonly <20
Viruses	Enteroviruses	<100 but commonly <20
Protozoa	Cryptosporidium (oocysts)	>360
Helminths	Ascaris lumbricoides (eggs)	>180

Soil parameters that modify survival of non-indigenous bacteria include soil pH, soil water content, organic matter content, soil texture, temperature, availability of nutrients, adsorption properties of the soil (MacLean, 1983). MacLean found that soils containing clays with a large surface area can adsorb bacteria. Bacteria can survive longer in cold soils than in warm soils, and longer in fine textured than in coarse-textured soils. The survival in soil of faecal coliforms, particularly *E. coli* (and including *E. coli* 0157:H7) has received particular attention. In cold soils (<5°C) the bacteria can survive for up to 100 days. Survival of *Enterococcus spp.* Was longer than that of *E. Coli* at 5°C, while the opposite was true at 15° C and 25°C (Cools *et al.*, 2001).

However, these authors also reported that while *Enterococcus spp.* survived longer in loamy soils than sandy soils at 25°C, the reverse was true for *E. coli*. Gagliardi *et al.* (2000) reported that *E. coli* 0157:H7 was able to replicate in and migrate through cores of various soil types. Numbers of the pathogen in leachate correlated with ammonia and nitrate levels, and the numbers exceeded inoculum levels in all treatments (i.e., soil types, tilled and no till, and rainfall amounts) except in intact clay loam cores (Gagliardi *et al.*, 2000). Survival of non-pathogenic *E. coli* exceeded 60 days at 25°C and 100 days at 4°C (Bogosian *et al.*, 1996) and may be extended beyond that by residing within soil protozoa (Barker *et al.*, 1999). Survival periods are shorter in coarse-textured soils than in finer-textured soils (Figure 7).

It is likely that at least part of the effect of texture is related to the water-holding capacity of these different soils (Sadovsky *et al.*, 1978). Cools *et al.* (2001) found that the g-1t survival of *E. coli* and *Enterococcus spp.* occurred in soils close to field capacity. Mubiru *et al.* (2000) showed that at the same gravimetric water content, the matric potential was lower in a silt loam with a clay content of 0.25 g. g<sup>-1</sup> than one with 0.12 g. g<sup>-1</sup>, and the survival of *E. coli* and *E. coli* 0157:H7 was also shorter. *Campylobacter* species appear to have somewhat shorter survival times than *E. coli*. *C. jejuni* survived in soil for at least ten days but this number could double when the ambient temperature decreased to 6°C (Lindenstruth and War, 1948).



**Figure 7.** Survival of *E. coli* 0157:H7 in soils of different texture. Redrawn from Fenlon *et al.*, 2000.

Sunlight can reduce the survival of bacteria in soil directly through the effect of ultraviolet light and as a result of drying (Sinton *et al.*, 1999; Gerba and Bitton, 1984; Mubiru *et al.*, 2000). *Campylobacter* has been shown to be able to remain viable in a range of environments at 4°C for up to 7 days (HMSO, 1993). Other environmental constraints include its inability to tolerate desiccation, low pH (< pH 5), exposure to O<sub>2</sub> and ultra violet light (HMSO, 1993).

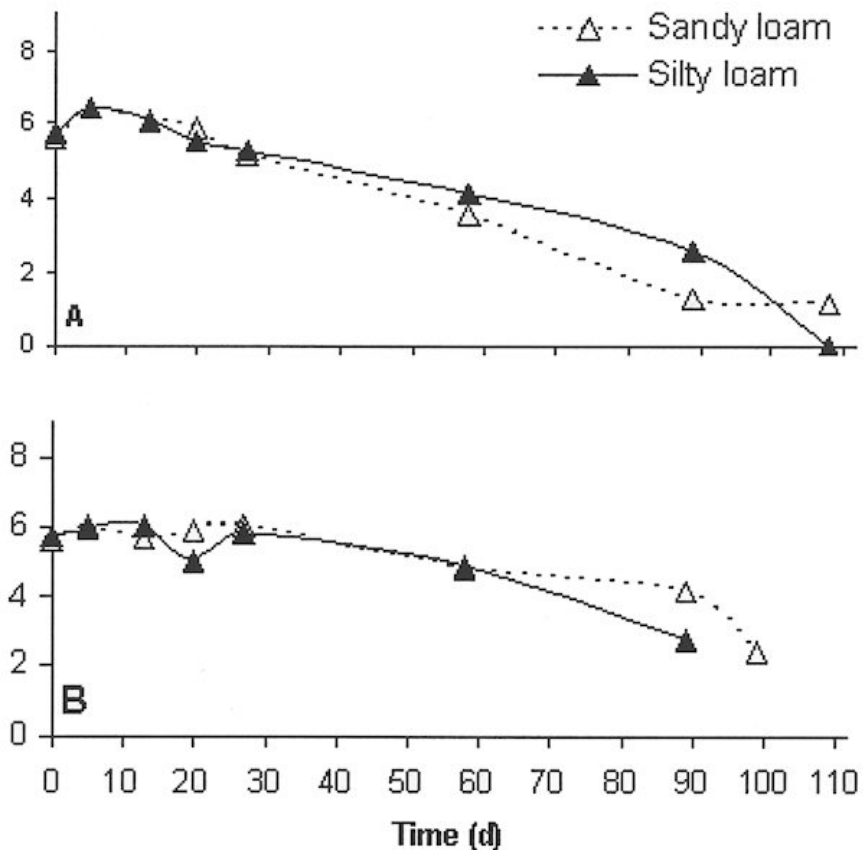
Survival of viruses also appears to depend on the water content of soils, as they are inactivated by dessication (Yeager and O'Brien, 1979). As with bacteria, soil texture, pH, and organic matter content all affect the longevity of viruses in soil (Gerba *et al.*, 1975). Viruses near the soil surface are rapidly inactivated by the combination of stresses imposed by sunlight, soil drying, predation, and other soil-based factors such as pH. Kowal (1985) reviewed the literature on virus survival. Moisture content appears to be a major factor once the virus has penetrated the soil surface. About 100 days is the longest survival time reported for enteric viruses.

Cools *et al.* (2001) reported that increased organic matter levels enhanced the survival of coliform bacteria, and suggested that this might be related to a variety of factors that organic matter influences. These include water retention, formation and stabilization of soil aggregates, and the formation of microhabitats.

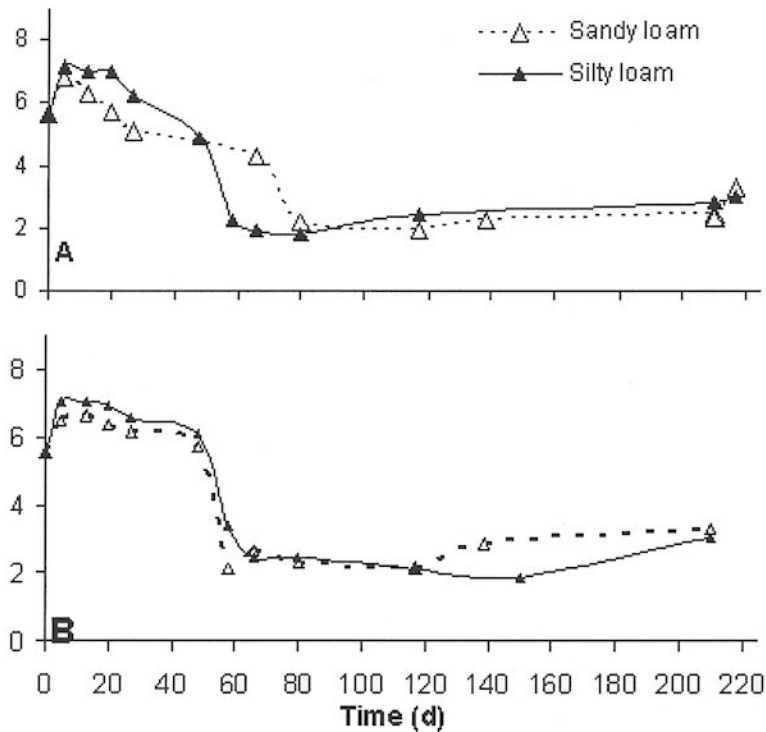
The water availability overrides the impact of other factors (Gerba and Bitton, 1984, Mubiru *et al.*, 2000). The initial interaction between faecal material and soil, which takes place as a result of land spreading, can have a marked effect on the retention of bacteria close to the

soil surface and their subsequent transport through the soils (Unc, 2002). However, while most of the information in the literature provides a good description on the long-term effect of organic waste on soil properties and nutrient cycling (Peacock *et al.*, 2001), little information is available about the immediate impact of manure application on the same features.

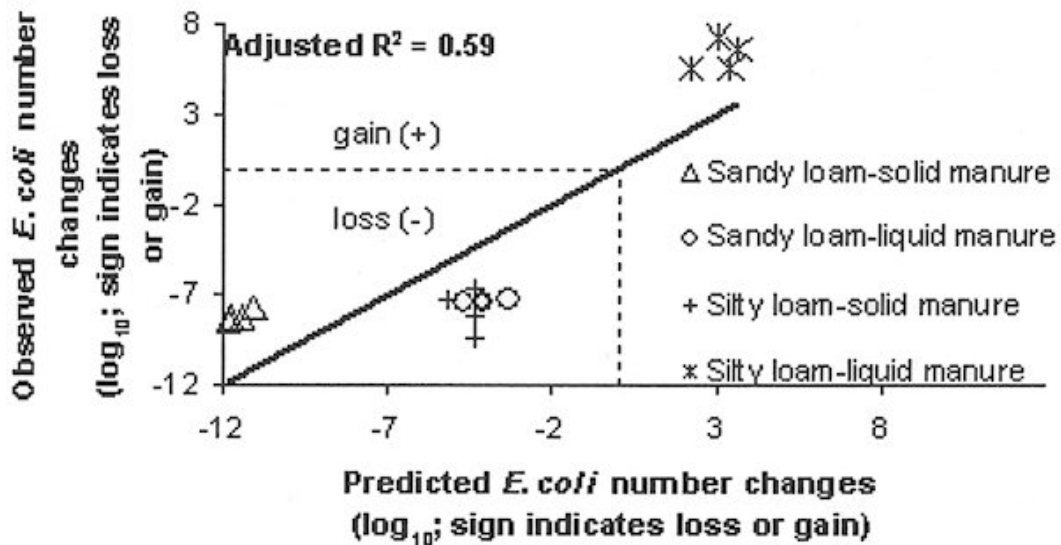
Experimental results suggest that the properties of the residual material with which the bacteria reach the field can have a significant impact on the post-application survival of these organisms. The survival potential of *E. coli* in the absence of water stress was shown to be at least 200 days and was not dependent on the initial concentration of *E. coli* in the applied manure (Unc, 2002, Figures 8 and 9). Solid beef manure accelerated microbial activity immediately after application and increased the initial number of *E. coli*, but also shortened the survival length while liquid swine manure resulted in a smaller number of *E. coli*, but increased their survival time in soil (Figure 8 and 9). Solid manure led to decreased survival at all incubation temperatures considered including freezing (Figure 8, 9 and 10). The impact of liquid manure on bacterial survival through freezing seems to vary with soil type (Figure 10).



**Figure 8.** Number of manure *E. coli* (log<sub>10</sub>) over time, in non-autoclaved soil-manure mixtures; A. solid beef soil-manure mixture; B. liquid swine soil-manure mixture. The initial numbers of *E. coli* (log<sub>10</sub>) in soil were 4.5 and 5.19 for the sandy loam and silt loam, respectively for 100 g of soil (dry weight).



**Figure 9.** Numbers of manure *E. coli* (log<sub>10</sub>) over time, in autoclaved soil manure mixtures; A. solid beef soil-manure mixture; B. liquid swine soil-manure mixture. No detectable *E. coli* in soils following autoclaving.



**Figure 10.** Changes of *E. coli* numbers (log<sub>10</sub> 100g<sup>-1</sup> soil) following freezing. (Values relative to bacteria concentration at the start of the experiment.)

Soil fauna can also be effective predators of bacterial pathogens and pathogenic strains associated with manure may not accumulate in soils containing earthworms. After 48 h, a population of *Salmonella* introduced to soil containing earthworms was reduced by a factor of four compared with *Salmonella* in a worm-free soil. Earthworms also caused a small reduction in the population of the normal bacteria. Free living protozoa, nematodes, and the soil bacterium *Bdellovibrio* are also predators of bacteria in the soil (Peterson and Ward, 1989). Presence of these organisms may reduce or limit the numbers of other bacteria. Nonetheless, introduced bacteria may still be able to survive for an extended period after manure application. On average, 10% of faecal coliforms and faecal streptococci were still present in the soil 11 and 14 days respectively after application of pig manure (Chandler *et al*, 1981).

One area of concern is manure from animals routinely treated with antibiotics. There is no evidence that bacteria in soils subject to regular manure applications have developed more antibiotic resistance because of the feeding of subtherapeutic antibiotic doses to enhance growth of livestock and poultry (Topp, 2000). This suggests that, if manure is properly applied, land application does not pose an additional threat to water resources from antibiotic-resistant bacteria. Nonetheless, the impact on the survival of enteric pathogenic microbes due to the use of antibiotics in feed has not been evaluated.

Viruses near the soil surface are rapidly inactivated by the combination of stresses imposed by sunlight, soil drying, predation, and other soil-based factors such as pH. Kowal reviewed the literature on virus survival (Kowal, 1985). Moisture content appears to be a major factor once the virus has penetrated the soil surface. About 100 days is the longest survival time reported for enteric viruses.

The movement of viruses from manure in surface runoff has not received significant attention. Movement to groundwater has been investigated in model systems or has been inferred from studies on wastewater application. Penetration of virus particles was deeper in sandy soil (with movement to 17.4 m) than in loamy or clay soils. It was also greater under conditions of saturated flow than under unsaturated flow (Lance and Gerba, 1984).

### **3.2.2.3 Retention of pathogens in soil after release from septic systems**

Of concern in terms of ground and surface water contamination is the quality of the effluent from the septic tank portion of the system, and the efficiency of constituent removal in the soil underlying the soil absorption system. In considering ground and surface water contamination from septic systems, attention must be directed to the transport and fate of biological contaminants (bacteria and viruses) from the soil absorption system through underlying soils and into ground and possibly surface water resources. Unfortunately, the factors influencing the transport, survival and fate of bacteria and viruses from the septic system environment to the subsurface environment and onward are inadequately characterized. Nevertheless, it is increasingly recognized and is documented that the majority of drinking waterborne outbreaks recorded in North America are due to contaminated groundwater with viruses being the main etiologic agents (MMWR, 1992). In most of these

outbreaks contamination was from malfunctioning on-site sewage treatment septic systems. Despite the documented microbial risks of groundwater contamination from septic systems, there is still limited data on and poor understanding of the factors controlling the efficacy of treatment (Scandura and Sobsey, 1997). This has highlighted the need for additional and ongoing research on the potential risks associated with septic systems and the underlying factors influencing the survival and transport of microorganisms to water resources.

While bacteria can survive outside host environments, there are numerous physical, chemical and biological removal mechanisms that may occur in both the soil and ground water systems. Several general factors influencing microbial reductions have been identified and include temperature, residence time in the septic tank (Hain and O'Brien, 1979), rainfall, soil saturation, distance to the water table in unsaturated soils (size of the vadose or unsaturated zone), horizontal distance from the distribution trench and the presence of a confining (impervious) soil layer (Brown *et al.*, 1979). In general, microbial reductions are greater at higher temperatures, in the absence of rainfall, in finer textured soils (but not impervious clays), in unsaturated soils with larger vadose zones and at increasing horizontal distances from the distribution trenches (Scandura and Sobsey, 1997). Reddy *et al.* (1981) summarized published information on the half-life of a number of faecal indicator and pathogenic bacteria. Values for bacteria range from about 2 to more than 500 h. Limited information on viruses seem to be of the same order (Table 15).

A study by Scandura and Sobsey examined the movement and fate of viruses and other contaminants in four septic systems located in sandy soils with shallow water tables. The results of this study lead to some specific suggestions. In particular, it was claimed that the risks of viral contamination of groundwater by on-site wastewater treatment systems are greatest in the most coarse (sandy) soils, when water tables are most shallow (shallowest vadose zones or unsaturated soils) and in the winter when temperatures are the lowest. It is recommended that extensive reductions of enteric viruses, bacteria and nutrients can be achieved by on-site septic systems in sandy soils if the clay content of the drainfield soil is 15% or more, if the depth of the vadose zone is 1 m or more and if the drainfield distribution lines do not become submerged in the groundwater. Poor reductions of viruses by on-site septic systems in sandy soils are indicated by increases in normally acidic ground water pH levels and by the presence of phosphorous and reduced nitrogenous compounds in ground water (Scandura and Sobsey, 1997).

The study by Scandura and Sobsey and a number of others look at the impacts of septic systems on aquifers in homogeneous sand. While septic systems do provide effective treatment of wastewater under the conditions of the above mentioned study, we are increasingly installing systems in regions where numerous topographic limitations exist.

**Table 15.** Range of half-lives of some faecal bacteria and viruses (based on Reddy et al.(1981).

Microorganism	Half-life (hours)
<b>Bacteria</b>	
<i>E.coli</i>	2.6 (pH =12) to 110.9 (water at 5°C)
Faecal coliforms	1.8 (on alfalfa leaves) to 237.6 (coliforms from poultry manure in clay-loam soil)
Total coliforms	41.6 (water)
Enterococci	21.9 (water)
Faecal streptococci	4.3 (raw sewage) to 520 (water)
<i>Aerobacter aerogenes</i>	57.4 (storm water)
<i>Shigella spp.</i>	22.5 to 26.8 (well water at about 10°C)
<i>Vibrio cholerae</i>	7.2 (well water)
<i>Staphylococcus aureus</i>	97.8 to 118.8 (waste water)
<i>Salmonella</i>	7.2 to 184.8 (sand)
<b>Viruses</b>	
Poliovirus	7.5 (water) to 415.8 (in soil)
Coliphage	6.6

#### 3.2.2.4 Fate of pathogens in soil

Most faecal bacteria reach the soil in the biosolid material that contained them. Both hydrophobic and hydrophilic components of the biosolids can interact with hydrophobic and hydrophilic loci on the soil and microbial surfaces, thereby influencing the electrochemical interactions between organisms and the surfaces of soil minerals and organic matter. Hence the retention and consequently the transport of bacterial cells are dependent on the hydrophobic and hydrophilic interactions between the cell surface, soil mineral and organic surfaces and the soluble and insoluble suspended components in the soil solution. Investigations conducted by Unc (2002) on bacterial transport through soils following land application of liquid swine manure and sold beef manure indicate that initial retention of faecal bacteria in soils can be enhanced at high ionic strength of the suspending solution after land application of manure. Subsequent dilution of the soil solution by incoming rain or irrigation favours re-suspension of initially retained microbial cells. However, presence of biosolids colloidal matter in suspension cancelled some of the effects of the increased ionic strength, favouring particle transport through the vadose zone.

Thus, despite the complexity of the interactions between bacterial cells soils and suspending solution, biosolids organic matter lowers the variability in the retention behaviour given by the intrinsic properties of charged particles (i.e. bacterial cells). Hence charged particles are more likely to remain in suspension and penetrate deeper into the soil profile in the presence of suspended organic matter.

Factors influencing the effectiveness with which soils retain viruses include cation concentrations, clays, soluble organic concentrations, pH, isoelectric point of the viruses, and general chemical composition of the soil. Essentially, they are similar to those identified for enteric bacteria (Table 16).

### **3.2.3 Contamination of water resources**

The likelihood of bacteria moving into water resources declines with time because the organisms die off. The shortest period of survival would be expected for bacteria from manure or land-applied biosolids would be expected with applications made in the summer. However, applications, especially manure made later than the time of side-dressing for corn might reduce the likelihood of bacterial contamination, but increase the risk of nitrate contamination of ground water.

The Ontario Farm Groundwater Quality Survey found that the proportion of wells contaminated with bacteria was significantly greater on farms where manure was spread than where only mineral fertilizers were used (Rudolph *et al*, 1998). Soil type was important in this result: less contamination resulted under coarse, gravelly soils and fine-textured soils than under loams (Goss *et al*, 1998). The results of the survey, which included a study using freshly inserted monitoring wells, also showed that contamination of drinking water wells was similar to that under fields where the farmers were carrying out their normal cropping practices (Rudolph *et al*, 1998). This clearly indicated that groundwater could be contaminated by bacteria moving through the soil, rather than by surface water entering poorly-maintained wells. Evidence of repeated groundwater contamination has been observed under land where manure was regularly applied (Figure 11).

**Table 16.** Factors in the retention and survival of enteric bacteria and viruses in soil.

Factor	Enteric bacteria	Viruses
Moisture content	Greater survival time in moist soils and during times of high rainfall	Some viruses persist longer in moist soils than dry soils
Moisture holding capacity	Survival time is less in sandy soils than in soils with greater water-holding capacity	Soils with a larger water holding capacity will retain moisture longer than those with a smaller capacity
Temperature	Longer survival at low temperatures; longer survival in winter than in summer	Viruses survive longer at lower temperatures
pH	Shorter survival time in acid soils (pH 3- 5) than in alkaline soil	Most enteric viruses are stable over the pH range 3 to 9: survival may be prolonged at near neutral values
Sunlight	Shorter survival time at soil surface	Dessication will reduce survival
Organic matter	Increased survival and possible regrowth when sufficient amounts of organic matter are present	Presence of organic matter may protect viruses from inactivation: others have found that it may reversibly retard virus infectivity
Antagonism from soil microflora	Increased survival time in sterile soil	Some viruses are inactivated more readily in the presence of certain micro-organisms: but, adsorption to the surface of bacteria can be protective
Salt species and concentration	Salt concentration is important for the initial retention of bacteria in soil	Some viruses are protected from inactivation by certain cations: the reverse is also true
Association with soil	Adsorption onto soil particles is important for bacterial retention	In many cases, survival is prolonged by adsorption to soil: however, the opposite has been observed
Aggregation of microbes	Reduces their mobility and hence increases the retention of bacteria in soil	Enhances survival
Soil properties	The mobility, and hence the residence time of bacteria, is very dependent on the surface properties of soil particles	Effects on survival are probably related to the degree of virus adsorption
Microbe physiology	Different pathogenic bacteria vary widely in their response to environmental stress.	Different virus types vary in their susceptibility to inactivation by physical, chemical and biological factors

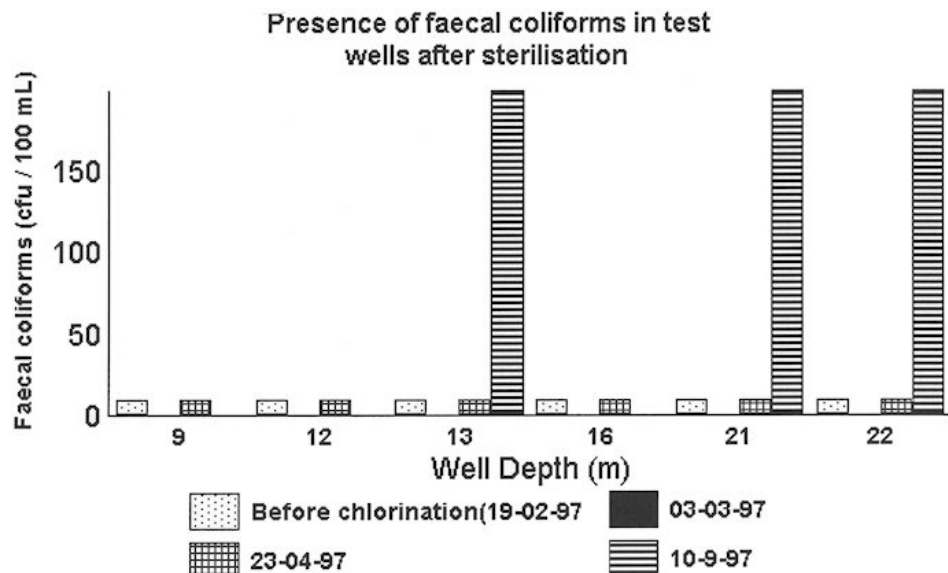
Source: Gerba et al. 1975.

Source: Yates and Yates 1988.

Liquid manure adversely affected tile-water quality when applied to the land following current farming guidelines. Of the manure spreading events investigated, 75% resulted in water quality impairment (Dean and Foran, 1991). Bacteriological contamination from tile drains can be greater after injection than after surface spreading (Dean and Foran, 1991). It is difficult to determine an acceptable rate of liquid manure application, due to the numerous factors which affect the contamination of watercourses (Foran *et al*, 1993). The importance of soil macropores for the rapid transport of bacteria to tile drains was highlighted in their studies. The likelihood of bacteria moving into water resources declines with time after manure application because the organisms die off, but this takes longer in manure applied in late fall, shortly before freeze-up. Application as a side dressing for corn (which generally occurs in mid-June, when soils are relatively dry and warm) results in the shortest period of survival. Later applications might further reduce the likelihood of bacterial contamination, but increase the risk of nitrate contamination of groundwater because the crop has insufficient time to acquire the nutrient from the soil.

A major problem is associated with applying manure to land with tile drains. After liquid manure application, bacteria move rapidly to the tile drains if the soil is close to field capacity.

Septic systems were not identified as a source of well contamination in the Ontario Farm Groundwater Quality Survey. However, more detailed studies using nalidixic acid resistant *E.coli* as a tracer have identified that a significant proportion of chronically contaminated wells had either the domestic or the milk house septic system as the source of the bacterial contaminants (Conboy, 2000. Goss *et al.*, 2003).



**Figure 11.** Presence of faecal coliforms in test wells after sterilisation (Goss and Unc, unpublished).

### 3.2.3.1 Transport Processes

Substances in manure move to water resources by a variety of transport processes (Addiscott and Wagenet, 1985; Jarvis *et al*, 1991). Contaminants originating in manure that affect water resources can be divided into three basic classes:

- simple inorganic ions (e.g.,  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{H}_2\text{PO}_4^+$ ),
- more complex organic molecules (e.g., phytates and endocrine-disrupting substances),
- particulates (e.g., microorganisms).

The concentration of simple inorganic ions is controlled by the equilibrium between the solids and their solution phases in soil water. This may involve the formation of sparingly soluble precipitates and adsorption reactions with soil particles. For organic molecules and some inorganic species such as  $\text{NH}_4^+$ , the final concentration of contaminants in soil water depends on their vapour pressure and their solubility in water and in soil organic matter. In contrast, particulates are generally affected by surface charge. In all cases, transport varies greatly depending on soil structure, especially the size distribution and continuity of soil pores. For inorganic nitrogen compounds and bacteria, the soil is itself a source and may also contain one or more sinks.

#### 3.2.3.1.1 Water partitioning at the soil surface

As water is the primary factor determining the movement of contaminants, its partitioning at the soil surface into runoff and infiltration (drainage) is of fundamental importance. During precipitation (rainfall or irrigation), the surface of the soil becomes wet and water starts to move down through the soil. If the rate of precipitation exceeds the ability of the soil to transmit water to its depths, ponding occurs.

Ponding allows water to fill very large pores at the soil surface, therefore promoting preferential flow (flow in areas of the soil that offer the least resistance — see section 3.2.6). On land with any slope, the depth of ponding is likely to be very small before the water starts to flow down the slope.

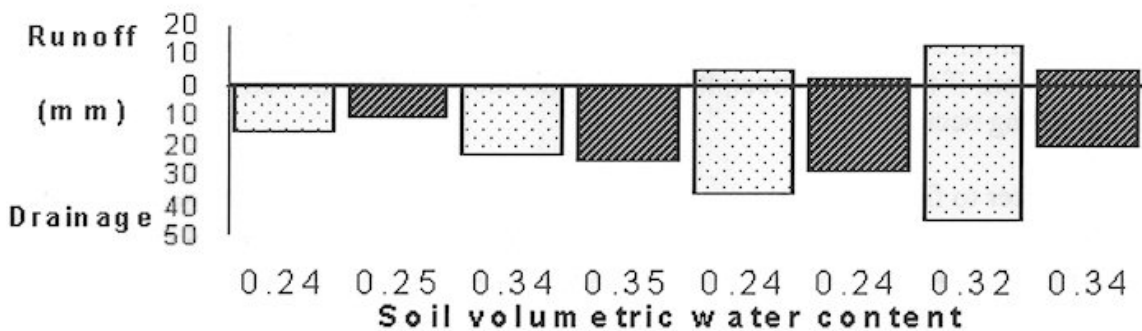
The rate of flow of surface runoff can be slowed by crop residues and soil clods. As the flow slows, the depth of water increases or the ponded area gets larger. In either case it enhances infiltration into the soil and restricts transport off the field. Increased infiltration into vegetated buffer strips also increases their efficiency of contaminant removal from surface runoff (Coyne *et al*, 1998).

All faecal coliform bacteria were removed by passage through a 6.1-m vegetated buffer strip. A tool (VFS\_INT) to design vegetative filter strips (VFS) based on site specific characteristics of the upland area contributing flow of sediments and phosphorus has recently been developed (Rudra, 2003). It is very user-friendly and program documentation includes a *User's manual*, *Help files*, and *Sample project data sets* which can help new users to get

acquainted with input data preparation and model execution. The design mode of developed model helps user to design vegetated filter strips based on user defined criteria such as trapping efficiency and pollutant (sediment and phosphorous) load to streams. The tool is undergoing further development to account for the removal of bacteria and other pathogens. In developing the design tool it was also found that filter strips of 5m width were sufficient to trap sediment particles greater than 40  $\mu\text{m}$ . However, for finer sediments, generally those transported in the water eroding fine textured soils, either longer filter strips or induced infiltration techniques such as a french drain were required.

Runoff generation areas were able to be identified from parameters such as hydraulic conductivity at field saturation ( $K_{fs}$ ), bulk density, elevation and field slope during summer period and  $K_{fs}$ , bulk density, elevation field slope and initial soil water content during the fall.

Manure can affect the partitioning of water in the period immediately after land application, but the direction of the change depends on both the manure type and the soil type. In coarse-textured soils, there is no effect because rainfall intensity is not likely to exceed the infiltration rate. In loamy and finer-textured soils, the application of dilute liquid manure can both encourage surface runoff and enhance preferential flow. Until solid manure has been incorporated, it acts as a mulch and encourages infiltration rather than surface runoff (Figure 12).



**Figure 12.** Partitioning of precipitation into surface runoff and drainage after the application of liquid swine and solid beef manure. After Unc and Goss (2000).

### 3.2.4 Basic equations governing transport through the soil

Water movement through the unsaturated zone towards an aquifer can be described by the Richards equation assuming one-dimensional flow in a homogeneous soil:

$$\frac{\partial \theta}{\partial t} = \frac{\partial [K(\theta) \frac{\partial \phi}{\partial z}]}{\partial z} - U(z, t) \quad (1)$$

where  $\theta$  is volumetric water content,  $K(\theta)$  is the water content-dependent hydraulic conductivity,  $\frac{\partial \phi}{\partial z}$  is the hydraulic gradient,  $U$  is an extraction term for uptake of water by plant roots,  $z$  is depth, taken as positive downwards, and  $t$  is time. To develop a solvable form of the equation a term called the hydraulic diffusivity,  $D(\theta)$ , is introduced, defined as:

$$D(\theta) = K(\theta) \frac{\partial \phi}{\partial \theta} \quad (2)$$

where  $\phi$  is the matric potential, and  $\frac{\partial \phi}{\partial \theta}$  is the slope of the moisture release characteristic curve. Equation 1 becomes:

$$\frac{\partial \theta}{\partial t} = \frac{\partial [D(\theta) \frac{\partial \theta}{\partial z} - K(\theta)]}{\partial z} - U(z, t) \quad (3)$$

A quantitative description of the transport of contaminants that only dissolve in water usually assumes the convection-dispersion equation (CDE):

$$q_s = q_w C_l - D_e \left[ \frac{dC_l}{dz} \right] - D_h \left[ \frac{dC_l}{dz} \right] \quad (4)$$

where  $q_s$  is the mass of the solute moving through unit cross sectional area per unit time;  $C_l$  is the concentration of the solute in the soil water;  $D_e$  is the effective diffusion coefficient of the solute in the soil and has been adjusted for the water content and tortuosity of the pore system;  $D_h$  is the mechanical dispersion coefficient that includes the effect of local variation in the velocity of water in large and small pores;  $q_w$  is the water flux density.

Thus, the transport of contaminants depends on factors governing their concentration in the soil solution and the flux of water available to move them. If a contaminant undergoes transformations in the soil, is subject to die-off, or is absorbed by plants, additional sink terms have to be added to the continuity equation, as in equation. Further refinements are needed if the exchange of a contaminant between the liquid and solid phases in the soil takes place.

### 3.2.5 Contaminant characteristics relevant to their transport

It is important to consider the features of different contaminants that affect their transport. Transport of particulates such as microorganisms follows that of colloids, although viruses exhibit little filtration and are adsorbed onto low-molecular weight organic molecules in soil (Kowal, 1985). It is reasonable to assume that the concentration of a solute will tend to become uniformly distributed within each pore space, but this may not be true of colloids. Bacteria are much larger than nitrate ions and their movement is more likely to be affected

by the flow associated with the pore size in which they are transported. They have variable surface charge which allows stronger adsorption of the bacteria to soil particles. Bacteria also have very large surface-area-to-volume ratios that provide a large proportion of sites for adsorption. A third consideration with microorganisms is that their populations are dynamic. They are alive and influenced by factors that affect their survival. Many are also motile.

### **3.2.6 Preferential flow and solute transport**

Soil pore characteristics are important for transport. However, the transport of contaminants in soils with strongly aggregated structures or with large and continuous pores is not well described by models based on equation 5, because preferential flow occurs (Thomas and Phillips, 1979; Wagenet, 1990). Preferential flow is the process whereby water, and materials contained in it, move by preferred pathways through a porous medium. This means that part of the matrix is effectively bypassed.

The term preferential flow does not itself convey a mechanism for the process (Helling and Gish, 1991), whereas the often-used term "macropore flow" implies transport through relatively large pores, channels, fissures, or other semi-continuous voids within the soil. Although there is no standardized definition for macropores, some pore classification has been proposed. Luxmoore suggested the classes of micro-, meso-, and macropore, defined by equivalent pore diameters of less than 10  $\mu\text{m}$ , 10 to 1000  $\mu\text{m}$ , and more than 1000  $\mu\text{m}$  (1 mm), respectively (Luxmoore, 1981). Skopp defined macroporosity as that pore space which provides preferential paths of flow so that mixing and transfer between such pores and the remaining pore space is limited (Skopp, 1981).

Some other classifications of soil pore size and their functions with respect to water movement or root penetration have been summarized by Helling and Gish (Helling and Gish, 1991). Pore size and the corresponding capillary potential was given by Beven and Germann (Beven and Germann, 1982). Macropores may develop by physical (e.g., swell-shrink, freeze-thaw, or tillage) or biological (e.g., burrowing by earthworms, insects, and other soil fauna or the growth of roots) processes in the soil. Continuous macropores can be formed by the activity of soil macro-fauna, especially earthworms (Ehlers, 1975).

In soils with significant swell-shrink behaviour, cracking may be important in the development of a preferential flow domain, and the extent of crack development is generally related to water extraction by roots. The channels created by roots can also dominate the transport process once the original roots have decayed (Barley, 1954). Freeze-thaw cycles may also result in fractures. The installation of tile drains also provides some continuous porosity between the soil surface and the drain. The macropores therefore provide a rapid conduit between the field and the surface water body into which the tile drains discharge. Macropore flow allowed manure liquids to move into subsurface drains within an hour after application (Fleming and Bradshaw, 1991, 1992a, 1992b).

The essential feature of preferential flow is that percolating water can bypass a large fraction of the soil matrix, thus moving deeper and with less displacement of the initial soil solution

than would have been predicted by piston displacement (Beven and Germann, 1982; Bouma, 1981; Quisenberry and Phillips, 1976, 1978, Quisenberry *et al*, 1993; Shirmohammadi *et al*, 1991) Watson and Luxmoore found that under ponded conditions, 73% of the flux was conducted through macropores (pore diam. >1 mm) (Watson and Luxmoore, 1986). Furthermore, they estimated that 96% of the water was transmitted through only 0.32% of the soil volume. As much as 70-90% of applied chemicals maybe moving preferentially through macropores (Ahuja *et al*, 1993).

Preferential flow may occur even in coarse-textured soils that are considered to be homogeneous (Kung and Donohue, 1991). Macropore flow commenced at the tilled-untilled boundary in a cultivated Maury silt loam (Quisenberry and Phillips, 1976) and in a Cecil sandy clay loam in the Piedmont of South Carolina (Hatfield, 1988). Transport of bacteria is concentrated in regions of preferential flow. Unc and Goss applied liquid swine manure to an undisturbed column of clay-loam soil (Unc and Goss, 2000). They found that about 90% of bacteria moved through only 15% of the available cross-sectional area. The same proportion from solid beef manure were transported through less than 25% of the cross-sectional area of soil (Figure 13). These values were not necessarily directly correlated with the amount of water that penetrated the cores indicating that the bacterial contaminant flow was very concentrated. Nevertheless more water penetrated the cores following the application of liquid manure (Figure 6).

Jardine *et al*. found that solutes were transported by convection and diffusion from small-pore to large-pore regions in undisturbed soil as a result of hydraulic and concentration gradients, respectively (Jardine *et al*, 1990). Small pores were a major source of the solute transported rapidly by large pores. A diffusion-based mechanism described by Luxmoore (Luxmoore, 1991), in which new water entering a soil gains the chemical attributes of old water, could explain the results reported by Jardine *et al.*, 1990. According to Luxmoore, a large surface area of interaction, combined with a short diffusion path between mesopore channels and micropores, allows diffusion to be a significant contributor to chemical transport during preferential flow events (Luxmoore, 1991).

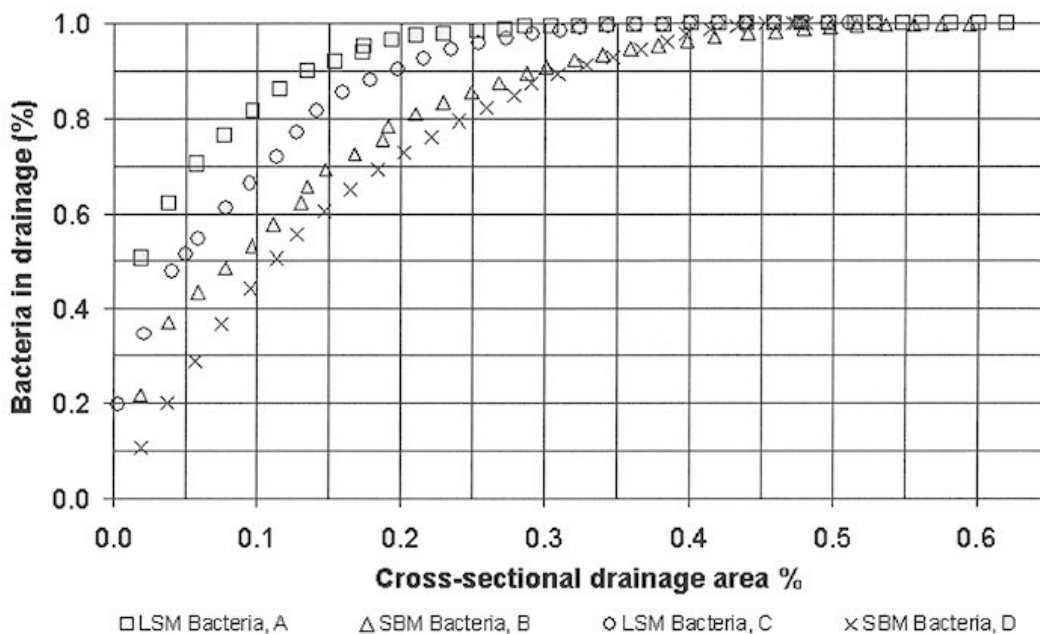
Helling and Gish (1991) described some factors affecting the process of preferential flow, including soil porosity, pore characteristics, structure, initial moisture content, and soil management. Flow through tubes is proportional to the fourth power of their radii, therefore drainage is much more rapid through large continuous macropores than through pores of smaller diameter. Mouldboard ploughing may destroy the continuity of pores between the plough layer and the deep horizons. Long-term no-tillage plots, on the other hand, often develop a high density of continuous, relatively large vertical channels (Goss *et al*, 1993).

Manure application may encourage the activity of earthworms which may result in a greater continuity of macropores. Hence, contaminants may break through faster than predicted (Munyankusi *et al*, 1994). A relatively large water content at the time of application might result in deeper movement of contaminants (Quisenberry and Phillips, 1976), but the opposite effect has also been reported (White *et al*, 1986). Understanding the mechanism of bypass flow through convection and diffusion from regions with small pores to those with large pores

may help to explain such differences.

It is not sufficient to describe the transport of contaminants in the water phase without first identifying other factors that can affect mobility.

For microorganisms in manure, preferential flow is important to their transport through the soil and hence into water resources. Bacteria can impact surface water through runoff and in tile drainage. They can also be transported to groundwater.



**Figure 13.** Importance of preferential flow for movement of bacteria from liquid swine (LSM) and solid beef (SBM) manure through undisturbed monolith lysimeters containing clay soil

### 3.2.7 Transport of pathogens from manure, biosolids and septic systems

Microbial contaminants have a wide variety of physical and biological characteristics, including wide ranges in size, shape, surface properties, and die-off rates. The distance of travel of bacteria through soil is of considerable significance in relation to contamination of water supplies and the associated health hazard. In particular, the identification of a source of contamination becomes an increasingly difficult undertaking as the distance of travel increases. Little detailed information is available on the transport of pathogens from manure and organic waste.

Only limited information is available on the transport of contaminants from manure other than N and P. However, the greater the initial soil water content, the deeper the penetration within the macropores because less water moves laterally into the micropore system (Beven and Germann, 1982), and hence the deeper contaminants are likely to move. When the soil water content is close to field capacity, the micropore space is filled with water and application of more solution, such as liquid manure, tends to encourage flow in the macropore space (Unc and Goss, 2000).

Bacterial transport is affected by soil pH. Long-term land application of cattle or pig manure can result in a decrease in soil pH (Chang *et al*, 1991; Bernal *et al*, 1992). This potentially reduces bacterial transport due to an increase in the number of binding sites available for bacterial adsorption. It may also affect bacterial survival. Cattle manure induced smaller soil pH changes compared with pig manure.

The moisture content of the soil prior to rainfall is another important factor in water movement and consequently contaminant transport. Abu-Ashour *et al*. conducted a series of experiments to determine factors influencing bacterial transport through soil (Abu-Ashour *et al*, 1994a). Their findings indicated that initial soil moisture was the critical variable in determining the extent of bacterial migration. In dry soil, none of the marked bacteria (biotracer) were detected below 87.5 mm. However, the biotracer travelled the full length of the soil columns (175 mm) if the soil was wet. If water was added after biotracer inoculation, the biotracer moved deeper into the soil. The actual depth depended upon how close to saturation the soil became after the addition of a given volume of water. The water may have increased the soil water content sufficiently to allow the bacteria to move through the soil with percolating water. However, bacteria can be transported to depth even if the soil is not saturated (McMurry *et al*, 1998; Unc, 1999).

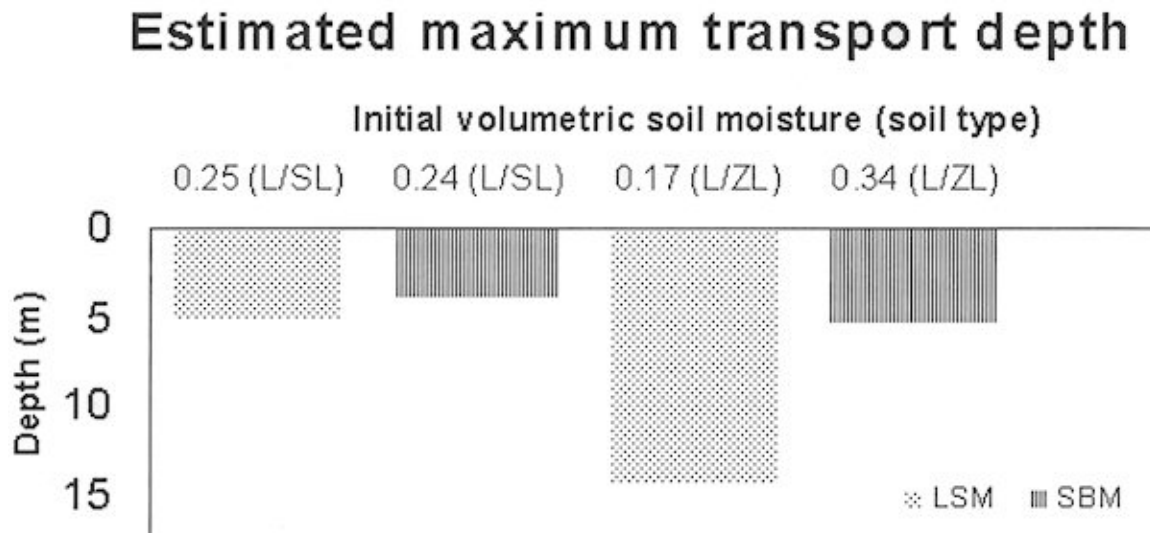
A further factor influencing bacteria transport following liquid manure application is the large concentration of salts present in manure. The salts may act as "bridges," allowing negatively charged bacteria to adsorb to negatively charged soil particles. High salt concentrations can also decrease the thickness of the diffuse double layers around soil colloids, thereby allowing bacteria access to surfaces to which they can adhere. The addition of rainwater dilutes the salt concentration, thus increasing the thickness of the double layer and possibly causing the flushing out of adsorbed bacteria (Yan *et al*, 1991). This increases the number of bacteria that remain mobile in the soil solution and increases the risk to groundwater. Harvey has shown that the transport of bacteria may be faster, slower, or similar to that of tracers, such as chloride or bromide, that are not transformed into other compounds (Harvey, 1991). Bacteria move through soils and aquifers by several mechanisms, including continuous, discontinuous, and chemotactic migration (Bitton and Harvey, 1992). Much of the modelling effort has treated transport as a continuous process, which assumes passive transport of bacteria. However, bacterial movement through the subsurface, especially over substantial distances, maybe discontinuous because of processes that temporarily remove bacteria from solution. Bacteria are removed from the flowing water by straining or by reversible sorption on solid surfaces. They are remobilized later.

Discontinuous transport creates an apparent retardation of the bacteria relative to conservative tracers. Retardation factors as large as 10 have been reported for bacterial populations travelling through porous aquifers (Matthess *et al*, 1988). Bacteria may also travel significantly faster than chloride or bromide due to motility.

Movement due to taxis (self-propulsion) is faster than that caused by random thermal (Brownian) motion. Motile bacteria penetrated Berea sandstone cores in the presence of a nutrient gradient up to eight times faster than non-motile ones (Jenneman *et al*, 1985). Bacteria may also appear to travel faster than conservative tracers for other reasons. Bacterial transport is restricted to macropores, whereas conservative tracers diffuse into the soil matrix as well as the larger pores. This may cause the average peak in bacterial concentrations to appear earlier than that of the conservative tracer. The bacteria are exploiting faster paths but can travel only during peak flow, whereas the average tracer concentration, moving through the soil matrix and macropores, would not peak until the majority had infiltrated through the soil matrix (Bitton and Harvey, 1992).

The impact of preferential flow on the velocity of bacterial transport, relative to the average pore water velocity, was investigated by Unc (2002). Near the soil surface, where the structure was more uniform because of tillage, bacteria moved at a rate similar to the average pore water. Deeper in the soil profile, bacterial movement was much faster than the average pore water because they were concentrated in the preferential flow paths. In addition to transport processes, the kinetics of bacterial population growth and decay must be considered in relation to the timing and numbers of organisms reaching a water resource. Microorganisms adsorbed to soil particles may survive longer than those in the liquid phase, as organic substrate and nutrients are more readily available to them (Sobsey, 1983).

The Ontario Farm Groundwater Quality Survey demonstrates the importance of preferential flow for bacterial transport since bacteria were found in properly maintained drilled wells greater than 30 m deep (Goss *et al*, 1998). Preferential flow can thus facilitate the transport of contaminants to aquifers at depths that might be expected to remain unaffected by surface contaminants. This presents an important concern for predictions of bacterial transport. For example, when flow parameters in the theoretical model described by Corapcioglu and Haridas were taken to the permissible limits, the predicted extent of bacterial transport through unsaturated soil over 2 weeks was 0.2 m (Corapcioglu and Haridas, 1985). Tamasi reported that *E. coli* and *Salmonella typhimurium*, applied in liquid manure, rarely penetrate deeper than 1.6 m in packed columns of either a sand or a garden soil (Tamasi, 1981). But Smith *et al.* observed that *E. coli* penetrated through a column of undisturbed soil to a depth of 0.3 m in 20 minutes (Smith *et al*, 1985). Harvey *et al.* observed that bacterial-sized microspheres were transported through several metres of aquifer material (Harvey *et al*, 1989) Unc estimated the depth of soil necessary to filter out bacteria from manure applied under field conditions (Unc, 1999). Based on bacterial counts measured at 0.75m, values ranged from 0.1 m to more than 20 m (Figure 14), demonstrating the importance of preferential flow paths. The vertical distribution of beneficial microorganisms applied to the soil also depends on preferential flow (Natsch *et al*, 1996).



**Figure 14.** Variation in the depth of soil required to filter bacteria in liquid swine manure (LSM) and solid beef manure (SBM) in loam over sandy-loam (L/SL) and loam over silt-loam (L/ZL) soils. From Unc 1999.

The transport of protozoa has been studied in far less detail than has bacterial transport. The movement of *Cryptosporidium parvum* oocysts through saturated columns of glass spheres, coarse sand, or shale aggregate has been modelled. The oocysts (from dairy calves) did not adhere to sand or glass spheres, moving throughout the system of pores between the particles. The oocysts moved preferentially in the larger pores between shale aggregates. Sand was more effective at removing oocysts than were the other particles, probably by filtration. The authors suggested that their results indicated that significant transport was possible in both surface runoff and with infiltrating water (Brush *et al*, 1999).

Based on the information presented, a number of environmental factors are known to influence the transport rate, including rainfall; soil moisture, temperature and pH; and availability of organic matter. Environmental factors affecting the survival of enteric bacteria in soil include soil moisture content and holding capacity, temperature, pH, sunlight, organic matter, and antagonism from soil microflora. The physical process of straining, and the chemical process of adsorption appear to be the most significant mechanisms in bacterial removal from water percolating through soil.

### 3.2.8 Predicting contamination of water resources by components of manure and biosolids

Although the presence of pathogenic bacteria in manure, biosolids and the release from septic systems, and in receiving waters is well documented, and the phenomena influencing bacterial transport are known, the actual effect of the material in which they enter the resource on the quality and quantity of bacterial contamination is less well understood.. Aspects of bacterial transport have been studied, but mainly under laboratory conditions. Such experiments have mostly evaluated the effects of various individual factors on the bacterial transport. Thus, while many predictive models for the transport of soluble forms of contaminants have been developed, few models exist that describe bacteria transport. Those that are available are still in the formative phase (e.g., the LEACHB routine of the LEACHM program (Hutson and Wagenet, 1992). No account is taken of the potential effect of the manure on the bacterial transport, despite the fact that manure is one of the major sources of pathogens contaminating groundwater and surface waters. The possibility for preferential flow needs to be included in such models. The microbial model in LEACHB considers microbial dynamics as affected by substrate availability and prey-predator interaction. Bacterial growth and distribution can influence the transport of solutes. The model, however, does not refer to physical movement of bacteria. The only movement between locations is considered in the context of the distribution of available substrates. The model also does not allow for a bacterial population to die. All these characteristics make the model suitable for describing the movement of indigenous soil bacteria that are in a dynamic equilibrium, but not the transport of faecal bacteria introduced in an application of manure.

The MACRO model (Jarvis, 1994; Jarvis *et al.*, 1999; McGechan *et al.*, 2002) provides an alternative approach to describing the movement of contaminants when macropore or preferential flow is an important feature of the transport. The model assumes a two-domain system of water movement: macropores (interaggregate pores) and micropores (intraggregate pores). Flow in the macropores is assumed to be gravity driven, and flow in the micropores is governed by capillary forces and uses the Richards equation (Equation 1). Division of flow between the two domains depends on the soil water potential at which air entry occurs in the macropores. Movement of particulate or colloidal material is subject to straining and filtration processes, and this will occur where flow moves into micropores, including necks in macropores. Deep bed filtration models are used to describe these processes. Particulate contaminants can undergo sorption onto soil particles or onto mobile colloids from the manure or biosolid. McGechan (2002) reported on the use of MACRO to describe leaching of soluble and particulate inorganic the phosphorus from liquid manure. The paper concluded that the model was 'highly suited to the representation of P leaching processes'.

Reddy *et al.* (1981) proposed a first order decay model to describe the decline in viral activity in soil. The concentration of virus,  $M$ , at time,  $t$ , was given by:

$$M = M_0 e^{-kt} \quad (5)$$

where  $M_0$  is the initial virus concentration, and  $k$  is the rate coefficient for viral decay. The main factor modifying the rate coefficient,  $k$  ( $10\log_{10} d^{-1}$ ), was found to be temperature (Yates and Yates, 1988). These same authors obtained an expression for  $k$ , given by:

$$k = -0.181 + 0.0214 T \quad (6)$$

where  $T$  is the temperature ( $^{\circ}C$ ).

Based on equation 6, the time required to reduce the virus concentration by three orders of magnitude at  $10^{\circ}C$  would be about 90 days.

Factors influencing the transport of bacteria and viruses are very similar (Table 17), the major difference being the reduced transport of viruses in finer textured soils compared with the greater likelihood of movement for bacteria in clay soils.

A number of models have been developed to describe the transport of bacteria in runoff from agricultural land. Many are steady-state models, and are not sensitive to rainstorm events (Fraser *et al.*, 1998)

Scaling is a fundamental problem associated with modelling processes associated with surface and ground water hydrology. When modelling processes are investigated at the small spatial scale (soil cores in the laboratory, undisturbed soil monoliths, microplots) it has become common to suggest that this allows for representative modelling of hydrologic processes and parameters at larger scales (field to watershed). This is not appropriate as hydrologic processes like infiltration; evaporation and runoff are highly non-linear and spatially auto-correlated. Application of theories developed at the laboratory scale to the vadose zone comprising heterogeneities on many different scales, encounters a scale issue. One consequence of the scale issue is that estimates of the partitioning of precipitation into surface runoff and infiltration determined in experiments at a small scale can underestimate the runoff component at the watershed scale.

To deal with this issue, two approaches have evolved: the systems approach and the physical approach. The system approach treats the vadose zone as a low pass filter, and its governing principle is determined by the relationship between its input and output histories whereas physical approach is based on the up scaling of laboratory-based theories to the whole vadose zone. The system approach has been widely applied by soil scientists, but often is criticized for its empiricism and lack of physical principles. Hydrologists have tried the physical approach by applying the concept of Control Volume (CV), Darcian Continua (DC) and Representative Elementary Volume (REV) to study the water flow and solute transport in porous media. However, this concept is not applicable to scale up hydrologic processes or parameters because size of CV and REV is scale dependent. Approaches being adopted are:

- Identification of dominant hydrologic processes and parameters and then scaling up,
- Changing the structure of model as scale increases to represent dominant

- hydrologic processes,
- Aggregation and averaging of hydrologic parameters or properties measured at small scale to represent notional properties at larger scales with the same model structure,
- Scaling distributions of hydrologic parameters or properties to larger areas with the same model structure,
- Calibrating simpler model structures for larger areas, using small area simulations that are more physically based and verifiable.

Use of geographic information systems to link digital terrain and soil data can help to identify the deterministic component of scaling issues as well as that stemming from stochastic causes.

Most models related to pathogen transport have been developed and tested at the laboratory scale, or at least at small scale. Current collaborative work between Agriculture Agri-Food Canada and the Universities of Guelph and Ottawa aim to improve understanding at the field scale, but by including undisturbed monoliths it is anticipated that this will allow greater understanding of the changes in dominant processes and parameters as the scale of measurement increases.

There has been relatively little effort devoted to developing new models or modifying existing models to describe pathogen transport at a watershed or basin level. Early models were based on deterministic relationships which provided estimates of bacterial concentrations in runoff. Moore *et al.* (1983) proposed a mass balance approach that considered both management and critical environmental factors (soil, weather) for bacterial transport in surface runoff. MWASTE, an event-based model was developed by Moore *et al.* (1988) to describe bacterial movement from land applied manure as surface runoff. This model again only provided an approximation of faecal coliform concentrations in runoff because of the simplified assumptions used to account for population dynamics Sadeghi and Arnold (2003). Furthermore, it does not allow the population of microbes to be determined under variable weather and soil conditions with different management practices.

Sadeghi and Arnold (2003) developed a continuous, process-based bacterial transport module for the Soil and Water Assessment Tool (SWAT) that can be used at the watershed and basin level, and contains functional relationships for growth and die-off of organisms. SWAT is a watershed scale model for nutrient and sediment loss from watersheds in different geographical locations. The hydrologic component estimates the runoff for each sub-watershed separately, and routes it to obtain the total for the whole watershed. Runoff volume is estimated for daily rainfall by using the USDA curve number method. Peak runoff is predicted using a modified Rational formula, while sediment yield is estimated from the modified universal soil loss equation. The model assumes that pathogen presence will be suggested by the presence of faecal coliform bacteria. However, the authors recognized that some pathogens can cause disease in man when only small numbers are present, so they assume that there are two groups of bacteria present in the source material, and that these groups have contrasting die-off and regrowth characteristics.

**Table 17.** Comparison of factors influencing the movement of bacteria and viruses.

Factor	Transport	
	Bacteria	Viruses
Moisture content	Movement more likely if soil initially close to field capacity	Generally, virus migration increases under saturated flow conditions
Temperature	Reduced survival reduces likelihood of movement	
pH	pH changes will affect retention	Generally, low pH favours adsorption and high pH results in virus desorption from soil particles
Sunlight	Reduced survival reduces likelihood of movement	
Organic matter	Presence of colloidal material will enhance transport	Soluble organic matter competes with viruses for adsorption sites on soil particles
Antagonism from soil microflora	Reduced survival will reduce transport	Reduced survival will reduce transport
Salt species and concentration	Changes in water quality, particularly variations in salt content, can modify retention	Generally, increasing the concentrations of ionic salts and increasing cation valencies enhance virus adsorption
Association with soil	Sorption, straining and filtration reduce transport	Virus movement through the soil is slowed or prevented by association with soil
Aggregation of microbes	Reduces the likelihood of transport because of straining and filtration	Retards movement
Soil properties	Transport greater in finer textured soils because of structural porosity	Greater virus migration in coarse textured soil: there is a high degree of virus retention by the clay fraction of soil
Microbe physiology	Formation of small cells under environmental stress likely to increase movement	Virus adsorption to soils is probably related to physiochemical differences in virus capsid surfaces
Hydraulic conditions	Transport increases with the volume of liquid applied to a field and the incident rainfall	Generally, virus migration increases with hydraulic loads and flow rates

The model allows bacteria to be added to soil with manure applications and from point sources along the river network. Bacteria can be incorporated into the soil by tillage operations, after which they are no longer available to be transported in surface runoff water or attached to sediment. Bacteria die off or regrow according to a first order decay equation. Movement from the soil surface can take place in infiltrating water as well as in runoff.

Once bacteria enter a stream they can be carried freely with the water or be attached to suspended particulate material. The bacteria attached to suspended material travels with that material, and are uniformly distributed across all suspended material. The bacteria are subject to the same die off and regrowth equations as in the soil. Bacteria in the water phase are only subject to die off.

A watershed scale model is under development at the Scottish Agricultural College (see Section 5).

### **3.3 Alternative Indicator Organisms**

Appropriate surrogate indicators of the presence of pathogens in water resources and the identification of sources of contamination are key to the development and testing of any index system. The appropriateness of various bacterial indicators for the assessment of water quality, has been a debated issue. In particular, the ability of a bacterial indicator to assess the presence of pathogens in water and predict associated health risks is inadequately understood.

The World Health Organization stated that 'Examination for faecal indicator bacteria in drinking water provides a very sensitive method of quality assessment' (WHO, 1996). It is well recognized that the disease-producing potential of drinking and recreational water can be assessed using bacterial indicators that are positively correlated with the presence of faecal contamination. It is believed that *E. coli* best fulfills the requirements of a suitable indicator and for this reason is the most widely accepted indicator of bacterial water contamination. *E. coli* is present in extremely high numbers in the faeces of all mammals, it does not appreciably multiply in the environment outside its host, methods to detect it are inexpensive, simple, sensitive, and specific, and it survives long enough under a broad range of drinking water conditions, so that in almost all circumstances a cost-effective sampling protocol can be developed (Edberg *et al.*, 2000).

While faecal indicator organisms, particularly total coliforms and faecal coliforms, specifically *E. coli* are relied on for the assessment of water quality, there is concern that these groups of microorganisms do not necessarily correlate well with the presence of pathogenic organisms. The lack of significant correlations between the presence of traditional indicators and pathogens (Grabow *et al.*, 1989; Araujo *et al.*, 1990) and the ability of pathogens to assume a viable but non-culturable state (Grimes *et al.*, 1986) has highlighted the potential inadequacy of indicator systems in predicting water quality and associated health risks (Ferguson *et al.*, 1996).

Alternatives to the traditional water quality indicators have been suggested. For example several studies claim that faecal streptococci and *Clostridium perfringens* are the most promising alternatives (Bisson *et al.*, 1979). Further aspects of alternative indicator organisms are considered in Section 7.

### **3.4 Risk Evaluation**

Based on the review of the literature on the biophysical aspects of pathogens on farms and their likelihood of impacting water resources, the following points provide a summary of the risks. For pathogens present in confined systems, risks relate to:

- Failures of structures and pipes
- Inadequate capacity to confine manure, especially to collect runoff from solid manure stores or to store liquid manure in prolonged wet springs
- Inadequate protection of on-site waste water treatment facilities leading to saturation or ponding

For pathogens present in unconfined conditions, risks relate to:

- Excess applications due to spills or poor management leading to temporary ponding or runoff
- Non-compliance with recommendations for the land application of manure or biosolids, or with standards for the operation of septic systems
- Adverse weather conditions after land application
- Permitting animals direct access to water courses
- Failure to prevent erosion of soil to which manure or biosolids have been applied, or around waste treatment facilities

#### **3.4.1 Risk Evaluation Models**

##### **3.4.1.1 OSRAS**

One of the most comprehensive risk assessment models associated with septic systems has been developed for the New South Wales Department of Local Government, Australia. Their On-site Sewage Risk Assessment System (OSRAS) is focussed on the hazard associated with the failure of on-site facilities and the cumulative impact and consequent downstream risk from sewage pollution. It exists as conceptual framework for the collation, classification and analysis of spatial data relevant to the management of on-site facilities. This framework will be considered in some detail because of the possibilities for considering it in the broader context of an agro-ecosystem indicator system.

The framework uses the concept of the natural hazard associated with a site, which is a measure of the relative risk of contaminant release due to the natural features of soil, landscape (including slope), and climate. Five classes of hazard are identified:

1. Minimum
2. Minor
3. Moderate
4. High
5. Severe

#### **3.4.1.1.1 Soil material**

A number of features of soils are identified as posing risk:

- Swell-shrink behaviour in the top metre
- Sodicity and dispersivity in the top metre
- Small water holding capacity in the top metre
- Slow permeability in the top metre
- Fast permeability in the top metre
- Salinity in the top 0.5 m limiting plant growth
- Poor fertility in the top 0.5 m limiting plant growth

To expand this to a broader indicator system suitable for Canada, the depth of frost penetration would need to be added to deal with runoff with spring melt when there are frozen soil layers.

An expanded list of soil limitations has also been developed:

- Structurally weak soils with limited load bearing
- Soils with excess organic matter
- Soils of high erodibility
- Hard-setting surface soils
- Presence of plant toxicity
- Acidity
- Acid sulphate
- Aluminium
- Alkalinity

#### **3.4.1.1.2 Landscape**

A significant number of landscape limitations are identified, many related to indication of the local and area hydrology. Risks are associated with:

- Area susceptible to flooding
- Area being a receiving site for runoff
- Area subject to persistent waterlogging
- Area subject to seasonal waterlogging; watertable encroaches seasonally within the top 0.6 m
- Elevated watertable <1.2 m
- Soil depth <1 m
- Rock outcrops
- Regolith within 1 m of soil surface
- Slope class - limitation is considered to be associated with the uneven distribution of materials leading to runoff (it would also provide some additional indication of the different threat to ground water.
  - <6% small risk
  - 6-12% minor risk
  - 12-20% moderate risk

- 20-25% high risk
- >25% severe risk

These classes would need to be revised to take account of typical agricultural topographic conditions in Canada.

### 3.4.1.1.3 Climate

Climatic risk is assessed according to the variability in precipitation, based on the observation that for septic systems the most common failure results from surcharge to surface and ground water resources because of rainfall exceeding design criteria.

The variability in rainfall is calculated as the difference between the mean annual value and the 90<sup>th</sup> percentile value (mm). The climate risk is then classified as:

- Low; variability < 1000 mm
- Moderate; variability 1000 - 1500 mm
- High; variability > 1500 mm

The onsite natural hazard classes can be constructed from a matrix of soil and climatic limitations (Tables 18a, b and c).

**Table 18a.** On-site natural hazard class matrix—climate classification low variability.

Soil classification	Slope classification				
	1 (<6%)	2 (6-12%)	3 (12-20%)	4 (20-25%)	5 (>25%)
Little limitation	1	1	1	3	5
Minor limitation	1	1	2	4	5
Moderate limitation	1	2	3	5	5
High limitation	3	3	4	5	5
Severe limitation	5	5	5	5	5

**Table 18b.** On-site hazard class matrix—climate classification moderate variability.

Soil classification	Slope classification				
	1 (<6%)	2 (6-12%)	3 (12-20%)	4 (20-25%)	5 (>25%)
Little limitation	1	1	2	4	5
Minor limitation	1	2	3	5	5
Moderate limitation	2	2	4	5	5
High limitation	3	4	5	5	5
Severe limitation	5	5	5	5	5

**Table 18c.** On-site hazard class matrix—climate classification high variability.

Soil classification	Slope classification				
	1 (<6%)	2 (6-12%)	3 (12-20%)	4 (20-25%)	5 (>25%)
Little limitation	1	2	4	5	5
Minor limitation	2	3	5	5	5
Moderate limitation	3	4	5	5	5
High limitation	4	5	5	5	5
Severe limitation	5	5	5	5	5

#### 3.4.1.1.4 Export hazard assessment

This assessment allows areas where waste water is applied to land to be evaluated, and considers the likelihood that contaminants will leave the site. It combines site natural hazard and the human hazard factors in assessing the risk of contaminant export. The assessment takes account of the land that is covered by impervious zones (e.g. roads, buildings). Calculations compare the amount of contaminant and the area for its application and assimilation. For nitrogen, N, it calculates the area under vegetation required to utilize the N entering the land base from on-site treatment systems. The calculation assumes that vegetation will assimilate  $140 \text{ kg N ha}^{-1} \text{ y}^{-1}$ . The average septic system generates

approximately 20 kg N ha<sup>-1</sup>y<sup>-1</sup>, and hence requires a seventh of 1 ha (1429 m<sup>2</sup>) for utilization. The steps for determining the critical land area for the export hazard assessment are:

- determine local water quality objectives
- determine physical characteristics of the site
- characterise on-site treatment and disposal facilities
- determine average failure rate of the facilities
- calculate dimensions of the disposal facilities
- determine typical concentrations of contaminants
- determine pollutant discharging from standard disposal facility
- determine volume of runoff from the catchment
- estimate losses and retention of pollutants
- determine typical background levels of pollutants
- calculate allotment density that will result in an average pollutant concentration equal to the water quality objective.

Where there is no chance that a contaminant can leave the chosen site and impact the environment, the risk is assessed as small. The availability of water that could increase the risk of waterlogging, runoff, or leaching, is used to provide a modifier of the risk. The framework assumes that risk is reduced if the site is more than 100 m from a water course.

#### **3.4.1.1.5 Environmental sensitivity**

Drinking water and recreational water can become contaminated as a result of the runoff or leaching of pathogens. OSRAS assumes that the impact of sewage-related contaminants depends the relative quantity or concentration of the contaminant that can reach a receptor, and that it also depends on the 'sensitivity' of the receptor to those contaminants. Sensitivity can be considered as the relative degree to which the receptor is affected by sewage contaminants. For pathogens, humans and human health are of primary importance. The concern is the introduction of pathogens such as viruses, bacteria, protozoa and helminths to drinking water sources, aquatic food production areas and recreational areas in contact with water.

Environmentally sensitive areas, include those in proximity to a waterway or shallow groundwater within a drinking water catchment and those within the catchment of an estuary intermittently open to the sea.

##### **3.4.1.1.5.1 Sensitivity thresholds**

For analysis of risk of impacts at a receptor, the probability of contamination is the likelihood of contaminant reaching the receptor. The consequence or magnitude of impact of contaminant reaching a receptor is a function of its sensitivity to the contaminant. The processes by which contaminants reach a receptor are complex, and depend on catchment characteristics together with the number, nature and location of hazards and the cumulative effects that catchments provide, the transport characteristics and the physical, biological and

chemical processes that influence contaminants during transport to the receptor.

“Sensitivity” to contamination can be considered as the relative impact on a receptor if a “threshold” level of a key contaminant reaches that receptor. Thresholds or guideline values need to be developed. Contamination with pathogens of source water for potable supplies can constitute significant risk, particularly if a large population is involved. However, drinking water (including limits for intake water to treatment plants) and recreational water guidelines can serve as the appropriate indicative guidelines (Table 19).

**Table 19.** Selected water quality indicators relevant to on-site sewage disposal for various environmental values.

Environmental value	Selected relevant water quality indicators	Indicative guideline
Raw drinking water	Faecal coliform bacteria Total coliform bacteria Blue-green algae counts	<1 cfu/100 mL Up to 10 cfu/100 mL Up to 5,000 cells/mL
Edible fish, crustacean and shellfish	Faecal coliform bacteria	(14 cfu/100 mL and 10% to be <43 cfu/100 mL)
Primary contact recreation	Faecal coliform bacteria Enterococci Protozoans Blue-green algae	150 cfu/100 mL with 4 of 5 samples <600 cfu/100 mL) 35 cfu/100 mL 0 cells/100 mL
Secondary contact recreation	Faecal coliform bacteria Enterococci	<1,000 cfu/100 mL <230 cfu/100 mL
Aquatic ecosystems	Total phosphorus (freshwater systems) Nitrate (estuarine and ocean) Chlorophyll- <u>a</u>	Rivers and streams 0.01-0.1 mg/L Lakes and reservoirs 0.005-0.05 mg/L Estuaries 0.01-0.1 mg/L, Coastal waters 0.01-0.06 mg/L

Ground water resources are sensitive to impacts from pathogen and nutrient-related factors. Ground water movement is generally far slower than surface water movement, and while potable groundwater supplies can be affected by pathogens, the spatial sensitivity of such resources is likely to be less than that for surface water supplies. Separation distances are generally recommended between potentially contaminating activities and groundwater bores. Larger distances are usually recommended for regional, as opposed to local supplies. Other factors also influence the risk of groundwater contamination, including the depth from which the groundwater is drawn ('shallow/deep') and the permeability of the surrounding soil matrix ('high/low'). Guidelines for selected environments that are sensitive to pathogens together with the required reductions from the key sources are required. An example for the protection of aquatic environments from septic system is given in Table 20.

**Table 20.** Indicative environmental sensitivity to pathogens (surface water focus).

Pathogen sensitivity class	Examples of sensitive environments	Threshold guidelines to faecal coliform bacteria (cfu/100 mL)	Relative reduction required from septic tank effluent
Very high	Raw water supplies	<1	$10^5$ - $10^7$
High	Aquatic foods (oyster leases/shellfish production areas)	<14	$7 \times 10^3$ - $7 \times 10^5$
Moderate	Primary contact recreation	<150	$7 \times 10^2$ - $7 \times 10^4$
Low	Secondary contact recreation areas	<1,000	$10^2$ - $10^4$

The sensitivity of aquatic food species — edible fish, crustacea and other sea food — depends on the flushing characteristics of their location and the time of exposure to contaminants during their life cycle.

Notional partitioning of distances potentially provides some indication of risk for categories subject to a spatial risk assessment (Table 21).

**Table 21.** Notional distances.

Distance class	Notional distance
Catchment	0-50 km
Subcatchment	0-10 km
Local	0-2 km
Neighbour	0-500 m
Immediate	0-100 m

Analysis of risk attenuation based on distance alone assumes all contaminants, such as pathogens, move through and survive in the environment in similar ways. Consequently, sensitivity to the most 'robust' pathogens must be assessed in the conservative environment of risk assessment. As information regarding pathogen transmission improves, these factors may be separated to provide additional detail. For example, differentiation may be possible between viruses, bacteria and helminths, based on their significantly different sizes or rates of survival in water or soil. Those with the potential to affect human health would be a priority. In most instances, shallow (e.g. alluvial) groundwater movement also follows surface water movement direction; however, exceptions exist. For example, geological layering, folding and faulting can lead to shallow groundwater movement in different directions to

surface water movement. This influence can become increasingly important for deeper groundwater reserves.

Catchments and drainage information provides a starting point in the assessment of the fate of emitted pollutants. Catchment boundaries also help clarify cumulative contributions of hazards to downstream receptors. This is where the capability of geographic information systems (GIS) can be used to identify the pathways for transport and the environments at risk.

Drainage lines are usually identified through the interpretation of contour information and other resources, such as aerial photographs. Some GIS and other spatial analysis packages, can interpret digital elevation models (DEMs) to analyse drainage patterns by assessing cumulative flow from one grid cell into surrounding cells. In such GIS, 'flow accumulation' is defined as the number of cells flowing to a point, which when multiplied by the cell area, provides a direct measurement of the catchment area at that point. Generally, the larger the catchment the larger the capacity of the drainage line. Through the application of similar GIS tools, catchment boundaries can also be mapped.

Flow accumulation is a particularly important GIS application in the context of OSRAS as it can also be used to assess 'hazard accumulation'. Hazard accumulation is a flow accumulation grid weighted with a grid defining hazards. This can be used to assess the cumulative number of grid cells containing potential pollutant export sites within the catchment of a sensitive receptor.

OSRAS contains all the elements necessary for an agroenvironmental risk assessment process. It has the flexibility to include both confined and unconfined sources.

#### **3.4.1.2 Bacteria Leaching Risk**

An index system for the risk of bacterial pathogen leaching was developed by the University of Guelph as part of its decision support system MCLONE 4 (Manure Cost Labour Odour Nutrients Environment). It comprises two spreadsheet analysis packages. One deals with contamination of ground water, the other with surface water. The calculations are based on information for bacteria, and need to be reassessed to decide whether they are adequate for protozoa such as *Cryptosporidium parvum*. Only the calculation for risk of groundwater contamination has been added into the manure management software.

The essence of the two spreadsheets is that they consider the likelihood of surface ponding. For surface runoff, the duration of ponding is assumed to indicate the extent of runoff. For the contamination of ground water, surface ponding is required to develop macropore transport. Bacteria are too large to move through the soil matrix except via macropores. Risk assessment for the bacterial contamination of groundwater was developed based on experimental data (Unc, 1999) and use of the SHAW model for predicting surface ponding. It includes factors for season, field characteristics, manure type and management practice. For example, if liquid manure is surface applied in June and the ground water table is at 4 m

depth and soil water status is moist, the risk level is moderate. If the manure is incorporated however and the soil is dry, the risk level would be low (Table 22).

#### **3.4.1.2.1 Probability of Deep Percolation Exceeding 30 or 60 mm**

Estimates of seasonal deep percolation for predicting leaching risk are long term averages based on deep percolation estimates by the SHAW model when run using daily weather data for 1954 to 1987. A scale number is assigned to the average value as part of the N leaching risk calculation. The categories of deep percolation amount used for assignment of scale numbers can also be assigned a probability of occurrence, based on frequencies in the SHAW output data set for 1954 to 87. The number of years in which deep percolation exceeded 30 mm (moderate amount) or 60 mm (high amount) for each season were calculated. Logistic regression was used to derive relationships between these frequencies and slope, cover, and clay content for three regions of Ontario. The probabilities for the season with the greatest leaching risk can be output to help the user better assess the leaching risk. For example, for Guelph in the fall, if slope = 2%, residue cover = 0.3, and clay content = 25%, the probability that deep percolation equal or exceed 30 mm is 30%, and exceed 60 mm is 14%. The user should interpret this as meaning a moderate leaching risk in the fall could be expected about one year in three and a high risk about one year in seven, assuming a moderate amount of N available for leaching.

#### **3.4.1.2.2 Estimation of Deep Percolation Between Any Two Days**

Also to help in judging the leaching risk after a manure or fertilizer application, equations were developed for predicting the amount of percolation expected during a time period chosen by the user. For example, this period might be from application until crop uptake begins, or until the end of leaching in early summer, or until soil freeze-up. Values exceeding 30 or 60 mm could be interpreted as moderate or high risk for N leaching. These estimates would provide a check on the original seasonal leaching risk by allowing the user to evaluate the risk for a specific time period that they might judge to be more appropriate than the seasonal evaluation.

The prediction equations were developed using the long-term average daily amounts of deep percolation predicted by the SHAW model. Polynomial equations were fitted to the cumulative daily percolation values for the combination of slope, cover, and clay content that gave the greatest annual percolation, and the combination that gave the least annual percolation. For the Guelph site (i.e. Central region), the difference between minimum and maximum cumulative values was very small, and so the average value was used. The user should judge where their situation would fall within the range based on the drainage properties of their soil. Use of this estimation tool might help the user develop a greater interest in managing the N leaching risk.

### 3.4.1.2.3 Probability of a Daily Rainfall Exceeding 38 mm

**Table 22.** Assignment of scale numbers and risk levels in evaluating the bacteria leaching risk.

Factor Levels		Scale Number
F1)	Ground water table depth (m)	
	0 - 2	4
	2 - 5	2
	below 5	1
F2)	Soil moisture status	
	wet	4
	moist	2
	dry	1
F3)	Season	
	winter	4
	spring	2
	summer and fall	1
F4)	Manure type	
	liquid	8
	slurry	4
	solid	1
F5)	Management practice	
	surface applied, not incorporated	2
	incorporated	1
Assignment of Risk Levels:		
	$\text{Log}_2 (F1 * F2 * F3 * F4 * F5)$	Risk
	0	None
	1-3	Low
	4, 5	Moderate
	6, 7	High
	8-10	Very high

Rainfall causes leaching mainly in the spring and fall because the soil profile is wet due to greater precipitation than evapotranspiration. During these times, light rainfall interrupted by days without rain will result in minimal leaching, but a heavy rainfall will leach nitrate through the soil matrix. Also a heavy rainfall under drier soil conditions can cause very rapid leaching of surface applied nutrients by transport through macropores. This macropore flow can carry not only soluble nutrients such as nitrate but also less mobile nutrients such as ammonium and phosphorous and also manure bacteria. It is especially of concern when manure has been applied to the soil surface on tile drained land that has not been recently tilled, because contaminants in the macropore flow can directly enter the tile drains.

A first step in quantifying the leaching risks associated with a heavy rainfall is to know the probability of such a rainfall event occurring in a given time period. Using daily rainfall data for 1961 to 1990, the frequency of days with a rainfall amount exceeding 38 mm (1.5 inches) was determined for 36 periods (9 to 11 days in length) during the year. The value of 38 mm was chosen because it is within the range of moderate risk for seasonal deep percolation values, and because a greater value would result in very low frequencies of occurrence. Polynomial equations were fit to the cumulative frequency for each of the three sites. The equations can be used to calculate the probability of a daily rainfall exceeding 38 mm between any two days in a year or consecutive years. This risk calculation is also relevant to concerns about runoff of surface applied manure. A rainfall event of this magnitude will likely cause some overland flow, especially in the spring or fall when the soil is already wet.

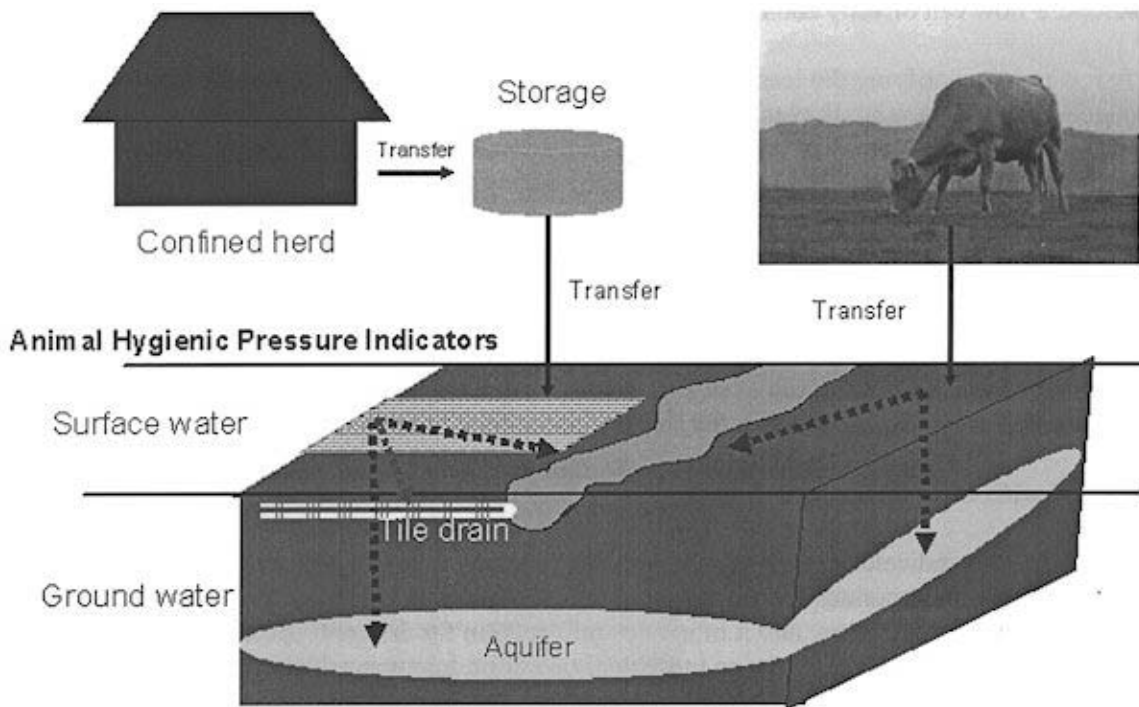
#### **3.4.1.3 Health Canada Animal Hygienic Pressure Indicators**

Health Canada's indicator system takes a similar approach to that in MCLONE4, with the exception that it incorporates die-off information for pathogens in storage and after faeces have entered the soil water system, and it integrates information for different species within a livestock operation and integrates values for an individual operation into watershed or regional locations.

The data required to produce the summary indicator is all considered to be that which is currently available. The unit of assessment is each livestock operation. For each animal group (e.g. beef cattle, dairy cattle, swine, layer hens, broiler chicken) the housed animals and those given free access to the fields are considered separately, and the information finally summed. The information for each livestock operation within an accounting unit (e.g. watershed, geographic region, public health area) is also summed.

For an animal component, information is required for:

1. Excretion of pathogens by housed animals
2. Excretion of pathogens by grazing animals
3. Survival of pathogens during storage of manure
4. Survival of pathogens after manure from housed animals has been removed from storage and land applied
5. Survival of pathogens after faeces from grazing animals have been excreted onto the land



**Figure 15.** Schematic diagram showing the components of the Animal Hygiene Pressure Indicator system. Pathogens associated with animals in the barn, and grazing in fields together with the stored manure and manure applied to the land (directly by excretion, or indirectly as part of agronomic practices) comprise the animal component. The soil component includes the pathogens present in the transporting medium, either surface runoff or infiltration water.

Excretion of pathogens,  $A_1$ , is given by

$$A_1 = N \times P \times V \times C$$

where  $N$  is the number of animals,  $P$  is the true prevalence of pathogens within the herd,  $V$  is the volume of manure excreted per animal per day, and  $C$  is the concentration of pathogens in manure.

Survival time (days) of pathogens,  $A_2$ , during storage of manure is given by

$$A_2 = \int_{t=0}^{t=t_d} e^{-k_f \times t} dt$$

where  $k_f$  is the net die-off rate of pathogens in faeces,  $t$  is the time, and  $t_d$  is the duration of manure storage.

Survival of pathogens,  $A_{3h}$ , after manure has been land applied is given by

$$A_{3h} = e^{-k_s \times t_{1r}}$$

where  $k_s$  is the net die-off rate of pathogens in soil, and  $t_{1r}$  is the interval between manure spreading and the first rainfall event that occurs afterwards

Survival time (days) of pathogens,  $A_{3g}$ , following excretion by grazing animals is given by

$$A_{3g} = t_g / f_r \int_{t=0}^{t=f_r} e^{-k_s \times t} dt$$

where  $k_s$  is the net die-off rate of pathogens in soil,  $t$  is time,  $t_g$  is the time on pasture, and  $f_r$  is the interval between rainfall events during the grazing season.

For a soil component, information is required for:

- pathogen runoff into surface water
- pathogen infiltration down to ground water

In both cases, there is a sub-component related to grazing and one related to manure that has been land applied.

Pathogen runoff,  $S_r$ , is given by

$$S_r = \frac{(M_r \times P \times D_s)}{A} \times P$$

where  $M_r$  is the proportion of pathogens that are available for runoff,  $P$  is the rainfall in the period (monthly value for those months when manure is spread, or the time on pasture for grazing animals) when manure is applied or deposited,  $D_s$  is the proportion of precipitation partitioned to surface runoff,  $A$  is the area of land, and  $p$  is a zero or unity factor covering incorporation of the manure.

Pathogen infiltration,  $S_i$ , is given by

$$S_i = \frac{(M_i \times P \times D_i)}{A} \times P_p \times \tau$$

where  $M_i$  is the proportion of pathogens that are available for infiltration,  $P$  is the rainfall in the period (monthly value for those months when manure is spread, or the time on pasture for grazing animals) when manure is applied or deposited,  $D_i$  is the proportion of precipitation partitioned to infiltration,  $P_p$  is a zero or unity factor covering pre-tillage, and  $\tau$  is a zero or unity factor for the presence of a sub-soil drainage system.

The surface water indicator,  $SI_{ahp}$ , is calculated as the integration for each livestock operation and each livestock species in the operation, of each component:

$$SI_{ahp} = \sum_{livestock-operation} \sum_{Species} [\log (A'_1 \times A'_2 \times A_{3h} \times S'_\tau) + \log (A''_1 \times A''_2 \times A_{3g} \times S''_\tau)]$$

Where  $A'$  and  $S'$  indicate the component for land applied manure, and  $A''$  and  $S''$  indicate the component for grazed land.

The ground water indicator,  $GI_{iahp}$ , has a similar formulation.

## 4. Best Management Practices and Associated Government Instruments

### 4.1 Best Management Practices in Canada

Best or beneficial management practices (BMPs) are designed to be practical, affordable approaches to conserving soil, water, and other natural resources in rural areas. Nutrient management issues are covered by a variety of BMPs for soil and water management, as well as livestock and poultry waste management. The management of sewage and other biosolids that can be applied to agricultural land is included under these same BMPs. The BMPs cover activities relating to the collection, transfer, storage, and land application of waste materials, plus restriction of livestock access to watercourses. In general, few BMPs are specifically directed towards the protection of water resources from pathogens. Importantly it is recognized that manure applied at excessive rates, or that leaches or runs off following applications, can damage the environment through:

- contamination of water with disease-causing organisms,
- poisoning of fish and other aquatic organisms from ammonia toxicity,
- oxygen depletion of water from the addition of organic matter, and
- physical and biological damage from organic material

BMPs have been developed for manure handling in the barn, long-term storage, and land application. There are recommendations about the transfer of manure from the barn to the storage facility. In addition to the BMPs, regulations and guidelines may apply that determine the appropriate siting and setbacks (minimum distance separation) for barns and storages, and general biosafety on farms. The general framework for Ontario is presented in OMAF's *Guide to Agricultural Land Use* (OMAF, 1995a). Specific regulations are being developed in association with the Nutrient Management Act, 2002.

Ontario's MDS I guidelines provide minimum distance separation for new non-agricultural development from existing livestock facilities (OMAF, 1995b). Ontario's MDS II guidelines provide minimum distance separation for new or expanding livestock facilities from existing or approved development (OMAF, 1995c). The differences between MDS I and MDS II applications are based on whether the proposed change in land use is due to a non-agricultural development in an area with existing agricultural operations (MDS I) or on whether the change is due to proposed changes in livestock operations in the area of an existing or already approved non-agricultural land-use activity. Thus, the distinctions recognize rights of prior uses.

#### **4.1.1 Manure handling and storage**

Farmers are made aware that potential pollutants from manure include:

- coliform bacteria that can contaminate water supplies;
- pathogenic bacteria that can cause disease in humans and livestock in extreme cases;

Manure can pollute air and water in a variety of ways, such as:

- contaminated liquid can run from storage areas and exercise yards into surface and groundwater;
- manure stored on gravelly soils or shallow, cracked bedrock can pollute groundwater;
- BMP requires at least 240 days storage capacity

#### **4.1.2 Land application**

A given field's suitability for manure and biosolids application depends on a combination of topography, soil type, and vegetative cover. Application rates should include consideration of the total nutrient management requirements for the farm, according to crop and soil fertility. BMPs suggest the optimal timing of applications, as summarized in Table 23. In general, factors to be considered when spreading manure and biosolids include:

- preventing the loss of nutrients in surface runoff;
- reducing the loss of nitrogen into the atmosphere;
- minimizing soil compaction and problems with soil structure;
- eliminating oversupply of nutrients in soil caused by spreading manure on same ground each year;
- preventing leaching of nitrate into groundwater;

- reducing pollution of waterways by manure runoff or direct livestock access;
- slowing the build-up of nutrients and bacteria in ponds, wells, and other waterways;
- spreading of manure on forage and pasture appropriately to avoid rejection by animals.

To avoid risk of bacterial contamination of wells and groundwater, the following guidelines for separation distances are followed:

- 15 m (50 ft.) for drilled wells with a steel casing greater than 30 m (100 ft.) in depth, and
- 30 m (100 ft.) for all other wells. In fields with shallow soil (<1 m) over bedrock, or if the water table is less than 1 m from the soil surface at the time of manure application

**Table 23.** Summary of General BMPs for applying nutrients to crops.

Purpose	Practice
For Production and Profit	Apply exactly what crops need when they need it. Maximize the benefit of nutrients by reducing losses and apply where the nutrients will be used most efficiently.
For Practicality	Rotation may use nutrients not taken up by previous crops.
For Protection of the Environment	If the crop's needs are met by applying nutrients in the right amount at the right time, there is no detriment to the environment.

**Source:** Canada, Agriculture and Agri-Food Canada (AA-FC), Ontario, Ministry of Agriculture, Food and Rural Affairs (OMAFRA), and Ontario Federation of Agriculture (OFA), 1996, *Best Management Practices, Livestock and Poultry Waste Management* (Toronto, ON: OFA; Canada, AA-FC et al.).

#### 4.2 Best Management Practices (BMPs) Recommended in the United States

The USA strategy does not prescribe specific best management practices, but rather specifies "management measures," defined as "economically achievable measures to control the addition of pollutants to our coastal waters, which reflect the greatest degree of pollutant reduction achievable through application of the best available non- point pollution control practices, technologies, processes, siting criteria, operating methods, or other alternatives." (NACAC, 2000). The emphasis is to develop coordinated groups of affordable management practices that can be used together as a system to achieve comprehensive goals at specific sites, without recommending any particular practice outside the context of an overall goal. A group of practices is termed a "management measure." The US General Accounting Office (1999) suggests that the factors that should influence the choice of management practices for a management measure include:

- site-specific factors, type and volume of waste, and proximity to surface or groundwater;
- cost considerations;
- state and local regulations

Recommended components of management measures for animal feeding operations include:

- *Divert clean water:* Siting or management practices should divert clean water (run-on from uplands, water from roofs) from contact with holding pens, animal manure, or manure storage systems
- *Prevent seepage:* Buildings, collection systems, conveyance systems, and storage facilities should be designed and maintained to prevent seepage to ground and surface water
- *Provide adequate storage:* Liquid manure storage systems should be (a) designed to safely store the quantity and contents of animal manure and wastewater produced, contaminated runoff from the facility, and rainfall from the 25-year, 24-hour storm, and (b) consistent with planned use and schedules. Dry manure should be stored in production buildings or storage facilities, or otherwise covered to prevent precipitation from coming into direct contact with the manure
- *Application:* Apply manure in accordance with a nutrient management plan that meets the performance expectations of the management measure
- *Address lands receiving wastes:* Areas receiving manure should be managed in accordance with the erosion and sediment control, irrigation, and grazing management measures as applicable, including practices such as crop and grazing management practices to minimize movement of applied materials, and buffers or other practices to trap, store, and 'process' materials that might move during precipitation events
- *Consider the full range of environmental constraints and requirements:* When siting new facilities or expanding existing ones, operators should consider the proximity to surface waters, areas of high leaching potential, and sink holes or other sensitive areas.

Waste management practices include:

- limit runoff by cementing and curbing animal confinement areas or planting grassed buffers around these areas;
- collect and store waste, e.g., with scraping or flushing systems, storage tanks, or retention ponds;
- alter or treat waste, e.g., by reformulating feed mixes or composting;
- use waste, e.g., as an organic fertilizer, as an additive to animal feed, or for on-farm energy generation, using methane produced from anaerobic decomposition of wastes in covered lagoons or tanks (NACAC, 2000)

A barn or waste lagoon cannot be located in a 100-year floodplain or a jurisdictional wetland, nor within 150 feet of a lake or river. Land application of livestock waste is not allowed within 150 feet of water wells. Waste management system operators who intend to apply liquid hog manure must take soil samples from the fields to be treated and complete an analysis of swine waste nutrient content. Land application is not allowed on saturated ground, during precipitation, or on frozen ground, and waste must not be applied at a rate that exceeds infiltration. All swine waste application areas must have a filter strip on its lowest side.

### **4.3 Actions in Canada**

#### **4.3.1 New Brunswick**

The setback restricts establishment of any new agricultural land use within 75 m of a watercourse, and allows no agricultural activity, including grazing livestock, within 30 m. Tillage must be managed to prevent surface runoff from entering the watercourse. The recommended minimum separation distances are in part based on hydrogeological information such as groundwater sources, quality, and quantity; depth to the water table; depth to bedrock; and surface slope. The guidelines provide recommendations on the spreading of manure to optimize crop performance while minimizing water contamination. Requirements for a minimum land-base and application rates are supplied in tabular form. Other actions aim at preventing spillage on public properties.

#### **4.3.2 Quebec**

The goal is to minimize environmental impacts of animal agriculture by providing for leak-proof storage of livestock waste and regulating spreading activities on cultivated land (Environment Quebec, 2001). Livestock waste spreading is restricted to growing periods before October 1 and after March 1, and the use of sprinklers and liquid manure cannons has also been prohibited. At no time is animal waste accumulating in a livestock raising facility allowed to come into contact with the soil. Animal waste storage facilities must be leakproof, and the floor must be above the highest level of the water table. Storage facilities must be situated so as to prevent infiltration by runoff. Certain solid manure storage facilities are exempt from leak-proof requirements. These facilities can only be used where there are smaller livestock populations than the limits provided by the regulation. Storage facility capacity must allow accumulated waste for a minimum period of 250 days, and 200 days for facilities built before 1997. The maximum amount of waste material that can be stored cannot exceed a facility's limit or the quantity that can be spread on the land at the facility's disposal. Surplus waste must be transported in a closed watertight container to a manure management organization. The regulation specifies conditions under which solid manure coming from a building is exempt from watertight storage requirements. These include a minimum distance of 300 m from groundwater sources or municipal water supplies; 150 m from a lake, watercourse, natural marsh, swamp or pond; and 30 m from a ditch. In addition, such facilities must be secure from runoff infiltration, on a slope of less than 5%, not located in the 20-year floodplain of a watercourse or lake, and not located on the same site for two consecutive years. A waterproof covering must cover manure from a group of facilities comprising 35 or more animal units.

#### **4.3.3 Ontario**

*Protection of water resources from land application* All operations need to consider the protection of water resources.

*Surface water* Runoff causing soil erosion carries particulate and dissolved substances into

surface water. Protection of surface water depends on preventing water erosion Table 24 summarizes the relationships between soil type, land topography and the potential for surface water contamination from runoff and contamination from manure and biosolids (Canada, AA-FC, OMAF and OFA, 1998).

To deal with surface runoff farmers are recommended to:

- construct erosion-control structures, e.g., grassed waterways, water and sediment control basins, and diversion terraces, to manage concentrated flows of water,
- use strip cropping and buffer strips. Strip cropping is the practice of planting alternating strips of row crops with forages or cereal crops,
- till and plant crops across the slope where possible or use a system of contour cropping.

**Table 24.** Potential for surface water contamination from manure runoff.

Soil Infiltration Rate (Soil Texture)	Surface Water Contamination Potential			
	Topography (land slope) within 150 m (500 ft.) of water			
	<0.5%	0.5-2%	2-5%	>5%
Fast (sand)	VL	VL	VL	L
Moderate (loam)	VL	L	L	M
Slow (clay loam)	L	M	M	H
Very slow (clay)	M	H	H	H

Sources: Canada, AA-FC *et al.*, 1996, 1998

More general BMPs that help to reduce the susceptibility of soils to erosion are associated with:

- Improved residue cover that moderates soil temperature and encourages higher earthworm populations which benefit the soil structure
- Incorporation of crop rotations that alternate forage or cereal crops with row crops. The forage or cereal crops leave less soil exposed over the year, whereas the row crops leave the soil exposed for much of the year and return little residue to the soil
- Install subsurface drainage in wet fields. Where possible, soils that are naturally low lying or have high water tables and need drainage. Drainage also benefits crops and adds value to agricultural land. Surface drains remove water in shallow open ditches but have limited effect on the water table. They are usually used in fine-textured soil. Subsurface drains (tile drains) remove excess water from the soil profile. Water moves down to the tile drains by gravity

*Groundwater* The main approach of BMPs is to carry out good nutrient management planning. It is recommended that farmers:

- schedule nutrients to meet crop needs,
- use lower rates,

- pre-till to reduce excess percolation, and
- monitor tile outflow carefully following application

Some fields are naturally slowly draining, and the water table can be found within the top metre of soil in the spring. BMPs that deal with such soils recommend that farmers:

- install drainage tile and/or surface drains;
- grow crops suited to wetter soil conditions or crops that are planted later in the growing season (e.g., soybeans, winter wheat);
- use a reduced tillage system such as ridge tillage, which creates a zone of drier soil for plant growth;
- use tillage carefully to expose soil to the air for evaporation and soil warming;
- use crop rotations;
- include deep-rooted crops such as alfalfa, clover etc.;
- encourage earthworm population for macropore development by leaving residue on the soil surface;
- use timely tillage and field operations;
- minimize the tillage passes to reduce compaction; and
- timely tillage and field operation: stay off wet fields, soil should be at proper moisture conditions at tillage depth;
- in winter, manure should go into storage. Winter application should only be considered if the storage is full (all recent livestock operations should have adequate storage).

There is considerable risk of runoff with snowmelt, and no nutrient demand from crops at this time. The following must be taken into consideration:

- manure should not be spread on frozen bare (no cover crop) land;
- manure should not be spread when it is likely to run off, e.g., if a period of mild temperatures, rainfall, or wet snow is forecast for the ensuing 48 hours; and
- manure should be spread on a level field and kept away from water courses.

It is recommended that separation distances between applied manure and surface water sources based on surface water contamination potentials should be doubled when spreading is done in winter.

*Buffer zones and setbacks for watercourses* Buffer strips or buffer zones are permanent borders on field boundaries or along watercourses that help reduce soil input into streams.

## **5. Current Studies Undertaken in Other Jurisdictions in Canada, North America, Europe and Australia to Develop an Index System for Assessing the Risk of Pathogens from Agricultural Operations Contaminating Water Resources**

Contacts were made by electronic mail to experts in zoonotic diseases across Canada, the U.S., England and Wales, Scotland, Italy, Germany, Denmark, and Australia. In addition a search of relevant Websites on the Internet was undertaken and Dr Goss visited the Scottish

Agricultural College in Edinburgh in December 2002. Responses were obtained from all countries with the exception of Italy. A listing of all respondents is presented in Appendix 1.

In Canada, the program closest to the development of an agroecosystem indicator for pathogens from agricultural operations is a joint project led by M. Bigras-Poulin. The program starts from the position that infections by enteropathogenic microorganisms linked to agroenvironmental contamination represent a significant threat to urban and rural communities. To characterize and manage this risk better, the researchers consider that it is necessary, not only to describe accurately the enteric illnesses occurring over time or across regions, but also to assess correctly exposure attributable to this environmental pollution. New agroenvironmental hygienic pressure indicators (AHPis) are being developed to synthesis relevant data expressing this exposure. They will be derived from a conceptual framework for developing sustainable agriculture indicators and specifically adapted for describing the microbial risk of water contamination by livestock operations. The proposed indicators include two components, and five attributes whose values are calculated at the livestock operation level from a set of available data related to the fields of microbiology, animal production, agronomy, hydrology, and meteorology. They will then be aggregated at a higher geographical level to better express exposure of populations to potential of water contamination by zoonotic enteropathogens. The indicators will be calculated separately for zoonotic enteropathogens, and for water source (surface or groundwater). They take into account the various animal species within each livestock operation. When validated, the proposed indicators will allow decision-makers and public health officials to better manage crucial issues in the area of water safety and agriculture. A full account is provided in Section 3.4.1.3.

In The U.S.A., an agroecosystem indicator review was carried out, starting in 1994. The concepts were to develop a model for identifying and using indicators to assess ecosystem conditions (Ecological, economic, and social). However, no other references were found that suggested that a pathogen indicator has been developed. Current research is focussed on source tracking (see Section 7) and on the development of molecular methods to identify the presence of specific pathogens in environmental samples (see Section 7.1). There is a recognition that multiple organisms will need to be used as indicator species for source tracking, and the main interest is on their detection. Specific programs are investigating the use of animal viruses (bovine phage (genotyping male-specific RNA coliphage, Bacteriodes phage and Bifidobacterium phage). For *E. coli*, antibiotic resistance markers (tetracycline, sulfamethoxazole, cephalothin, ampicillin), chemical markers (methanogen fluorescent compounds), PCR methods (ribotyping). For protozoa, genotyping is the approach of preference.

Information from Australia suggested that the approach there is similar to that in the U.S.A. with the exception that an indicator system for on-site sewage risk assessment (OSRAS) has been developed (see Section 3.4.1). In Europe, the approach has been to develop models that describe the movement of (bacterial) pathogens from animal manure and sewage biosolids to surface water courses and to resort beaches. Much of this activity is linked to the need to deal with preventing untreated sewage entering shallow seas, such as the North and

Irish Sea. One model under development provides a generic microbial risk assessment for the spreading of biosolids on land.

Indicator organisms being investigated include those identified above, but in addition somatic coliphages and bacteriophages infecting *Bacteriodes fragilis* have also been considered.

In Scotland, pilot schemes have been initiated to study measures to reduce livestock pollution of the water environment (D. Merrilees and M. Aitken, SAC). Four schemes are in place involving 56 farms. An assessment has been made of the risk that each farm poses to the contamination of water resources. Mitigation measures have been identified to deal with the risk on each farm. The loading of contaminants from each farm was assessed and, together with the suggested mitigation measures and other baseline data, was entered onto a geographical information system (GIS) database. Between 95% and 99% of all faecal indicators in streams was associated with conditions of high rates of flow. This observation is significant as it highlights the fact that most contamination is driven by storm events, and that monitoring regimes have to be responsive to such conditions. Appropriate schemes have been put in place to monitor the impact of the imposition of the mitigation measures.

Underpinning this study is a multidisciplinary research program on particulate modelling and measurement of the fate and movement of microorganisms (PAMIMO). It involves a detailed study of pathogen dynamics at spatial scales from laboratory to catchment. Included in the deliverables on the program are a field-scale model and a distributed catchment scale model of transport and fate of microbes. The models aim to consider both direct defecation by grazing animals as well as the spreading of manure from housed animals (D. Lewis, SAC). The transport through soil is a layered soil approach and comprises transport through the matrix as well as by fracture flow. During transport, microbes undergo sorption, straining and filtration. Microbe numbers are determined by population growth, mortality and predation, and are influenced by water content, temperature, carbon to nitrogen ratios of potential substrates, and by pH. The transport at the catchment level assumes a simple delivery of microbes from fields. It calculates the water balance and routes runoff water according to topography. The infiltrating water moves through soil with two groups of pores, and field drains are included as a means of repartitioning the water to surface and ground waters. Microbes entrained within the flux of water through the soil are subject to decay. The model is tightly coupled to a GIS system. The flow of microbes in surface runoff or from tile drains then acts as input to a river transport module. This module partitions rivers into reaches with a minimum size of 200 m. It includes decay, sedimentation and entrainment of microbes within each reach. It allows for point sources to discharge into each reach.

## **6. Identification of Components of Existing Agroecosystem Indicators that could Contribute to an Agroecosystem Indicator for Pathogens**

Assessment of the probability of surface runoff required in the pathogen indicator is also a component for a Phosphorus indicator and the assessment of the probabilities associated with preferential flow regimes is likely to be an important component of an agroecosystem indicator on Nitrogen. Information on other agroecosystems was insufficient to identify

possible common components with the requirements of the pathogen indicator.

## 7. Approaches to Enhancing the Effectiveness of Agro-ecosystem Indicators

Agro-ecosystem indicators can highlight the risk to natural resources of contamination with plant nutrients, crop protection agents, soil and pathogenic organisms, but the quality of the resource, especially that of water has also to be monitored. Considerable research activity surrounds the development of approaches to monitor the quality of water resources, and establish the nature and source of any microbial contaminants present. The source can help identify measures to prevent or reduce contamination to below the total maximum daily load appropriate for the resource. Such measures may range from repairs to manure storages or correction of faulty domestic septic systems to the establishment of appropriate BMPs such as riparian buffer strips.

### 7.1 Microbial Source Tracking Methods

Four approaches are being used to track sources of microbial contamination:

1. Speciation - finding species that are indicative of a given source. Four bacterial genera have been used - *Escherichia* (particularly *E. coli*), *Enterococcus spp*, *Bacteroides spp*, and *Bifidobacterium spp*. *E. coli* and *fecal enterococci* have been isolated and cultured from different faecal sources. An important limitation of these methods is that they depend on culturing techniques, but these tend to underestimate the density of bacteria in a source sample. Non-culturing approaches have involved *Bacteroides spp*, and *Bifidobacterium spp*, not least because *Bacteroides spp*. require highly specialized culturing facilities. Because of their ubiquity, it is possible that bacteria like *E. coli* and faecal enterococci are not the most suitable indicator species. Research with oocysts of *Cryptosporidium spp* has shown that there are genotypes specific to different animals. Oocysts have been used to identify the probable source of faecal contamination in storm water (Xiao *et al.*, 2000). Human, cattle and some wild animal sources were identified in this way (Xiao *et al.*, 2001). Coliphages (viruses that infect *E. coli*) have been shown to form four groups of male specific (F+) types. Group 1 are found in both human and animal faeces. Group 2 and 3 are largely associated with human faeces, and Group 4 are mainly found in animal faeces. Genetic discrimination between coliphages does not seem to allow differentiation of animal faeces. Bovine enteroviruses are also being proposed for use in distinguishing between different animal sources (Ley *et al.*, 2002).
2. Biochemical tests - testing for biochemical reactions or products typical of microbes from the source. Antibiotic resistance analysis (ARA) is the most commonly used biochemical method. It uses antibiotic resistance patterns for faecal streptococci, enterococci, or *E. coli* to identify faecal sources. The assumption is that human enteric organisms will have greater resistance to antibiotics than those from animals. Large numbers of isolates can be tested for resistance to a range of antibiotics over short periods of time (Hagedorn, 2002). The profiles for sample isolates are compared with

those from known sources within the region. ARA is susceptible to bias because of the transfer of plasmids and other genetic material carrying multiple antimicrobial resistance genes. Acquisition or loss of such material changes the resistance profile of an isolate. The average rate of correct classification of an isolate is smaller for isolates from an unknown source than for those from known sources. It has been stated that ARA may be difficult to interpret where animal manure is applied to land (Simpson *et al.*, 2002). Another factor is that the antibiotic resistance capability declines if the selective pressure is not maintained. Analysis of the fatty acid methyl ester (FAME) content of bacterial capsule material provides another chemical method that allows an environmental isolate to be compared with a data in a library of bacteria from known sources. Carbon utilization profiling (CUP) tests the ability of isolates to use a variety of carbon sources. A commercially available BIOLOG system allows the utilization of 96 carbon sources to be tested over a short period of time. The disadvantage with the technique is that many factors in the environment can modify nutrient requirements and therefore change the profile relative to profiles of bacteria from a library connection.

3. Establishment of assemblages of microbes that together can indicate the presence of genes of microbes from different sources. Conboy and Goss (1997) proposed an assemblage of micro-organisms consisting of *E. coli*, total coliforms, *Clostridium perfringens*, and faecal streptococci to identify the source and timing of ground water contamination. Presence of *C. perfringens* and *E. coli* signalled animal manure as the source of contamination in well water, but contamination by *E. coli* alone indicated a human source. The ratio of faecal coliforms to faecal streptococci has been used to distinguish between human and animal sources. Human faeces normally has a ratio of 4 or more, whereas ratios below 0.7 are associated with animal faeces. However, differences in faecal enterococci densities have been found in individuals with different diets and environmental factors differentially influence the survival of the two groups of organisms (Simpson *et al.*, 2002). The ratio failed to allow the discrimination of human and domestic animal sources (Howell *et al.*, 1995)
4. Molecular methods to identify the genomes or phylogenetic genes of microbes from the contaminated resource and link them to a particular source. These methods largely depend on polymerase chain reaction (PCR) techniques, and have the potential to detect the presence of indicator species without relying on culturing the organisms. PCR *coupled* with detection by the use of fluorescence markers allows near real-time monitoring of bacteria present in environmental samples. A limitation to adoption of these techniques is the presence of substances such as humic acid and cations can inhibit the development of fluorescence

Several methods rely on universal genetic markers to discriminate between bacteria from different sources. Ribotyping consists of generating sequences of 16SrDNA by restriction fragment length polymorphism (RFLP). Essentially, fingerprints are constructed based on the size differences of fragments of DNA related to the location of specific gene sequences. Parveen *et al.* (1999) and Carson *et al.* (2001) used

ribotyping to distinguish between *E. coli* of human origin and from a mix of isolates from non-human sources. However, the average rate of correct classification of an isolate was small when more than three faecal sources were involved. Natural populations of *E. coli* exhibit a wide diversity of genetic variation, so future studies using *E. coli* ribotyping will require more isolates if the applicability of the method is to be evaluated for source tracking.

Length Heterogeneity-PCR and Terminal Restriction Fragment Length Polymorphism are based on the detection of fluorescently labelled 16S rDNA PCR products using an automated DNA sequencer (Bernhard and Field, 2000). There is significant diversity in Bacteroides - Prevotella 16S rDNA sequences present in animals faeces, and this allows discrimination of human and cattle faeces (Bernhard and Field, 2000).

Repetitive PCR, uses conserved sequences in repetitive elements as PCR primers to distinguish between different strains of the same species. In bacteria there are three major repetitive elements that have been used to generate fingerprints: REP, ERIC and BOX. These repetitive elements are present in most Gram negative bacteria. BOX primers are more helpful in determining the separation of human from animal bacteria (Simpson *et al.*, 2002).

Denaturing Gradient Gel Electrophoresis is a technique capable of discriminating between different PCR products of similar size based on changes in electrophoretic mobility induced by the melting properties of DNA fragments. It has been used in distinguishing between enteric microbes from human and animal sources, but has yet to be applied successfully to source tracking within a watershed.

Pulsed-field Gel Electrophoresis involves digesting DNA from pure cultures of bacteria using a series of restriction enzymes. The digestion takes place in agarose plugs, which are then embedded into specialized gels and subjected to electrophoresis for up to 2 days. Alternating currents are used to create the fingerprints. The technique is slow and the number of isolates that can be tested at any one time is limited, but the technique has been used in a clinical setting to identify the source of contamination (Simpson *et al.*, 2002) Amplified Fragment Length Polymorphism combines the use of RFLP with PCR amplification to generate between 50 and 100 DNA fragments that are then base sequenced. The unknown isolate is then compared with records from a library of microbes from the potential sources in a watershed. There are problems associated with the use of these molecular techniques for watershed monitoring. These relate to detection limits, the temporal and spacial variability of markers of a given population, and the reproducibility of the assays.

There is a real need to support research into the use of these techniques in association with agroecosystem indicators as a means of identifying key sources of contamination within a watershed, and developing measures to reduce their impact on surface and ground water quality.

## 8. General Assessment and Conclusions

For pathogens present in confined systems, the main risks relate to:

- Failures of structures and pipes,
- Inadequate capacity to confine manure, especially to collect runoff from solid manure stores or to store liquid manure in prolonged wet springs,
- Inadequate protection of on-site waste water treatment facilities leading to saturation or ponding.

In general, these risks would be expected to be much smaller than those associated with pathogens present in unconfined locations.

For pathogens present in unconfined conditions, major risks relate to:

- Excess applications due to spills or poor management leading to temporary ponding or runoff,
- Non-compliance with recommendations for the land application of manure or biosolids, or with standards for the operation of septic systems,
- Adverse weather conditions after land application,
- Permitting animals direct access to water courses,
- Failure to prevent erosion of soil to which manure or biosolids have been applied, or around waste treatment facilities,
- Further risks can result from the failure to carry out any treatment of manure or biosolids sufficient to reduce pathogen numbers to the levels anticipated.

For housed animals, assuming that a given pathogen is being shed by a proportion of the animals, the total amount of manure will likely be contaminated. The concentration of pathogens will reflect the dilution resulting from mixing the manure from infected with that from uninfected individuals. The same will be true of septage and other biosolid materials that enter or are applied to the soil. Management practices, especially those related to biosecurity, will influence the likelihood of animals within a herd becoming infected. Within a watershed, both the total number of animals and the number of herds will likely need to be combined to provide an assessment of the risks that pathogens are present. Current census polygons are too large to reflect the density of animals that could contribute pathogens to a given surface water body.

Knowledge of the survival and transport of pathogens under field conditions is very limited, and largely qualitative, especially with regard to the influence of weather, particularly the interaction between weather and soil physical and chemical conditions. Whether there are separate effects of soil texture and water content needs to be established, as does the relative importance of soil organic matter as a modifier of the water content-water potential relationship and as a substrate for bacterial growth. The effect of pH changes in the soil following the application of materials rich in ammoniacal N does not appear to have been quantified. Much of our knowledge of transport has come from the application of pure cultures to the soil, or from experiments where cultures of pathogens have been added to organic

wastes immediately before land application. The pathogens have generally not had time to accommodate to the conditions in the waste prior to mixing with soil. Nonetheless, some models for transport being developed appear to be promising.

Models are being developed and evaluated that could provide the means to assess the risk to ground and surface water from individual fields, but models that are appropriate for assessing the risks at the watershed scale need to be properly evaluated, especially under Canadian conditions. The appropriateness of scaling approaches for pathogen transport needs to be evaluated.

Much of the investigation of risk to water resources from pathogens present on agricultural holdings has been based on the use of bacterial indicators such as *Escherichia coli*. There is some evidence that this is not a satisfactory approach, even for bacterial pathogens, and that other indicator organisms should be considered, especially in the context of tracking the source of contaminant species in environmental water samples. Some success has been achieved in source tracking using assemblages of indicators. Much of the current research effort in the USA and the EU is being devoted to developing and assessing the use of biochemical and molecular methods for real-time monitoring of water resources that have been identified as being contaminated regularly at levels that cannot be accommodated within the watershed.

No significant effort to develop an agro-ecosystem indicator of the risk of pathogen contamination of water resources has been identified in jurisdictions outside of Canada. The program being developed by Health Canada is unique in that it considers the risk associated with both housed and freely grazing animals. However, it does not consider direct defecation into a water course. Die-off and precipitation frequency are the two factors that most affect the risk to water resources. Soil factors are assumed to influence the die-off of pathogens and the partitioning of water into surface runoff and infiltration. The bacterial index formulated at the University of Guelph as part of its MCLONE4 manure management decision support system, focusses on soil type and the likelihood of surface runoff and macropore flow immediately after application of manure. No contribution from grazing animals is included. It assumes that attenuation rather than die-off governs the risk to water resources. An indicator system has been developed in New South Wales, Australia, for contamination derived from septic systems. The latter approach seems admirably adaptable to consider non-point source contamination from agricultural holdings. Again, because release of microbes from the septic system is continuous, it also focusses on attenuation rather than die-off. The potential for use of GIS with this approach is inherent in its construction.

## 9. References

- Abu-Ashour, J., C. Etches, D.M. Joy, H. Lee, C.M. Reaume, C.B. Shadford, H.R. Whiteley, and S. Zelin. 1994a. *Field Experiment on Bacterial Contamination from Liquid Manure Application: Final Report for RAC Project No. 547G*. Toronto: Ontario Ministry of Environment and Energy.
- Abu-Ashour, J., D.M. Joy, H. Lee, H.R. Whiteley, and S. Zelin. 1994b. "Transport of microorganisms through soil." *Water, Air, & Soil Pollution*. 75:141-158.
- Addis, P.B., T. Blaha, B. Crooker, F. Diez, J. Feirtag, S. Goyal, I. Greaves, M. Hathaway, K. Janni, S. Kirkhorn, R. Moon, D.E. Morse, C. Phillips, J. Reneau, J. Shutske, and S. Wells. 1999. *Generic Environmental Impact Statement on Animal Agriculture: A Summary of the Literature Related to the Effects of Animal Agriculture on Human Health*. University of Minnesota, Minnesota, USA. pp. 134.
- Addiscott, T.M., and R.J. Wagenet. 1985. "Concepts of solute leaching in soils: A review of modelling approaches." *Journal of Soil Science*. 36:411-424.
- Ahuja, L.R., B.B. Barnes, and K.W. Rojas. 1993. "Characterization of macropore transport studied with the ARS root zone water quality model." *Transactions of the American Society of Agricultural Engineers*. 36:369-380.
- Angus, K.W. 1987. "Cryptosporidiosis in domestic animals and humans." *In Practice*. 9:47-49.
- Anonymous. 1991. "The many views of composting." *The Biocycle Guide to the Art and Science of Composting*.
- Aramini, J., M. McLean, J. Wilson, J. Holt, R. Copes, B. Allen, and W. Sears. 2000. *Drinking Water Quality and Health Care Utilization for Gastrointestinal Illness in Greater Vancouver*. Ottawa: Health Canada.
- Araujo, R.M., R. Pares and F. Lucena. 1990. "The effect of terrestrial effluents on the incidence of *Aeromonas* spp. in coastal waters." *Journal of Applied Bacteriology*. 69:439-444.
- Atabay, H.I., and J.E.L. Corry. 1998. "The isolation and prevalence of campylobacters from dairy cattle using a variety of methods." *Journal of Applied Microbiology*. 84:733-740.
- Azevedo, J., and P.R. Stout. 1974. "Farm animal manures: An overview of their role in the agricultural environment." *Manual (California Agricultural Experiment Station)*. 44. Davis, CA: Univ. Calif.
- Baldwin, C.S. 1981. *A Barnyard Manure Story: A Summary of 20 Years Research*. Ridgetown, ON: Soil Section, Ridgetown College of Agricultural Technology.

- Barker, J., T.J. Humphrey, and M.W. Brown. 1999. "Survival of *Escherichia coli* 157 in a soil protozoan: implications for disease." *FEMS Microbiology Letters*. 173:291-295.
- Barley, K.P. 1954. "Effects of root growth and decay on the permeability of a synthetic sandy loam." *Soil Science*. 78:205-210.
- Barrington, S.F., and M. Piche. 1992. "Research priorities for the storage of solid dairy manures in Quebec." *Canadian Agricultural Engineering*. 34:393-399.
- Barrington, S.F., J. Denis, and N.K. Patni. 1991. "Leakage from two concrete manure tanks." *Canadian Agricultural Engineering*. 32:137-141.
- Barrington, S.F., P.J. Jutras, and R.S. Broughton. 1987a. "The sealing of soils by manure. I. Preliminary investigations." *Canadian Agricultural Engineering*. 29:99-103.
- \_\_\_\_\_. 1987b. "The sealing of soils by manure. II. Sealing mechanisms." *Canadian Agricultural Engineering*. 29:105-108.
- Beauchamp, E.G. 1983. "Response of corn to nitrogen in preplant and side dress applications of liquid dairy cattle manure." *Canadian Journal of Soil Science*. 63:377-386.
- \_\_\_\_\_. 1986. "Availability of nitrogen from three manures to corn in the field." *Canadian Journal of Soil Science*. 66:713-720.
- Benfield, D.A., I. Stotz, R. Moore, and J.P. McAdaragh. 1982. "Shedding of rotavirus in feces of sows before and after farrowing." *Journal of Clinical Microbiology*. 16:186-190.
- Bergen, W.G., and D.B. Bates. 1984. "Ionophores: their effect on production efficiency and mode of action." *Journal of Animal Science*. 58:1465-1483.
- Bernal, M.P., A. Roig, A. Lax, and A.F. Navarro. 1992. "Effects of the application of pig slurry on some physico-chemical and physical properties of calcareous soils." *Bioresource Technology*. 42: 233-239.
- Bernhard, Anne E. and Katharine G. Field. 2000. "Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes." *Applied and Environmental Microbiology*. 66(4):1587-1594.
- Beven, K., and P. Germann. 1982. "Macropores and water flow in soils." *Water Resources Research*. 18:1311-1325.
- Bisson, J.W. and V.J. Cabelli. 1979. "Membrane filtration enumeration method for *Clostridium perfringens*." *Applied and Environmental Microbiology*. 37:55-56.

- Bitton, G., and R.W. Harvey. 1992. "Transport of pathogens through soil and aquifers." *Environmental Microbiology*. R. Mitchell (ed.). Toronto, ON: Wiley-Liss Inc. p. 103-124.
- Blackie, M., 2000. [Personal communication.] Agricultural Impact Specialist, Ontario Ministry of the Environment, London, Ontario.
- Blaser, M.J., D.N. Taylor, and R.A. Feldman. 1983. "Epidemiology of *Campylobacter jejuni* infections." *Epidemiological Reviews*. 5:157-176.
- Bogosian, G, L.E. Sammons, P.J. Morris, J.P. O'Neil, M.A. Heitkamp and D.B. Weber. 1996. "Death of the *Escherichia coli* K-12 strain W3110 in soil and water." *Applied and Environmental Microbiology*. 62:4114-4120.
- Bouma, J. 1981. "Soil morphology and preferential flow along macropores." *Agricultural Water Management*. 3:235-250.
- Brown, I.H., and D.J. Alexander. 1998. "Influenza." *Zoonoses. Biology, Clinical Practice, and Public Health Control*. S.R. Palmer, Lord Soulsby and D.I.H. Simpson (eds.). Oxford: Oxford University Press. p. 365-386.
- Brush, C.F., W.C. Ghiorse, L.J. Anguish, J.Y. Parlange, and H.G. Grimes. 1999. "Transport of *Cryptosporidium parvum* oocysts through saturated columns." *Journal of Environmental Quality*. 28:809-815.
- Buckhouse, J.C., and C.C. Bohn. 1983. "Response of coliform bacteria concentration to grazing management: Livestock grazing systems in relation to fecal contamination of rangelands, watersheds, runoff, non-point source pollution, stream monitoring." *Research in Rangeland and Management*. Special Report 682. Agricultural Experiment Station. Oregon State University. p. 17.
- Busato, A., D. Hofer, T. Lentze, C. Caillard, and A. Burnens. 1999. "Prevalence and infection risks of zoonotic enteropathogenic bacteria in Swiss cow-calf farms." *Veterinary Microbiology*. 69:251-263.
- Buswell, C.M., Y.M. Herlihy, C.W. Keevil, P.D. Marsh and S.A. Leach. 1999. "Carbon Load in aquatic ecosystems affects the diversity and biomass of water biofilm consortia and the persistence of the pathogen *Campylobacter jejuni* within them." *Journal of Applied Microbiology*. 85: S161- S167.
- Buswell, C.M., Y.M. Herlihy, L.M. Lawrence, J.T.M. McGuiggan, P.D. Marsh, C.W. Keevil and S.A. Leach. 1998. "Extended survival and persistence of *Campylobacter* spp. In water and aquatic biofilms and their detection by immunofluorescent- antibody and -rRNA staining." *Applied and Environmental Microbiology*. 64:733-741.

- Byrd, J.J., H.S. Xu and R.R. Colwell, 1991. "Viable but non-culturable bacteria in drinking water." *Applied and Environmental Microbiology*. 57:875-878.
- Canada. Agriculture and Agri-Food Canada (AA-FC). 1995. *Dairy Animal Improvement Statistics*. Ottawa: Market and Industry Services Branch.
- \_\_\_\_\_. 2000. *Medium Term Policy Baseline - International and Domestic Markets*. Ottawa: AAFC.
- Canada. Agriculture and Agri-Food Canada (AA-FC), Ontario. Ministry of Agriculture, Food and Rural Affairs (OMAF), and Ontario Federation of Agriculture (OFA). 1996. *Best Management Practices, Livestock and Poultry Waste Management*. Toronto, ON: OFA.
- \_\_\_\_\_. 1998. *Best Management Practices, Nutrient Management Planning*. Toronto, ON: OFA. Canada.
- Canadian Commission on Building and Fire Codes. 1995a. *National Building Code of Canada, 1995*. 11<sup>th</sup> ed. [Ottawa]: National Research Council of Canada, Institute for Research in Construction.
- \_\_\_\_\_. 1995b. *National Farm Building Code of Canada, 1995*. 8th ed. Ottawa. National Research Council of Canada. Institute for Research in Construction.
- Canadian Council of Ministers of Environment (CCME). 1999. "Canadian water quality guidelines for the protection of agricultural water uses: Summary table." *Canadian Environmental Quality Guidelines, 1999*. Winnipeg: CCME.
- Canter L.W. and R.C. Knox. 1988. *Septic Tank System Effects on Ground Water Quality*. Lewis Publishers, Inc. Chelsea, Michigan.
- Carson, C. Andrew, Brian L. Shear, Mark R. Ellersieck and Amha Asfaw. 2001. "Identification of fecal *Escherichia coli* from humans and animals by ribotyping." *Applied and Environmental Microbiology*. 67(4):1503-1507.
- Chandler, D.S., I. Farran, and J.S. Craven. 1981. "Persistence and distribution of pollution indicator bacteria on land used for disposal of piggery effluent." *Applied and Environmental Microbiology*. 42:453-460.
- Chang, C., T.G. Sommerfeldt, and T. Entz. 1991. "Soil chemistry after eleven annual applications of cattle feedlot manure." *Journal of Environmental Quality*. 20:475-480.
- Chauret, C., N. Armstrong, J. Fisher, R. Sharma, V.S. Springthorpe, and S.A. Sattar. 1995. "*Cryptosporidium* and *Giardia* in water in the Ottawa(Canada) region: Correlation with microbial indicators of water quality." *Journal of the American Water Works Association*. 87:76-84.

- Chen, S., R.E. Franklin, and A.D. Johnson. 1997. "Clay film effects on ion transport in soil." *Soil Science*. 162:91-96.
- Chen, S., R.E. Franklin, V. Quisenberry, and P. Dang. 1999. "The effect of preferential flow on the short- and long-term spatial distribution of surface applied solutes in a structured soil." *Geoderma* 90:229-241.
- Clark, E.A. 1998. "Landscape variables affecting livestock impacts on water quality in the humid temperate zone." *Canadian Journal of Plant Science*. 78:181-190.
- Clinton, N.A., R.W. Weaver, L.M. Zibilske, and R.J. Hidalgo. 1979. "Incidence of salmonellae in feedlot manure." *Journal of Environmental Quality*.8:480.
- Collins, E. R. Jr.. 1996. Composting dead poultry. Virginia Cooperative Extension: Virginia Tech and Virginia State University. Publication Number 442-037. [www.ext.vt.edu/pubs/poultry/442-037/442-037.html#L7](http://www.ext.vt.edu/pubs/poultry/442-037/442-037.html#L7).
- Conboy, M.J. 1999. Factors affecting bacterial contamination of rural drinking water wells: comparative assessment. In: *Advances in Planning and Management of Watersheds and Wetlands in Eastern and Southern Africa*. J.E. FitzGibbon (ed.), Weaver Press, University of Guelph. Canada. p. 155-168.
- Conboy, M.J., and M.J. Goss. 2001. "Identification of an assemblage of indicator organisms to assess the timing and source of bacterial contamination in groundwater." *Water, Air, & Soil Pollution*. 29:101-118.
- \_\_\_\_\_. 1997. "Is *Escherichia coli* an effective predictor of health risks and source of water contamination for rural populations?" *Toxicology & Ecotoxicology News* 4:156-157.
- Cools, D., R. Merckx, K. Vlassak and J. Verhaegen. 2001. "Survival of *E. coli* and *Enterococcus spp.* derived from pig slurry in soils of different texture." *Applied Soil Ecology*. 17:53-62.
- Corapcioglu, M.Y., and A. Haridas. 1985. "Microbial transport in soils and groundwater: A numerical model." *Advances in Water Resource*. 8:188-200.
- Coyne, M.S., R.A. Gilfillen, A. Villalba, Z. Ihang, R. Rhodes, L. Dunn, and R.L. Blevins. 1998. "Fecal bacteria trapping by grass filter strips during simulated rain." *Journal of Soil and Water Conservation*. 53:140-145.
- Crane, S.R., J.A. Moore, M.E. Grismer and J.R. Miner. 1983. "Bacterial pollution from agricultural sources: a review." *Transactions of the American Society of Agricultural Engineers*. 858-872.
- Davies, C.M., J.A. Long, M. Donald, and N.J. Ashbolt. 1995. "Survival of fecal microorganisms in marine and freshwater sediments." *Applied and Environmental Microbiology*. 61:1888-1896.

- Davies, P.R., W.E.M. Morrow, F.T. Jones, J. Deen, P.J. Fedorka-Cray, and I.T.Harris. 1997. "Prevalence of *Salmonella* in finishing swine raised indifferent production systems in North Carolina, USA." *Epidemiology and Infection*. 119:237-244.
- Dean, D.M., and M.E. Foran. 1991. *The Effect of Farm Liquid Waste Application on Receiving Water Quality. Final Report RAC Projects 430G and 512G*. Exeter, ON: Ausable-Bayfield Conservation Authority.
- \_\_\_\_\_. 1992. "The effect of farm liquid waste application on tile drainage." *Journal of Soil and Water Conservation*. 47:368-369.
- De Graaf, D.C., E. Vanopdenbosch, L.M. OrtegaMora, H. Abbassi and J.E. Peeters. 1999. "A review of the importance of cryptosporidiosis in farm animals. *International Journal for Parasitology*. 29. 1269-1275.
- Dowe, M.J., E.D. Jackson, J.G. Mori and C.R. Bell. 1997. *Listeria monocytogenes* survival in soil and incidence in agricultural soils. *Journal of Food Protection*. 60:1202-1207.
- Duncan, I.J.H. 1996. *Observations of Cattle at Four Sites in Ontario during Summer 1995: Interim Report*. [unpublished report]. Guelph, ON: Animal and Poultry Science, University of Guelph.
- Duncan, I.J.H., E.A. Clark, and K. Maitland. 1998. *Livestock Behaviour in and near Watercourses in Ontario: 3 Year Summary*. [unpublished report]. Guelph, ON: Animal and Poultry Science and Plant Agriculture, University of Guelph.
- Edberg, S.C., E.W. Rice, R.J. Karlin and M.J. Allen. 2000. *Escherichia coli*: the best biological drinking water indicator for public health protection. *Journal of Applied Microbiology*. 88:106116.
- Ehlers, W. 1975. "Observations on earthworm channels and infiltration on tilled and untilled loess soil." *Soil Science*. 119:242-249.
- Endale, D.M., M.H. Young, D.S. Fisher, J.L. Steiner, K.D. Pennell, and A. Amirtharajah. 2000. "Subsurface transport of *Cryptosporidium* from pastures to surface waters: 1. Rationale and site description." *Annual Meeting Abstracts, ASA, CSSA, SSSA*, Minneapolis, Minnesota, November 59. p. 206.
- Environmental Law Institute (ELI). 1997. *Enforceable State Mechanisms for the Control of Nonpoint Source Water Pollution*. Washington, D.C.  
[www.epa.gov/OWOW/NPS/elistudy/index.html](http://www.epa.gov/OWOW/NPS/elistudy/index.html).
- Estes, M.K., and J. Cohen. 1989. "Rotavirus gene structure and function." *Microbiological Reviews*. 53:165-169.

- Feachem, R.G. D.J. Bradley, H. Garelick and D.D. Mara. 1983. "Sanitation and Disease: Health Aspects of Excreta and Wastewater Management. Chichester: John Wiley & Sons.
- Feder, I., F.M. Wallace, J.T. Gray, P. Fratamico, P.J. Fedorka-Cray, R.A. Pearch, J.E. Call, R. Perrine, and J.B. Luchansky 2003. Isolation of *Escherichia coli* 0157:H7 from intact colon fecal samples of swine. *Emerg Infect Dis* [serial online] 2003 Mar. Available from: URL <http://www.ncbi.nlm.nih.gov/pubmed/12643837>
- Fenlon, D.R., I.D. Ogden, A. Vinten, and I. Svoboda. 2000. "The fate of *Escherichia coli* and *E. coli* 0157 in cattle slurry after application to land." *The Society for Applied Microbiology*. 88: 149S-156S.
- Feresu, S.B. and J. Van Sickle. 1990. "Coliforms as a measure of sewage contamination of the River Zambezi. *Journal of Applied Bacteriology*. 68:397-403.
- Ferguson, C.M., B.G. Coote, N.J. Ashbolt and I.M. Stevenson. 1996. "Relationships between indicators, pathogens and water quality in an estuarine system. *Water Research*. 30:2045-2054.
- Fleming, R., and H. Fraser. 2000. *Impacts of Winter Spreading of Manure on Water Quality: Literature Review*. Ridgeway, ON: Ridgeway College, University of Guelph.
- Fleming, R., J. Johnston, and H. Fraser. 1999. *Leaking of Liquid Manure Storages: Literature Review [for Ontario Pork]* . Ridgeway, ON: Ridgeway College, University of Guelph.
- Fleming, R., J. McLelland, D. Alves, D. Hilborn, K. Pintar, and M. MacAlpine. 1997. *Cryptosporidium in Livestock Manure Storages and Surface Waters in Ontario. Final report to Ontario Federation of Agriculture*.
- Fleming, R.J., and S.H. Bradshaw. 1991. *Macropore Flow of Liquid Manure*. Saskatoon, SK: Canadian Society of Agricultural Engineers. Paper No. 91-241.
- \_\_\_\_\_. 1992a. *Detection of Soil Macropores Using Smoke*. Saskatoon, SK: Canadian Society of Agricultural Engineers. Paper No. 92-103.
- \_\_\_\_\_. 1992b. *Contamination of Subsurface Drainage Systems during Manure Spreading*. St. Joseph, MI: American Society of Agricultural Engineers. Paper No. 92-2618.
- Foran, M.E., D.M. Dean, and H.E. Taylor. 1993. "The land application of liquid manure and its effect on tile drain water and groundwater quality." *Agricultural Research to Protect Water Quality: Proceedings of the Conference*. February 21-24, Minneapolis, MN, Ankeny, IA, USA. Soil and Water Conservation Society. p. 279-281.

- Fraser, Robert H., Paul K. Barten and David A.K. Pinney. 1998. "Prediction Stream Pathogen Loading from Livestock using a Geographical Information System-Based Delivery Model." *Journal of Environmental Quality*. 27:935-945.
- Fricker, C.R. and R.W.A. Park. 1989. "A 2-year study of the distribution of thermophilic *Campylobacters* in human, environmental and food samples from the reading area with particular reference to toxin production and heat-stable serotype. *Journal of Applied Bacteriology*. 66: 477490.
- Frost, J.P., R.J. Stevens, and R.J. Laughlin. 1990. "Effect of separation and acidification of cattle slurry on ammonia volatilization and on the efficiency of slurry nitrogen for herbage production." *Journal of Agricultural Science*. 115:49-56.
- Gagliardi, J.V. and J.S. Karns. 2000. "Leaching of *Escherichia coli* 0157:H7 in diverse soils under various agricultural management practices." *Applied and Environmental Microbiology*. 66:877-883.
- Gamal-El-Din, H. 1986. "Biogas production from antibiotic-contaminated cow manure." *Biogas, Technology, Transfer and Diffusion.: proceedings of the international conference held at the National Research Centre, Cairo, Egypt, 17-24 November 1984 on biogas technology, transfer, and diffusion: State of the Art*. M.M.E1-Halwagi (ed.). New York: Elsevier Applied Science Publishers, Commission of the European Communities. Directorate-General of Science, Research, and Development, Luxembourg . p 480-487.
- Garber, L.P., S.J. Wells, L. Schroeder-Tucker, and K. Ferris. 1999. "Factors associated with fecal shedding of verotoxin-producing *Escherichia coli*0157 on dairy farms." *Journal of Food Protection*. 62:307-312.
- Gary, H.L., S.R. Johnson, and S.L. Ponce. 1983. "Cattle grazing impact on surface water quality in a Colorado front range stream." *Journal of Soil and Water Conservation*. 38:124-128.
- Gerba, C. P. and G. Bitton. 1984. Microbial pollutants: their survival and transport pattern to groundwater. In *Groundwater pollution microbiology*. Charles P. Gerba Gabriel Bitton (eds.), A Wiley-Interscience publication. John Willey & Sons, New York, NY. 66-88.
- Giddens, J., and A.P. Barnett. 1980. "Soil loss and microbiological quality of runoff from land treated with poultry litter." *Journal of Environmental Quality*. 9:518-520.
- Girardin, P., C. Bockstaller, and H. Van der Wed. 1999. "Indicators: tools to evaluate the environmental impacts of farming systems." *Journal of Sustainable Agriculture* 13(4):5-21.
- Goss, M. J. 1994. "Biophysical criteria for the evaluation of intensive cropping and livestock management systems." Proceedings of the International Workshop on Sustainable Land Management for the 21st Century. Volume 2: Plenary Papers. R.C. Wood and J Dumanski (eds.). Agriculture Institute of Canada, Ottawa, Canada. p 189-201.

- Goss, M.J., D.A.J. Barry, and D.L. Rudolph. 1998. "Groundwater contamination in Ontario farm wells and its association with agriculture. 1. Results from drinking water wells." *Journal of Contaminant Hydrology*. 32:267-293.
- Goss, M.J., W.E. Curnoe, E.G. Beauchamp, P.S. Smith, B.D.C. Nunn, and D.A.J. Barry. 1995a. *An Investigation into the Management of Manure Nitrogen to Safeguard the Quality of Groundwater*. COESA Report No. LMAP-013/95 prepared for Research Branch. Agriculture and Agri-Food Canada. [http://agrienvarchive.ca/gp/gpres/download/rep4\\_2.pdf](http://agrienvarchive.ca/gp/gpres/download/rep4_2.pdf)
- Goss, M.J., K.R. Howse, P.W. Lane, D.G. Christian, and G.L. Harris. 1993. "Losses of nitrate-nitrogen in water draining from under autumn crops established by direct drilling or mould board ploughing." *Journal of Soil Science*. 44:35-48.
- Grabow, W.O.K., G.K. Idema, P. Coubrough and B.W. Bateman. 1989. "Selection of indicator systems for human viruses in polluted seawater and shellfish." *Water Science and Technology*. 21(3):111-117.
- Graczyk, T.K. R. Fayer, J.M. Trout, E.J. Lewis, C.A. Farley, I. Sulaiman and A.A. Lal. 1998. "*Giardia* sp. Cysts and infectious *Cryptosporidium parvum* oocysts in the faeces of migratory Canada geese (*Branta canadensis*). *Applied and Environmental Microbiology*. 64:2736-2738.
- Guidi, G., and G. Poggio. 1987. "Some effects of compost on soil physical properties." *Compost: Production, Quality and Use*. M. De Bertoldi, M.P. Ferranti, P. L'Hermite, F. Zucconi (eds.). New York: Elsevier Applied Science, Commission of the European Communities. Directorate-General of Science, Research, and Development, Luxembourg. p577-583.
- Gyles, C. 2000. "*E. Coli* 0157:H7 — Global perspective." Canadian Cattleman's Association (CCA) *E. Coli* 0157:H7 Workshop. 27 and 28 November 2000, Calgary, Alberta. p. 9-23.
- Hain, K.E. and O'Brien. 1979. The Survival of Enteric Viruses in Septic Tanks and Septic Drain Fields. New Mexico State University Water Resources Research Institute Report # 108. New Mexico State Univ., Las Cruces.
- Harvey, R.W. 1991. "Parameters involved in modelling movement of bacteria in groundwater." *Modelling the Environmental Fate of Microorganisms*. C.J. Hurst (ed.). Washington, D.C.: American Society for Microbiology. 89-114.
- Harvey, R.W., L.H. George, R.L. Smith, and D.R. Leblanc. 1989. "Transport of microspheres and indigenous bacteria through a sandy aquifer: Results of natural and forced-gradient tracer experiments." *Environmental Science & Technology*. 23:51-56.
- Hatfield, W.A. 1988. *Water and Anion Movement in a Typic Hapludult*. Ph.D. dissertation. Clemson, SC: Clemson Univ.

- Heckrath, G., P.C. Brookes, P.R. Poulton, and K.W.T. Goulding. 1997. "Phosphorus losses in drainage water from an arable silty clay loam." *Phosphorus Loss from Soil to Water*. H. Tunney, O.T. Carton, P.C. Brookes, and A.E. Johnston (eds.). Wallingford, UK: CAB International. p. 367-369.
- Helling, C.S., and T.J. Gish. 1991. "Physical and chemical processes affecting preferential flow." *Preferential Flow*. T.J. Gish and A. Shrimohammadi (eds.). St. Joseph, MI: American Society of Agricultural Engineers. p. 77-86.
- Herriott, D.E., D.D. Hancock, E.D. Ebel, L.V. Carpenter, D.H. Rice, and T.E. Besser. 1998. "Association of herd management factors with colonization of dairy cattle by shiga toxin-positive *Escherichia coli* 0157 ." *Journal of Food Protection*. 61:802-807.
- HMSO. 1993. "Interim report on *Campylobacter*. Advisory Committee on the Microbiological Safety of Food. HMSO, London, 107pp.
- Holland, R.E. 1990. "Some infectious causes of diarrhea in young farm animals." *Clinical Microbiology Review*. 3:345-375.
- Holter, J.B., and W.E. Urban Jr. 1992. "Water partitioning and intake prediction in dry and lactating Holstein cows." *Journal of Dairy Science*. 75:1472-1479.
- Howell, J.M., M.S. Coyne and P. Cornelius. 1995. "Fecal bacteria in agricultural waters of the bluegrass region of Kentucky." *Journal of Environmental Quality*. 24:411-419.
- Humphrey, T.J. and P. Beckett. 1987. *Campylobacter jejuni* in dairy cows and raw milk. *Epidemiology and Infection*. 98:263-269.
- Hutchison, M.L., A.K. Ashmore, K.M. Crookes, D.W. Wilson, S.J. Groves, B.J. Chambers, C.W. Keevil and A. Moore. "Enumeration of pathogens in livestock wastes and factors affecting their survival. *Proceedings of the Joint CIWEM and Aqua Enviro Technology Transfer 7' European Biosolids and Organic Residuals Conference*. Session 3, Paper 15: 6pp.
- Hutson, J.L., and R.J. Wagenet. 1992. *LEACHM — Leaching estimation and chemistry model, Version 3. Research Series No. 92-3*. Ithaca NY: Dept of Soil, Crop and Atmospheric Sciences. Cornell University.
- Iserson, K.V., 1994. *Death to Dust: What Happens to Dead Bodies*. Galen Press, Ltd., Tucson, AZ, U.S.A.
- Issac-Renton, J., W. Moorehead, and A. Ross. 1995. "*Giardia* cyst concentrations and infectivity: Longitudinal community drinking water studies." *Protozoan Parasites and Water*. W.B. Betts, D. Casemore, C. Fricker, H. Smith, and J. Watkins (eds.). Cambridge : Royal Society of Chemistry, UK.

- Jackson, S.G., R.B. Goodbrand, R.P. Johnson, V.G. Odorico, D. Alves, K.Rahn, J.B. Wilson, M.K. Welch, and R. Khakhria. 1998. "*Escherichia coli* 0157:H7 diarrhoea associated with well water and infected cattle on an Ontario farm." *Epidemiology and Infection*. 120:17-20.
- Jardine, P.M., G.V. Wilson, and R.J. Luxmoore. 1990. "Unsaturated solute transport through a forest soil during rain storm events." *Geoderma*.46:103-118.
- Jarvis, J. 1994. "The MACRO model-Technical description and sample simulations." *Reports and Dissertations* 19. Swedish University of Agricultural Sciences, Department of Soil Sciences. Uppsala: 51pp.
- Jarvis J, K.G. Villholth, and B. Ulen. 1999. "Modelling particle mobilization and leaching in macroporous soil." *European Journal of Soil Science*. 50:621-632.
- Jarvis, N.J., P.E. Jansson, P.E. Dik, and I. Messing. 1991. "Modelling water and solute transport in macroporous soil. I. Model description and sensitivity analysis." *Journal of Soil Science*. 42:59-70.
- Jenneman, G.E., M.J. McInerney, and R.M. Knapp. 1985. "Microbial penetration through nutrient-saturated Berea sandstone." *Applied Environmental Microbiology*. 50:383-391.
- Jofriet, J.C. 1992. *Structural Components for Concrete Manure Storage Tanks*. Report to OMAF, Guelph, ON.
- Johnson, J., and D. Hilborn. 1999. *Interim Recommendations Regarding Tile Drains and Manure Storage Structures*. Infosheet, September, 1999. Guelph, ON: OMAF.
- Jones, D.L., G. Campbell and C.W. Kaspar. 2002. "Human enteric pathogens in the farming environment." *Agriculture, Hydrology and Water Quality*. P. Haygarth and S. Jarvis (eds.). Wallingford UK:CAB International. p. 133-153.
- Jones, K., M. Betaieb and D.R. Telford. 1990. "Correlation between environmental monitoring of thermophilic *Campylobacters* in sewage effluent and the incidence of *Campylobacter* infection in the community." *Journal of Applied Bacteriology*. 69:235-240.
- Jongbloed, A.W. 1991. "Developments in the production and composition in manure from pigs and poultry." *Mest & Milieu in 2000*. H.A.C. Verkerk(ed.). Wageningen, Netherlands: Dienst Landbouwkundig Onderzoek.(Dutch).
- Kearney, T.E., M.J. Larkin, J.P. Frost, and P.N. Levett. 1993. "Survival of pathogenic bacteria during mesophilic digestion of animal waste." *Journal of Applied Bacteriology*. 75:215-219.

- Keener, H.M., D.L. Elwell and M.J. Monnin. 2000. Procedures and equations for sizing of structures and windrows for composting animal mortalities. *Applied Engineering in Agriculture*, v16, n 6, p 681-692.
- King, D.J., G.C. Watson, G.J. Wall, and B.A. Grant. 1994. *The Effects of Livestock Manure Application and Management on Surface Water Quality. Summary Technical Report*. London, ON: GLWQP-AAFC Pest Management Research Centre, Agriculture and Agri-Food Canada.
- Kirchmann, H. 1985. "Losses, plant uptake and utilisation of manure nitrogen during a production cycle." *Acta Agriculturae Scandinavica, Supplementum*. 24:77pp.
- Koenraad, P.M.F.J., F.M. Rombouts and S.H.W. Notermans. 1007. "Epidemiological aspects of thermophilic *Campylogacter* in water-related environments: A review. *Water Environment Research*. 69:52-63.
- Kowal, N.E. 1985. *Health Effects of Land Application of Municipal Sludge*. Triangle Park, NC: Health Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency.
- Kudva, I.T., K. Blanch and C.J. Hovde. 1998. "Analysis of *Escherichia coli* 0157:H7 survival in ovine or bovine manure and manure slurry. *Applied and Environmental Microbiology*. 64:3166-3174.
- Kung, K.J.S., and S.V. Donohue. 1991. "Improved solute-sampling protocol in a sandy vadose zone using ground-penetrating radar." *Soil Science Society of America Journal*. 55:1543-1545.
- Lance, J.C., and C.P. Gerba. 1984. "Virus movement in soil during saturated and unsaturated flow." *Applied and Environmental Microbiology*. 47:335-337.
- Lance, J.C., C.P. Gerba, and D.S. Wang. 1982. "Comparative movement of different enteroviruses in soil columns." *Journal of Environmental Quality*. 11:347-351.
- Lewis, D. 2002. SAC. [Personal Communication]. December.
- Ley, V., J.A. Higgins and R. Feyer. 2002. Bovine enteroviruses as indicators of fecal contamination. *Applied and Environmental Microbiology* 68:3455-3461.
- Lim, T.T., D.R. Edwards, S.R. Workman, B.T. Larson, and L. Dunn. 1998. "Vegetated filter strip removal of cattle manure constituents in runoff." *Transactions of the American Society of Agricultural Engineers*. 41:1375-1381.
- Lindenstruth, R.W., and B.Q. Ward. 1948. "Viability of *Vibrio fetus* in hay, soil and manure." *Journal of the American Veterinary Medical Association*. 113:163.

- Luxmoore, R.J. 1981. "Micro-, meso-, and macroporosity of soil." *Soil Science Society of America Journal*. 45:671-672.
- \_\_\_\_\_. 1991. "On preferential flow and its measurement." *Preferential Flow*. T.J. Gish and A. Shrimohammadi (eds.). St. Joseph, MI: American Society of Agricultural Engineers. p. 113-121.
- MacGowan, A.P., K. Bowker, J. Mclauchlin, P.M. Bennett and D.S. Reeves. 1994. "The occurrence and seasonal-changes in the isolation of *Listeria* spp in shop bought food stuffs, human faeces, sewage and soil from urban sources. *International Journal of Food Microbiology*. 21: 325-334.
- MacLean, A.J. 1983. "Pathogens of animals in manure: Environmental impact and public health." *Farm Animal Manures in the Canadian Environment*. Ottawa: National Research Council of Canada Associate Committee on Scientific Criteria for Environmental Quality. p. 103-107.
- Madison, R.J., and J.O. Brunett. 1985. "Overview of the occurrence of nitrate in groundwater of the United States." *National Water Summary 1984: USGS Water Supply Paper No. 2275*. Washington, D.C.: U.S. Govt. Printing Office. p. 93-105.
- Mafu, A.A., R. Hjiggins, M. Nadeau, and G. Cousineau. 1989. "The incidence of *Salmonella*, *Campylobacter*, and *Yersinia enterocolitica* in swine carcasses and the slaughterhouse environment." *Journal of Food Protection*. 52:642-645.
- Manitoba, 2001. Dead animal disposal. Chapter 7.
- Manser, P.A., and R.W. Dalziel. 1985. "A survey of *Campylobacter* in animals." *Journal of Hygiene*. (London) 95:15-21.
- Matthess, G., A. Pekdeger, and J. Schroeter. 1988. "Persistence and transport of bacteria and viruses in groundwater: A conceptual evaluation." *Journal of Contaminant Hydrology*. 2:171-188.
- Mawdsley, J.L., RD. Bargett, R.J. Merry, B.F. Pain, and M.K. Theodorou. 1995. "Pathogens in livestock waste, their potential for movement through soil and environmental pollution." *Applied Soil Ecology*. 2:1-15.
- McGechan, M.B. 2002. "Effects of Timing of Slurry Spreading on Leaching of Soluble and Particulate Inorganic Phosphorus explored using the MACRO Model." *Biosystems Engineering*. 83:237-252.
- McMurry, S.W., M.S. Coyne, and E. Perfect. 1998. "Fecal coliform transport through intact soil blocks amended with poultry manure." *Journal of Environmental Quality*. 27:86-92.

- Meng, X.J., R.H. Purcell, P.G. Halbur, J.R. Lehman, D.M. Webb, T.S. Tsareva, J.S. Haynes, B.J. Thacker, and S.U. Emerson. 1997. "A novel virus in swine is closely related to the human hepatitis E virus." *Proceedings of the National Academy of Science*. 94:9860-9865.
- Merrilees, D. and M. Atiken. 2002. SAC. [Personal Communication]. December. Millner, P. 2003. [Personal Communication]. March.
- MMWR (1992). CDC surveillance summaries: waterborne-disease, 1989-1990. MMWR, 40 (SS-3), 1-20.
- Monteith, H.D., E.E. Shannon, and J.B. Derbyshire. 1986. "The inactivation of bovine enterovirus and a bovine parvovirus in cattle manure by anaerobic digestion, heat treatment, gamma irradiation, ensilage and composting." *Journal of Hygiene*. 97:175-184.
- Morris, J.R., T. O'Connor, F. Kains and H. Fraser. 1997. Composting Livestock Ontario Ministry of Agriculture and Food, Division of Agriculture and Rural History: Original Factsheet 97-001.
- Mubiru, D. N. Coyne M. S. and J. H. Grove. 2000. Mortality of *Escherichia coli* 0157:H7 in two soils with different physical and chemical properties. *Journal of Environmental Quality*. 29:1821-1825.
- Munroe, D.L., J.F. Prescott, and J.L. Penner. 1983. "*Campylobacter jejuni* and *Campylobacter coli* serotypes isolated from chickens, cattle, and pigs." *Journal of Clinical Microbiology*. 18:877-881.
- Munyankusi, E., S.C. Gupta, J.F. Moncrief, and E.C. Berry. 1994. "Earthworm macropores and preferential transport in a long-term manure applied Typic Hapludalf." *Journal of Environmental Quality*. 23:733-784.
- Murphy, D.W., and L.E. Cam 1991. Compositng dead birds. Maryland Cooperative Extention. University of Maryland. Fact Sheet 537.
- Natsch, A., C. Keel, J. Troxler, M. Zala, N. Von Albertini, and G. Defago. 1996. "Importance of preferential flow and soil management in vertical transport of a biocontrol strain of *Pseudomonas fluorescens* in structured field soil." *Applied and Environmental Microbiology*. 62:33-40.
- The Netherlands Ministry of Agriculture, Nature Management and Fisheries. 2001. [online]. [Cited August 10, 2001] <http://english.minlnv.nl/>
- New York State Soil and Water Conservation Committee and the New York State Department of Agriculture and Markets. 2000. *Guide to Agricultural Environmental Management in New York State*. <http://www.dec.ny.gov/> .

- Ngoddy, P.O., J. Haper, R.K. Robert, G.D. Wells, and F.A. Heidar. 1971. *Closed System Waste Management for Livestock*. Washington, D.C.: U.S. Environmental Protection Agency.
- Nodar, R., M.J. Acea, and T. Carballas. 1992. "Poultry slurry microbial population: Composition and evolution during storage." *Bioresource Technology*. 40:29-34.
- \_\_\_\_\_. 1990. "Microbial composition of poultry excreta." *Biological Wastes*. 33:95-105.
- Olson, M. 2000. "Transmission and survival of *Esherichia coli* 0157:H7." Canadian Cattleman's Association (CCA) *E. coli* 0157:H7 Workshop. 27 and 28 November 2000, Calgary, Alberta. p. 28-33.
- Olson, M.E., C.L. Thorlakson, L. Deselliers, D.W. Morck, and T.A. McAllister. 1997. "*Giardia* and *Cryptosporidium* in Canadian farm animals." *Veterinary Parasitology*. 68:375-381.
- On-site Sewage Management Risk Assessment System (OSRAS).  
[http://www.dlg.nsw.gov.au/dlg/dlghome/dlg\\_osras.asp](http://www.dlg.nsw.gov.au/dlg/dlghome/dlg_osras.asp)
- Ontario. Ministry of Agriculture and Food. Ministry of the Environment, and Ministry of Housing. 1976. *Agricultural Code of Practice*. Toronto:OMAF, OME, OMH. Ontario. Ministry of Agriculture, Food and Rural Affairs
- (OMAF). 1994a. *Agricultural Pollution Control Manual*. Toronto: Queen's Printer.
- \_\_\_\_\_. 1994b. "Earthen storage design and construction." *Agricultural Pollution Control Manual*. Toronto: OMAF.
- \_\_\_\_\_. 1995a. *Guide to Agricultural Land Use*. Toronto: Queen's Printer.
- \_\_\_\_\_. 1995b. *Minimum Distance, Separation I (MDS I)*. Toronto: Queen's Printer.
- \_\_\_\_\_. 1995c. *Minimum Distance, Separation II (MDS II)*. Toronto: Queen's Printer.
- \_\_\_\_\_. 1999a. *Field Crop Recommendations 1999-2000*, Publication 296. Toronto: Queen's Printer. p. 154.
- \_\_\_\_\_. 2000. *Nutrient Management, 2000 (NMAN2.1 updated)* [software]  
<http://www.omafra.gov.on.ca/english/nm/nman/software.htm>
- \_\_\_\_\_. 2001. *Discussion Paper on Intensive Agricultural Operations in Rural Ontario [online]*. [Cited February 12, 2002; no longer available].
- OMAF, 2001. Managing on-farm mortalities.

- Pacheco, A. and B.A. Matos. 2002. Cemeteries and the environment. Universidade de Sao Paulo, Instituto de Geociencias. <http://www.igc.usp.br/subsites/cemiterios/cemit2.php>
- Parkin, G.W., C. Wagner-Riddle, D.J. Fallow and D.M. Brown. 1999. Estimated seasonal annual water surplus in Ontario. *Canadian Water Resources Journal* 24:277-292.
- Orskov, E.R., C. Frazer, V.C. Mason, and S.O. Mann. 1970. "Influence of starch digestion in the large intestine of sheep on caecal fermentation, caecal microflora and faecal nitrogen excretion." *British Journal of Nutrition*. 24:671-682.
- Ostling, C.E., and S.E. Lindgren. 1991. "Bacteria in manure and on manured and NPK fertilized silage crops." *Journal of the Science of Food and Agriculture*. 55:579-588.
- Oxford County. 1999. *Nutrient Management Strategy for Oxford County: 1999*. Woodstock, ON.
- Pain, B.F., S. Jarvis, and B. Clements. 1991. "Impact of agricultural practices on soil pollution." *Outlook on Agriculture*. 20:153-160.
- Parveen, Salina, Kenneth M. Portier, Kevin Robinson, Lee Edmiston and Mark L. Tamplin. 1999. "Discriminant analysis of ribotype profiles of *Escherichia coli* for differentiating human and nonhuman sources of fecal pollution." *Applied and Environmental Microbiology*. 65:3142-3147.
- Patni, N.K., and P.Y. Jui. 1984. *Changes in Mineral Content of Dairy Cattle Liquid Manure during Anaerobic Storage*. Paper No. 84. Saskatoon, SK: Canadian Society of Agricultural Engineers.
- \_\_\_\_\_. 1987. "Changes in solids and carbon content of dairy-cattle slurry in farm tanks." *Biological Wastes*. 20:11-34.
- \_\_\_\_\_. 1991. "Nitrogen concentration variability in dairy-cattle slurry stored in farm tanks." *Transactions of the American Society of Agricultural Engineers*. 34:609-615.
- Peacock, A. D. Mullen M. D. Ringelberg D. B. Tyler D. D. Hedrick D. B. Gale P. M. and D. C. White. 2001. Soil microbial community responses to dairy manure or ammonium nitrate applications. *Soil Biology & Biochemistry* 33:1011-1019.
- Peer, D., and W. Merritt. 1997. *Water Quality and Pig Performance. Factsheet*. Guelph, ON: Ontario Ministry of Agriculture, Food and Rural Affairs. Agdex# 400.
- Pell, A.N. 1997, "Manure and microbes: Public and animal health problem?" *Journal of Dairy Science*, 80, p. 2673-2681.
- Peterson, T.C., and R.C. Ward. 1989. "Development of a bacterial transport model for coarse soils." *Water Resources Bulletin*. 25:349-357.

- Phillips, C.A. 1995. "Incidence, epidemiology, and prevention of foodborne *Campylobacter* species." *Trends in Food Science and Technology*. 6:83-86.
- Phillips, R.E., V.L. Quisenberry, J.M. Zeleznik, and G.H. Dunn. 1989. "Mechanism of water entry into simulated macropore." *Soil Science Society of America Journal*. 53:1629-1635.
- Quisenberry, V.L., and R.E. Phillips. 1976. "Percolation of surface-applied water in the field." *Soil Science Society of America Journal*. 40:484-489.
- \_\_\_\_\_. 1978. "Displacement of soil water by simulated rainfall." *Soil Science Society of America Journal*. 42:675-679.
- Quisenberry, V.L., B.R. Smith, R.E. Phillips, H.D. Scott, and S. Nortcliff. 1993. "A soil classification system for describing water and chemical transport." *Soil Science*. 156(5):306-315.
- Read, I.A., and I.F. Svoboda. 1995. "The effect of aerobic treatment on the survival of *Cryptosporidium parvum* oocysts in cattle slurry." *Protozoan Parasites and Water*. W.B. Betts, D. Casemore, C. Fricker, H. Smith, and J. Watkins (eds.). Cambridge: Royal Society of Chemistry, UK.
- Reddy, K.R., R. Khalfft and M.R. Overcash. 1981. "Behaviour and transport of microbial pathogens and indicator organisms in soils treated with organic wastes." *J. Environmental Quality*. 10 (3):255-266.
- Ritter, W.F., A.E.M. Chirnside, and R.W. Scarborough. 1990. "Soil nitrate profiles under irrigation on coastal plain soils." *Journal of Irrigation and Drainage Engineering*. 116(6):738-751.
- Rollins, D.M. and R.R. Colwell. 1986. "Viable but nonculturable stage of *Campylobacter jejuni* and its role *Applied and Environmental Microbiology* 52:531-538.
- Roszak, D.B. and Colwell, R.R. 1987. "Survival strategies of bacteria in the natural environment." *Microbiological Reviews*. 51:365-379.
- Rowell, J.G., M.H. Miller, and P.H. Groenevelt. 1985. "Self-sealing of earthened liquid manure storage ponds: II. Rate and mechanism of sealing." *Journal of Environmental Quality*. 14:539-543.
- Rudolph, D. L. 2003. Performance Evaluation of Existing Concrete Manure Storage Facilities and Associated Environmental Risks. Report to Ontario Pork, University of Waterloo.
- Rudolph, D.L., D.A.J. Barry, and M.J. Goss. 1998. "Contamination in Ontario farmstead domestic wells and its association with agriculture: 2. Results from multilevel monitoring well installations." *Journal of Contaminant Hydrology*. 32:295-311.

- Russell, J.B., F. Diez-Gonzalez, and G.N. Jarvis. 2000. "Effects of diet shifts on *Escherichia coli* in cattle." *Journal of Dairy Science*. 83:863-873.
- Sadeghi, Ali M. and Jeffrey G. Arnold. 2002. "A SWAT/Microbial sub-model for predicting pathogen loadings in surface and groundwater at watershed and basin scales. *Environmental Regulations: Proceedings of the March 11-13 2002 ASAE Conference*. Fort Worth, Texas, U.S.A. p. 56-63. <http://asae.frymulti.com/abstract.asp?aid=7529&t=2>
- Santos, N., R.C.C. Lima, C.M. Nozawa, R.E. Linhares, and V. Gouvea. 1999. "Detection of porcine rotavirus type G9 and of a mixture of types Gland G5 associated with Wa-like VP4 specifically: Evidence for natural human-porcine genetic reassortment." *Journal of Clinical Microbiology*. 37:2734-3736.
- Scandura, J. E. and M.D. Sobsey. 1997. Viral and Bacterial Contamination of Groundwater from On-Site Sewage Treatment systems. *Water Science and Technology*. 35:141-146.
- Scott, C.A., H.V. Smith, and H.A. Gibbs. 1994. "Excretion of *Cryptosporidium parvum* oocysts by herd of beef suckler cows." *Veterinary Record*. 134:172.
- Shere, J.A., K.J. Bartlett, and C.W. Kaspar. 1998. "Longitudinal study of *Escherichia coli* 0157.H7 dissemination on four dairy farms in Wisconsin." *Applied and Environmental Microbiology*. 64:1390-1399.
- Shirmohammadi, A., T.J. Gish, A. Sadeghi, and D.A. Lehman. 1991. "Theoretical representation of flow through soils considering macropore effect." *Preferential Flow*. T.J. Gish and A. Shirmohammadi (eds.). St Joseph, MI: American Society of Agricultural Engineers. p. 233-243.
- Simpson, Joyce M., Jorge W. Santo Domingo and Donald J. Reasoner. 2002. "Microbial Source Tracking: State of the Science." *Environmental Science and Technology*. 36(24):5279-5288.
- Sinton, L. W. Finlay R. K. and A. P. Lynch. 1999. Sunlight inactivation of fecal bacteriophages and bacteria in sewage-polluted seawater. *Applied and Environmental Microbiology* 65, no. 8: 3605-3613.
- Skopp, J. 1981. "Comment on "Micro-, meso-, and macroporosity of soil." *Soil Science Society of America Journal*. 45:1246.
- Skovgaard, N. and C.A. Morgen. 1988. "Detection of *Listeria* spp in faeces from animals, in feeds, and in raw foods of animal origin. *International Journal of Food Microbiology*. 6:229-242.
- Skovgaard, N. and B. Norrung. 1989. "The incidence of *Listeria* spp in faeces of Danish pigs and in minced pork meat. *International Journal of Food Microbiology*. 8:59-63.

- Smith, K.A., R.J. Unwin, and J.H. Williams. 1985. "Experiments on the fertilizer value of animal waste slurries." *Long Term Effects of Sewage Sludge and Farm Slurries Applications*. J.H. Williams et al. (eds.). New York: Elsevier Science.
- Sobsey, M.D. 1983. "Transport and fate of viruses in soils." *Microbial Health. Considerations of Soil Disposal of Domestic Wastewaters*. L.W. Canter, E.W. Akin, J.F. Kreissl, and J.F. McNabb (eds.). Cincinnati, OH: U.S. Environmental Protection Agency. p. 175-197.
- St. Jean, R. 1997. *On-farm Manure Composting Techniques: Understanding Nitrogen and Carbon Conservation*. Research Report 1.3. COESA Report No. RES/MAN-003/97. Prepared for Agriculture and Agri-Food Canada, London Research Centre, London, Ontario. Prepared by Ecologistics Limited, Waterloo, Ontario.  
<http://agrienvarchive.ca/gp/gpres/report/rep13sum.html>
- Stanley, K.N., J.S. Wallace, J.E. Currie, P.J. Diggle and K. Jones. 1998a. "Seasonal variation of thermophilic *Campylobacters* in lambs at slaughter. *Journal of Applied Microbiology*. 84:1111 - 1116.
- Stanley, K.N., J.S. Wallace, J.E. Currie, P.J. Diggle and K. Jones. 1998b. "The seasonal variation of thermophilic *Campylobacters* in beef cattle, dairy cattle and calves. *Journal of Applied Microbiology*. 85:472-480.
- Steenhuis, T.S., J.-Y. Parlange, and M.S. Andreini. 1990. "A numerical model for preferential solute movement in structured soils." *Geoderma*. 46:193-208.
- Stelma, G.N., and L.J. McCabe. 1992. "Nonpoint pollution from animal sources and shellfish sanitation." *Journal of Food Protection*. 55:649-656.
- Strauch, D. 1987. "Hygiene of animal waste management." *Animal Production and Environmental Health*. D. Strauch (ed.). Amsterdam: Elsevier Science. p. 155-202.
- \_\_\_\_\_. 1988. "Krankheitserreger in Fakalien and ihre epidemiologische Bedeutung." *Tierärztliche Praxis, Suppl.* 3:21-27.
- \_\_\_\_\_. 1991. Survival of pathogenic micro-organisms and parasites in excreta, manure and sewage sludge. *Rev. Sci. Techn. Off. Int. Epiz.* 10:813-846.
- Tamasi, G. 1981. "Factors influencing the survival of pathogenic bacteria in soils." *Acta Veterinaria Academiae Scientiarum Hungaricae*. 29:119-126.
- Terzieva, S.I., and G.A. McFeters. 1991. "Survival and injury of *Escherichiacoli*, *Campylobacter jejuni* and *Yersinia enterocolitica* in stream water." *Canadian Journal of Microbiology*. 37:785-790.

- Thelin, R., and G.F. Gifford. 1983. "Fecal coliform release patterns from fecal material of cattle." *Journal of Environmental Quality*. 12:57-63.
- Thomas, C., D.J. Hill, and M. Mabey. 1999. "Evaluation of the effect of temperature and nutrients on the survival of *Campylobacter* spp. in water microcosms." *Journal of Applied Microbiology*. 86:1024-1032.
- Thomas, G.W., and R.E. Phillips. 1979. "Consequences of water movement in macropores." *Journal of Environmental Quality*. 8(2):149-152.
- Thompson, R.B., J.C. Ryden, and D.R. Lockyer. 1987. "Fate of nitrogen in cattle slurry following surface application or injection to grassland." *Journal of Soil Science*. 38:689-700.
- \_\_\_\_\_. 1990. "Ammonia volatilization from cattle slurry following surface application to grasslands. I. Influence of mechanical separation, changes in chemical composition during volatilization and the presence of the grass sward." *Plant and Soil*. 125:109-117.
- Tietjen, C. 1987. "Influence of faecal wastes on soil, plant, surface water and groundwater." In: *Animal Production and Environmental Health*. D.Strauch (ed.). Amsterdam: Elsevier Science. p. 203-217.
- Topp, E. 2000. [Personal Communication].
- Trick, J.K., B.A. Klink, P. Coombs, J. Chambers, D.J. Noy, J. West and G.M. Williams. 2001. Groundwater impact of Danescourt Cemetery, Wolverhampton. British Geological Survey, Internal Report IR/01/104.
- Unc, A. 1999. *Transport of Faecal Bacteria from Manure through the Vadose Zone*. M.Sc. thesis, University of Guelph, Ontario.
- Unc, A., and M.J. Goss. 2000. "Effect of manure application on soil properties relevant to bacterial transport." Paper presented at the *ASA, CSSA, SSSA Annual Meetings*, 5-9 November 2000, Minneapolis, Minnesota. Madison, WI: American Society of Agronomy.
- USFDA. 1992. *Campylobacter jejuni. Bad Bug Book - Foodborne Pathogenic Microorganisms and Natural Toxins Handbook*. U.S. Food and Drug Administration, Washington D.C., 5 pp.
- Van Horn, H.H., A.C. Wilkie, and W.J. Powers, 1994, "Components of dairy manure management systems," *Journal of Dairy Science*, 77:2008-2030.
- Van Rentergham, B., F. Huysman, R. Rygole and W. Verstraete. 1991. "Detection and prevalence of *Listeria monocytogenes* in the agricultural ecosystem." *Journal of Applied Bacteriology*. 71: 211 - 217.

- Van Stappen, R., F. Huysman, and W. Verstraete. 1990. "Land application of piggery manure: The need for adequate expert systems to evaluate and control manuring practices." *Fertilization and the Environment*. R.Merckx, H. Vereecken, and K. Vlassak (eds.). Leuven, Belgium: Leuven University Press. p. 264-274.
- Veira, D.M., A.S. Zimmerman, and D.R. Corkal. 2003. Meeting the water requirements of a prairie livestock industry. Paper 9 Canadian Society of Animal Science Annual Meeting. June 10-13, 2003 University of Saskatchewan, Saskatchewan, Canada.
- Wagenet, R.J. 1990. "Quantitative prediction of the leaching of organic and inorganic solutes in soil." *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences*. 329:321-330.
- Walker, J. 2001. Deadstock fees prompting more interest in composting. Ontario Farmer, Tuesday, January 9, 2001.
- Wang, G., T. Zhao, and M.P. Doyle. 1996. "Fate of enterohemorrhagic *Escherichia coli* 0157:H7 in bovine feces." *Applied and Environmental Microbiology*. 62:2567-2570.
- Watson, K.W., and R.J. Luxmoore. 1986. "Estimating macroporosity in a forest watershed by use of a tension infiltrometer." *Soil Science Society of America Journal*. 50:578-582.
- Webber, L.R., and T.H. Lane. 1969. "The nitrogen problem in the land disposal of liquid manure." *Cornell University Conference on Agricultural Waste Management*. p. 124-130.
- Weijtens, M.J.B.M., R.D. Reinders, H.A.P. Urlings and J. Van der plas. 1999. "*Campylobacter* infections in fattening pigs; excretion pattern and genetic diversity." *Journal of Applied Microbiology*. 86:63-70.
- Weijtens, M.J.B.M., J. Van der Plas, P.G.H. Bijker, H.A.P. Urlings, D. Koster, J.G. Van Logtestijn, and J.H. Veld. 1997. "The transmission of *Campylobacter* in piggeries; an epidemiological study." *Journal of Applied Microbiology*. 83:693-698.
- Weiss, J. and H.P.R. Seelinger. 1975. "Incidence of *Listeria monocytogenes* in nature." *Applied Microbiology*. 30:29-32.
- Welshimer, H.J. 1968. "Isolation of *Listeria monocytogenes* from vegetation." *Journal of Bacteriology*. 95. 300-303.
- White, R.E., J.S. Dyson, Z. Gerstl, and B. Yaron. 1986. "Leaching of herbicides through undisturbed cores of a structured clay soil." *Soil Science Society of America Journal*. 50:277-283.
- Whiteley, H. 1998. *Effects of Cattle Access on Bacteria Concentrations in Streams*. [unpublished report]. School of Engineering, University of Guelph.

- Wild, A. 1972. "Nitrate leaching under bare fallow at a site in northern *Nigeria*." *Journal of Soil Science*. 23:315-324.
- World Health Organization (WHO). 1996. World Health Report 1996: fighting disease, fostering development. Geneva. World Health Organization.
- Xiao, L., R.P. Herd, and G.L. Bowman. 1994. "Prevalence of *Cryptosporidium* and *Giardia* infections on two Ohio pig farms with different management systems." *Veterinary Parasitology*. 52:331-336.
- Yates, M.V. and S.C. Yates. 1988. "Modelling microbial fate in the subsurface environment. *CRC Critical Reviews in Environmental Control*. 17:307-344.
- Yeager, J.G., and R.T. O'Brien. 1979. "Enterovirus inactivation in soil." *Applied and Environmental Microbiology*. 38:694-701.

**Appendix 1.** E-Mail Contacts for Scoping Study for Agroecosystem Indicator for Pathogens

Contact	e-mail	Information Provided
Dr. Lyn Abbott School of Earth and Geographical Sciences - Soil Science Faculty of Natural and Agricultural Sciences University of Western Australia, Australia	labbott@cyllene.uwa.edu.au	- land application - conditions of soils - indicator organisms
Dr. Wolf-Rainer Abraham GBF - German Research Centre for Biotechnology Head of Chemical Microbiology Research Group Germany	wab@gbf.de	- real-time PCR technique for quantification of pathogens in soil
Dr. Soren Thiele-Bruhn Institut f. Bodenkunde & Pflanzenernahrung University of Rostock Rostock, Germany	soeren.thiele@auf uni- rostock.de	- input, fate and effects of antibiotics on soil
Dr. Mary Ann Bruns Assistant Professor of Soil Microbiology Dept. of Crop and Soil Sciences Pennsylvania State University, U.S.	mvb10@psu.edu	- molecular approaches to finding pathogens in water - E. coli from biosolids-amended mine spoils
Dr. Philippe Cantin Biologie et Microbiologie Centre & expertise en analyse environnementale du Quebec Ministere de l'environnement, Quebec	philippe.cantinmenv.gouv. qc.ca	- well contamination in Quebec; agricultural land versus non-agricultural land
Dr. Brian J Chambers Principal Research Scientist ADAS Gleadthorpe Research Centre Nottinghamshire, UK	brian.chambers@adas.co.uk	- factors affecting survival of pathogens in livestock waste - safe application of biosolids to agricultural soils

Dr. Caroline Cote Research and development for the agri-environment (IRDA) Saint-Hyacinthe, Quebec	caroline.cote@irda.qc.ca	- microbiological hazards associated with use of manure in vegetable production systems
Dr. John Doran Soil Scientist - USDA-ARS/ Department of Agronomy University of Nebraska NE, U.S.	j_doran1@unl.edu	- contacts in USDA-ARS
Dr. James Dosman Director - Institute of Agricultural Rural and Environmental Health University of Saskatchewan, SK	dosman@sask.usask.ca	- agricultural medicine
Victoria L. Edge Epidemiologist/Statistician Foodborne, Waterborne and Zoonotic Infections Division Health Canada	victoria-edge@hc-sc.gc.ca	- identification of links with Health Canada projects - herd versus animal members - risk assessment
Dr. Joeseeph Eisenberg, PhD Adjunct Assistant Professor Epidemiology and Environmental Health Sciences University of California, U.S.	eisenber@socrates.berkeley .edu	- food risk clearing house
Dr. Jeanette Thurston-Enriquez Environmental Microbiologist USDA-ARS University of Nebraska NE, U.S.	jthurston1@unl.edu	- alternative indicators for pathogens - animals versus herds as likely risk factors - analyzing pathogens and indicators in bioaerosols
Dr. Rob Gordon Department of Engineering Nova Scotia Agricultural College, Nova Scotia	rgordon@nsac.ns.ca	- burial of deadstock and microbial contamination of groundwater

Dr. Sue Grayston Principal Scientific Officer Plant Ecophysiology & Rhizosphere Processes Macaulay Land Use Research Institute Aberdeen, UK	sgrayston@macaulay.ac.uk	- Macaulay website - PCR ELISA to detect Campylobacter
Dr. Mansell Griffiths DFO/NSERC Chair in Dairy Microbiology and Director, Canadian Research Institute for Food Safety University of Guelph, Ontario	mgriffit@uoguelph.ca	- identification of indicator species
Dr Phil Haygarth (Co- ordinator) Institute of Grassland and Environmental Research North Wyke Research Station, Devon, UK	phil.haygarth@bbsrc.ac.uk	- role of agriculture on pathogen loadings in streams and water environment
David L. Jones School of Agriculture and Forest Studies University of Wales, UK	d.jones@bangor.ac.uk	- turbidity - farm waste storage areas high risk - survival in freshwater - human health risks
Dr. Ellen Kandeler Department of Soil Science University of Honenheim, Germany	kandeler@uni-hohenheim.de	- soil enzyme activity and pathogen detection
Dr. Ken Killham Department of Soil Science University of Aberdeen, UK	k.killham@abdn.ac.uk	- methodology to identify the potential for pathogen transport through soils
Dr Denise C. Lambkin School of Human and Environmental Sciences Department of Soil Science University of Reading, UK	d.c.lambkin@reading.ac.uk	- provided U.K. contacts

Kit Macleod Institute of Grassland and Environmental Research North Wyke Research Station, Devon, UK	kit.macleod@bbsrc.ac.uk	- role of agriculture on pathogen loadings in streams and water environment
Dr. Tim McAllister, Lethbridge Research Centre, Alberta	mcallister@agr.gc.ca	- E. coli in feedlot - animal, manure, compost and land application
Dr. Scott McEwan Department of Population Medicine University of Guelph, Ontario	smcewen@uoguelph.ca	- discussions on the potential list of pathogens in manure in Ontario - herd versus animal numbers as a component of risk analysis
Dr. Pascal Michel Health Canada St-Hyacinthe Unit Faculte de medecine veterinaire University de Montreal, Quebec	pascal.michel@umontreal.ca	- development of agroenvironmental hygienic pressure indicator system
Dr. J.J. (Jim) Miller Research Scientist/Water Quality Theme Agriculture and Agri-Food Canada Lethbridge, Alberta	millerjj@agr.gc.ca	- monitoring manure, compost, water and crops for persistence of total coliforms and generic E. coli; looking at BMPs to minimize their persistence in the environment.
Dr. Patricia D. Millner Sustainable Agricultural Systems Laboratory and Animal Waste Pathogens Laboratory USDA-ARS-BARC MD, U.S.	millnerp@ars.usda.gov	-technologies for disinfection of organic residuals in an era of emerging and re-emerging pathogens
Cindy Nakatsu Department of Agronomy Purdue University Indiana, U.S.	cnakatsu@purdue.edu	- alternative indicators for assessing water quality

David Oliver Research Student Institute of Grassland and Environmental Research Devon, UK	david.oliver@bbsrc.ac.uk	- role of agriculture on pathogen loadings in streams and water environment
Dr. David Powlson Head, Soil Science Department IACR-Rothamsted. Hertfordshire, UK	david.powlson@bbsrc.ac.uk	- list of contacts in Europe
Andre Ravel - Faculte do medecine veterinaire	andre_ravel@hc-sc.gc.ca	- development of agroenvironmental hygienic pressure indicator system
Dr. Jorge Santo Domingo US Environmental Protection Agency NRMRL/WSWRD/MCCB Ohio, U.S.	santodomingo.jorge@epamail.epa.gov	- modelling of contamination risks - microbial source tracking
Dr. Daniel Shelton Animal Waste Pathogen Laboratory - USDA MD, U.S.	sheltond@anri.barc.usda.gov.	- alternative indicators
Dr. Don Stoeckel Hydrologist/Microbiology USGS-WRD OH, U.S.	stoeckel@usgs.gov	- modelling of contamination risks
Dr. Joanne Van Kessel Animal Waste Pathogen Lab ARS-USDA MD, U.S.	jkessel@anri.barc.usda.gov	- links to USDA - AWPL
Dr. Jack Watson Professor of Soil Science State Program Leader for Agriculture and Natural Resources, and Assistant Director of Penn State Cooperative Extension University of Pennsylvania, U.S.	jackwatson@psu.edu	- identified linkages in North Eastern U.S.

Dr. Steve Weisberg SCCWRP California, U.S.	steve@scw.org	- reproductability of regional pathogen findings
Dr. Phil Whiting Director - Centre for Research in Environmental Science and Engineering University of Western Ontario, Ontario	pwhiting@eng.uwo.ca	- identified linkages in Canada
Franceska Wilde USGS Office of Water Quality VA, U.S.	fwilde@usgs.gov	- suggested Website links
Dr. Jeff Wilson Chief of Foodborne and Enteric Diseases, Bureau of Infectious Diseases, LCDC, HPB Department of Population Medicine University of Guelph, Ontario	jwilson@ovenetuguelph.ca	- linkages to Health Canada activities
Robert Wright National Program Leader Soil Management USDA-ARS MS, U.S.	rjw@ars.usda	

## Appendix 2. Websites Searched

AA-FC Agriculture and Agri-Food Canada, Canada	<a href="http://www.agr.gc.ca/">www.agr.gc.ca/</a>
ASAE American Society for Engineering in Agricultural, Food and Biological Systems (U.S.)	<a href="http://www.asabe.org/">http://www.asabe.org/</a>
Food Safety Risk Analysis Clearinghouse U.S.	<a href="http://foodrisk.org/">http://foodrisk.org/</a>
Health Canada Canada	<a href="http://www.hc-sc.gc.ca/english/">www.hc-sc.gc.ca/english/</a>
Macaulay Institute U.K.	<a href="http://www.macaulay.ac.uk/">http://www.macaulay.ac.uk/</a>
Manure Management Planner Page Purdue University, U.S.	<a href="http://www.agry.purdue.edu/mmp/">www.agry.purdue.edu/mmp/</a>
NRCS - USDA Natural Resources Conservation Service - United States Department of Agriculture, U.S.	<a href="http://www.nrcs.usda.gov/">www.nrcs.usda.gov/</a>
OMAF Ontario Ministry of Agriculture and Food, Ontario	<a href="http://www.omafra.gov.on.ca/">http://www.omafra.gov.on.ca/</a>
OSRAS On-Site Sewage Management Risk Assessment System - Septic Safe Program, Australia	<a href="http://www.dlg.nsw.gov.au/dlg/dlghome/dlg_osras.asp">http://www.dlg.nsw.gov.au/dlg/dlghome/dlg_osras.asp</a>
SCCWRP Southern California Coastal Water Research Project, U.S.	<a href="http://www.sccwrp.org">www.sccwrp.org</a>
Scottish Executive	<a href="http://www.scotland.gov.uk/library5/environment/pepf-02.asp">www.scotland.gov.uk/library5/environment/pepf-02.asp</a>
SEPA Scottish Environment Protection Agency, U.K.	<a href="http://www.sepa.org.uk/">http://www.sepa.org.uk/</a>
Source Molecular U.S.	<a href="http://www.sourcemolecular.com/">http://www.sourcemolecular.com/</a>
UFZ Centre for Environmental Research Germany	<a href="http://www.ufz.de/index.php?en=11382">http://www.ufz.de/index.php?en=11382</a>

USDA - ARS <http://www.ars.usda.gov/>  
United States Department of Agriculture - Agriculture Research Service, U.S.

USDA - AWPL [http://www.ars.usda.gov/main/site\\_main.htm?modecode=12-65-00-00](http://www.ars.usda.gov/main/site_main.htm?modecode=12-65-00-00)  
Animal Waste Pathogen Laboratory. U.S.

USDA - ERS [www.ers.usda.gov/amberwaves/](http://www.ers.usda.gov/amberwaves/)  
United States Department of Agriculture - Economic Research Service

US Environmental Protection Agency [www.epa.gov](http://www.epa.gov)  
U.S.

USGS [www.usgs.gov/](http://www.usgs.gov/)  
Bureau of the Department of the Interior, U.S.