TRANSPORT OF FECAL BACTERIA IN A RURAL ALASKAN COMMUNITY

By

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TRANSPORT OF FECAL BACTERIA IN A RURAL ALASKAN COMMUNITY

A

THESIS

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ABSTRACT

People living without piped water and sewer can be at increased risk for fecal-oral diseases. One Alaskan village that relies on hauled water and honeybuckets was studied to determine the pathways of fecal contamination of drinking water and the human environment so that barriers can be established to protect health. Samples were tested for the fecal indicators *Escherichia coli* and *Enterococcus*. Several samples were also tested for the pathogens *Giardia lamblia* and *Cryptosporidium parvum*. All terrain vehicle (ATV) use and foot traffic transported bacteria within the village and into the home. Surface water flow transported bacteria within the community during spring thaw, but flow from the dump did not appear to contribute to contamination in town. Within the home, viable fecal bacteria were found on water dippers, kitchen counters and floors, and in washbasin water. *Giardia* was found at the dump, but not in water from the river adjacent the community. Exposure to fecal contamination could be reduced by cleaning up after dogs, careful disposal of honeybucket bags and gray water, and by protecting stored drinking water.

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Introduction

Many residents of rural Alaskan communities lack piped water and sewer services and therefore must haul water from a watering point and haul buckets of sewage to a designated area for disposal. Due to financial constraints and climatic challenges some residents may never be served by piped water and sewer systems. Some people may also choose traditional water and wastewater management for cultural and other reasons. It is in the face of these realities that this study seeks to determine the pathways of fecal contamination of drinking water including the spread of fecal contamination in the environment.

Many diseases are spread by the fecal-oral route and these are strongly related to unimproved water and sanitation. Waterborne diseases are a concern in the absence of piped water or sewer and when drinking water is untreated or stored long enough to lose effective levels of residual disinfectant. Water-washed diseases, or diseases that occur because of a lack of proper sanitation or hygiene, are also an issue when an insufficient supply of water is available or consumed. Although large outbreaks of waterborne diseases are not currently occurring in rural Alaska, a disease burden is still likely and risk is not negligible. By determining the pathways of contamination within the village, this research aims to provide residents with information useful for protecting public health.

In addressing the objective of determining pathways of fecal contamination, the following hypotheses are tested and discussed.

- Human fecal bacteria are present in the community and not limited to the dump.
- Objects such as tires and shoes can carry fecal contamination within the village and into the home.
- Surfaces within the home and school are contaminated with fecal bacteria, indicating that hands are likely transporting bacteria to these places.
- Fecal contamination is present in the environment and moving during spring thaw.
- Drinking water is insufficiently protected and people are at risk for fecal-oral diseases.

Background

Rural water and sanitation.

Currently, most or all of the residents in at least 18 villages in the Yukon-Kuskokwim Delta region haul water and use honeybuckets (Table 1, RUBA 2005, ADCA 2005). Other villages have partial or nearly complete water and sewer services. Even in these villages, however, some households cannot feasibly be served by the systems or are not yet served. Whether residents are unserved in a village that has piped water and sewer or are waiting for the funding for, or completion of a water and sewer system upgrade, they still must deal with hauling water and waste. The health issues associated with individual hauling of water and sewage are therefore still relevant in rural Alaska, and probably will be for years to come. This research was intended to provide useful information to a broader audience than just the study community. Table 1 lists some villages that may benefit from findings presented here. Additional villages in the state face similar sanitation situations, though topography affects the nature of the problem, so the list was limited to the Yukon-Kuskokwim region.

Table 1. Yukon-Kuskokwim Delta villages lacking fully piped water and sewer.*

Most or all residents haul water and use honeybuckets or pit privies		
Akiachak	Kasigluk	Nunam Iqua
Atmautluak	Kipnuk	Pitka's Point
Chefornak	Kongiganak	Quinhagak
Chuathbaluk	Kwethluk	Shageluk
Crooked Creek	Kwigillingok	Tuluksak
Eek	Newtok	Tununak
Partial improvements, some haul water and use honeybuckets or pit privies		
Akiak	Marshall	Nunapitchuk
Kotlik	Napaskiak	
Large portion of community served or full service coming soon		
Napakiak	Tuntutuliak	

*Sources: RUBA 2005, ADCA 2005, VSW 2000. All villages listed had population estimates of 100-700 residents in 2004 and per capita income between $6495 and $10,487 in 2000.

Water sources in the Yukon-Kuskokwim Delta region include lakes, rivers, wells, rain and ice. Some communities or some within a community choose the continued use of traditional water sources. Others may use them because treated water is not as acceptable, affordable, or convenient (Appendix B). Certain lakes or rivers around a village may be selected as water sources. Ice is collected from these bodies of water in winter or water may be taken from above the ice in spring. Many households collect rainwater from their roofs in a large container such as a garbage can positioned under the downspout. A piece of cloth may filter out debris. Some households have tanks inside their homes that are fed by the gutter system. Essentially all communities have at least a central watering point, though sites such as fish camps, with few or no year-round inhabitants, generally lack public water systems. Private wells or traditional

sources are used instead (RUBA 2005). Treated water is therefore available, but quality varies based on source and treatment, storage, and distribution system.

Water storage techniques vary within and among villages. Those households with flush tank systems have a tank built in for water storage. Some other homes also have tanks designed for water storage, but those who use plastic garbage cans (~32 gallon) and 5-gallon buckets with or without plastic bag liners still remain (Appendix B). Even if covered, these receptacles with large openings are at risk for contamination particularly when water is dipped from them. Contamination during storage has been documented in various settings (Clasen and Bastable 2003, Genthe et al. 1997) and even if the water was piped from a treated source, residual disinfectant is quickly lost if the distribution system is damaged or subject to periods of low water pressure (Semenza et al. 1998).

Gray water disposal in villages without piped sewer is not tightly regulated. The city officials of a rural Alaskan community may give no direction with respect to disposal of household gray water. Sewage disposal receives more attention. In addition to specified honeybucket areas at dumps, villages often have a honeybucket haul system where residents deposit honeybucket bags in a hopper and a worker empties the hopper, controlling where the majority of the sewage goes. Likewise, with the flush tank system, the operator removes sewage from homes and deposits it in a dump or lagoon. Villages with more advanced sanitation may have true sewage treatment lagoons or wetlands.

State and regional health

Epidemic disease is not rampant in rural Alaska. Although water may be considered to be at risk, and a disease burden does exist, reports of waterborne diseases are few and for the most part, waterborne diseases are not among the infectious diseases of greatest concern to public health workers.

Early in the post-contact history of Alaska and through the 19th century, epidemics of influenza, pneumonia, mumps, measles, typhus, scarlet fever, smallpox, possible diphtheria and poorly defined respiratory disease took a toll on the health of Alaska Natives (Fortuine 1989). Tuberculosis was established early on and continued as a significant contributor to mortality in the 20th century (Fellows 1934). Other conditions described by early European contacts included ear, throat and respiratory infections, boils, and diarrhea in the summer (Fortuine 1989). Between 1926 and 1930, 77 deaths among Alaska Native people due to gastrointestinal illness were recorded, a figure dwarfed by the 982 tuberculosis deaths and 423 attributable to pneumonia and influenza (Fellows 1934). Later in the 20th century, infectious diseases contributing significantly to morbidity and mortality among Alaska Natives included upper and lower respiratory diseases, otitis media, and tuberculosis (Maynard et al. 1967, Brody 1965, Reed et al. 1967, Fleshman 1968). Fleshman (1968) noted that diarrhea had not been a significant problem except for an *E. coli* outbreak in 1965 and minor outbreaks at breakup attributable to *Shigella* or unknown etiological agents. Incidence of invasive pneumococcal disease has been high among Alaska Natives

relative to nonnatives and other US groups, but most of the observed serotypes are included in available vaccines (Davidson et al. 1989, 1994).

Historically and presently, health in Alaska is different than the developing world of circa 2000. While higher than ideal, incidence of diarrhea is not a large contributor to mortality. Incidence of diseases spread person to person, like tuberculosis, shows that interaction among people within close proximity is such that this transmission route is not cut off. Similarly, prevalence of water-washed diseases such as skin infections would indicate inadequate hygiene, possibly due to water conservation (McJunkin 1982).

Most recently, attention has been focused on *Streptococcus pneumoniae,* foodborne botulism, alveolar hydatid disease, viral hepatitis, *Heliobacter pylori*, *Haemophilus influenzae* type b, respiratory syncytial virus (RSV), and *Staphylococcus aureus* infections (Butler et al. 1999, Parkinson and Butler 2001). Of these diseases, hepatitis A virus (HAV) can be waterborne, but most are spread primarily by person to person contact or other pathways. *S. pneumoniae* is transmitted person to person and causes several conditions including pneumonia, bacteremia, otitis media, meningitis and sinusitis (CDC 2004a, Koneman et al 1997). *H. influenzae* type b causes meningitis, cellulitis, sepsis, and bacterial pneumonia and is spread by contact with respiratory droplets of an infected person (CDC 2004b). RSV causes bronchiolitis and pneumonia and is spread by close contact, respiratory secretions, and contaminated objects (CDC 2005a). Prevention through hand washing and not sharing dishes is recommended for RSV (CDC 2005a), so presumably this is a disease that is more readily spread when water is used too sparingly. However, person to person contact may overshadow the water-washed nature of this disease since other person to person diseases appear to spread readily in rural Alaska (e.g. tuberculosis: Table 2 and Appendix C). *S. aureus* can cause skin infections (pimples and boils), pneumonia, and wound and bloodstream infections. Hygiene is recommended for protection against *S. aureus* infections (CDC 2005b). Antibiotic resistance is an additional concern with *S. aureus* (CDC 2005b).

Currently, incidence of some reportable diseases is higher in Alaska or the smaller study region than in the rest of the United States (Table 2, top), but others are reported less frequently (Table 2, bottom). The diseases reported less often in Alaska than the rest of the US may actually reflect better health or reduced exposure as lifestyle and location preclude some risks such as fast food and runoff from feed lots. On the other hand, cases may not be recorded as self limiting diarrhea may be less likely to trigger a visit to the doctor in rural Alaska where doctors are not often present within the community. In the case of hepatitis A, aggressive vaccination in reaction to a previous problem is probably responsible for the lower incidence in Alaska.

Though not among the leading causes of morbidity and mortality in rural Alaska, diseases that are potentially food or waterborne have a presence (Fig. 1). Between 1994 and 1996 the gastrointestinal death rate for the Alaska Indian Health Service (IHS) service area was more than twice that of the US (IHS 2004). Digestive system disease ranked 4th in reason for hospitalization in FY 1997 accounting for

Table 2. Comparative infectious disease incidence, 2003.*

Disease	***Incidence per 100,000***		
	Southwest Alaska	*Alaska*	*United States*
Tuberculosis	47.57	8.79	
		8.89	5.17
Giardiasis	15.02	13.72	
		13.88	6.84
Botulism	7.51	0.46	
		0.47	0.04
Haemophilus influenzae,			
type b, invasive	5.01	0.46	
all serotypes, invasive		3.28	0.70
Meningococcal disease		1.09	0.61
Shigellosis	NR	1.72	8.19
Salmonellosis	2.5	14.95	
		14.98	15.16
AIDS	2.50	2.47	
		2.65	15.36
Cryptosporidiosis	NR	0.16	1.22
Hepatitis A, acute	2.50	1.54	
		1.56	2.66

*Regional versus state incidence is based on the 2003 annual infectious disease report for Alaska (State of Alaska Epidemiology, 2004) and state versus US incidence is based on national MMWR 2003 (CDC 2005c). Some differences are due to different reporting categories while others reflect the counting of confirmed or prospective cases or slightly different population estimates. NR = no cases reported in 2003.

9.6% of hospital discharges, following obstetric deliveries and complications of pregnancy, respiratory system diseases, and injury and poisoning (IHS 2004). Gastrointestinal illness is affecting Alaska Natives even if it is not the most serious problem they currently face. In addition, gastrointestinal illness is classically underreported (Mac Kenzie et al. 1994, Gordon et al. 1956, Jenkerson, and Middaugh 1990). In remote areas, mild and self limiting cases may be treated at home without diagnosis and therefore without reporting. Also, many diseases reflective of inadequate water and sanitation such as non-specific gastroenteritis, impetigo, and other skin infections (McJunkin 1982) are not included in epidemiology bulletins in the state of Alaska.

As will be discussed next, residents may have considerable immunity to familiar pathogens present in the family or community, so exposure to those pathogens is not as large a risk. However, Alaska Native communities are not completely isolated. Residents travel to municipal centers for medical care and shopping and to other villages to visit family and friends. People from outside the communities come to teach, build, and provide medical care. Some communities are on rivers, downstream from other communities and many have considerable contact with wildlife. All of these contacts make it possible and likely that new pathogens will periodically enter the community. Once a fecal-oral, waterborne, or water-washed pathogen is introduced it can spread quickly if barriers do not prevent it.

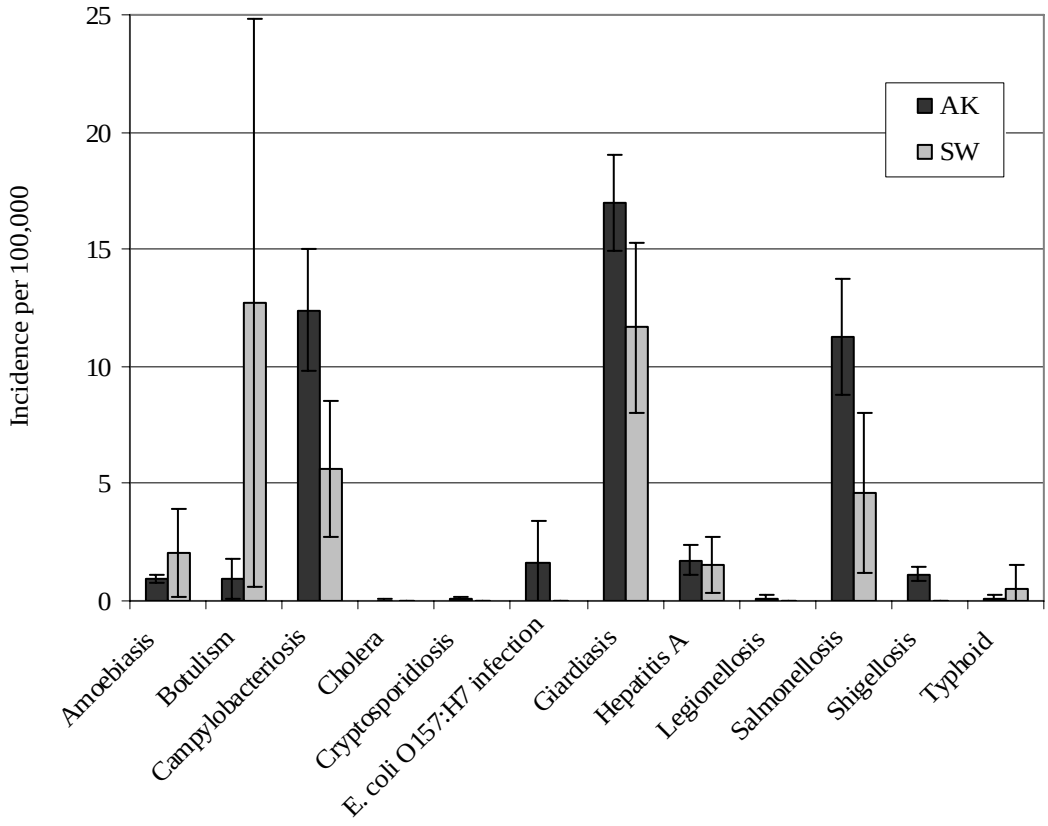

Figure 1

Food and waterborne disease incidence 2000-2004. Bar height represents the mean incidence per 100,000 in Alaska (AK) or Southwest Alaska (SW) over 5 years. Error bars showing a 95% confidence interval (CI) reflect the variable incidence from year to year (e.g. botulism) or relative stability (e.g. campylobacteriosis or hepatitis A). For comparison, tuberculosis incidence for the same period was 9.8 ± 3.8 statewide and 46.5 ± 26.9 in the southwest region. Data come from State of Alaska Epidemiology (2001-2005).

Fecal-oral disease transmission

While a variety of pathogens can be transmitted by the fecal-oral route, the most common disease or syndrome is diarrhea (Byers et al. 2001). By their very nature, pathogens that cause gastrointestinal disease are often voided in large numbers by an infected individual making the link between symptom (diarrhea) and transmission route (fecal-oral) logical. Worldwide diarrhea causes a significant health burden. While much of this is due to the sanitation conditions in the developing world, risk factors for fecal-oral diseases also include some developed world conditions like daycares and nursing homes, exposure to children, and exposure to animals (Byers et al. 2001). As illustrated by Esrey and Habicht (1985) fecal-oral disease transmission involves any range of pathways from hands and flies to water, food, and other fomites. Furthermore, water quality interventions do not necessarily prevent fecal-oral disease

transmission. Likewise, sufficient water quantity is instrumental in preventing the consumption of fecal pathogens in food and transport of pathogens by the hands. What these authors leave out in terms of types of interventions (water quality, water quantity and excreta disposal) are education and immunization which will be discussed later.

Fecal-oral diseases can be either endemic or epidemic. While common source outbreaks within a population with many susceptible individuals are considered epidemic, disease within a community exhibiting a continual presence of infection is endemic. The epidemiology of the two situations differs even if the pathogen is the same. For example, if a community water system in the contiguous United States was contaminated with HAV, or if a population was exposed to contaminated food, many people would get sick because most of the population is susceptible. In a developing community with endemic hepatitis A, children are exposed to the virus either through drinking water or household contacts at a young age when infection is less serious and there are therefore very few susceptible adults (Purcell 1994). As such, HAV in the drinking water may not cause an outbreak. Based on intervention and recreational water studies that will be discussed later, some conclude that endemic transmission is responsible for more of the disease burden than epidemic transmission, even in developed communities (Eisenberg et al. 2002).

As outlined by Eisenberg et al. (2002), waterborne diseases can follow 3 transmission pathways: (1) person to person, which can be associated with hygiene, (2) person to environment to person, which is important when drinking or recreational water is contaminated, and (3) environment to person, as would be the case when a pathogen comes from an animal that is contaminating the environment. In rural Alaska all of these three are possible. Water use is limited, human waste is released in the environment without treatment, and traditional water sources are not protected from wildlife.

Indicators

Because there are a host of pathogens and these specific pathogens are not always present in any given population's feces, good fecal indicators are necessary to effectively and economically evaluate the microbial quality of water. Ideally, a fecal indicator would be present in large numbers in feces and therefore outnumber the pathogens of interest, would not reproduce in the environment or come from a non-fecal source, would survive as long as the pathogens of interest under environmental conditions, and would be inexpensive and safe to detect (Medema et al. 2003).

Total coliform, *E. coli* and *Enterococcus* are three indicators commonly used in evaluating drinking and recreational water. Total coliform is a broad group consisting of 80 gram-negative species that ferment lactose at 35-37°C, produce acid, gas and aldehyde in 24-48 hours, and are oxidase-negative and non-spore-forming among other characteristics (Leclerc et al. 2001). Because the group can also be defined by ß-galactosidase activity, substrate based tests such as the Colilert® method allow easy, sensitive, and specific detection at a reasonable cost (Payment et al. 2003, Yakub et al. 2002, Edberg et al.

1991, Eckner 1998). While numerous in feces of warm-blooded animals, total coliform is not a specific fecal indicator. Total coliform can come from environmental sources, reproduce in paper pulp plants and other environments, and is often found in the absence of fecal contamination (Leclerc et al. 2001). The potential for false-positives in substrate based tests is also a concern with total coliform as an indicator because ß-galactosidase activity is not exclusively found within the group. *Aeromonas* spp. have been shown to trigger a false positive in the Colilert® test towards the end of the product's shelf life (Landre et al. 1998). Multiple plant and algal species have exhibited ß-D-galactosidase activity (Davies et al. 1994). While plant and algal interference may be an issue when sampling puddles, ponds, and water barrels in the village, *Aeromonas* poses less of a methodological issue because total coliform is not a fecal indicator and *Aeromonas* is a pathogen (Moyer 1999a), so conclusions are not undermined by the interference of *Aeromonas*. Despite the lack of specificity, total coliform is not a useless group of bacteria. By definition, they are present at least as frequently as *E. coli* and adequate treatment should inactivate total coliform (Payment et al. 2003).

E. coli is a species within the total coliform group. *E. coli* is well accepted as a specific fecal indicator. The work of Roger Fujioka and his colleagues is often acknowledged as the exception to the rule that *E. coli* does not reproduce in the environment. Fujioka (1988) first observed high levels of fecal indicators in tropical streams without obvious fecal sources nearby. Byappanahalli and Fujioka (1998) then grew *E. coli* on soil extract agar. Hardina and Fujioka (1991) observed *E. coli* growth in stream water incubated in the lab and in dialysis bags in a stream and observed *E. coli* at 36 cm depth in the soil. Fujioka et al. (1999) concluded that soil was the source of the *E. coli* found in the streams of Hawaii and Guam. While these studies showed that there is the potential for *E. coli* growth outside the host, they did not eliminate the possibility of input from animal sources in the field soil experiments to prove that soil is the primary contributor to stream pollution. Also, the degree to which *E. coli* in the soil was an established and productive population relative to an establishment of a population through recent fecal contamination was not sufficiently addressed. Additional laboratory incubations of soil samples at field temperatures over longer time periods would aid in supporting a soil-source hypothesis. While the use of *E. coli* as an indicator in tropical areas may be called into question by these findings, temperatures in the subarctic village of this study are most likely too low for *E. coli* reproduction outside of a warm blooded host. In temperate climates *E. coli* is regarded as a specific fecal indicator (Leclerc et al. 2001, Payment et al. 2003). Like total coliform, *E. coli* can be detected by substrate based tests that function on the ß-glucuronidase activity of *E. coli* (Payment et al. 2003).

Enterococcus is a genus of bacteria mostly of fecal origin (Payment et al. 2003). Enterococci compose a sub-group of what were called the fecal streptococci, or group D streptococci, and are tolerant of saline environments, high pH, desiccation, and more tolerant of chlorination than *E. coli* (Payment et al. 2003). Fecal streptococci, the group to which enterococci belong, have been found to outlast coliforms and

thermotolerant coliforms at points far from a fecal source (Cohen and Shuval 1973). Fecal streptococcal survival was also closer than that of total or thermotolerant coliform to viral survival. Fecal streptococci are also less affected by freezing than total and thermotolerant coliforms (Parker et al. 2000). Presence of fecal streptococci in the absence of the other indicators may be due to lack of specificity, but in areas known to be fecally polluted, these trends probably do actually represent differential survival. Enterococci's tolerance of saline environments and treatment processes as well as its better correlation with swimming associated gastroenteritis than other indicators has resulted in its being the preferred indicator for marine swimming beaches (Jin et al. 2004, Miescier and Cabelli 1982, EPA 1986). In addition, fecal streptococci better modeled the die-off of enteroviruses than did the shorter lived coliforms on the Mediterranean coast (Fattal et al. 1983). Substrate based detection of *Enterococcus*, such as Enterolert®, is also available based on ß-glucosidase, yielding a positive in the presence of 6 *Enterococcus* species (*E. faecalis*, *E. faecium*, *E. avium*, *E. gallinarum*, *E. casseliflavis*, and *E. durans*, IDEXX 2005).

Enterococcus is known to be of avian as well as mammalian origin. Kuntz et al. (2004) isolated *E. faecalis*, a species thought to be of avian and human origin, and other enterococci from broiler, owl, robin, seagull and turkey feces but not deer feces. *E. faecalis* has also been found in dog feces but not in the Canada goose, cattle, deer or swine (Wheeler et al. 2002). Other enterococci were isolated from the deer. Baele et al. (2002) also found *E. faecalis* in pigeon feces as well as *E. faecium*, *E. gallinarum*, and *E. casseliflavus*, but the two enterococci detected most frequently in the pigeon feces were *E. columbae* and *E. cecorum*, bacteria not detected by the substrate based method used in this study. The main sources expected in the study village (human, dog, and bird) should shed some *Enterococcus* species detected by the Enterolert® method even if it was not the predominant species of *Enterococcus* in the source's intestines.

Other indicators are also common, but were not employed in this study. Thermotolerant coliforms are the basis of recreational water regulation in Alaska (DEC 2003). This group is sometimes referred to as 'fecal coliform', includes *E. coli*, and is not specifically of fecal origin, although it is more specific than total coliform (Payment et al. 2003). *Clostridium perfringens*, heterotrophic plate counts, phages, Bacteroidetes and other microbes have been used as water quality parameters with different degrees of specificity to fecal contamination (Payment et al. 2003).

Source tracking

Knowing the source of fecal contamination is important to remediation decision making and because of the different risks associated with sources. If, for example, fecal contamination in a community is from dog waste, millions of dollars of sewage improvements will not change the contamination situation. Also, human pathogens will be found most often in human feces relative to the other animal contributors to the observed fecal load. Human contamination poses a greater threat to humans than most other animal

feces. There are, however, many zoonotic diseases that people can get from pets or wildlife (or livestock in different settings), so any fecal contamination warrants some degree of concern.

Source tracking, microbial source tracking (MST), or bacterial source tracking (BST), is a developing field. Some methods are further along than others, but none is yet truly the clear solution. MST methods generally fall into two categories: genotypic and phenotypic. Genotypic methods use molecular techniques to detect a genetic sequence thought to be specific to bacteria from a specific host species. These tend to be presence/absence tests based on the whole water sample, not bacterial isolates. A water sample may have molecular markers from bacteria thought to come from dogs and from bacteria thought to come from humans, but the proportional contribution is unknown. Phenotypic methods such as antibiotic resistance analysis (ARA) or carbon source utilization compare the profile of an isolate to the profiles in a library. By this method 20 isolates from a water sample may classify as dog isolates, 30 as human isolates, and 10 as wildlife, in which case the scientist may conclude that the human contribution is 50%. Due to uncertainty of classification, uncertainty as to whether the library is representative and stable, and because a large number of isolates may be needed to represent the bacteria in the contaminated water, such quantitative use is questionable. Genotypic methods can also be library based. In a comparative study of multiple MST methods, library based genotypic methods resulted in false positive rates as high as 57% (Myoda et al. 2003). Library independent genotypic identification with Bacteroidetes distinguished human and non-human sources as well as some other sources, but sample matrix affected detection (Field et al. 2003). Carbon source utilization and ARA had the highest false positive rates, enterovirus detection had the highest false negative rate, and the host-specific polymerase chain reaction (PCR) method using Bacteroides and *Prevotella* minimized false positives and negatives for humans as a source (Griffith et al. 2003). However, the latter method depends on availability of known specific primers for a source (Bernhard and Field 2000). Similar to the PCR method with Bacteroides, a molecular marker in *Enterococcus faecium* has been shown capable of differentiating between human and non-human waste (Scott et al. 2005).

Diseases

Various bacterial, viral, and protozoan pathogens can be waterborne, water-washed or related to fecal contamination. A few brief examples of each etiology follow.

Campylobacter is a genus of bacteria that causes campylobacteriosis. *C. jejuni* is the most common *Campylobacter* in human infection and *Campylobacter* infection is the most common cause of bacterial diarrhea in the US (CDC 2005d). *Campylobacter* can be carried by birds, cows, pigs, cats, dogs and other animals (Waldenström et al. 2002, Korhonen and Martikainen 1991a, CDC 2005d). *C. jejuni* survives better at 4°C than 20°C and studies involving freezing chicken meat and skin at -20°C for 2 weeks found a maximum of 3.39 $\log_{10}$ *C. jejuni* reduction (Korhonen and Martikainen 1991a, Bhaduri and Cottrell

2004). While most *Campylobacter* infections are related to raw poultry handling, *Campylobacter* can also be waterborne and spread from person to person via the fecal oral route (CDC 2005d).

E. coli was discussed previously as an indicator. As an indicator and a pathogen, the survival characteristics of this bacterium are significant. Maule (2000) summarized *E. coli* O157 survival and noted that *E. coli* could be detected in excess of 2 weeks after inoculating surfaces stored at 4°C. Showing its value as an indicator, *E. coli* was found to outlast *C. jejuni* in lake water experiments (Korhonen and Martikainen 1991b).

Many other bacteria pose threats and relate to water and sanitation. *Shigella* spp. cause gastroenteritis and dysentery and are characterized by their low infectious dose which results in their water-washed nature (Moyer 1999b, CDC 2005e). *Salmonella* spp. cause gastroenteritis, enteric fever (including typhoid and paratyphoid fevers), and septicemia and can be carried by livestock, birds, dogs, cats and other animals (Covert 1999). *Salmonella* are also spread by person to person and environment to person fecal-oral routes (Covert 1999).

In addition to hepatitis A (discussed elsewhere), viral concerns in water include adenoviruses, enteroviruses, rotaviruses and others. Rotaviruses are thought to be the world's most common cause of severe childhood diarrhea, with a significant contribution from adenovirus (Mahin and Pancorbo 1999). Adenovirus is resistant to UV disinfection and causes a variety of symptoms (Crabtree et al. 1997, Enriquez 1999). Noroviruses, a subset of Caliciviruses, are also indicted by some as the most significant cause of infectious intestinal disease in developed communities because they cause a significant burden among the adult population (Carter 2005). Enteroviruses count polio-virus among their numbers and can be spread by the fecal-oral route (Gerba 1999). Poliovirus-1 provides an example of infectious pathogens outlasting indicator bacteria in the environment (Skraber et al. 2004). Many other viruses also exhibit prolonged viability in a variety of environmental conditions and can remain infective for a year or longer when cold and sheltered from UV radiation (Carter 2005).

Cryptosporidium and *Giardia* receive a great deal of attention as waterborne protozoan pathogens. *Cryptosporidium* oocysts are buoyant, small, and resistant to chlorination (Mahin and Pancorbo 1999, Rose et al. 2002). An infectious dose (ID_{50}) as low as 10 oocysts has been found for some strains of *Cryptosporidium* (Rose et al. 2002). Though speciation of *Cryptosporidium* is a current study topic, many species have been identified and associated with host ranges that do not include humans (Zhou et al. 2004). *Cryptosporidium parvum* genotypes I and II are a hazard to human health, type I being the 'human' strain and type II being the 'cattle' strain, both of which can infect humans (Rose et al. 2002). *Cryptosporidium* is susceptible to heat and desiccation (Sterling and Marshall 1999). Freeze-thaw cycles contribute to *Cryptosporidium* inactivation, but cold alone (-10°C) was found to result in a 2 $\log_{10}$ inactivation within 50 days in water or 19-23 days in relatively dry soil (3% water, Kato et al. 2002).

Giardia lamblia is a commonly identified waterborne pathogen with robust cysts and zoonotic as well as person to person transmission (Rose et al. 1991, Schaefer 1999). *Giardia* cyst viability is reduced by freezing and thawing (Erlandsen et al. 1990). Boiling effectively inactivates *Giardia* cysts (Schaeffer 1999). Both *Cryptosporidium parvum* and *Giardia lamblia* can be transported by flies from sources of fecal contamination (Szostakowska et al 2004).

Feces, public health, sanitation and water supply

The world situation is such that in 2001 and 2002 the annual toll from diarrhea was estimated to be between 1.4 and 2.5 million childhood deaths or 1.8 million total deaths (WHO 2004, Kosek et al. 2003, Parashar et al. 2003). World Health Organization estimates attribute 88% of diarrheal disease to water, sanitation and hygiene, so the lack of improved water for 1.1 billion people and lack of improved sanitation for 2.6 billion people supports the continuance of this large disease burden (WHO 2004). While diarrheal mortality estimates are declining over the years, morbidity is not following this downward trend (Kosek et al. 2003). Oral rehydration efforts are partly responsible for reducing mortality but not morbidity, but clearly there are still gains to be made in the areas of water, sanitation, and hygiene (Feachem et al. 1983). Esrey et al. (1985) reviewed the diarrheal intervention literature and logically concluded that, in general, interventions have more impact on mortality than morbidity, more impact on severe than mild diarrhea, and more impact on diseases caused by high infectious dose (ID) pathogens than low ID pathogens. In other words simple interventions focusing on the most severe problems have reduced death rates and incidence of the easiest to prevent diseases.

Water related disease is not limited to the classically waterborne cholera, typhoid, dysentery and general gastroenteritis. It has long been understood that the volume of water available for domestic use has a significant impact on gastroenteritis, trachoma and other skin and eye infections, demonstrating the water-washed etiology of these conditions (White et al. 1972). While true waterborne diseases are little affected by volume and truly water-washed diseases are little affected by purity, maximal health requires sufficient clean water. The effects of water availability are also dependent on hygiene practices, as evidenced by successful educational interventions that involved no increase in water supply. Interventions can target water quantity or availability, water quality, sanitation or excreta disposal facilities, and/or personal and domestic hygiene through education.

Water availability has been categorized by some as primarily a rural concern (Schneider et al. 1978), but the rural or urban nature of adequate availability clearly depends on location and what resources can be accessed with the available technology. However, when water is not available on demand, as in many urban or developed world settings, use of an insufficient amount of water has a negative impact on health. Interventions improving water availability and comparisons between households with and without adequate water supply have shown a more significant reduction in diarrhea when water quantity, rather

than water quality is increased (Huttly et al. 1997, van Zijl 1966). Using collection time as a proxy for quantity, disease was reduced in houses where water was available close to or in the home (Imo State Evaluation Team 1989, Esrey and Habicht 1986). Since water is often contaminated in collection and storage, one would expect this health impact to be a result of improved hygiene instead of drinking water quality. That expectation is supported by the finding that water washed diseases (e.g. trachoma) were decreased when a water source was close to home (Esrey et al. 1991).

Despite the relative importance of water availability regardless of quality as discussed above, interventions improving water quality show that in many cases drinking water is responsible for a significant portion of the gastrointestinal disease burden. Home chlorination alone has resulted in significant diarrheal disease reduction in some cases (Quick et al. 2002, Mahfouz et al. 1995). Such improvements indicate that the drinking water was contaminated at some point before consumption in both studies. Either the source was contaminated or the water was contaminated during collection, storage and handling. Contamination between the source and point of use has been well documented (Clasen and Bastable 2003, Genthe et al. 1997, Wright et al. 2004, Swerdlow et al. 1992, Jagals et al. 1997). An increase between source and consumption is most obvious when the source water is of higher quality (Wright et al. 2004). When more fecal bacteria are found in household water than source water, storing water in the same vessel throughout storage and using a spout or spigot has shown to be protective (Clasen and Bastable 2003). Water quality interventions therefore often combine treatment and storage. A narrow necked vessel and chlorine or mixed oxidant in-home treatment have shown to improve water quality and reduce diarrheal illness in many cases (Quick et al. 1999, Sobsey et al. 2003, Deb et al. 1986). In some cases, a water quality intervention protects from some, but not all diarrheal diseases. In one treatment and storage intervention, general diarrhea was not clearly related to water quality at the point of use, but the intervention did reduce the incidence of cholera (Gundry et al. 2004). In another intervention, where drinking water was treated by solar disinfection (water bottles placed on roof), children drinking treated water were only modestly protected from general diarrhea, but significantly protected during a cholera outbreak relative to control children (Conroy et al. 1996, 1999, 2001). These interventions demonstrate that (1) drinking water may be one of several exposures to fecal pathogens, so only a portion of the disease burden will be lifted by water quality improvements, and (2) interventions that improve water quality but fall short of complete disinfection can protect against high ID pathogens, but have little or no impact on low ID pathogens.

Also among the water quality considerations is the source of contaminants. Schneider et al. (1978) suggests that water quality is more important than quantity in dense populations where classical waterborne pathogens are more common, but this likely assumes an adequate quantity. A study of preschool children found that those who did report diarrhea were more likely to be using water from a public tap than a private indoor or outdoor tap (Genthe et al. 1997). Such a finding supports the argument

that pathogens from outside the family are more of a problem than those shared within the family (VanDerslice and Briscoe 1993).

Appropriate excreta disposal or sanitation is important to human health because it removes a large number of fecal pathogens from the immediate human environment and also prevents diseases spread by flies from excrement to food. Esrey and his colleagues conducted multiple extensive reviews of the water and sanitation literature. A collection of well conducted studies supported the hypothesis that improved sanitation impacts morbidity, mortality and growth more than water quality or quantity do (Esrey and Habicht 1985, 1986, Esrey et al. 1985, 1991). An early study also found small health improvement with basic sanitation and a significant reduction in diarrhea with complete sanitation (van Zijl 1966).

Finally, hygiene, as impacted by education, is important to disease reduction. Cairncross (2003) argues that endemic diarrheal disease is not often waterborne, raising the issue of hygiene and explaining the lack of health improvement in some water quality interventions. Interventions involving education (e.g. promoting hand washing) can lead to substantial health improvements even without increasing water quality or quantity (Esrey et al. 1991). Often hygiene education is included in interventions with storage and treatment components.

While the sanitation situation in rural Alaska is not quite like the communities in which most of these interventions were implemented, the availability of piped water lowered infant diarrheal mortality relative to homes without piped water in a metropolitan area of Brazil (Victoria et al. 1988).

An example that may be relevant to communities where piped water quality is questionable or piped service is not complete but waste disposal and hygiene are reasonable comes from Uzbekistan. In this study the water system had undetectable chlorine residuals at more than 30% of the connected homes. Among the study groups, those who had no piped water but used a narrow necked vessel and chlorinated their water exhibited less diarrheal disease even than those with piped water (Semenza et al. 1998). The authors therefore concluded that the endemic disease burden was from the piped water supply.

Waterborne and water-washed diseases are common and a few types of interventions control the bulk of the disease burden, but one size does not fit all. Optimal health and protection from all water related pathogens requires proper waste disposal, an adequate supply of clean water, and good personal and domestic hygiene practices. Protection against specific pathogens, however, can be achieved through targeted interventions such as treated drinking water for cholera (see previous) or vaccination for hepatitis A (see below).

Hepatitis A provides an interesting glimpse into the nature of Alaskan public health. Hepatitis A virus (HAV) is a pathogen transmitted by the fecal-oral route. This route is possible for hepatitis A and E but not the other hepatitis viruses because hepatitis A and E are nonenveloped. Being nonenveloped, these viruses can remain infective after exposure to bile salts and acid in the digestive tract (Hollinger 1996). HAV has been indicted in waterborne outbreaks but is thought to be transmitted person to person most of

the time (Sobsey 1999, Purcell 1994). Infection with HAV results in nausea, vomiting, malaise, jaundice, inflammation of the liver and/or other symptoms and is described as acute, not chronic like hepatitis B or C (Sobsey 1999, Hollinger 1996). After recovery, a person is immune and serum samples reveal an anti-HAV antibody (Sobsey 1999). A study of serum samples collected 1980-1986 from Alaska Natives born before 1980 showed that overall, about half the sampled population was anti-HAV positive (Bulkow et al. 1993). Of the sampled population, 7% of individuals born since 1975 and 85% of those born before 1935 were positive for the antibody (Bulkow et al. 1993). This means that 85% of the individuals older than 50 years of age in 1985 had been exposed and developed immunity whereas only 7% of those under 10 years of age had developed antibodies. Bulkow et al.'s study found that infection rates were high enough within communities that the last outbreak could be determined by the birth year that divides the anti-HAV positive and negative samples with few misclassified samples. Since past outbreaks have thoroughly infected most communities and peaks in HAV infection persisted through the early 1990s, it appears that the fecal-oral route of disease transmission is intact.

Anti-HAV positive rates were not as high at as young an age as in many developing countries, but higher than expected in developed countries (Bulkow et al. 1993). An effective vaccine against HAV was developed in the 1980s and early 1990s with clinical trials beginning in 1988 and first licensing in Europe in 1991 (André et al. 1990, Purcell 1994). This vaccine proved immunogenic among Alaska Native children and adults and effective in controlling outbreaks in rural Alaskan communities in 1993 (McMahon et al. 1995, 1996). With health care provided by the Indian Health Service (IHS), Alaska Natives now have access to protection from HAV. In Alaska, vaccination against HAV is recommended at age 2 and required for entrance to daycare, head start, or public school grades K-12 (Gilbertson et al. 2005, National Network for Immunization Information 2005). The school and daycare vaccination requirement began with the 2001-2002 school year (Anon 2000). Recent reductions in HAV infection may reflect both improved sanitation and vaccination, but vaccination has likely had the stronger influence as sanitation is a slower change and control was rapid and coincided with vaccine development and application (Fig. 2). Outbreaks occurred in the mid-1970s, in late 1986, and are thought to be cyclic, occurring every 10 – 12 years (Bulkow 1993). As incidence has been low since 1995 on a state and regional level, it appears that vaccination has effectively controlled HAV outbreaks. Though there may be some susceptible individuals, immunity of a large portion of the population through vaccination or previous exposure prevents outbreaks and in turn protects the susceptible few.

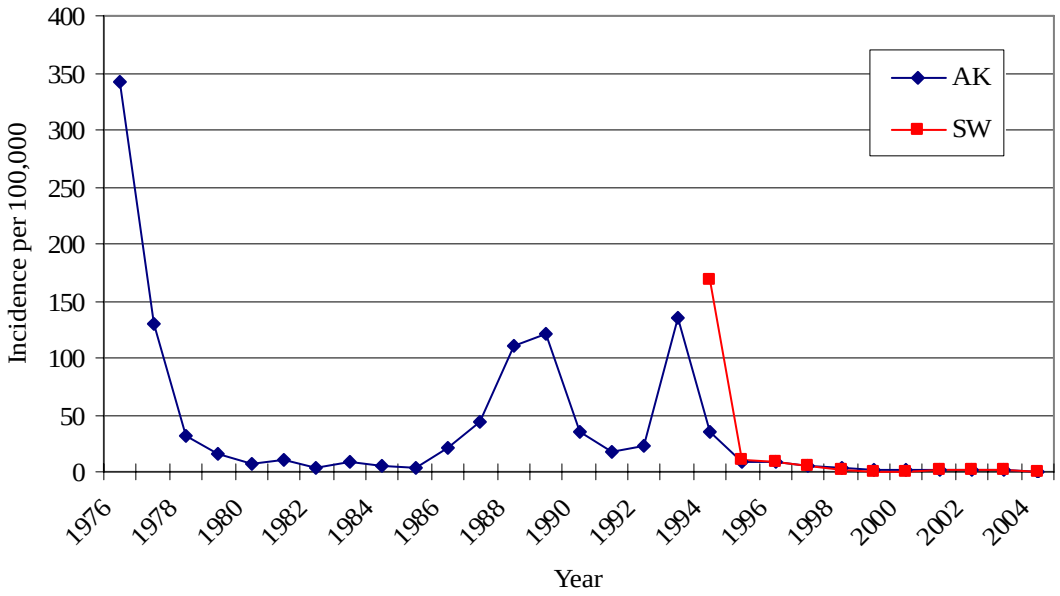

Figure 2

Hepatitis A incidence 1976 – 2004. Outbreaks are apparent in the late 1970s, 1986-1991, and 1993 – 1995. As HAV is a fecal-oral pathogen, the outbreak pattern reflects the water and sanitation situation. Use of an effective HAV vaccine since the mid-1990s has since controlled HAV incidence, masking the effects of poor sanitation and person to person transmission. Vaccination has been required for school children since the 2001-2002 school year. Data come from State of Alaska Epidemiology (1977-2005). AK includes all reported cases in the state. SW includes reported cases in the southwest region of Alaska.

As seen in the example of HAV, the sub-optimal sanitation allows diseases to spread in the ways of developing world epidemiology, but the higher level of health care available in rural Alaska sets it apart.

In developed countries outbreaks still occur. Pathogens in water systems and recreational exposure cause illness, showing that the problem of fecal contamination has not been eliminated. In rural Alaskan communities that lack the containment of sewage in pipes or flush tanks until treatment and/or safe disposal, the presence of feces in the environment creates a greater potential for exposure to pathogens.

Risk: microbes in water, drinking water outbreaks, recreational contact

Even in developed countries, waterborne diseases are not absent and contact with fecal contamination is not completely prevented. Water treatment systems fail, allowing pathogens and indicators to be distributed to consumers, and some pathogens are hardier than the indicators and resistant to the treatment methods. These realities mean that waterborne disease is still a possibility. Nor are inhabitants of developed countries completely isolated from sewage. Contact with diapers is not avoided in the best of sewage treatment systems. Sewage may also be discharged into bodies of water used for

recreation or into septic systems that fail to prevent the contamination of runoff. Pets and wildlife also contribute to the fecal load of runoff and bodies of water with which people come into contact.

Aside from the knowledge of the source of a group of bacteria as the earlier discussion of indicators covered, there is practical regulatory value in drawing connections between indicator presence and illness upon exposure. While regulations specify permissible levels of bacterial parameters, it is difficult to link risk with a specific level of any particular parameter. Likewise, monitoring of drinking water is intended to prevent outbreaks, but connections between indicators and pathogens are loose enough that problems with a water supply are often not realized until the onset of disease. Examples illuminating the meaning of indicators follow.

Total coliform is used for regulation of drinking water, yet this group of bacteria may have very little to do with health risk. Up to 5% of a month's samples can test positive for total coliform (EPA 2003), so drinking water in compliance might occasionally contain total coliform. In one study where water met bacteriological quality standards the presence or absence of total coliform was not associated with the endemic gastrointestinal illness observed (Payment et al. 1993). In a review of outbreaks, total coliform was detected in 34 of 65 community systems and 67 of 77 non-community systems in which there was an outbreak (Craun 1997). In the same study, the finding that there were no maximum contaminant level (MCL) violations in the 28 community outbreaks reviewed indicates that either total coliform is inadequate as an indicator for many waterborne pathogens or the MCL is too permissive. In a river water study, however, total coliform, thermotolerant coliform and *Clostridium perfringens* each significantly correlated with *Cryptosporidium*, *Giardia* and human enteric viruses (Payment et al. 2000). LeChevallier et al. (1991) also found that total and thermotolerant coliform and turbidity correlated with *Giardia* and *Cryptosporidium* densities. In Finish lakes and rivers studied by Hörman et al. (2004), the presence or absence of indicators (*E. coli* and thermotolerant coliforms) was a better predictor of enteropathogen presence or absence than indicator level.

Indicator bacteria and pathogens can be present in source and treated water and in some places there is an appreciable background level of gastrointestinal illness from the drinking water. Surface water is very likely to contain pathogens. One study found *Giardia* in 85% and *Cryptosporidium* in 87% of raw water samples from surface water sources (LeChevallier et al. 1991). Another study detected pathogens (*Campylobacter*, *Giardia*, *Cryptosporidium*, and/or noroviruses) in 41% of samples from lakes and rivers in Finland (Hörman et al. 2004). Raw water from 46 sites on the Saint Lawrence River (Canada) contained *Cryptosporidium* at 43 sites, *Giardia* at 42 sites, and enteric viruses at 43 sites at least once during a year (Payment et al. 2000). The fact that some pathogens survive treatment processes is demonstrated by the occurrence of outbreaks. Under normal operations Vivier et al. (2004) sampled water at two plants and found viable enteroviruses in 11% and 16% of the finished water samples from these plants. Considering the presence of pathogens in the environment and the variety of treatment levels employed, it is not

surprising that a background level of waterborne disease is evident even in developed countries. In Wisconsin researchers were able to detect an association between viral and bacterial diarrhea and the density of septic tanks within sections of 640 and 40 acres respectively (Borchardt et al. 2003). When the raw water source was a contaminated surface water, Payment et al. (1991, 1993) were able to detect a 25-35% reduction in gastrointestinal illness if tap water was further treated by reverse-osmosis in the home, even though the tap water met drinking water regulations. These studies were criticized as not being blind (the subjects knew they had reverse osmosis-units installed) and a similar, but blinded, study in Australia failed to detect a reduction of highly credible gastrointestinal illness (vomiting, diarrhea with fever or disabling, or nausea or stomachache with fever, Hellard et al. 2001). Hellard et al., however, note that the source quality was higher for the Australian study.

Outbreaks prove the limits and weaknesses of treatment systems in developed countries for disease prevention. The inability of indicators to adequately predict pathogen presence in a timely manner is problematic. During the 1993 *Cryptosporidium parvum* outbreak in Milwaukee, Wisconsin, water plants experienced increased turbidity, but there were no violations of microbiological or physiochemical parameters (Mac Kenzie et al. 1994). This outbreak resulted in an estimated 403,000 cases of watery diarrhea and $31.7 million in medical costs (Corso et al. 2003).

In Bennington, Vermont, 1978, no coliform was detected, yet a *Campylobacter* outbreak was attributed to the water supply, which was unfiltered and did not successfully maintain a sufficient free chlorine residual (Vogt et al. 1982). Insufficient chlorination was also blamed for a *Campylobacter jejuni* and *E. coli* O157:H7 outbreak in Walkerton, Ontario in 2000 (Hrudey 2002). In both of these latter cases, rainfall and/or spring runoff was a factor. A *Campylobacter* outbreak in Greenville, Florida, 1983 was also blamed on a chlorination failure (Sacks 1986).

Some pathogens, however, are not affected by chlorination alone. Such is the case with *Giardia*, which caused an outbreak in the chlorinated water supply of Penticton, British Columbia (Moorehead et a. 1990). Between 1961 and 1983 Geldrich (1996) reported 52 *Shigella*, 51 hepatitis A, 84 *Giardia*, 37 *Salmonella*, 5 *Campylobacter* and 16 Norwalk virus water supply outbreaks in the US. In many cases the pathogen itself was not detected in the water during the outbreak investigation. Ice made during the suspect time frames in Milwaukee did contain *Cryptosporidium* oocysts (Mac Kenzie et al. 1994), but the outbreak pathogens were not isolated from water in Greenville or Bennington (Sacks et al. 1986, Vogt et al. 1982). Hänninen et al. (2003) summarized 3 outbreaks in 2000 and 2001 in Finland. In the first, the same serotype of *E. coli* as found in patients was detected in the water after the outbreak. In a second, the patients had the same serotype as was found in tap water. Coliforms were also present in raw, treated and tap water. The first two outbreaks were associated with rain. In the third, *C. jejuni* and *Campylobacter coli* were isolated from the groundwater and a duck pond, but not the tap. Hänninen et al. (2003) suggest using larger sample volumes (up to 10 L) than are customarily used for bacteriological sampling. These

examples revealed that (1) current indicators and regulations are insufficient to prevent every outbreak, (2) pathogen and indicator detection during an outbreak investigation can easily miss the contamination event and (3) modern methods can link outbreaks to a contaminated water supply if a water sample is preserved (e.g. Milwaukee ice) or investigation is sufficiently prompt. Also, rainfall events are a common factor in an outbreak either due to the drainage of excess pathogens into the source water or the overloading of the treatment processes with increased turbidity. While indicators are often related to pathogens in drinking water, one can ingest total coliform without getting sick, or get sick from water meeting total coliform requirements.

The preceding drinking water outbreak and intervention studies assist in understanding the significance of indicator bacteria in drinking water, but much of what residents of the study village might be exposed to is not in their drinking water, but in their environment. To better understand the risks of fecal contamination in the human environment, let us now focus on the relationships between indicators and health in recreational water exposure.

Recreational water exposure studies are very involved because of the multitude of confounding factors, heterogeneity of microbial exposure over time and space, the low attack rates that necessitate large samples, and the requirement of human subjects. While some studies may be less than ideal, together they give an impression of the health risk associated with microbial parameters as sampled within the general area of contact.

Standards for recreational water are based on several indicator bacteria with the goal of keeping swimmer risk at an acceptable level. Swimming, or full body contact recreation, recommendations given by the US EPA limit the geometric mean of at least 5 samples over 30 days to 126 *E. coli* or 33 enterococci per 100 mL in fresh water or 35 enterococci/100 mL at marine beaches based on reviews of multiple epidemiologic studies (EPA 1986, Dufour 1984). The Environmental Health Directorate of Canada has also supported the use of *E. coli* or *Enterococcus* for freshwater and *Enterococcus* for marine monitoring based on the published data (Robertson 1993). Enterococci have been proposed as the metric of the World Health Organization's health based recreational water guidelines (Kay et al. 2004). One later review concluded that enterococci or fecal streptococci were appropriate for fresh or marine water, *E. coli* was appropriate for fresh water monitoring, and detectable increases in symptoms among swimmers occurred with 30 or fewer indicator bacteria per 100 mL (Prüss 1998). Despite EPA recommendations, states set their own recreational water criteria. In Alaska, fresh or marine contact recreation water can have monthly geometric means of 100 thermotolerant coliforms with no more than 10% of samples exceeding 200 thermotolerant coliforms per 100 mL and secondary recreation water limits are twice the contact recreation values (DEC 2003). Thermotolerant coliforms, though sometimes related to health risk, are limited as indicators because of potential non-fecal sources such as paper pulp effluent in which *Klebsiella* reproduces (Caplenas and Kanarek 1984).

Examining some of the studies reviewed while developing guidelines and some conducted since, one can see the variety of correlations among symptoms and indicators and a range of conclusions regarding the choice indicator. Symptoms involved in swimming studies often include one or more of the following: ear infections, skin irritation, gastroenteritis, eye irritation and respiratory illness. Comparing swimmers to non-swimmers at a lightly fecally polluted lake (thermotolerant coliform ≤ 71/100 mL, fecal streptococci < 200/100 mL and usually under 100/100 mL), Hendry and Toth (1982) found an increase in ear infections due to swimming exposure. Swimmers at New York City beaches meeting 1976 standards (geometric mean thermotolerant coliforms < 200/100 mL, ≤ 10% of samples exceeding 400/100 mL) experienced more gastrointestinal, respiratory, and general disabling (staying home or staying in bed) symptoms after swimming at 'barely acceptable' beaches than at beaches well below regulations (Cabelli et al. 1979). Cabelli et al. (1982) also found that an attack rate of highly credible gastrointestinal diseases (vomiting, diarrhea with fever or disabling, or nausea or stomachache with fever) as high as 1% when as few as 100 enterococci or *E. coli* per 100 mL were detected, with enterococci correlating best to highly credible gastrointestinal illness.

Continuing with correlations, Preieto et al. (2001) found gastrointestinal symptoms and skin irritation to be more related to total coliform than thermotolerant coliform or fecal streptococci in a marine environment while total symptoms (gastrointestinal, skin, and respiratory) related to all three indicators. Studies by Seyfried et al. (1985a,b) found respiratory symptoms to be more common than gastrointestinal, eye, ear, or skin complaints, morbidity to be more related to staphylococcal levels than thermotolerant coliform or fecal streptococci, and sediment bacterial concentrations to be an order of magnitude greater than indicator levels in the water column of swimming lakes.

While the above examples found correlations between health effects and total coliform or *Staphylococcus*, others found more specifically fecal indicators to better predict health outcomes. More symptoms were reported at Hong Kong (marine) beaches barely meeting 1981 criteria (1000 *E. coli*/100 mL) than at beaches not approaching acceptability limits and *E. coli* was most related to adverse health effects. The geometric means of *E. coli* and *Enterococcus* at the beaches were 69-1714 and 31-248 bacteria per 100 mL respectively and a combination of *E. coli* and *Staphylococcus* monitoring was recommended because of the additional relationships between *Staphylococcus* and total illness and respiratory symptoms (Cheung et al. 1990). Adults swimming at beaches in Sydney, Australia (marine) most often reported respiratory effects which along with eye and ear symptoms were related to thermotolerant coliform and fecal streptococci (Corbett et al. 1993)

A more stringent marine study that controlled for temporal and spatial variation in microbiological quality found that fecal streptococci was a good predictor of swimming related gastrointestinal illness while thermotolerant coliform was not (Fleisher et al. 1993). These findings were in agreement with a Mediterranean (marine) study that concluded that *Enterococcus* was more predictive of enteric disease than

thermotolerant coliforms or *E. coli* (Fattal et al. 1987). Since then the work of Kay et al. (1994) has also affirmed the use of fecal streptococci with the added detail that adverse health effects were evident when fecal streptococci exceeded 32/100 mL.

In summary, non-drinking water exposure to fecal pathogens can result in gastrointestinal illness and other symptoms. The relationship between indicator levels and health risk depends on the nature of the contact as well as the environmental conditions (e.g. salinity). *E. coli* (member of thermotolerant coliform) and *Enterococcus* (subset of fecal streptococci) are not only fecal indicators, but epidemiologically linked to adverse health effects in the context of contact with contaminated water. Adverse health effects have been detected at 30 indicator bacteria per 100 mL and increase at higher contaminant levels.

Lagoons and natural wetland treatment

While the tundra pond used for honeybucket disposal in the study village is not a wastewater lagoon, some of the same processes take place. Within the wetland or pond environment, indicator and pathogenic microorganisms experience exposure to sunlight and predation. Sedimentation may also reduce the microbial concentrations in the pond water.

Ultraviolet irradiation contributes to indicator and pathogen reduction in water. The doses necessary for inactivation of some viruses, bacterial spores, and amoebic cysts are higher than what is necessary to kill off the indicator *E. coli* (Chang et al. 1985). Other wavelengths in sunlight can also cause a reduction in microbial populations in lake water (Whitman et al. 2004). In polar regions sunlight is highly seasonal and therefore die-off due to UV radiation is expected to be much higher in the summer (Hughes 2005). Jin et al. (2002) attributed the efficacy of a floating aquatic plant wetland to the increased UV exposure.

Other important processes that occur in treatment wetlands are filtration and sedimentation. Wetlands can be effective in removing sediments or suspended solids by filtration through plant and mineral material and by sedimentation (Manios et al. 2003, Davies and Bavor 2000, Coleman et al. 2001). The removal of sediments is relevant because microorganisms are often bound to particles and plankton and are found at higher levels in the sediments than in the water column (Davies et al. 1995, LaLiberte and Grimes 1982, Signoretto et al. 2004, Characklis et al. 2005).

Wetlands may also provide an environment in which predators prey upon some indicator and pathogenic bacteria as seen in the example of thermotolerant coliforms growing in cultures where protozoa were inhibited while dying off if protozoa were not inhibited (Davies et al. 1995). Korhonen and Martikainen (1991a,b) found that *Campylobacter* survived better in filtered water, showing that predators and competitors that are found in the natural environment reduce microorganism survival.

Although natural and constructed wetlands can have a wide range of efficacy in reducing microbial contamination, several basic processes should work against the fecal indicators and pathogens dumped at the honeybucket tundra pond in the study village.

The honeybucket pond in the study village also has another factor at work. Some residents put chemicals such as Aqua-kem® or other toilet and holding tank deodorizers in their honeybuckets to limit the odor. These products have ingredients like paraformaldehyde which should have a negative impact on the indicator organisms. The relative effect of these agents on indicators and pathogens is unknown.

Breakup and special concerns

Breakup refers to the period when the snow and ice melt in the spring. Breakup is a time of particular interest in this study because of the potential release of pathogens upon thawing and anecdotal evidence of increased illness each spring. As discussed previously, bacteria, and presumably other kinds of pathogens, can survive freezing and thawing (Parker et al. 2000). The survival of enteric pathogens through prolonged exposure to cold was cited as a breakup concern nearly 50 years ago along with observations of spring and summer diarrhea outbreaks in the arctic (Gordon et al. 1956). Visible flows of sewage at breakup have been historically noted (EPA 1995). Fleshman (1968) mentioned minor outbreaks at breakup attributable to *Shigella* or unknown etiological agents in his summary of Alaska Native children's health. More recently, the school administrator in the study village has noticed an increase in sickness among the students in fall and spring (Walker, personal communication 2004).

Increased risk at breakup is not unexpected. Outbreaks of waterborne disease in Norway and Canada have been associated with spring breakup or spring runoff (Melby et al. 1991, Hrudey et al. 2002, Moorehead et al. 1990). Also, breakup is probably the time of year when water is most abundant in puddles and ephemeral streams. Rain and runoff events have also been associated with increased total coliform, thermotolerant coliform and *E. coli* loads in two Canadian studies (Moorehead et al. 1990, Hyland et al. 2003).

Materials and Methods

Study site

This study was conducted in a community of about 300 people near Bethel, AK. The village lies within a tundra ecosystem and marine climate (Fig. 3). Tundra ponds and connecting marshy waterways (referred to hereafter as channels) limit high and dry areas for construction. A high water table precludes the use of outhouses or septic systems and the community has no wells. An intake in the adjacent river is the source of water, which is then filtered and chlorinated. A washeteria provides piped water to the school, laundry and shower facilities, and serves as a source of treated water for the community. Homes and city buildings lack piped water and sewer. Residents haul water from the washeteria or collect ice in winter and rain in summer. Sewage from the home is collected in honeybuckets (5-gallon bucket lined with plastic bag) until it is taken to a hopper or a designated tundra pond. When in operation, hoppers are

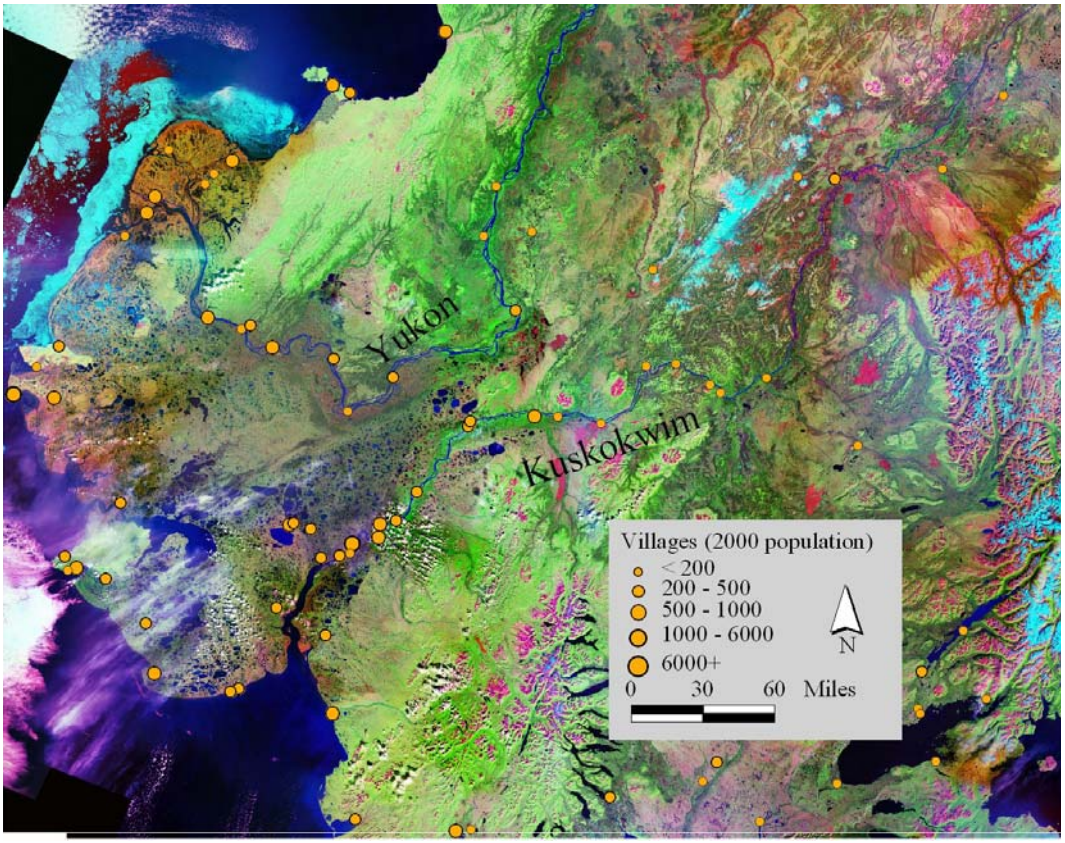

Figure 3

Study region. Landsat imagery of the Yukon-Kuskokwim Delta shows the abundance of surface water, lack of development, and limited high ground in much of the delta region. The false color image was produced around 1990 ± 3 years and colors are as follows: red, band 7 (mid-infrared); green, band 4 (near-infrared); and blue, band 2 (visible green). Villages are indicated by scaled markers. The image was adapted from NASA files and village data came from Alaska community information summaries (ADCA).

hauled to the designated tundra pond by an individual employed by the city. Hoppers are normally in service during the summer but not the winter months. When the dump pond is frozen residents usually drive onto the ice to deposit honeybucket bags within the pond area. However, when the pond thaws and the ground softens, honeybucket bags are dumped at the edge of the pond, sometimes within reach of the boardwalk access. Wastewater from the washeteria and school goes to a sewage lagoon. Household gray water is disposed of at the discretion of the household (Petluska, personal communication). Features of the study village are displayed in figure 4.

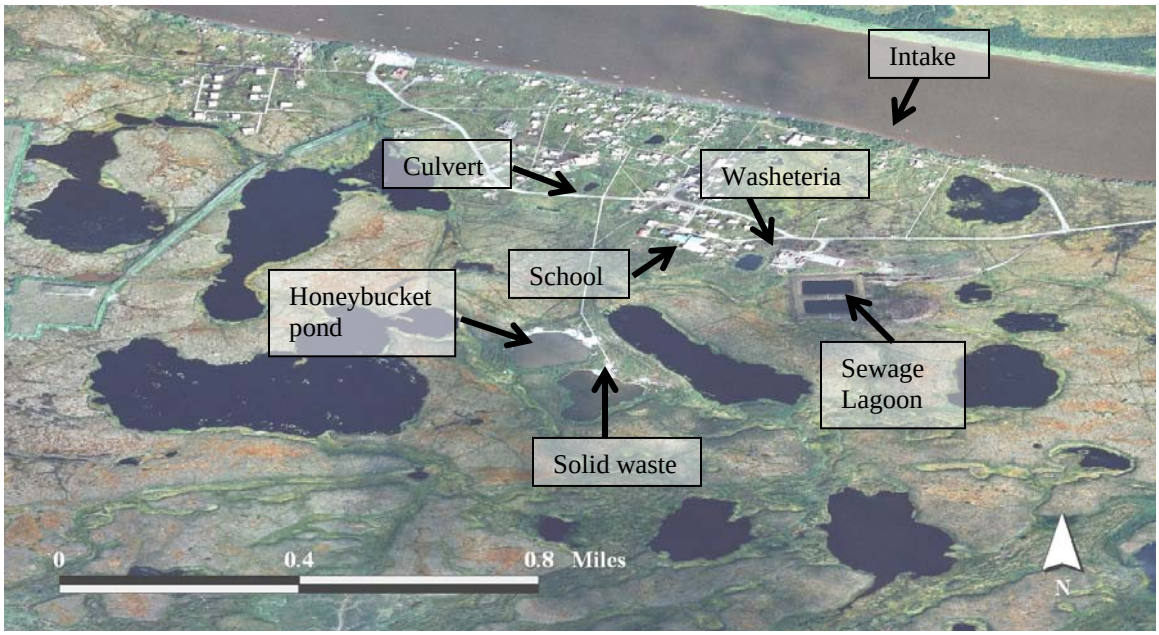

Figure 4

Study village. The image of the study village is modified from aerial photography done by AeroMap U.S. in August 1999. School and washeteria water is piped and waste from these sources feeds the sewage lagoon. All other sewage is deposited at the honeybucket pond. The new clinic under construction to the east of the washeteria (not pictured) will also have piped water and sewer. One main road runs through town while boardwalks connect homes and facilitate travel to the dump. The new air strip (west) is complete and the barge landing (end of road) allows for summer shipments.

The study village is primarily a subsistence community with limited cash economy. Median household income was $17,500 in 2000 with per capita income just below $9000 (ADCA 2005). With a limited tax base and ability to pay for water and sewer services, the community is dependent on outside funding for the construction of a water delivery system or installation of a flush tank system. However, gaming revenues are currently sufficient to subsidize the hauling of honeybucket hoppers (RUBA 2005). Being only accessible by barge in the summer or plane year round and because of the small scale, water system construction, maintenance, and operation is costly.

Since health data was not accessible for the study community, 2003-2004 school attendance data were obtained from community's one school (without student names). The percent of enrolled students

absent (excused) on each school day was calculated and then smoothed as a 3-day rolling average over the school year in an attempt to find times of increased absence and estimate the timing of potential spring breakup illness.

Materials.

Microbiological analysis of water and surface swab samples employed the Colilert®, Enterolert ®, and Quantitray®/2000 products from Idexx Laboratories (Westbrook, ME). Colilert® and Enterolert® are substrate based technologies. The detection of total coliform, *E. coli*, or *Enterococcus* relies on the enzymes characteristic of each group of bacteria. Total coliform bacteria have ß-D-galactosidase to digest o-nitrophenyl-ß-D-galactopyranoside (ONPG), *E. coli* have ß-D-glucuronidase to digest 4-methyl-umbelliferyl-ß-D-glucuronide (MUG), and *Enterococcus* has ß-glucosidase to digest 4-methyl-umbelliferyl-ß-D-glucoside. Each of these enzyme digestions results in a chromogenic or fluorogenic product, o-nitrophenol (total coliform) or 4-methylumbelliferone (*E. coli* and *Enterococcus*, IDEXX, Yakub et al. 2002). Quantitray®/2000 divides the 100 mL sample into 97 wells and yields a bacterial concentration as the most probable number (MPN) within the range 1 to 2419.6.

Because the target bacteria are not the only organisms possessing the enzymes on which the tests are based, interferences are limited by temperature and inhibitors within the formula. Colilert® samples were incubated at 35°C and Enterolert® at 41°C. *Aeromonas* spp. have the enzyme ß-D-galactosidase and can metabolize ONPG at 35°C, enabling them to trigger a false positive for total coliform with Colilert®. Inhibitors in the formula prevent this interference, but near the end of the product shelf life inhibition is reduced (Landre et al. 1998). Similarly, several plants and algae have been found to exhibit ß-D-galactosidase and ß-D-glucuronidase activity, creating the potential for total coliform and *E. coli* false positives in substrate based tests (Davies et al. 1994).

Stool samples were analyzed for *Giardia* and *Cryptosporidium* infection with ColorPac™ *Giardia*/*Cryptosporidium* (BD Diagnostic systems, Sparks, MD). ColorPac™ *Giardia*/*Cryptosporidium* is an immunoassay that simultaneously detects *Giardia lamblia* and *Cryptosporidium* antigens in human stool samples. In the case of *Giardia*, it is cyst wall protein 1 (CWP1) described by Boone et al. (1999) upon which the ColorPac™ assay is based. Since the subject of the assay is a protein on or from the cyst or oocyst wall, and not an immune-response product of the infected individual, the test kits were also used for dog stools in this study even though the kit was designed and marketed for human stool samples. If a strain or species of *Giardia* or *Cryptosporidium* not of human health importance affected the dog, that may not have been detected. However, the strains that infect humans should have triggered a positive result in both dog and human stool samples.

Sample collection and analysis

Water samples were tested for total coliform and *E. coli* or *Enterococcus* according to the manufacturer's instructions with Colilert® or Enterolert®. In brief, 100 mL samples were collected in sterile plastic bottles containing sodium thiosulfate for the removal of chlorine. Samples were most often processed upon arrival at the on-site laboratory. If immediate processing was not possible, samples were refrigerated for a maximum of 24 hours. Reagent packs were added to the samples. Presence/absence Colilert-18® samples were pre-warmed in 44.5°C water for 7 – 10 minutes. Enumerated samples were poured into Quanti-tray/2000® and sealed in the Quanti-tray sealer. Coliform/*E. coli* samples were incubated at 35°C for 24 – 28 or 18 – 22 hours, depending on whether Colilert® or Colilert-18® was used. *Enterococcus* samples were incubated for 24 – 28 hours at 41°C.

In order to sample surfaces and dry soil or vegetation, the above method had to be adapted. Surfaces were sampled by filling the sample bottles with clean water, moistening a clean cotton swab in the water, wiping on the surface, and swishing in the sample bottle for 1 minute. When soil samples were taken, a clean wooden spatula was used to scoop approximately 1 cm^3 into a sample bottle containing clean water.

Samples for fecal source tracking were taken from places of interest based on *E. coli* and *Enterococcus* results. Samples for analysis were approximately 1 L and came from the culvert where the spring drainage crosses the road (Fig. 5) and from the ponding around 2 houses where dog impact was not obvious and gray water straight-pipes appeared to be present (actual use of pipes was unknown). The prior was chosen because it was a site of heavy fecal load. Dog impact was clearly present, but human contribution was unknown. The latter sample was an attempt to determine if human fecal contamination was present in town close to a potential source if gray water was a significant contributor to fecal load. The positive control consisted of approximately 500 mL of deionized water to which samples from several freshly dumped honeybucket bags were added. Similarly, the negative control was made by adding stool samples from 4 dogs and a ptarmigan to 500 mL of deionized water. Samples and controls were labeled with a number to blind the laboratory and shipped overnight on ice packs to Source Molecular Corporation, Gainesville, FL. At the lab, polymerase chain reaction with primers specific to molecular markers in strains of *Enterococcus* and Bacteroidetes carried by humans identified presence or absence of human fecal contamination. *Enterococcus* was also enumerated in the samples with an upper limit of quantification of 10^5 CFU/100 mL.

Two water samples were tested for *Cryptosporidium parvum* and *Giardia lamblia*. One sample was 4 L and came from the raw water spigot at the washeteria, which has its intake in the river, under the ice. The other sample was 3 L and came from the area in the dump pond designated for honeybucket bag disposal. Samples were shipped overnight on ice packs to the Provincial Laboratory for Public Health, Calgary, AB, Canada. At the laboratory, *Giardia* and *Cryptosporidium* were analyzed according to EPA

method 1623 which involves filtration, immunomagnetic separation, and immunofluorescence microscopy (EPA 2001). With this procedure cyst walls fluoresce and are counted and examined microscopically.

Stool samples were analyzed on site with a ColorPac kit. Dog samples were each a composite of stools from 2 dogs. The freshest stools were taken from yards of consenting families. Recently deposited honeybucket bags found at the dump pond were opened and a sample was taken back to the on-site laboratory in a plastic bag. Theoretically, each honeybucket bag is a composite of the family and most honeybucket bag samples were a composite of 2 or 3 bags. However, a small amount of feces is used in the assay, so it may only represent 1 stool of 1 contributor despite attempted mixing. As per the manufacturer's instructions, the samples were diluted in distilled water, reagents were added, and the sample was placed on the sample card and read after 10 minutes.

June outdoor bacterial distribution

First the area from the old airport (east) to the new airport (west) was sampled on an approximate 500 ft. grid. When water was available within about 20 ft. of the intended grid point it was sampled. If no water was nearby a soil sample was taken. These samples were processed for presence/absence of total coliform and *E. coli*. Then narrowing the area to in and around the town, enumerated total coliform and *E. coli* samples were taken from puddles that were adjacent to boardwalks, in ATV trails, adjacent to the road, and away from obvious human impact (natural drainage). The portion of these samples that were in town was roughly spaced by sampling at the intersections of major boardwalks with additional samples in bodies of water and trails. The river was sampled in the middle along the length of the community by boat. Major ponds near the village were also sampled in triplicate from 1 point on the shore.

Transport on outdoor surfaces

Outdoor transport of indicator organisms on surfaces was tested on each visit. Preliminary swabs of dogs, shoes, tires, and boardwalk surfaces in June guided boot and tire experiments in August and additional tire experiments in April. Preliminary swabs were processed as presence/absence and did not cover a specific surface area.

The boot experiment took two forms. The bulk of the samples were taken at the conclusion of one of 20 walks around town where the walker took one step onto clean linoleum at an entrance to the school. The paths taken were intended to be logical paths that adults or children would walk. The walker did not intentionally get muddy, though some "children's" paths went off the boardwalks. Additional paths (5) started in a mud puddle at the intersection of two boardwalks and ended a distance along the boardwalk equivalent to the distance to the nearest inhabited residence. One step was then taken onto a piece of linoleum set out on the boardwalk. In all cases the linoleum was bleached, rinsed, and pre-swabbed to confirm the absence of indicator bacteria. The walker's boots were also cleaned with bleach and rinsed

before each walk. After 1 step on the linoleum the walker's boot and the linoleum were sampled for total coliform and *E. coli*. Samples from the 20 walks were presence/absence while the 5 'mud to linoleum' trials were enumerated.

Tires of ATVs were sampled on each trip. In June tire swabs were either from ATVs in town or ATVs on their way back from the dump, just as residents were using them. In August a more structured experiment consisted of swabs of a 4 in. x 4 in. square of boardwalk and of the front right tire at each of 8 stops along the orange path marked in figure 5. At stop 9 only the tire was swabbed. This path was run 5

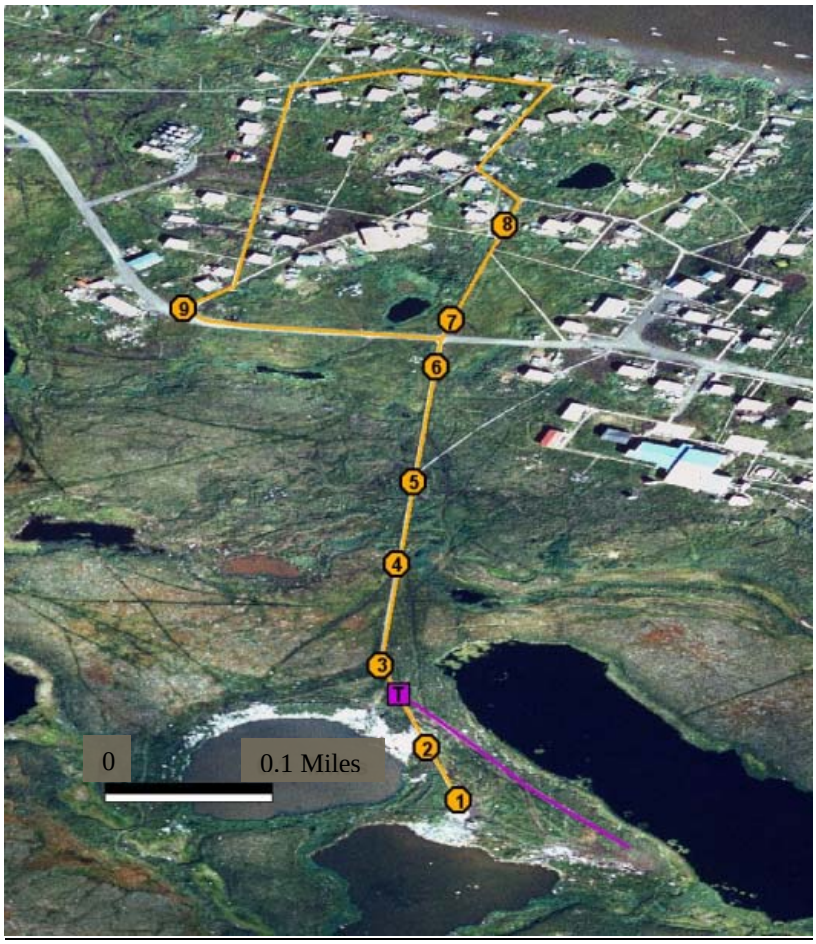

Figure 5

ATV experiment path. Stop descriptions are as follows. 1: End of boardwalk at solid waste disposal area, 2: honeybucket dump site, 3 and 4: intermediate stops, 5: hopper platform, 6: just before crossing road, 7: after crossing road, 8: into town, 9: far turn around point. The path was stops 1 – 9 with a return along the road and back down the boardwalk to stop 1. Turning around at stop 1 required driving off the boardwalk on the grass and dirt. The return trip used the opposite side of the boardwalk so as not to drive over portion of boardwalk tested in the 1 – 9 sequence. An additional 5 runs went along the purple path (trail) out to the current solid waste dumping site. This path has no boardwalk and was muddy at the time of the experiment. Tire and boardwalk swabs were done at stop "T" and the ATV was driven out to the road to turn around.

times. Also, trips along the trail to the solid waste dump (purple line, Fig. 5) were made and tire and boardwalk samples were taken at stop "T." For the 'trail' portion the ATV was driven out to the road and back between trials. In April another 5 runs were made along the trail to the solid waste dump, but instead of boardwalk samples, the ATV was driven across a tarp holding tap water and a water sample was taken from the pool on the tarp after each trip.

Breakup flow and bacterial distribution

The April 2005 trip was aimed at spring breakup when water is flowing and disease is thought to be a problem. Flow direction was observed and marked on an aerial photograph wherever it could be determined between April 28th and 29th of 2005. *E. coli* water samples were taken on an approximate grid within the community area, around the dump, and between the dump and town between April 24th and 28th, 2005. *Enterococcus* samples were then taken from water testing high in *E. coli* as well as some other places for comparison of the indicators between April 26th and 28th, 2005. *E. coli* and *Enterococcus* data aided in the selection of sample locations for source tracking methods.

Indoor transport

April sampling also included testing within homes, the school, and the clinic. Surfaces were swabbed for *Enterococcus* in the homes of 5 volunteer families. While some may have cleaned in preparation for the sampling, others did not. Ideally, sampling would have taken place with no special cleaning so that it would reflect normal conditions, but this was not specifically required of participants. At each house 11 surfaces were swabbed. The target surfaces were kitchen sink or spigot handle, bathroom door, front door, kitchen floor, kitchen counter, refrigerator or microwave door, TV remote, ATV or snowmachine handle, water container lid, phone, and dipper, but modifications to the list were made when one or more objects were not available or not in use. Participants reviewed the list before sampling and had the option of declining a sample or suggesting an alternative surface. With these swabs there was no specific surface area intended to be sampled. Instead, the purpose was to find the bacteria if it was on the surface, so swabs of floors and counters hit the dirty spots and included up to a several foot span of surface. Dipper swabs intentionally got into the grooves where accumulation most easily occurs and included dipper bottoms and sides and sometimes handles (unless the dipper handle was a separate sample). These samples were enumerated, but reported as presence/absence and summarized generally in order to protect the privacy of the participants.

Also in the 5 participating homes, samples of the washbasin water were analyzed for *E. coli* and *Enterococcus*. To test for the transfer of bacteria from the wash water to clean hands, hands were first washed with antibacterial soap and rinsed, then disinfected with hand sanitizer and allowed to air dry. One hand swab after disinfection was done to confirm the effective pre-cleaning of the hand. Hands were then

washed in the washbasin with the soap and water present and dried on the hand towel present if that was requested by the participant. A swab of one washed hand was then tested for *Enterococcus*. To protect the interest of the participants, hand washing experiments were conducted solely with my hands and the participants' hands were never swabbed.

Stored water was collected from 1 to 3 containers at each of 5 homes and tested for coliform and *E. coli*. At 2 of the homes 1 or more catchment surface was swabbed. Additional catchment testing, including catchment water samples, was limited by the weather. Most catchments were not yet in use and so would not reflect typical use conditions after a first flush, spring cleaning, and installation of a fabric filter that protects the barrel from debris.

At the school a wide variety of surfaces were swabbed. One set of surfaces was pre-cleaned, then sampled after 4 hours, 1 day or 6 days. Pre-cleaning included wiping the surfaces with a paper towel soaked in a 10% dilution of household bleach and rinsing with a tap water soaked paper towel after 5 minutes. After air drying, 4 surfaces were sampled to confirm the efficacy of the cleaning. Other surfaces were also swabbed with no pre-cleaning. Pre-cleaned surface samples were intended to show how quickly surfaces become contaminated, and non-pre-cleaned surface samples were to show what surfaces are prone to contamination.

In the clinic, water and surfaces were tested for fecal bacteria. The door, floor, and water were sampled for total coliform, *E. coli* and *Enterococcus*. The spigots and lids of the water coolers were swabbed for *Enterococcus*.

Data analysis

Spatial data was plotted with ArcView GIS 3.3 software. Shaded surfaces were made using second power inverse distance weighting using samples within 500 or 750 ft. as designated in figure captions. The larger distance was used for samples taken on a 500 ft. grid so as to include the 8 surrounding points in the region of a sample. Shading for quantified samples represents an estimate of the concentration of bacteria at any given point. Shading for presence/absence samples represents the likelihood that a sample taken at any given point would test positive for the bacteria.

Other figures were produced with Excel 2002. Means, confidence intervals, and other statistics were calculated manually, in Excel 2002, or with SPSS 13.0. Due to censored data, i.e. values above or below the enumeration range of the tests, parametric statistics are inappropriate for some sets of data. Nonparametric comparisons were also used when the parametric assumption of homogeneity of variance was not satisfied. For the purpose of calculating statistics and interpolated surfaces, the most probable number (MPN) < 1 was entered as 0 and >2419.6 was entered as 2420. While not numerically accurate with any certainty, such designations are sufficient for determining where there were high levels of fecal contamination. Presence/absence data were entered as 1s (present) and 0s (absent).

Results

June outdoor bacterial distribution

Presence/absence results from the airport to airport sweep of the village area show that *E. coli* was present in many areas, but was not ubiquitous (Fig. 6). Total coliform, however, was essentially ubiquitous (Fig. D1, appendix D). Several of the places that tested negative for *E. coli* or total coliform were dry ground and therefore soil samples instead of water samples. Although the interpolation from the presence/absence data suggests that much of the eastern part of the area was contaminated and that this contamination was connected, figure 6 shows only presence, not magnitude of fecal bacteria contamination, and interpolation is based only on distance with no regard for relief or barriers to flow.

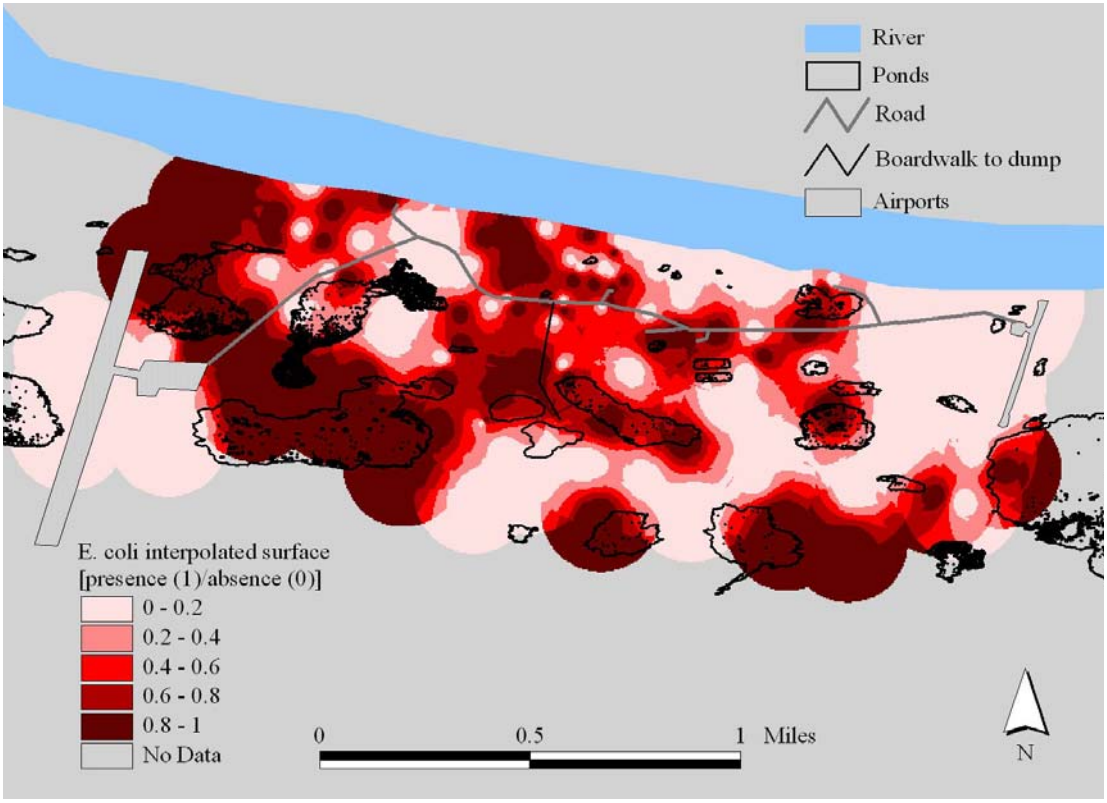

Figure 6
E. coli presence/absence, June 2004. Shading represents the likelihood that *E. coli* would be detected at points between samples. Interpolation by second power inverse distance weighting includes samples within 750 feet. Samples were taken at an interval of ~500 feet. Modified after Chambers et al. (2005).

Quantified samples show that the major ponds appearing to be contaminated in the presence/absence data indeed carried only a light fecal load (Fig. 7). *E. coli* was present but not abundant in much of the community area. Seven points had levels of *E. coli* above 2419.6 MPN/100 mL, and these were mostly within the residential portion of the community area.

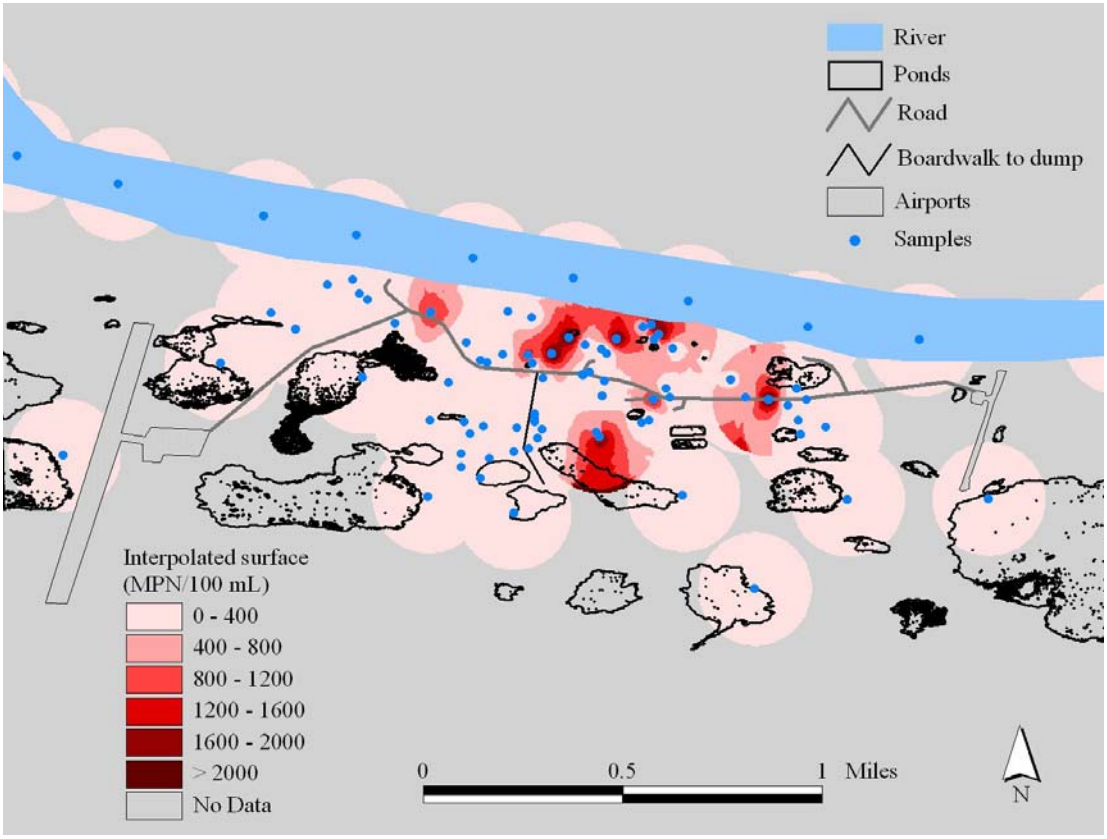

Figure 7

E. coli MPN, June 2004. Shading represents estimated concentration between sample points based on second power inverse distance weighting of samples within 750 feet. Topography is not accounted for as estimates are purely based on distance from samples and concentration at those points. Modified after Chambers et al (2005).

Considered categorically, larger bodies of water such as lakes, ponds, and the river had low levels of *E. coli* relative to most of the puddles sampled (Fig. 8). Differences among the lakes and river were statistically significant (Kruskal-Wallis $\chi^2 = 32.9$, d.f. = 11, p = 0.001), but there was little practical significance as all were below 21 *E. coli*/100 mL. The 'lakes' category included samples of the honeybucket pond taken opposite the main dumping area, yet *E. coli* did not exceed 21/100 mL. Also, *E. coli* was detected more often in puddles on the road than puddles adjacent to the road and *E. coli* was not usually detected in samples of road material in the absence of standing water (Fig. 9). There was a significant difference among the categories of figure 8 (Kruskal-Wallis $\chi^2 = 20.8$, $\nu = 6$, p = 0.02), but parametric post-hoc tests are not appropriate for this data set that lacks homogeneous variances. More detailed statistical comparisons are unavailable. In terms of practical differences, the lakes, river, and school puddles had lower levels of *E. coli* than the other puddle types. The data displayed categorically in figures 8 and 9 are included in the spatial displays of figures 7 and 6 respectively.

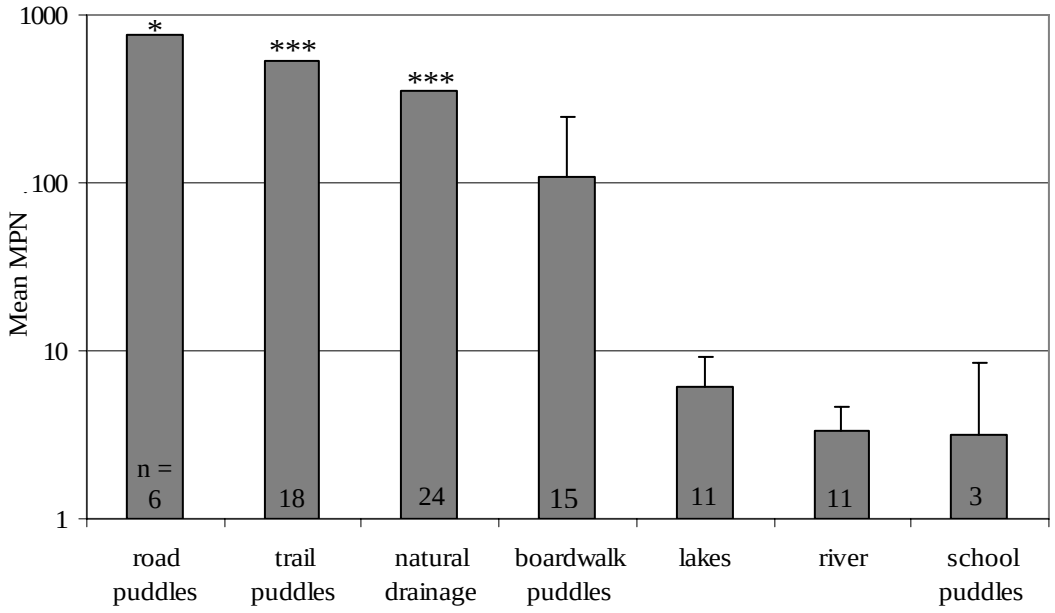

Figure 8

E. coli in bodies of water, June 2004. Error bars represent a 95% upper confidence limit and are not displayed when samples exceeded 2419.6 *E. coli* per 100 mL. Asterisks above the bars represent the number of samples > 2419.6 per 100 mL. Modified after Chambers et al. (2005).

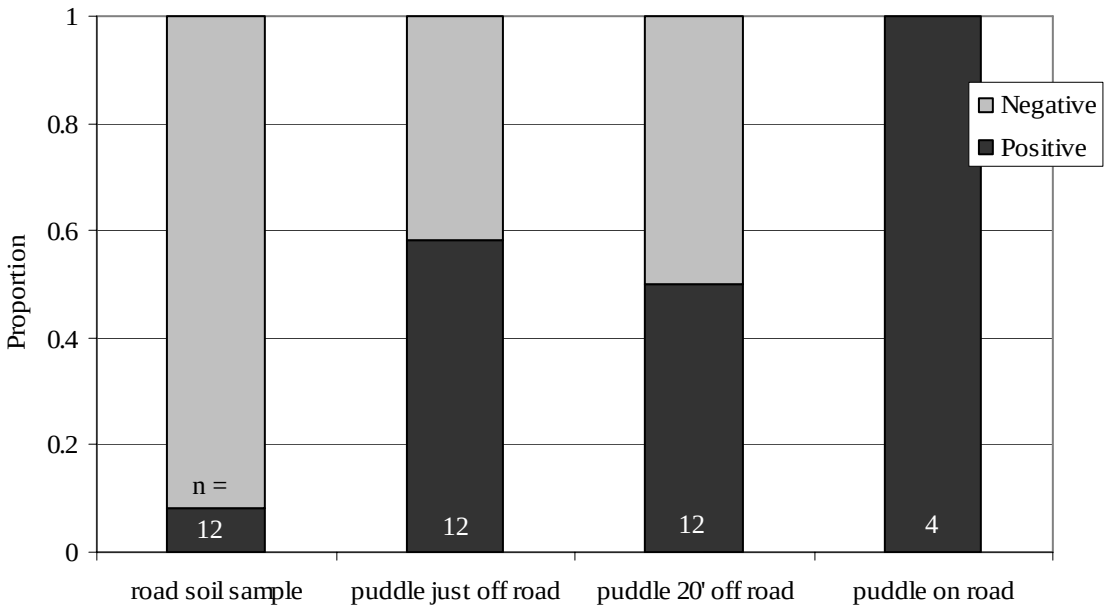

Figure 9

E. coli along road, June 2004. Samples were either dry road material added to clean, dechlorinated water or water samples from puddles on, adjacent to (just off), or 20 feet off the road.

Transport on outdoor surfaces

Swabs of surfaces in June 2004 showed that shoes, tires, and dog paws were potential carriers of fecal bacteria (Fig. 10). Since dogs are generally restrained, further attention was focused on shoes and tires of ATVs returning from the dump.

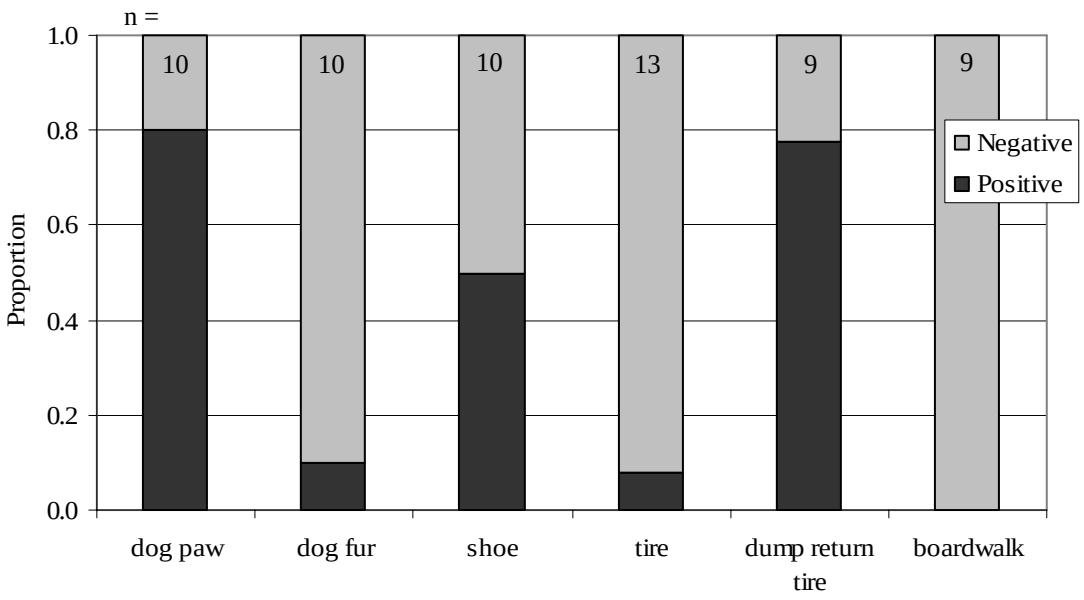

Figure 10

E. coli surface swabs, June 2004. Surfaces were sampled by swabbing with a clean cotton swab moistened in clean, dechlorinated water. Dogs were local pets tied to their posts. Shoes were on volunteer residents going about their business in town. Tires were on vehicles in town. Dump return tires were on ATVs met returning from the dump along the boardwalk. Boardwalk samples came from various places around town. Modified after Chambers et al. (2005).

Boot experiments

In August 2004, boots picked up coliform bacteria and *E. coli* on walks around town. The boots carried the microbial contamination and transferred a detectable amount of *E. coli* with a single step to clean linoleum 10.5% of the time (Fig. 11). This frequency of transport was without intentionally getting muddy, but simply walking logical paths in the community. In an extension of this experiment, the walker's boots carried bacteria from mud puddles at boardwalk intersections a distance equivalent to that of the nearest dwelling and transferred the bacteria to the piece of linoleum (Table 3). In this case boots carried *E. coli* 80% of the time and transferred *E. coli* to the linoleum in 40% of trials.

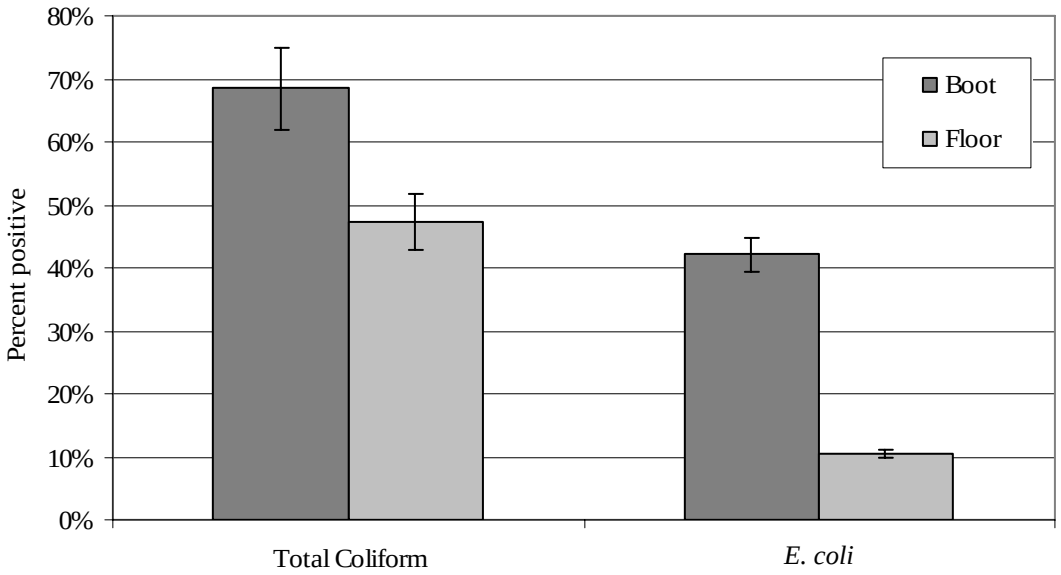

Figure 11

Walks around town, August 2004. Various paths expected to be traveled by children and adults ended at the school where 1 step was taken onto disinfected linoleum. The walker's shoe and the linoleum were then swabbed. Bar height represents the percent of 19 trials positive for total coliform or *E. coli*. Error bars represent the uncertainty based on the percent error found in replicated swabs of surfaces for total coliform or *E. coli*.

Table 3. August 2004 boot experiment—puddle to linoleum on boardwalk (outside).

Path	***Distance (m)***	***MPN Total Coliform***		***MPN E. coli***	
		Boot	*Linoleum*	*Boot*	*Linoleum*
1	77	> 2419.6	27.9	14.8	< 1
2	35	> 2419.6	290.9	3.1	2.0
3	12	142.1	22.3	4.1	< 1
4	30	> 2419.6	517.2	43.3	14.6
5	28	143.0	1.0	< 1	< 1

ATV experiments

E. coli was not frequently detected on the tires or boardwalk surface during the 9-stop experiment in August 2004 (Fig. 12). Most surprisingly, *E. coli* was not detected on the boardwalk at stop 2, the stop at which the honeybucket hoppers were routinely tipped. *E. coli* on the tires at stop 1 may have come from the grass and dirt at the end of the boardwalk where the ATV was necessarily driven to turn around and start again.

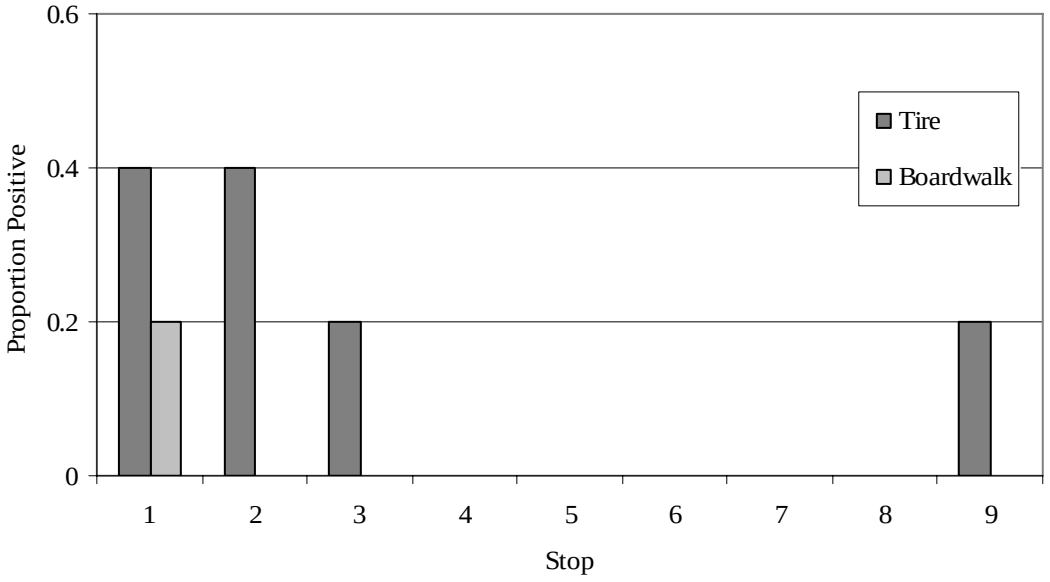

Figure 12

E. coli swabs of ATV tires, August 2004. Stop numbers refer to locations marked on figure 5. The path was traveled 5 times. A 4" x 4" square of the boardwalk or tire was swabbed with a clean, wet cotton swab which was then swished in the sample bottle for testing.

When the ATV was driven along the trail to the solid waste disposal site, through the mud and puddles on the trail, *E. coli* was more frequently detected on the tire, but still rarely transferred to the boardwalk (Table 4). Transfer to the boardwalk may not be as efficient as transfer to soft mud if the bacteria are sticking to the recessed patterns of the tires that do not contact the firm boardwalk. An additional experiment in April resulted in increasing total coliform count in the water held on a tarp and driven through with the ATV (Table 5). *E. coli* was not detected on the tire and rarely detected in the tarp water, but it was also absent from 2 of the puddles along the way and detected at a low level (8.4 *E. coli*/100 mL) in slush on the boardwalk that was driven through each time.

Table 4. August 2004 ATV dump trail results.

Run	***MPN Total Coliform***		***MPN E. coli***	
	Tire	*Boardwalk*	*Tire*	*Boardwalk*
1	727.0	3.0	< 1	1.0
2	1119.9	< 1	6.3	< 1
3	461.1	2.0	93.4	< 1
4	> 2419.6	1.0	> 2419.6	< 1
5	365.4	4.0	5.2	< 1

Table 5. April 2005 ATV dump trail results

Run	***MPN Total Coliform***		***MPN E. coli***	
	Tire	*Tarp water*	*Tire*	*Tarp water*
1	2	3.1	0	0
2	1	5.2	0	0
3	0	3.1	0	0
4	0	86.2	0	1
5	1	156.5	0	3.1

Breakup flow and bacterial distribution

Enterococcus results are overlain on shading representing an interpolation of *E. coli* results in figure 13, both from the end of April 2005. *Enterococcus* was either very low or very high in all the samples. As displayed by black squares on a light pink background, sometimes high levels of *Enterococcus* were present when *E. coli* levels were low or undetectable. High levels of fecal bacteria were again found in the middle of town where *E. coli* levels were high in June of 2004 (Fig. 7).

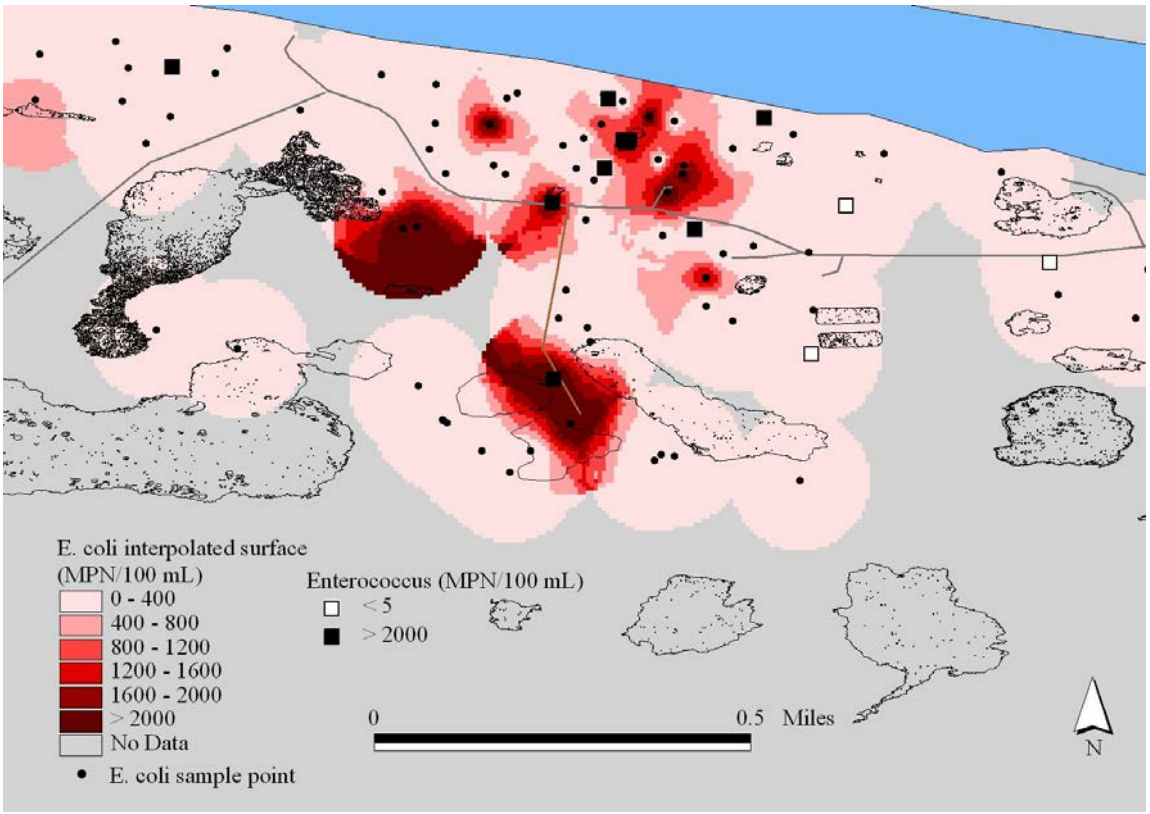

Figure 13

E. coli and *Enterococcus* concentrations, April 2005. Shading shows an estimate of *E. coli* concentration between sample points based on second power inverse distance weighting including samples within 500 feet. *E. coli* was sampled April 24 – 28, 2005. *Enterococcus* samples were either very high or very low. These are represented by squares on the map. *Enterococcus* samples were taken April 26 – 28, 2005.

Surface flow, a potential transport mechanism, is depicted in figure 14 as it was observed between the 28th and 29th of April 2005, during the period of spring runoff. Flow and bacterial concentrations are of particular interest in the middle of town because it is an area with high fecal bacteria load and at the dump because it is a potential source of human fecal contamination.

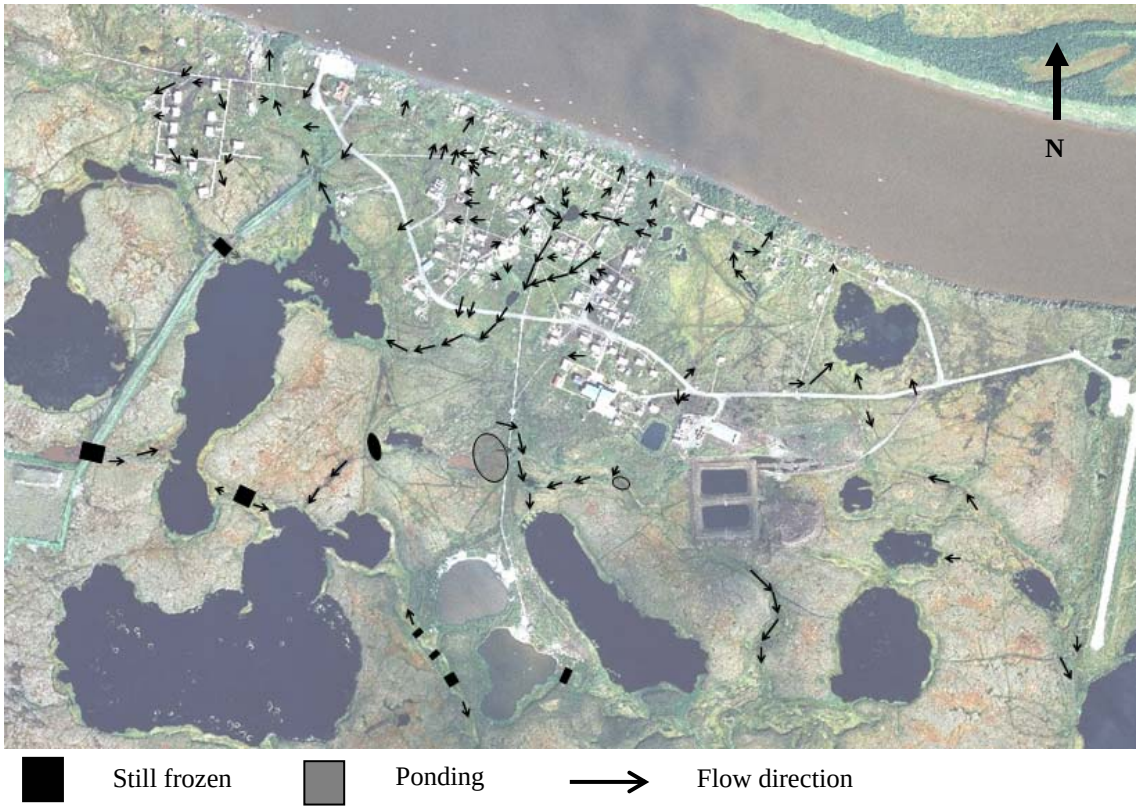

Figure 14
Flow, April 2005. Flow direction was observed April 28 – 29, 2005. Arrows represent direction, but not magnitude of flow. In areas of ponding (gray) direction could not confidently be determined.

In April 2005, melting snow created a stream through the middle of town. Water was flowing in this channel in June 2004 both from the northern pond in figure 15, south through the culvert at the road, and towards the southern end of the same pond from the WNW. By August 2004 the latter channel was dry and puddles on either side of the culvert were present, but too low for water to flow through the culvert. In April 2005 flow was greater than in June 2004 and the stream took the approximate path of the channel, but ran through ice and over the boardwalk. Water did not go through the culvert because of ice, but rather eroded the road material overlying and adjacent to the culvert. Once south of the road, the stream turned towards the west to a lake. Discharge from the other side of the lake continued northwest into the river. The approximate drainage area of the mid-town stream prior to crossing the road (i.e. the catchment area for any 'culvert' samples) is indicated by a solid black line in figure 15. Several water samples within this

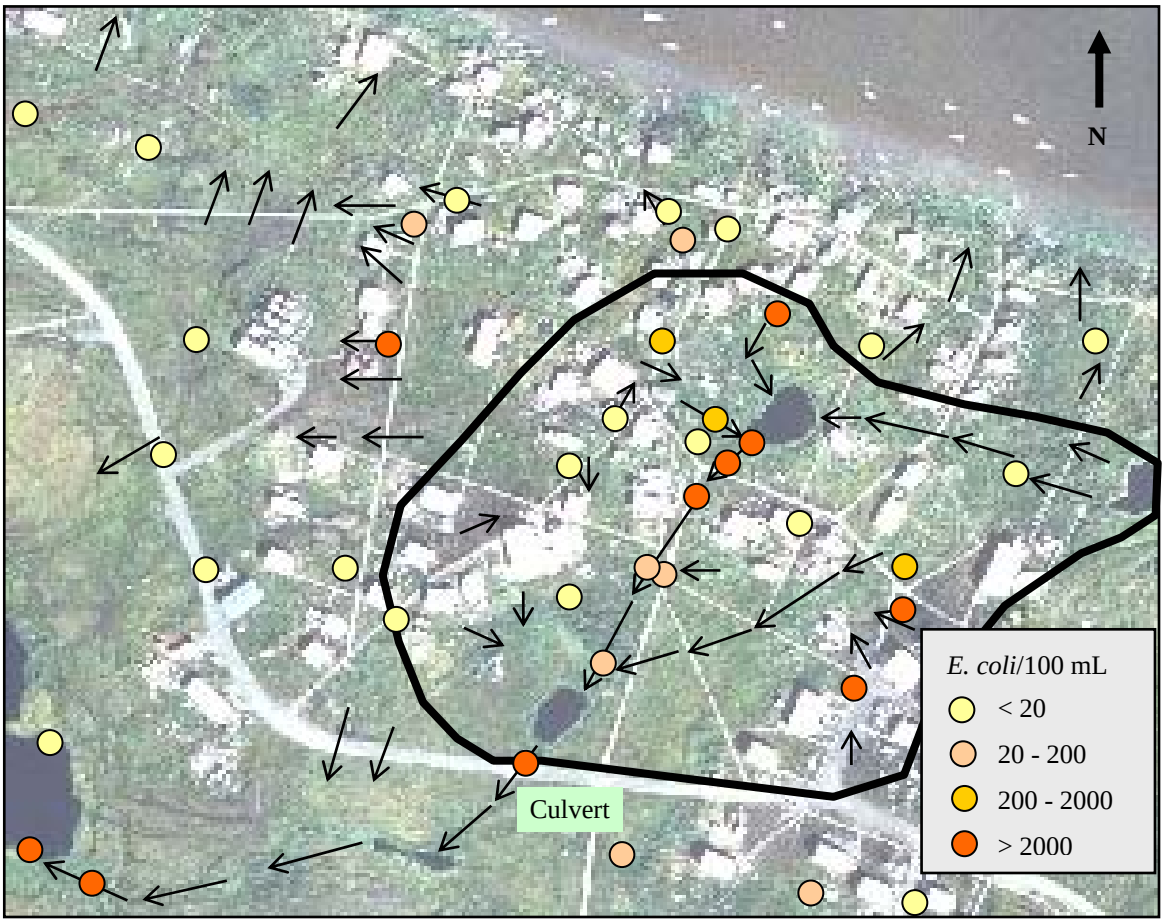

Figure 15

Mid-town drainage flow and *E. coli* concentrations, April 2005. Flow reflects observations 4/28 – 4/29. *E. coli* samples were taken 4/24 – 4/28. Sample points were marked with a GPS unit and overlain on a warped aerial photograph, but due to imperfect warping of the image, points were up to 30 feet off, usually to the ESE of features they should have matched. Points as shown were hand plotted to increase accuracy but errors of up to about 10 feet are possible.

area had high levels of *E. coli*. Dog feces were visibly present within the drainage area, but source tracking methods indicated that human fecal contamination was present as well in the culvert sample (Table 6). Also, those procedures revealed that *Enterococcus* was present at the culvert at concentrations greater than 10^5 CFU/mL. Though fecal bacteria were present, human fecal contamination was not detected in the ponding around houses (composite sample of two house areas) as might be expected if gray water discharged close to the house or material tracked into the yard were responsible for in-town contamination.

Table 6. Source tracking results, April 2005.*

	Enterococcus CFU/mL	*Human Enterococcus ID*	*Human Bacteroidetes ID*
Positive control	Inhibited	-	+
Negative control	$> 10^5$	-	-
Culvert sample	$> 10^5$	+	+
House pond sample	$> 10^5$	-	-

*Samples analyzed by Source Molecular Corporation, Miami, FL.

At the dump, high levels of *E. coli* were detected on the side of the ponds nearest the boardwalk, but levels were diminished on the other side and in adjacent bodies of water. The northern pond in figure 16 is the honeybucket pond and the area with the two high samples is the typical summer dumping area. During the winter people mostly dump there and on the northern end of the pond, though honeybucket bags were observed most of the way around the lake and some dispose of waste in the center when the ice is sufficiently thick. Also, honeybucket bags were found along the edges of the southern pond, though this is typically used for solid waste. The northeast shore of the southern lake was also an area of heavy dumping.

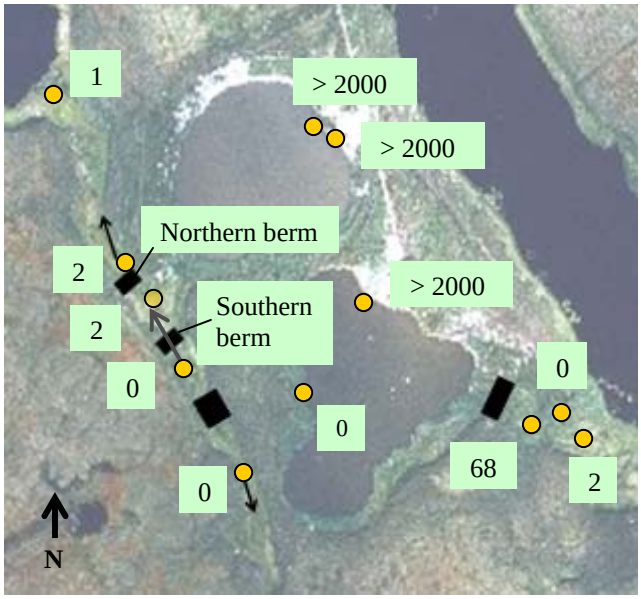

Figure 16

Dump pond flow April 28 – 29, 2005. Black arrows indicate flow direction April 28-29. Black rectangles are areas that were still frozen these dates preventing flow in either direction. The gray arrow shows flow into the pond area over the southern berm, starting April 27, but stopping within a day. Numbers are the MPN *E. coli*/100 mL of samples at the yellow dots.

Differences in *E. coli* concentration across the ponds were dramatic and movement of fecal bacteria out of the dump area by surface flow appeared unlikely. Although the sample with a value of 2 *E. coli*/100 mL at the end of the gray arrow appears to be on dry ground, the photograph is from August 1999, and at the time of sampling there was standing water at that point and it was a part of the honeybucket pond. On either side of the berms from that point, the water levels were higher than in the honeybucket

pond. These differences were visible in April 2005. The same pattern held in August 2004 when surveying showed the honeybucket pond portion of the channel to be 0.1 and 1.3 ft lower than in the channels to the north or south separated by berms. Across the northern berm water flowed north; not out of the honeybucket pond, but from the ponding on the northern side of the berm towards the lake at the northwest corner of the picture (Fig 16). Water did not flow across the southern berm during the general flow observation period (April 28th and 29th). Water had been flowing into the honeybucket pond on April 27th, but flow over or through the berm was not observed at any other time during the trip. On the last few days of the April trip, the areas indicated by black rectangles were still frozen and high enough to prevent connection of ponding areas in the channels. Water was not exiting either dump pond on the surface to the southwestern or southeastern channels. However, the apparent separation of the ponds is unlikely to prevent mixing of the ponds, especially when strong winds blow across the water sitting on top of the frozen ponds.

Breakup illness

School attendance data for the 2003-2004 school year show several peaks in absence throughout the year (Fig. 17). The largest peak occurred just before Thanksgiving 2003. Information on the reason for

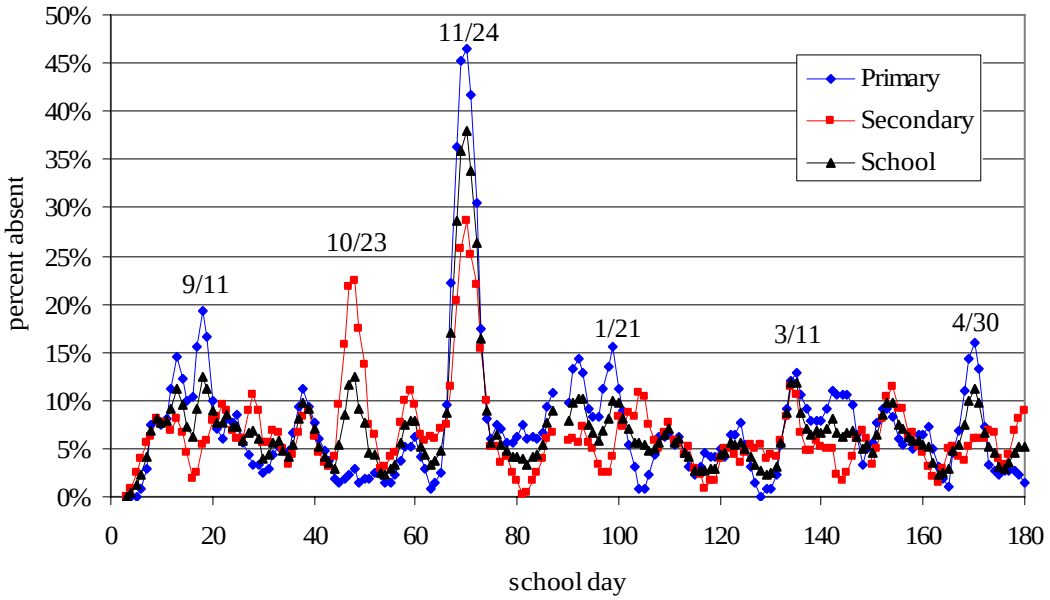

Figure 17

Excused absence rate, August 18, 2003 – May 14, 2004 school year. The plot is smoothed by a 3 day rolling average including the x-axis school day and the two previous school days. Primary includes children who do not change classes during the day. Secondary students change classes and attendance is recorded for each of 6 periods daily.

absence was not available, so this could have been illness, travel, or a community event. Peaks in absence were sometimes predominantly older students (around October 23) or younger students (around April 30) but sometimes a school-wide trend (around November 24 and March 11).

Pathogens

Stool and water samples were analyzed for *Giardia lamblia* and *Cryptosporidium parvum* to see if these pathogens were present in the environment or human or animal populations. No *Giardia lamblia* or *Cryptosporidium parvum* infection was detected in dogs or humans. These samples included 4 dogs, which contributed to two composite samples, and eight composite honeybucket bag samples. Raw water from the treatment plant intake located under the ice in the river had no *Giardia lamblia* or *Cryptosporidium parvum* in the 4 L sample. Water from the dumping area of the honeybucket pond had 5 intact *Giardia lamblia* cysts in the 3 L sample, indicating that some human or non-human contributor to the fecal load at that site was infected with *Giardia*. Additional broken cyst walls were also present in this sample. Honeybucket additives may have contributed to the destruction of the cysts.

Indoor transport

Swabs of surfaces in the school showed that *Enterococcus* was detectable on several, but not the majority of surfaces (Table 7). No more than 1 *Enterococcus* was detected per swab in the school. By comparison, as many as 13.5 enterococci were found on a swab of a university bathroom door in Fairbanks. These swabs in Fairbanks are the only available comparison because this is not a common method used in other literature. Table 7 shows all school surface swabs, whether the surface was pre-cleaned or not. Of 4 samples taken 4 hours after cleaning, none had detectable levels of *Enterococcus*. After 1 day, 3 of 15 samples were positive (bathroom stall handle, bathroom sink handle, and bathroom door) and after 6 days, 1 of 4 samples were positive (bathroom sink handle). Altogether, *Enterococcus* was detected on 5 of 35 surface swabs in the school. The 4 controls tested immediately after cleaning were negative.

Table 7. *Enterococcus* on school surfaces

No Enterococcus detected	***Enterococcus detected (positive/total samples)***
Gym and other doors (n = 5)	Bathroom sink handle (2/7)
Soap and paper towel dispensers (n = 3)	Bathroom stall handle (1/2)
Light switch (n = 2)	Bathroom door (1/4)
Table and computers (n = 3)*	Basketball (1/3)
Locker (n = 2)*	
Gym floor (n = 2)	
Volleyball and other gym balls (n = 2)*	

*Multiple computers, lockers or balls were swabbed for a single sample so the number of objects sampled is greater than n.

In the 5 volunteer homes, a total of 55 surfaces were swabbed. From the variety of surfaces sampled, *Enterococcus* was detected on kitchen counters, kitchen floors and water dippers (Table 8). Positive samples had an MPN ≤ 4.1. Very comparable results came from a cabin in the Fairbanks area with a dog and no running water where an MPN ≤ 5.2 was found in each positive sample and *Enterococcus* was detected on the counter and floor.

Table 8. *Enterococcus* on home surfaces

No Enterococcus detected	***Enterococcus detected at least twice***
Phone (n = 5)	Kitchen counter (n = 4)
TV remote (n = 5)	Kitchen floor (n = 5)
Refrigerator (n = 4)	Water dipper (n = 4)
ATV/snowmachine handle (n = 5)	
Microwave (n = 3)	
Front door knob (n = 5)	
Bathroom door (n = 4)	
Other surfaces* (n = 11)	

*Includes a stove top, handles of dippers if swabbed separately from dipper bottoms and sides, and other surfaces of water barrels such as lids, spigots, handles, and exterior surface.

Water in washbasins, despite the use of antibacterial soap by some families, contained bacteria. All samples contained high levels of total coliform. *E. coli* was rarely detected in the wash water, but *Enterococcus* was usually present at levels > 1000 MPN/100 mL (Table 9). When a clean hand was washed in the basin and then swabbed for *Enterococcus*, all samples were negative (n = 4). There was one case where there was no *Enterococcus* in the water, so only 3 cases where there was *Enterococcus* in the water that was not detected in a swab of the hand.

Table 9. Washbasin water and hands washed in the washbasins, April 2005.

Washbasin (water sample)			***Hand (swab)***
Total coliform (MPN/100 mL)	*E. coli (MPN/100 mL)*	*Enterococcus (MPN/100 mL)*	*Enterococcus (Swab of 1 hand)*
> 2419.6*	46.4*	> 2419.6	-
> 2419.6	0	1011.2	-
> 2419.6	0	> 2419.6	**
> 2419.6	0	0	-
> 2419.6	0	1011.2	-

*Only ~150 mL of water was available in the washbasin so the total coliform and *E. coli* sample was approximately half washbasin water and half dechlorinated tap water. The true MPN are therefore > 4849 for total coliform and ~93 for *E. coli*.
**Missed sample.

In the clinic, swabs of the door and floor and the water supply were negative for total coliform, *E. coli* and *Enterococcus*. The lid and spigot of the water container were also negative for *Enterococcus*.

Water supply

Samples from 11 water storage containers at 5 houses tested negative for *E. coli*. Nine of these, however, contained total coliform. Downspout, roof, and gutter samples tested negative for *Enterococcus* and the downspout was also negative for total coliform and *E. coli*. Multiple samples from the same house showed that total coliform concentration increased during storage and use. In one house with a catchment in use, the catchment was negative for *Enterococcus* and the catchment-fed tank was negative for coliforms. The concentration of total coliforms in the inside barrel (plastic garbage can with lid on) was approximately 2 orders of magnitude lower than the uncovered 5-gallon bucket lined with a plastic bag in use in the kitchen. In all 4 cases where multiple samples were taken from a home, the water closer to the point of use had higher concentrations of total coliform.

Indicator comparisons

Preliminary indicator comparisons were made in August 2004 when a swab was rolled in mud in a puddle by the dump and compared to a swab of the boardwalk surface adjacent to the honeybucket tip. As seen in figure 18, total coliform > *E. coli* > *Enterococcus* in the wet environment whereas *Enterococcus* is present in the absence of *E. coli* in the dry environment. Other indicator comparisons from the various experiments are summarized in table 10.

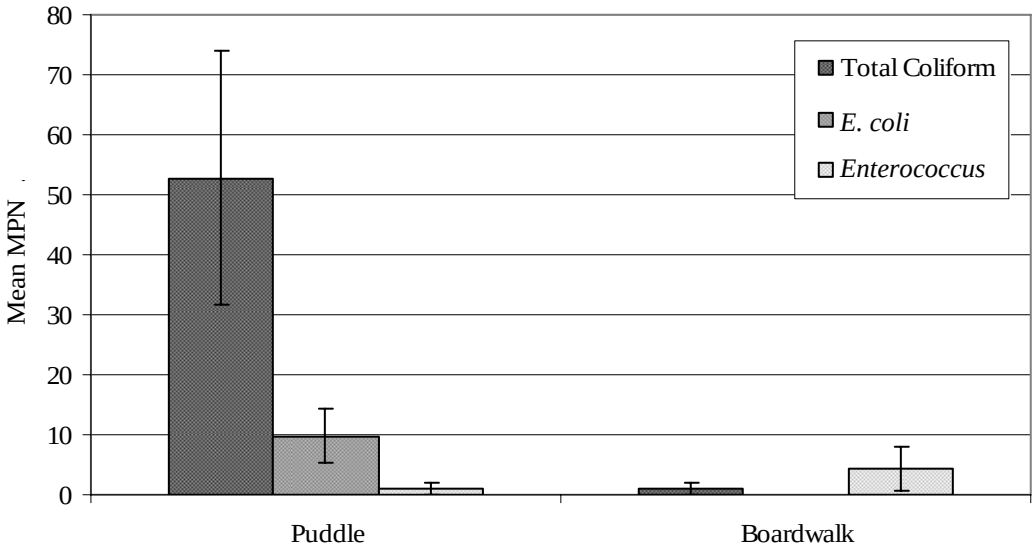

Figure 18

Indicator comparison in wet and dry environments, August 2004. Bar height represents the mean ± 90% CI (n = 3).

Additional paired samples tested with Colilert® and Enterolert® did not yield much information about relative abundance of *E. coli* and *Enterococcus* in the presence of low to moderate contamination because all samples were either very low or high in *Enterococcus* concentration (Table 10). The data still show, however, that total coliform can be present without other fecal indicators (k, v). Fecal contamination can be present when *E. coli* levels are low or absent (c, j, t, u, w). Yet at other times the three indicators have approximately comparable concentrations (a).

Table 10. Indicator comparison.

Sample	***MPN total coliform****	***MPN E. coli****	***MPN Enterococcus****	***Sample source/type***
a	1.6 ± 1.4 (n = 5)	0.2 ± 0.4 (n = 5)	1.7 ± 0.7 (n = 3)	outside water, breakup
b	> 2419.6	13.2	>2419.6	outside water, breakup
c	> 2419.6	1	>2419.6	outside water, breakup
d	> 2419.6	128.7	>2419.6	outside water, breakup
e	> 2419.6	332.5	>2419.6	outside water, breakup
f	> 2419.6	>2419.6	>2419.6	outside water, breakup
g	> 2419.6	>2419.6	>2419.6	outside water, breakup
h	> 2419.6	178.9	>2419.6	outside water, breakup
i	> 2419.6	>2419.6	>2419.6	outside water, breakup
j	34.1	0	>2419.6	outside water, breakup
k	>2419.6	0	0	outside water, breakup
l	12.2	2	2	outside water, breakup
m	>2419.6	>2419.6	>2419.6	culvert (breakup flow)
n	0	0	0	basketball swab
o	0	0	0	gym floor swab
p	0	0	0	clinic floor swab
q	0	0	0	clinic door swab
r	0	0	0	clinic water sample
s	> 2419.6**	46.4**	> 2419.6	gray water
t	> 2419.6	0	1011.2	gray water
u	> 2419.6	0	> 2419.6	gray water
v	> 2419.6	0	0	gray water
w	> 2419.6	0	1011.2	gray water
x	0	0	0	downspout swab

*Value is either a single measurement or a mean ± 95% CI.

**Water sample half dechlorinated tap water because not enough water was available to sample, so true concentration is approximately twice the tabulated MPN.

Discussion

June outdoor distribution

June sampling (Figs. 6-8) showed that there was *E. coli* present in the town, that *E. coli* levels were high in places, that *E. coli* levels were more than a background or natural level, and that *E. coli* was not uniformly distributed across the community area. Using recreational water guidelines for perspective, the fecal contamination in the community appears to be a significant problem as multiple samples exceed 126 *E. coli*/100 mL (EPA 1986). However, these levels are not unheard of. Samples from puddles in Fairbanks also reached the maximum enumerable number for the method and peaks averaging as high as about 1400 *E. coli*/100 mL were observed in the Oldman River basin (Alberta, Canada) during rain events (Hyland et al. 2003). Another consideration from figure 9 is that the large bodies of water (lakes and the river) had low levels of *E. coli* while the puddles (aside from the ones in the school yard) tended to have high numbers of *E. coli*. Since puddles are generally small and isolated from voluminous flow and fecal matter has many *E. coli* per gram, a puddle could easily reach the maximum detectable limit if a single boot or tire tracked small amounts of feces into it, if a bird defecated in it, or if it received runoff from a single dog's yard. The river, however, drains a large area inhabited by wildlife and receives some runoff from contaminated parts of the community, but has sufficient volume to dilute the fecal load. Likewise, the large ponds and lakes were not free of *E. coli*, but would have been considered swimmable.

Interpolation is admittedly a poor estimate of *E. coli* concentrations between samples because it factors in only sample values and distance from samples. A more accurate understanding requires consideration of connection and flow as addressed in April through observation of flow and connection of waterways.

The road samples in June showed that fecal bacteria were present in high levels on the road supporting tires as a mechanism of transport, but these data also bring up questions of indicator adequacy. All of the puddles on the road deep enough to sample had *E. coli* in them (Fig. 9) and road puddles had the highest average MPN of *E. coli* (Fig. 8). The presence and level of fecal indicators on the road make it a distinct possibility that goods (including water containers and anything else carried by ATV) could become contaminated by mud dispersed by tires. Since the puddles within about 20 ft. of the road tested positive only about half the time, the presence of *E. coli* in all of the puddles on the road supports the idea that fecal contamination was tracked along the road. Presence of indicator bacteria on tires returning from the dump also supports this proposed mechanism of transport (Fig. 10, Table 4). Absence of *E. coli* from most of the road soil samples could mean two things. Either (1) contamination was limited to the puddles where it is more likely to wash off of tires carrying fecal matter from the dump or dog yard or (2) contamination was present on the road but *E. coli* had died off, concealing this presence of fecal contamination. While a specific and widely accepted fecal indicator in water, *E. coli* is not necessarily an ideal indicator for dry

material or surfaces because of its susceptibility to desiccation compared to other indicators and pathogens (Payment et al. 2003). *Enterococcus* would be a better indicator for dry environments, though some pathogens may still be more resistant to desiccation.

Transport on outdoor surfaces

June swabs of outdoor surfaces provided a preliminary look at where fecal bacteria might be found under non-controlled circumstances. Finding *E. coli* frequently on shoes around town and ATV tires returning from the dump led to the boot and tire experiments conducted in August. *E. coli* was also frequently detected on dog paws (Fig. 10). No additional experiments were conducted on dog paws because the dogs are generally kept chained to posts and rarely get free to run around town, tracking fecal contamination. However, dogs will likely have fecal bacteria on their feet and those who bring their dogs inside risk allowing fecal contamination to be tracked into their homes.

August experiments showed that fecal contamination could be tracked into the home on shoes (Fig. 11, Table 3). While transfer of *E. coli* to the floor 10% of the time may seem insignificant, these experiments involved only one step onto the floor. If each person entering a house takes several steps on the floor before removing his or her shoes, enters the house multiple times a day, and is only one of several people to frequently enter the house, contamination of the floor is likely. For families with young children who play on the floor, fecal contamination would be a concern. Also, presence of fecal bacteria on the boots was more frequent than transfer to the floor and this contamination could be transferred to the hands when shoes are removed. As for the magnitude of contamination, the results in table 3 should be regarded as semi-quantitative. Swabs of the linoleum covered the area of the footprint and boot swabs included edges and soles, but in either case, the amount of mud on the boot affected how much was truly sampled. That is, all the mud on a boot could not be removed with a single cotton swab. Still, the relatively low numbers of *E. coli* found on the boots and linoleum make it clear that the walker did not step in a fresh pile of dog feces and track multiple-gram pieces onto the floor. Rather, it appears that runoff from feces in the area contaminated the puddles that served as starting points for the tabulated paths. Within the puddle, sediments (i.e. the mud) likely harbored the greatest numbers of *E. coli* (Characklis et al. 2005, Davies et al. 1995). The mud was also what was most likely to stick to the boots.

August and April ATV experiments supported tires as a potential transport mechanism, but gave little reason to believe material tracked from the dump accounted for the major patterns of fecal contamination observed in the community. The August boardwalk experiment (Fig. 12) showed that tires sometimes carried fecal contamination. However, the boardwalk at stop 2, immediately adjacent to the honeybucket tip site never had detectable *E. coli*, and this is where tires might be expected to pick up contamination. Instead, it seems more likely that contamination was picked up in turning around on the dirt and grass just before stop 1. Granted, honeybucket bags were avoided and tire tracks through bags

demonstrated that not every driver succeeds in avoiding the bags. Also, in one run *E. coli* was detected at stop 9 after six stops of it not being detected. The number of *E. coli* swabbed from the tire was also greater at stop 9 than at stops 1 or 2 where it was also detected on the tire. It may be that cases like this are due to the uneven distribution of contamination on the tire. At each stop a different section of the tire was swabbed to avoid swabbing an area that had been 'cleaned off' at a previous stop. Or it may be that contamination was picked up in town at some point before stop 9. The probability of the latter is increased by the fact that the highest number of *E. coli* on a tire swab prior to the dump trail experiment (discussed next) was the preliminary swab when the ATV was delivered to the school by the individual loaning it. At that point it had probably come from someone's front yard, not the dump.

The dump trail experiments showed that tires carried contamination and transferred it to a soft, wet environment more effectively than to the firm, dry boardwalk. High levels of *E. coli* were detected during some runs through dump puddles in the summer, but minimal transfer to the boardwalk was detected (Table 4). This was probably because mud containing the bacteria stayed in the grooves of the tire while bacteria on the treads were more quickly wiped off onto the dirt and grass. In April one must consider total coliform to discuss transport because *E. coli* was not as abundant in the puddles on the trail. Tires appear clean in the April results (Table 5) because the ATV had to go through slush after the puddles to get to the sampling point and the tires were swabbed after the ATV drove over the tarp. The prior was uncontrollable, but the latter was an oversight. Nevertheless, the total coliform concentration in the water on the tarp increased with ATV traffic, supporting the possibility of transport of contamination from the dump to front yards on ATV tires.

While tires can occasionally carry high numbers of fecal bacteria and these bacteria can wash off in puddles, it is not likely that the patterns seen in town were primarily the result of ATV traffic from the dump. Dump and in-town sources may contribute to road puddle fecal loads when tires move fecal material. Traffic from the dump may even be responsible for the presence of human contamination in the mid-town drainage. However, these data do not strongly support the hypothesis that the whole problem, or even a significant portion of it, is ATV traffic from the dump.

Breakup flow and bacterial distribution

In April 2005 as in June 2004, high levels of *E. coli* were found within the residential part of town. Multiple samples exceeded *E. coli* and *Enterococcus* guidelines for recreational water and not all of these highly contaminated locations were close to, or connected to the dump (Fig. 13 and 14). Samples from the ponding below the wastewater lagoon (two rectangular bodies of water visible in the eastern half of figure 13) had few or no *E. coli* and *Enterococcus*, showing that either the water ponded there was not from the lagoon or that it received sufficient treatment before exiting the lagoon. Flow patterns (Fig. 14) make that area as well as the dump unlikely sources of contamination in town. As seen in figure 15, multiple *E. coli*

'hot spots' were located within the area drained by the culvert. Most of the fecal load in this area was probably from dogs since human contamination was not always present in contaminated areas and dogs were numerous in the community. Water was not flowing from the dump (Fig. 16) to the culvert drainage, so the human fecal contamination found at the culvert had to come from another source or transport mechanism. Likely possibilities are gray water dumped within the drainage area, honeybucket spills, and/or bacteria tracked from the dump by vehicles and boots. The vast majority of fecal bacteria found in this drainage were probably from dog waste.

The pattern of fecal bacteria concentrations detected in the culvert drainage area supports the idea that the contamination was not from a single point source. While concentrations were high at and above the upper pond (Fig. 15), dilution and possibly a small amount of die off likely reduced the *E. coli* concentration further down the stream. Additions from other areas, such as drainage from the southeast portion of the basin and additional unsampled hot spots brought the concentration back up to the upper enumeration limit by the time the runoff reached the culvert.

Breakup illness

As for the health impacts of breakup, the school's site administrator did comment on an increase in sick students during the breakup research trip at the end of April (Walker, personal communication April 2005). School attendance data from the previous spring shows a small peak in absence of primary school children around April 30 (Fig. 17). While the peak is not as large as one might expect if breakup is the largest morbidity event of the year, the principal suggested that illness throughout the year was sufficient to mask the peak (Walker, personal communication 2004). Absence of primary school children probably reflects illness more accurately than older students because the younger kids are more susceptible to gastrointestinal illness and are also the ones most likely to play in the runoff streams.

Indoor transport

Indoor transport samples relied on the modification of the typical use of indicator bacteria. Total coliform, *E. coli*, and *Enterococcus* are routinely used in water quality monitoring, but using these indicators for surfaces is less routine. The swab samples, as in the shoe and tire experiments, can be semi-quantitative at best. Samples were run as enumerated samples for ease and accuracy in reading the results as well as a general understanding of the magnitude of contamination encountered on surfaces. While swabs were collected with the intention of finding contamination if it was present (i.e. swabbing large area and intentionally hitting grime and crevices) it is easier to be confident in a positive result than a negative one. Finding *Enterococcus* on the water dippers, counters and floors in the home causes concern because these are now known to be contaminated at least some of the time. The fact that *Enterococcus* was not detected on phones and other surfaces does not mean that these are definitely without fecal contamination.

Also, in terms of health importance, there may be non-fecal water-washed pathogens on these surfaces and these data do not attempt to address those concerns.

The main objective in swabbing surfaces in the homes and school was to determine if hands were transporting fecal contamination. Hands are often implicated in spreading disease and many studies have found improvements in health with increased hygiene education and hand washing (Esrey et al. 1991). Swabbing hands of people in the community would be too invasive so surfaces touched by hands were tested as a surrogate. Determining more accurately the role of hands in transport of fecal contamination would require thorough and direct testing of the hands by a method such as the 'glove juice' method. This method involves wearing a glove containing fluid, massaging the hand, and then sampling the fluid for the indicator or pathogen (Sickbert-Bennett et al. 2005). Results from the washbasin experiment suggest that a more thorough hand testing like the glove juice method is warranted to confirm that hands washed in contaminated washbasin water do not carry the indicator bacteria. The hand washing experiment presented here should be regarded as preliminary because swabs of hands were not very thorough and the sample size of hands washed in water containing the indicator was only 3. Relative survival of the indicators in the washbasins will be discussed later, however, it is important to note that many total coliform were found in every washbasin and *Enterococcus* was abundant in 4 of 5 basins even though some families used antibacterial soap. Although some soaps have antimicrobial ingredients such as triclosan, much of the effectiveness is attributable to the removal of pathogens from the hand rather than inactivation of the pathogen (Sickbert-Bennett et al. 2005).

Presence of fecal bacteria on surfaces (and hands) presents a health problem whether or not that fecal contamination reaches the drinking water because of the potential for hand contact with food or the mouth. In addition to waterborne diseases, water-washed diseases can be significant in the absence of piped water. One example that might be of concern is *Shigella* sp. infection because of a low infectious dose (Keusch 1979), however *Shigella* incidence in Alaska has been minimized in recent years (Appendix C, Fig. C5).

Water supply

Increased detection of total coliform in the water containers with increased proximity to the point of use supports the conclusion that water is contaminated during storage. Other studies have come to the same conclusion (Clasen and Bastable 2003, Genthe et al. 1997, Wright et al. 2004, Swerdlow et al. 1992, and Jagals et al. 1997) though usually in more grossly contaminated water supplies. The data collected offer no evidence that the contamination is fecal. However, not all pathogens are fecal, so the contamination is still a concern. If bacteria from the environment are introduced when water is dipped or transferred from one container to another, pathogens may enter the water. Total coliform bacteria can be considered acceptable in drinking water when specific fecal indicators are absent (Payment et al. 2003), and

the presence of total coliform is not nearly as alarming as the presence of *E. coli* or *Enterococcus* would be. However, increasing levels of total coliform towards the point of use showed that the water was insufficiently protected during storage.

Due to the limited extent of the catchment sampling, little can be said other than that no problems were detected on collection surfaces. More testing of catchment water during collection should be done to confirm the safety of that water supply. First flush samples would be of interest in terms of setting an upper limit of contaminant load, but one must recognize that those samples would not reflect the water typically collected for use.

Indicators

The samples taken as a part of this research have revealed some characteristics that affect the utility of the various bacterial indicators. Three main areas for discussing the indicators are (1) absence or low levels of *E. coli* at the far side of the dump pond, (2) hardiness of enterococci, and (3) utility of total coliform for drinking water.

In both June and April, *E. coli* levels in the honeybucket dump pond were surprisingly low. Natural processes such as UV inactivation, sedimentation and predation would be acting upon the *E. coli* population in the pond, but more seems to be going on. Some families add deodorizing substances to their honeybuckets which may be affecting *E. coli* survival. The absence or low levels of *E. coli* in the dump pond might have suggested that the water was rather benign; however *E. coli* might not be a sufficient indicator. Additional studies comparing pathogen and indicator survival in the presence of these deodorizing substances would allow for a better explanation of the risk associated with dump pond water.

Enterococcus appears to be the better indicator in dry environments, washbasins and in breakup water. In paired samples other than the puddle mud versus boardwalk swab comparison whenever *E. coli* was present, *Enterococcus* was present at comparable or greater concentrations. Multiple samples also showed that *Enterococcus* was abundant when *E. coli* was absent or present in low concentrations. The difference between the puddle mud swab and the other comparisons may be due to the time of year. In fresh human fecal matter *E. coli* would be more abundant that *Enterococcus* (Slanetz and Bartley 1957). Finding total coliform>*E. coli*>*Enterococcus* would therefore reflect more recent fecal contamination. *Enterococcus*, however, tends to survive better in high pH (as in soapy washbasins), freezing, and saline or dry environments (Payment et al. 2003). Therefore *Enterococcus* is a more reliable indicator when the fecal contamination has been outside of the host for a longer period of time or in those environments unfavorable to *E. coli*. Recreational studies discussed previously established correlation of *Enterococcus* and adverse health effects.

Total coliform, though lacking specificity, still holds some value for drinking water quality monitoring because it is more common. Simply monitoring for *E. coli* or *Enterococcus* would allow a

person to miss the fact that bacteria are being introduced to a water container if those bacteria were non-fecal. The fact that anything is being introduced increases the risk that pathogenic microorganisms will get into the drinking water.

Comparison of the three indicators under various circumstances and at various concentrations would be nice, but when both tests were in use most samples had either very high or very low levels of one or more indicators making quantitative comparisons difficult.

The source tracking methods employed in this study show promise. While inhibition, probably due to honeybucket additives (such as some paraformaldehyde containing deodorants), impacted the detection of the *Enterococcus* marker, the Bacteroidetes marker performed well on both controls (Table 6). Presence of human fecal contamination in town indicates that the current method of separating human waste from the community is insufficient. Fecal material is either being spilled within or tracked into the inhabited part of town. Gray water dumped near houses may also be a source of human fecal bacteria. The absence of human fecal bacteria in the presence of fecal contamination indicates that humans are not the only sources. Observation would indicate that humans are not likely the major sources as the dog population was distributed very similarly to the human population. As source tracking develops as a field and more tests are readily available, reliable, and less expensive, such tests should become popular in defining the problem in communities with pets, wildlife, and no piped sewer.

Application in other communities

Much of the information from this study appears to be site specific, but several principles are transferable. First is the issue of dump siting. In the study community the dump is not very far from town, but either the location turned out to be good by luck or intentional observation. Water was not flowing on the surface out of the dump. Nor was the water level in the dump higher than the adjacent channels. Except for one day of water flowing into the dump, the constructed berms were of sufficient height to prevent the movement of water into or out of the dump. Groundwater flow may be possible, especially as the thaw depth increases through the spring, and water levels may exceed the berms in very wet years, but in 2005 flow from the dump was not responsible for bacterial levels observed elsewhere. In most communities, sites for new tundra pond dumps are not currently being chosen, but rather lagoons and landfills are being permitted and built. However, should a community need to choose a location for waste disposal, observation of the movement of water, especially during spring breakup when water levels are high, can aid in selecting the best possible location.

Transport of contamination on tires and shoes is likely wherever there are tires or shoes and a source of fecal contamination. Without piped water and sewer, people must haul waste and the waste is not isolated or treated once it is disposed of. The presence and proximity of open sources of waste allows for the tracking of human fecal contamination on tires and shoes. However, even with piped water and sewer

or a closed haul system, the presence of dogs in the community will allow transport of fecal contamination on tires and shoes. Therefore, the knowledge that tires and shoes are capable of moving contamination is applicable in other communities.

While the presence of human fecal contamination cannot be observed with the naked eye, fecal contamination is not invisible in light of the findings presented here. An observer in any community can see where dogs are defecating. Since viable fecal bacteria were found in washbasins even when antibacterial soaps were used, gray water should be considered fecally contaminated. Household and community members can observe and influence where gray water is dumped. Likewise, community members can observe the frequency of honeybucket spills and make efforts to clean them up with disinfectant. These findings coupled with observation of flow patterns in any community can give a resident an idea of the potential risk of fecal contamination of their living environment.

Sickness at spring breakup is anecdotally reported in various places. If a community recognizes a pattern of sickness coinciding with spring breakup, observations within their home and community coupled with results of this study could be helpful. Although no fecal contamination was found in the drinking water tested in homes, progressive bacterial contamination was observed and dippers were observed to be contaminated. Drinking water may not be ideally protected, but it did not appear to be a major pathway for the transport of fecal-oral diseases since no specific fecal indicators were detected in drinking water samples. Attention is therefore turned to other ways of contracting fecal-oral diseases. This study found fecal contamination on several surfaces within the home and school as well as the stream that forms at breakup where children play. Household contacts are not dependent on topography, so similar contamination might be expected in other communities without piped water and sewer. Likewise, kids playing outside and dogs dispersed about the community are characteristics not limited to the study community.

Conclusions and recommendations

Distribution of fecal bacteria

The levels of fecal bacteria found at some points within the community were higher than background levels and human fecal contamination was present in the village. The human contribution to the fecal load might be from honeybucket spills, contamination tracked back from the dump, or from gray water dumped within the community. Care in honeybucket and gray water disposal might therefore reduce the human fecal load within the community. Since washbasin water was shown to contain viable fecal bacteria, gray water should be considered contaminated and disposed of away from homes or play areas, preferably where it will drain away from people. Since most of the fecal load in the community area is probably due to dog waste, removing the dog waste or relocating the dogs may prevent exposure to fecal-oral pathogens from the dogs.

Transport of fecal bacteria

Movement of fecal bacteria from the dump was possible on tires but flow from the dump was unlikely. In town flow carried fecal contamination at breakup. Shoes are capable of tracking fecal contamination into the home. Hands most likely carried fecal bacteria in the school to surfaces such as sink and door handles. Contaminated surfaces such as kitchen counters and dippers may compromise food and water safety. Indicator bacteria survived in home washbasins despite the use of soap. Removing shoes upon entering a house is a practice observed in the community that should help to keep pathogens from entering the house.

Indicator bacteria

Indicators of fecally contaminated water can be used on surfaces qualitatively but such application more legitimately shows the presence of fecal contamination than its absence. *Enterococcus* is preferable to *E. coli* for this purpose due to relative susceptibility to desiccation.

Safety of water supply

Water is relatively unprotected, so open sources of sewage are a legitimate concern. Drinking water was not necessarily the source of breakup illness, but water is still a potential risk factor in the case of an outbreak. Water could be protected by the use of small necked containers with spigots or water barrels with taps. Containers that dispense water to a pitcher or pan prevent the introduction of pathogens from a potentially contaminated dipper. In-home chlorination could also be used to ensure the safety of drinking water. In the very least, dippers should be handled more carefully. Contamination was observed on the dippers and counter tops, indicating that dippers need a safer storage place.

Non-drinking water exposure

The stream that flows at breakup is a source of non-drinking water exposure to fecal pathogens and household contacts may also spread fecal-oral disease. Parents should have their children wash their hands after playing in the streams that form as the snow and ice melt in the spring or after playing in the yard where ATVs may have tracked in contamination.

Suggestions for future work

Various aspects of this research could be expanded upon. Suggestions relating to washbasin and hand hygiene, indicator use, sampling in the environment, and public health studies follow.

Additional washbasin experiments could test for other indicators and pathogens under normal use conditions. Though already done with running water (Sickbert-Bennett et al. 2005), a hand-washing experiment testing the effect of soap on seeded pathogens and indicators could show the hazards of reusing wash water more accurately. Future hand experiments should use the 'glove juice' or other more thorough sampling method.

In future water sampling *Enterococcus* would probably be the better choice of indicator because of its hardiness relative to *E. coli*, especially when conditions are not favorable to *E. coli* survival (melt water, soapy water, saline water). *Enterococcus* is also better for surface testing than *E. coli*, though other options might also be explored. Testing surfaces for water-washed pathogens that cause diseases common in the community would be valuable to the community and shed light on the effects of water supply on health without the difficulty of obtaining health data. Total coliform should not be discarded completely as an indicator when testing drinking water.

While information on puddles in town is valuable, outside of town sampling should focus on the major bodies of water. *E. coli* and *Enterococcus* samples from transects across the lakes (especially the dump pond) and from different depths as well as samples at intervals along the connecting channels would better show the spread of contamination from a source than would a grid and interpolation that disregards topography. Testing the honeybucket pond for other bacteria and viruses might show that the indicators used do not survive long enough to model the presence of contamination. Laboratory experiments testing the effects of honeybucket additives on indicators and pathogens would further the understanding of the honeybucket pond situation.

Understanding of the health impacts of various water and sanitation service levels would help engineers and funding agencies design and fund projects that provide the greatest health benefit for the amount of money available. A study that could provide the information needed by these groups would have to involve health data, information not available for this study. One possible experiment that would not involve collecting health data would be to sample for indicator bacteria under current conditions and compare that with levels found in a community or several communities that choose to relocate or clean up after their dogs or to dispose of gray water away from housing. On the one hand researchers could see numerically the impact of the changed practice on the levels of indicator bacteria. At the same time community members would see in their family's health whether the change in practice reduced illness. While statistics are important to engineers and funding agencies, when a change requires effort on the part of the residents, personal observation of the benefits may be as important.

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Appendix A

Water storage and use survey form

1. What are your greatest environmental health concerns? Please number them in order of priority and list any others that may be missed.

____ Drinking water
____ Indoor air quality
____ Dump/solid waste
____ Adequate, affordable water for all daily needs
____ Honeybuckets and sewage

Other concerns:_____
_____

2. How does a lack of piped water and sewer affect your family?

_____
_____

3. Approximately how much water does your family use per person per day?
1-4 gallons____ 5-10 gallons____ 11-15 gallons____ 16-20 gallons____ 21-25 gallons____

Is that enough for your needs?_____
_____

4. Do you use treated water from the central water point?_____For what purposes? (Please circle all that apply)

(a) All purposes. (b) Drinking water only. (c) Cooking. (d) Washing & cleaning

5. Has your choice been affected by the opening of the new washeteria?
____ Yes ____ No

If so, why is that?_____
_____

6. How do you transport drinking water to your home? (Please check all that apply)

Open containers____ Containers with caps____ Containers with taps____
Other_____

7. Where and how do you store water inside the home? Please check all that apply.

____ Large galvanized or heavy duty polyethylene tank (food grade)
____ Dedicated 35 gallon dip bucket (non food grade)
____ Covered container with a tap

8. Approximately how long do you store drinking water before it is used up?
2-3 days____ 4-5 days____ 6-7 days____ longer____

9. About how often do you wash out water containers? Do you sterilize them with chlorine at that time?

_____
_____

10. Do you use a traditional water source (e.g. ice-melt/roof catchment) for drinking? Please indicate what source and at which time of year.

Traditional Drinking Source	Source location	Winter	Spring	Summer	Fall	
____ Natural Spring	____		___	___	___	___
____ River	____		___	___	___	___
____ Creek	____		___	___	___	___
____ Ice/Ice-melt	____		___	___	___	___
____ Tundra Pond	____		___	___	___	___
____ Lake	____		___	___	___	___
____ Rain catchment	____		___	___	___	___
_____Other (describe)	____		___	___	___	___

11. If you use a traditional water source for drinking, why not the treated water from the washeteria? Please number reasons in order of priority.

____ Cost ____ Taste
____ Appearance (color) ____ Ready access

Other reasons or
comments:_____
_____

12. Do you purify traditional drinking water (e.g. snow/ice melt, catchment etc.)? (Please check all that apply)
Boil____ Treat with Chlorine____ Use a water filter____ None____

13. Would you be more likely to treat the water if there were improved safe and practical ways of doing so?

_____

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Appendix B

Water storage and use survey result tally and discussion

Total respondents: 47, administered June 2004

Results are listed as a tally of respondents unless otherwise noted.

1. Greatest environmental health concerns, number of respondents giving a rank of 1 or 2, ranks averaged if checked instead of ranked by respondent.
   - Drinking water: 30.5
   - Indoor air quality: 5
   - Dump/solid waste: 20.5
   - Adequate, affordable water for all daily needs: 7
   - Honeybuckets and sewage: 32.5

2. Open ended, variety of responses.

3. Use per person per day
   - 1 – 4 gallons: 20
   - 5 – 10 gallons: 18
   - 11 – 15 gallons: 7
   - 16 – 20 gallons: 0
   - 21 – 25 gallons: 0

   Is that enough?
   - yes: 20
   - no: 9
   - sometimes/maybe: 6

4. Use treated water from central water point?
   - yes: 34

   For what purposes?
   - all purposes: 15
   - drinking water only: 3
   - cooking: 2
   - washing and cleaning: 20

5. New washeteria affecting choice
   - yes: 24
   - no: 21
   - blank: 2

6. Transporting water to home
   - open containers: 14
   - containers with caps: 33
   - containers with taps: 7
   - other or no transport: 7

7. Storage in home
    - Large galvanized or heavy duty polyethylene tank (food grade): 19
    - Dedicated 35 gallon dip bucket (non food grade): 25
    - Covered container with a tap: 11
    - Other: 1

8. Storage time
    - 2 – 3 days: 15
    - 4 – 5 days: 8
    - 6 – 7 days: 12
    - longer: 14

9. Frequency of washing: open ended, answers vary.

    Sterilize with chlorine
    - yes: 17
    - no: 13
    - blank: 17

10. Traditional water source use
    - Natural spring: 5
    - River: 19
    - Creek: 1
    - Ice/ice-melt: 39
    - Tundra pond: 5
    - Lake: 3
    - Rain catchment: 44
    - Other: 1

11. Reasons not to use washeteria water, respondents assigning rank of 1 or 2
    - Cost: 16
    - Appearance (color): 10
    - Taste: 20
    - Ready access: 13
    - Other: 3

12. Purification of water from traditional source
    - Boil: 21
    - Treat with chlorine: 2
    - Use a water filter: 14
    - None: 21

13. Likely to treat water with safe, practical method
    - yes: 30
    - no: 4
    - blank: 13

The survey form was distributed to the community prior to the June 2004 visit. Few responses were returned, so UAF researchers and high school assistants from the village took surveys door to door for completion. The local high school students involved in the project assisted in obtaining responses from the elders who spoke primarily Yup'ik. In total, 47 responses were obtained, not all of which were perfectly complete.

Weaknesses of the survey including phrasing of questions, text formatting, and misinterpretation led to some ambiguity. In questions asking for a rank of priority, some respondents simply checked those of interest or concern. In order to include these in the tally, an average of ranks was assigned to each checked response. For example, if 3 options were checked, each was assigned a priority of 2 (mean of 1, 2, and 3). This resulted in more than the 'possible' 94 first or second priority responses for question 1. While question 3 gives a glimpse of what the family thinks they use, it is not clear whether it is in-home water use or total water use including that consumed at the washeteria that is in question. Considering the water storage capacity of most homes, some probably over-estimated or answered for the whole family instead of per person. Question six was unclear or did not fit the situation well. Whether 'cap' refers to a screw on cap or a lid is not clear. Most water containers with screw on caps have a tap in that cap, so respondents claiming a cap probably have a lid. Others specified 'lid' under the category 'other.' Likewise question 7 should have included 'bucket' as an option. Misalignment in question 9 resulted in obvious seasonal discrepancies. The 'natural spring' option in question 10 was interpreted at least once as bottled water and 'river' may have been understood to include river ice. While other villages have springs, the study village does not. Bottled water should have been included as an option. Finally, the meaning of 'filter' in question 12 should not be understood as a filter acceptable for removal of all waterborne pathogens. Cloth filters between downspouts and rain barrels and Brita® or Pur® filters were among the 'filters' used. These perform on a range of levels but do not remove all bacteria, viruses, and protozoan cysts.

Responses to open ended questions could not be briefly summarized, so are not included here, though they were valuable to a fuller understanding of the community's water and sewer situation and needs.

Appendix C
Additional disease trends

Disease trend graphs are plotted from data available in State of Alaska Epidemiology Bulletin Annual Infectious Disease Reports (State of Alaska, 1977-2005). When the number of cases for a year changed between the first report and the following year (each annual report compares that year to the previous), the first reported value was used for consistency. The preparers of the Bulletin point out that the values reflect reported cases, not actual incidence, as not all cases are diagnosed and reported. Plots therefore represent trends and some of the trend may come from reporting trends as access to health care has changed. Incidence is also reported as cases per 100,000 population so that regional (southwest, SW) and statewide (AK) values could be compared. State population changed from 326,870 in 1976 to 655,435 in 2004. The population of the southwestern portion of the state ranged from 37,971 to 39,938. When population was not reported on the bulletin, estimates were based on bulletins shortly before and after the missing value. Since even the maximum population for the southwest region is well below 100,000, incidences as high as 2.5 or 2.6 can be the result of one reported case. Because of low incidences of some diseases and the small population under consideration, comparisons to other incidence rates must be made with caution. The region called ‘southwest’ approximately encompasses the Lower Yukon, Lower Kuskokwim, Kuspuk, Iditarod, Southwest Island, and Aleutian Region regional education attendance areas plus the Aleutians East and Lake and Peninsula Boroughs. A map of the regions can be found on any recent Annual Infectious Disease Report (State of Alaska Epidemiology Bulletin). Current regional divisions began in 1994. Some diseases were not reported over the full length of the record (e.g. giardiasis, campylobacteriosis) but the same x-axis scale was kept for easy comparison between graphs.

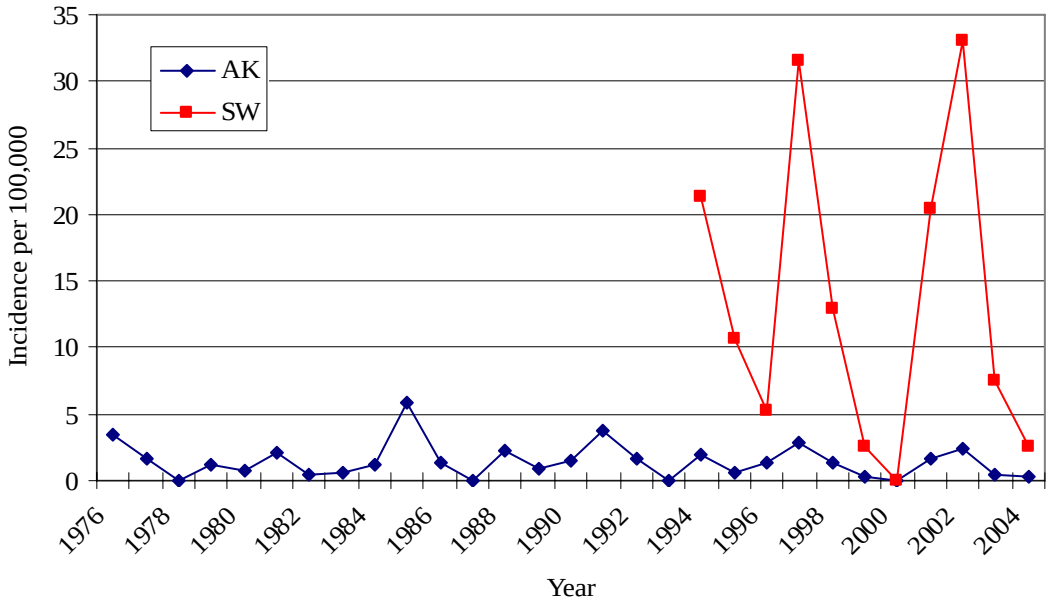

Figure C1
Botulism 1976-2004. Botulism is a food-borne illness.

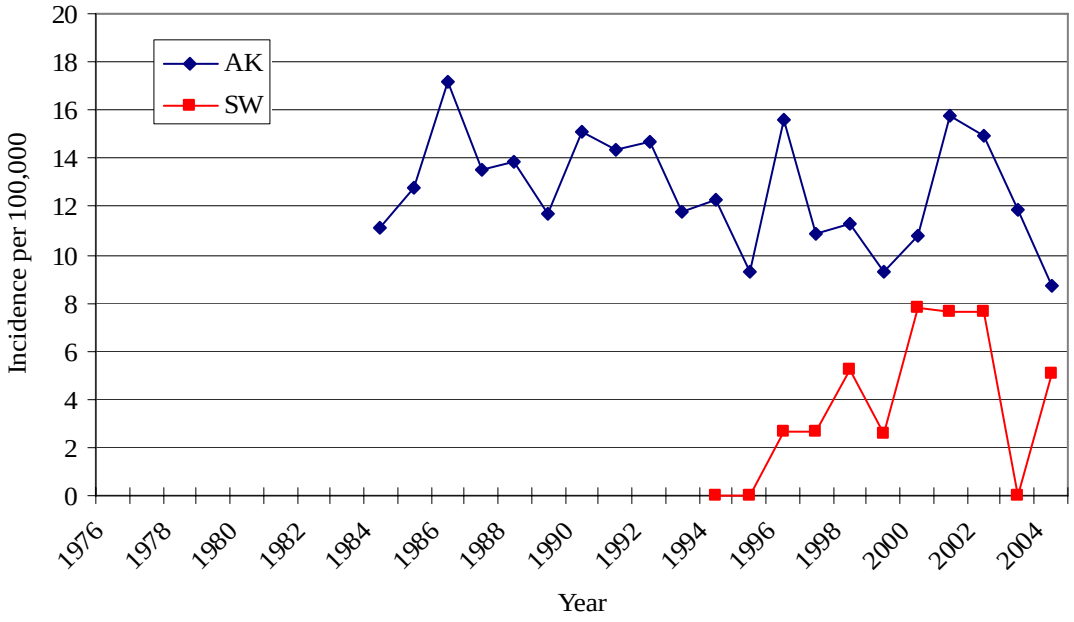

Figure C2
Campylobacteriosis 1984-2004. *Campylobacter* infection is acquired mainly by the fecal-oral route through contaminated food or water (Fricker 1999) and is a common cause of intestinal infection in developed countries (Hänninen et al. 2003).

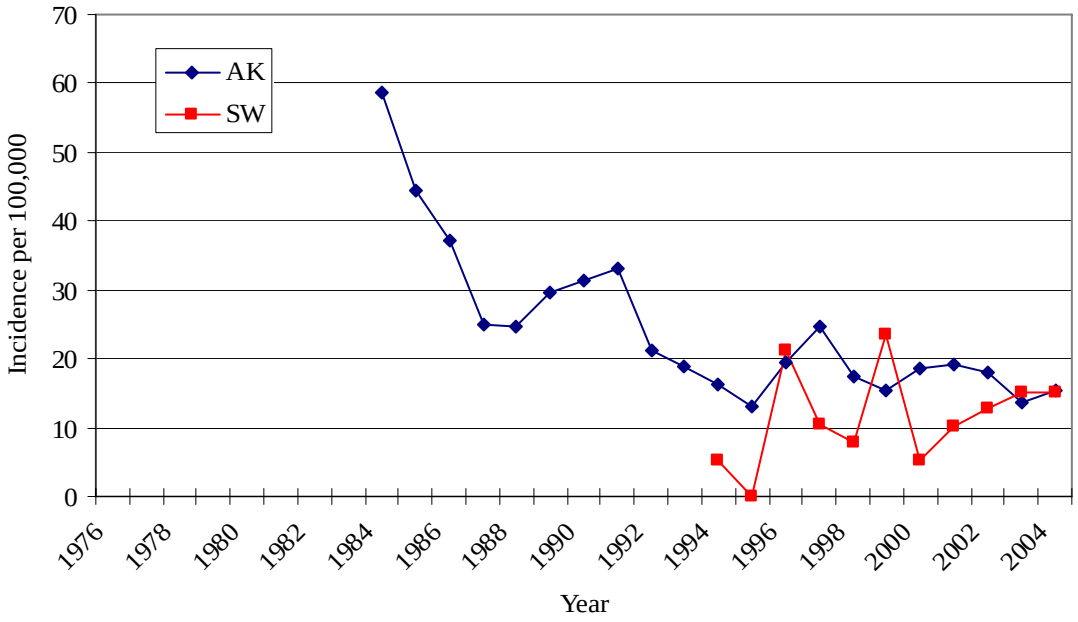

Figure C3

Giardiasis 1984-2004. *Giardia lamblia* is an intestinal protozoan parasite spread by the fecal-oral route through contaminated food or water as well as person to person contact (Schaefer 1999).

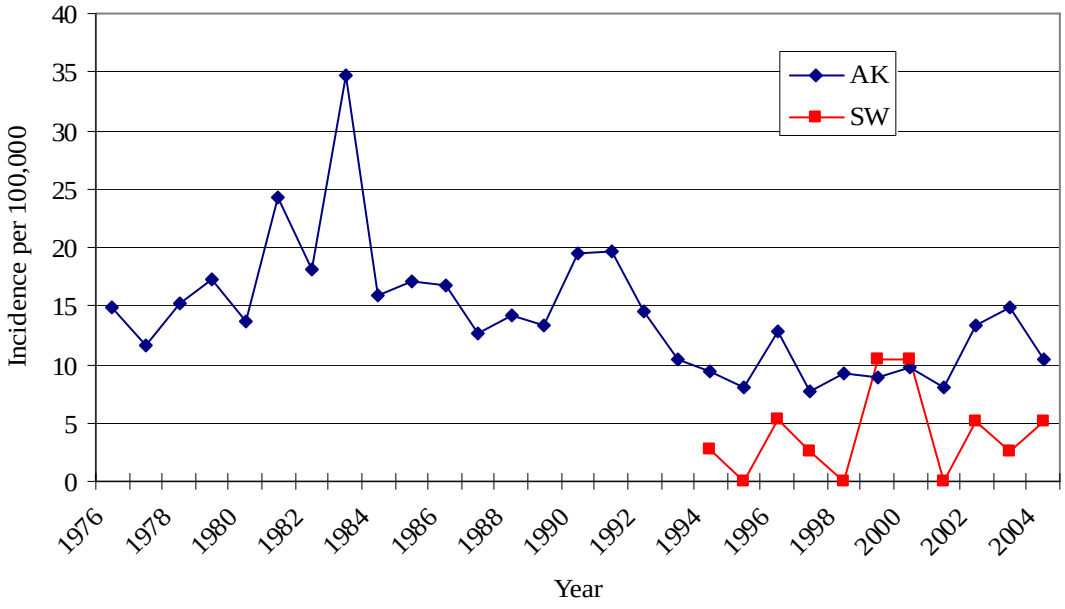

Figure C4

Salmonellosis or salmonella dysentery 1976-2004. *Salmonella* is spread by the ingestion of food or water contaminated with human or animal feces. Some species are specific to human hosts; others can come from animals including birds, dogs, and livestock (Covert 1999).

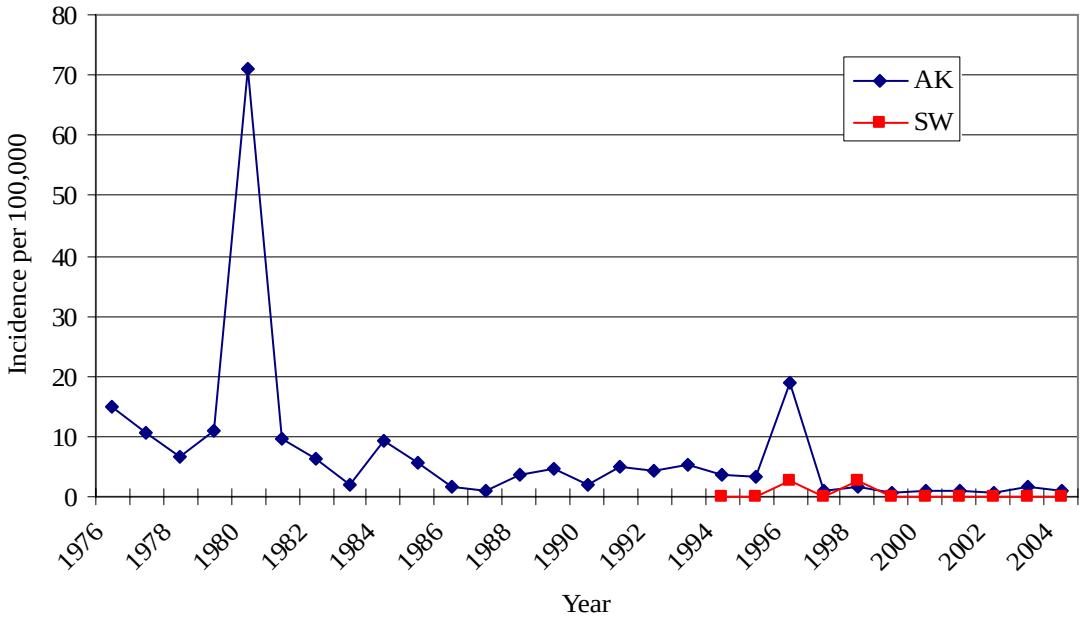

Figure C5

Shigellosis or shigella dysentery 1976-2004. *Shigella* infection occurs by the consumption of food or water contaminated with human feces and by more direct fecal-oral transmission (Moyer 1999b).

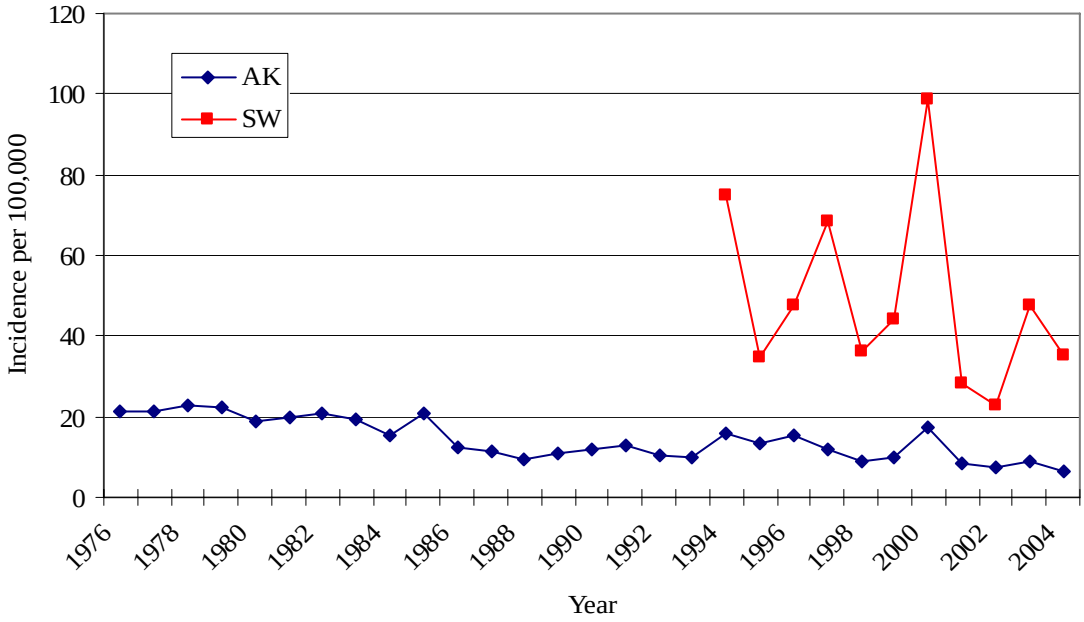

Figure C6

Tuberculosis 1976-2004. Tuberculosis is spread person to person through inhalation of airborne bacteria from an infected person.

Appendix D

Total coliform graphs

Total coliform is not a specific fecal indicator, but it is abundant in feces. By definition, total coliform concentration will be at least as great as *E. coli* concentration for a sample because *E. coli* is a member of the total coliform group. The following figures are the total coliform analogs of *E. coli* figures presented in the body of the thesis.

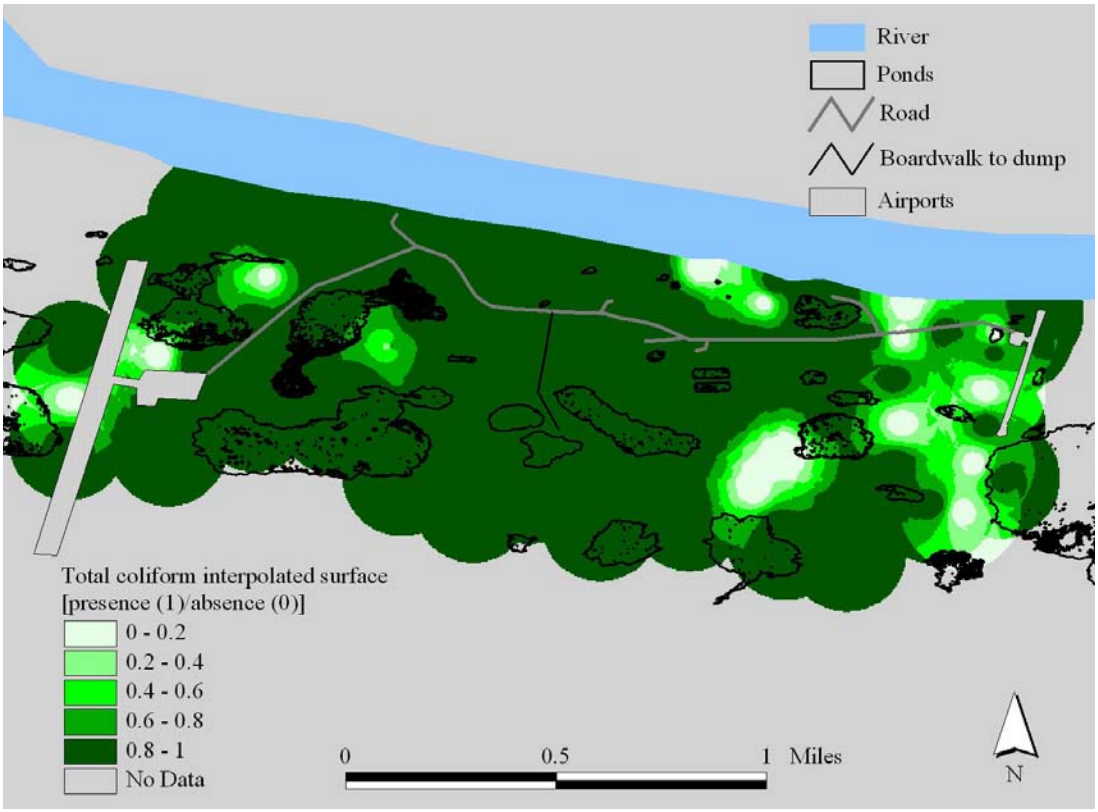

Figure D1

Total coliform presence/absence, June 2004. Shading represents the likelihood of detecting total coliform at points between sample locations. Interpolation is by second power inverse distance weighting including neighbors within 750 ft. Most samples were water samples, but when no water was near the grid point ~1 cm^2 soil was added to clean dechlorinated water. Most water samples contained total coliform. Samples were taken on a 500 foot interval grid. This is the total coliform analog of figure 6.

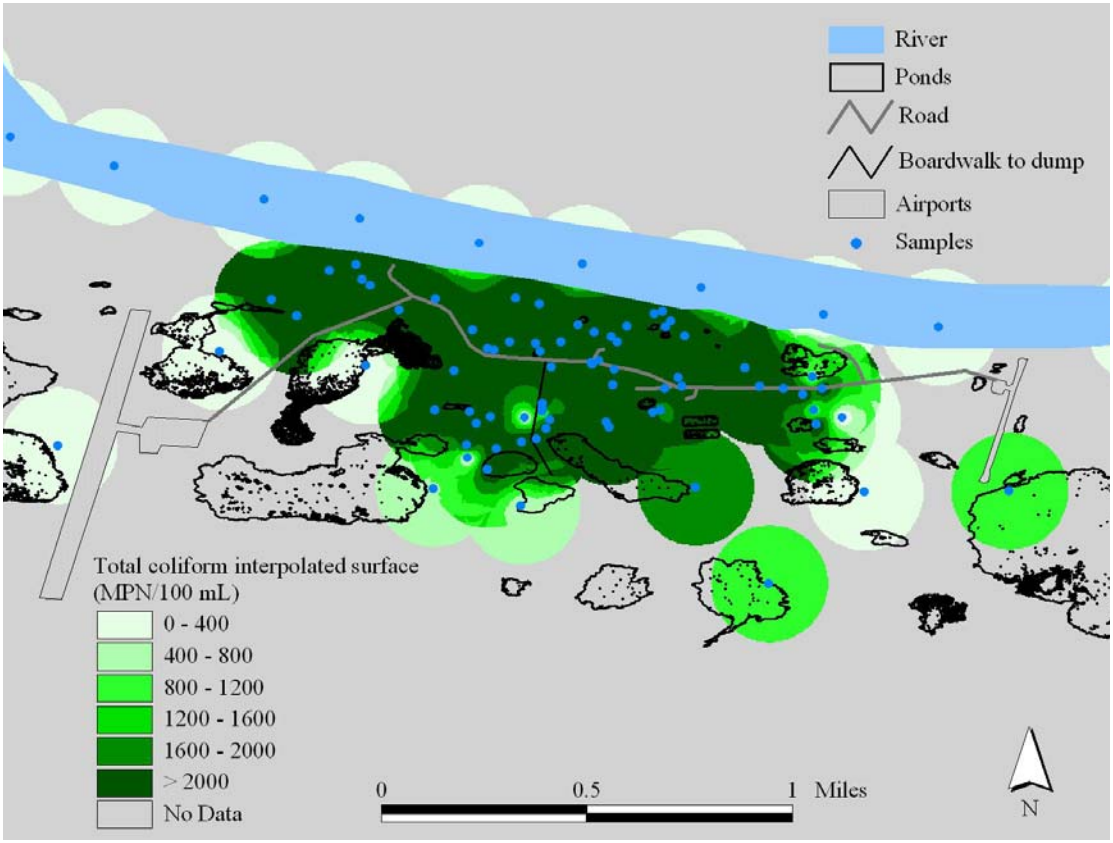

Figure D2

Total coliform MPN, June 2004. Shading represents an estimate of total coliform concentration between sample points. Interpolation is by second power inverse distance weighting of neighbors within 750 ft. This is the total coliform analog of figure 7.

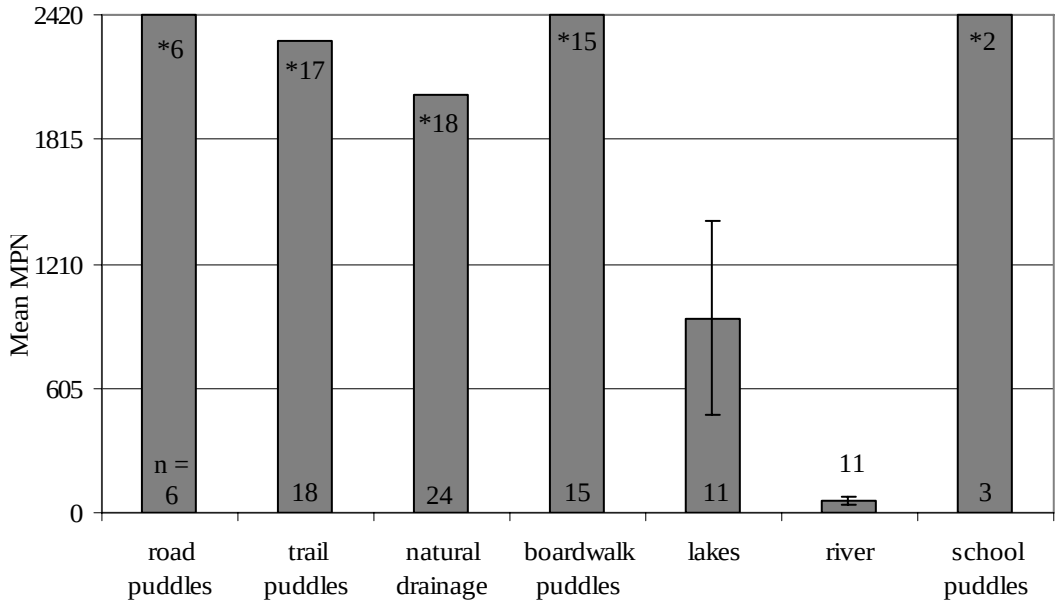

Figure D3

Total coliform in bodies of water, June 2004. Error bars represent 95% confidence interval (CI). Error bars were removed whenever samples exceeded 2419.6 total coliform/100 mL. Numbers by asterisks indicate samples over 2419.6/100 mL This is the total coliform analog of figure 8.

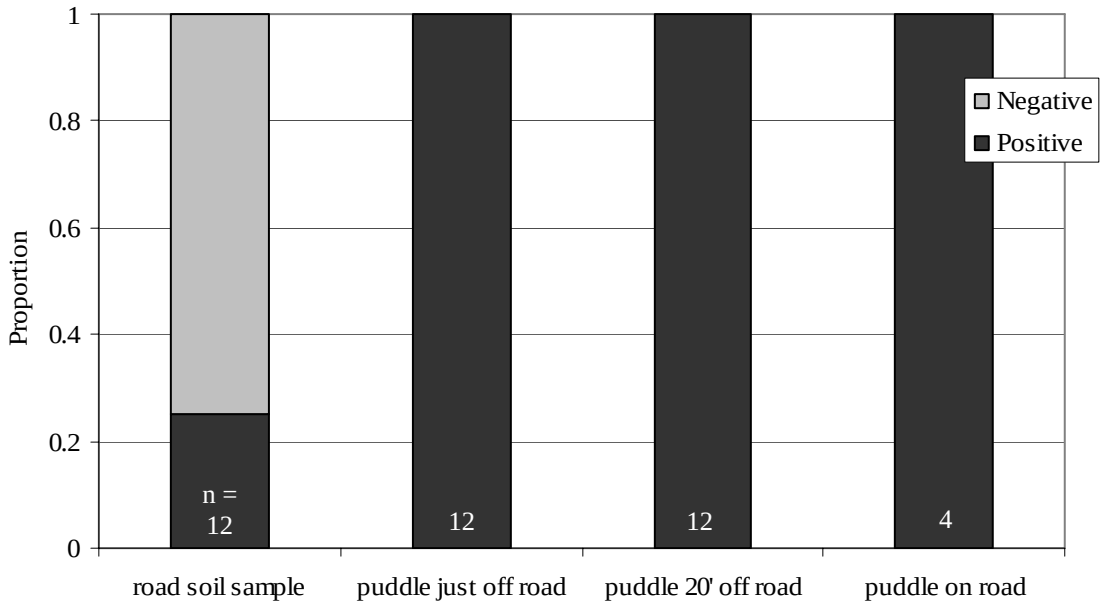

Figure D4

Total coliform along road, June 2004. This is the total coliform analog of figure 9.

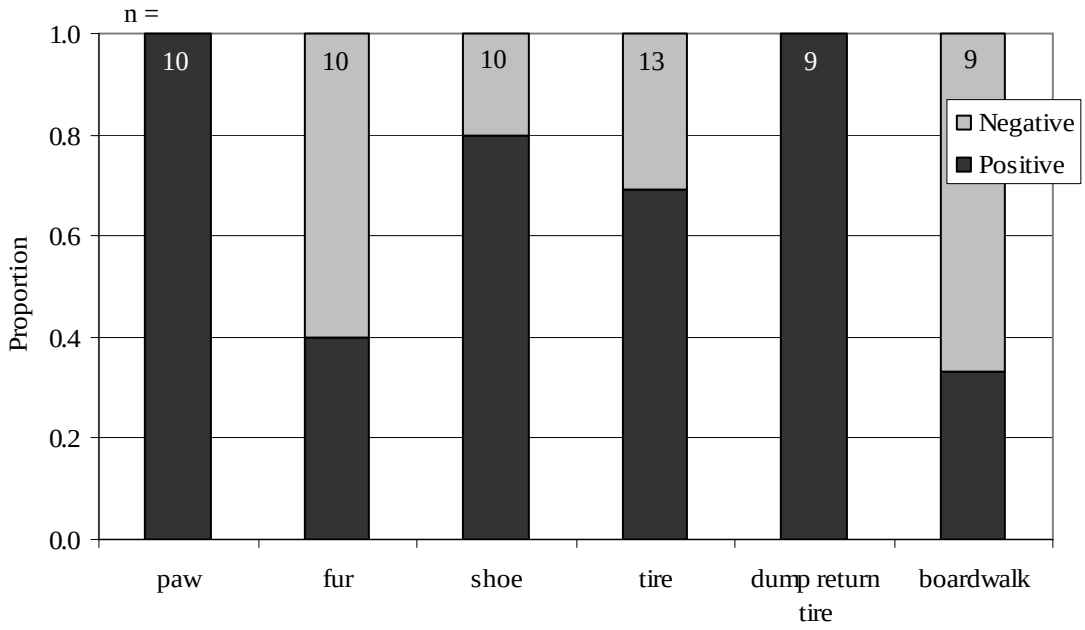

Figure D5
Total coliform surface swabs, June 2004. This plot is the total coliform analog of figure 10.

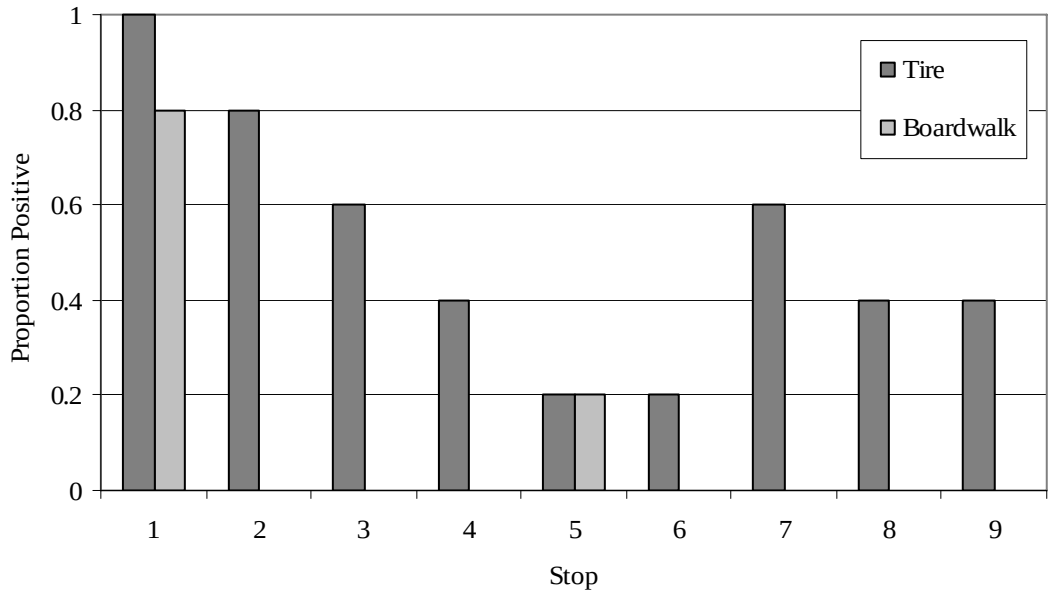

Figure D6
Total coliform swabs of ATV tires, August 2004. Stops refer to locations noted in figure 6. Swabs covered a 4"x4" square on the boardwalk surface or tire (including ridges and valleys). The path was run a total of 5 times. This is the total coliform analog of figure 12.