

Tampa Branch Laboratory/Virology

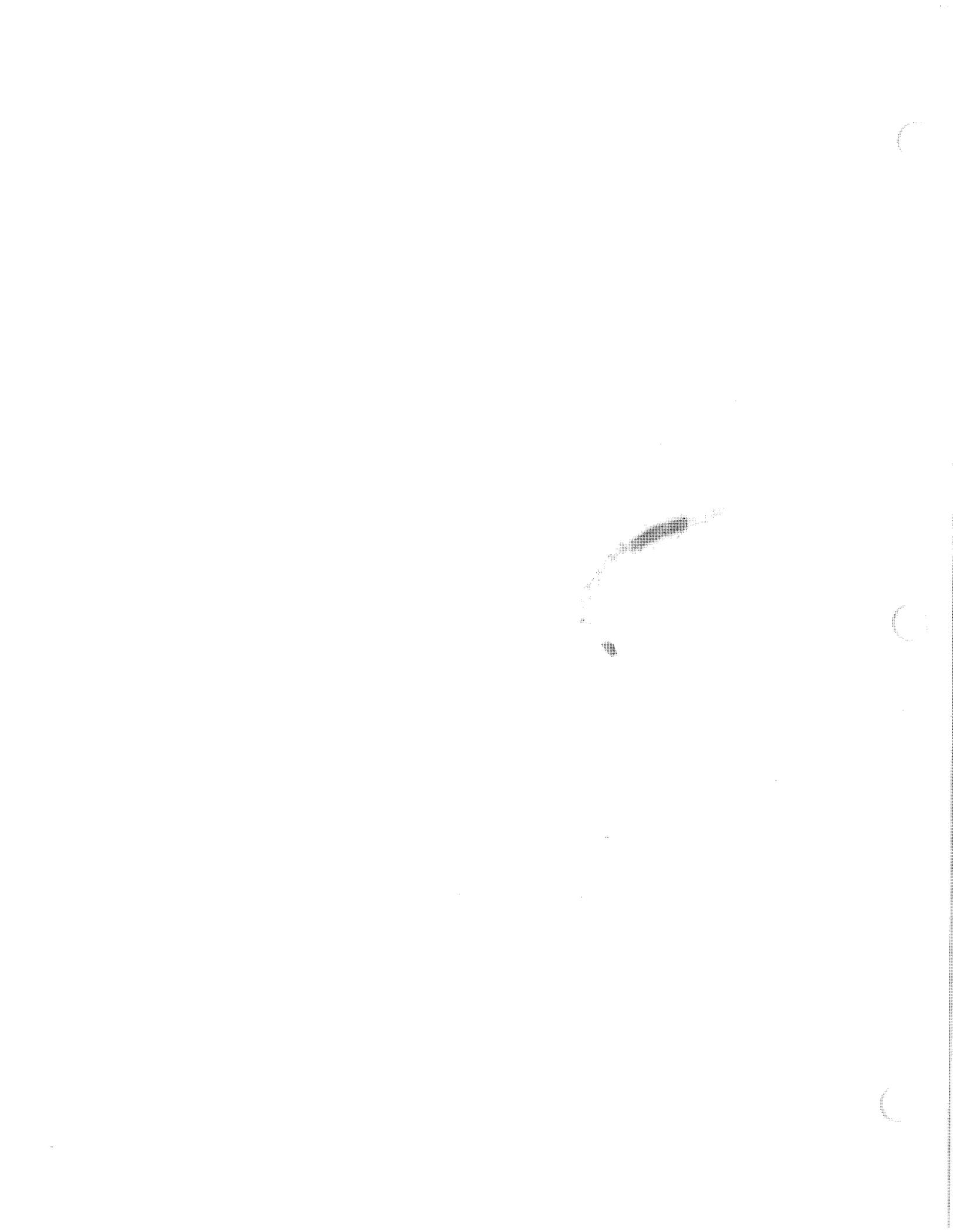
Florida OSDS Research Project

Viral Study Summary

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Executive Summary

The methods used to determine the fate of human enteroviruses in biological household waste scrutinized the circumstances and movement of these agents from households using onsite septic disposal systems (OSDS). These were conventional septic systems utilizing subsurface ground infiltration.

Initially soil specimens from four (4) study households were collected from the infiltration area beneath the drainfields at the initiation of the study. No enteroviruses were detected in these specimen soil cores which penetrated the unsaturated zone to a depth of four (4) feet in one community and two (2) feet in the other. These findings are of limited value due to a lack of prior knowledge of virus in either the feces of the household residents or in the effluent exiting the OSDS's of these households. Since soils aren't homogeneous, geography, climate, pH, soil type and moisture as well as ionic changes influence viral adsorption and elution in a drainfield differently. Even though the incidence of human enteroviral infection is year round in the study areas, a single specimen collection may have missed encountering any soil adsorbed virus due to factors which promoted rapid, en mass, elution through the soil infiltration area sampled.

Ground water, 1512 liters (400 gallons) per specimen, was collected from each of four (4) monitoring wells in the community, established downgradient of a

segment of the community's OSDSs. This effort was pursued monthly for fifteen (15) months. Even though contaminants usually associated with septic tank effluent were present in some wells, no enteroviruses were detected in 120 specimens examined. During this time enteroviral infections were noted in community householder's and the same viral serotypes detected exiting their OSDSs. However, this finding isn't necessarily indicative that ground water transport of enteroviruses can be ruled out. Instead, since previous study of ground water movement calculated only a velocity of 3.65-7.3 feet per year in one community and 0.03-0.05 feet per day at the other, there exists an extremely narrow prospect of viral plumes from a community's OSDS would reach the monitoring wells during the study period. Thus, an appreciable part of the subdivision's septic tank effluent that reached groundwater went unmonitored due to its sluggish movement down gradient toward the monitoring wells.

The large number, 10^6 - 10^{10} , of infective viral particles shed per gram of feces over at least several days are not all inactivated during transit through an OSDS. Monthly examination of householder's feces and their septic tank effluent yielded identical viral serotypes on numerous occasions. Quantification of the agents in the septic tank effluent revealed that a most probable number of infectious units that varied from 0.07 to greater than 58.57 per liter. As might be expected, the number of infectious units increased or declined with the length of time after infection was determined through fecal examination. A range of at least thirty (30) days for Coxsackievirus B2 to at least 137 for a reovirus was noted for for the detectable presence of these agents in septic tank effluent.

On an occasion Coxsackievirus A9 was detected in both a householder's feces and the septic tank effluent. Additional monitoring disclosed the presence of the same viral serotype in the effluent for at least fifty-seven (57) days, and at least twenty (20) days in a nearby newly placed monitoring well whose water level was four (4) feet nine (9) inches below the ground surface. An additional monitoring well at least ten (10) feet down gradient failed to yield the virus. This would infer that virus reached ground water but due to the ground water velocity, 0.05 feet per day, the viral plume intercepted well #1 but failed to reach the monitor well further down gradient. Thus, while low numbers of a most probable number of infectious units, 0.0024 and 0.00047 per liter, were detected in the ground water their transport was relatively limited under the conditions existing in the community.

Intensive efforts were also aimed at encountering viral adsorption to soil following detection of a coxsackievirus in a householder's feces and the septic tank effluent. Two (2) soil cores were collected adjacent to the drainfield measuring forty-four (44) and 30.5 inches below the gravel infiltration area thirty-eight (38) days after detection of the agent in specimens. Virus quantitated at 0.14 and 0.15 most probable number of infectious units per gram of soil was isolated only from the first four (4) inches of the core nearest to the infiltration area. This demonstrated adsorbed virus which, under the proper conditions, could elute and eventually migrate into ground water.

The data accrued show that human enteroviruses are present in OSDS effluent, adsorb to soil, can percolate through soil and reach ground water under the geographic, soil and climate conditions encountered in Florida. Due to many variables associated with monitoring under "real life conditions", data regarding the fate of viruses in the environment are difficult to obtain. Frequency of sampling is the most important aspect since viruses move rapidly and billions of virions can pass an area and be easily missed. Thus, it is difficult to speculate the length of time viruses remain viable in either soils or ground water.

A prospective cohort design approach was used in the epidemiological investigation of risk of enteric virus infection in children exposed to groundwater. The two communities which had previously been selected to participate in a study of the potentially polluting effects of septic tanks on the groundwater in their vicinity participated. This project ran concurrently with the environmental study. One community (exposed) had a high water table and private wells in addition to septic tanks on third acre lots; the other (control) had a low water table, septic tanks on quarter acre lots, and a chlorinated community water supply. Community demographics and family structures were very similar. Children less than 13 years of age, the group most susceptible to enteric virus infection, were enrolled in both communities to provide fecal specimens which were assayed for the presence of enteric viruses. In the control community 49 children provided 623 specimens for analysis over the three year study period. In the exposed community 31 children provided 466 specimens.

The null hypothesis was that exposure to groundwater from unchlorinated wells on lots with septic tanks does not increase risk of enteric virus infection. Both cumulative incidence and incidence density of enteric virus infections were determined for each community. Risk and rate ratios calculated from the data were such as to deny the null hypothesis. The study was hampered by small sample size in numbers of children participating, leading to risk ratios which were close to, but did not achieve, statistical significance (RR=1.346, p=0.068, all age groups combined). Nevertheless, there were significant differences found in the shorter time to first incident infection after enrollment (2.48 months, exposed, versus 5.57 months, controls, p= 0.031) as well as a higher incidence density ratio (rate ratio = 1.5079, p=0.0417) for the children less than 5 years of age in the exposed community. Regression analysis demonstrated that the community was a significant predictor of numbers of enteroviral infections experienced by young children, both in univariate and multivariate testing. Thus, the alternative to the null hypothesis, that there is a risk associated with exposure to groundwater from wells located on small lots (1/3 acre or less) with onsite sewage disposal systems, should be accepted.

Overall, the OSDS Virology project has demonstrated that enteric viruses excreted by family members enter the family septic tank systems, and were detected entering the OSDS drainfield for extended periods of time. These viruses were detected in the soil and water near the drainfield, although none were detected in groundwater monitoring wells situated some distance from the septic tank drainfields. Nevertheless, there was an increased risk for enteric virus infection in children from the community with private wells onsite along with septic tank systems as demonstrated in the epidemiological analysis. Hence, prudence dictates the need for caution when planning small lot use of septic tank systems.

Introduction

Study Aims

Groundwater is the source of 87% of Florida's public drinking water supplies and 94% of its private rural supply. Approximately 27% of the housing in Florida is being served by onsite sewage disposal systems (septic tanks). Many of these are located in high density developments which are beyond the service areas of municipal sewers. (Ayres & Associates, 1987) Because of the inability of local governments to meet the demand for appropriate sewage treatment systems for Florida's rapidly growing population, many more septic tank systems will be installed. Florida has unique hydrogeological and soil conditions. Concerns have been raised about the potential polluting effects of discharges from septic tank systems on our groundwater.

A major study concerning the impact of onsite disposal systems on groundwater was performed under the direction of the State of Florida Department of Health and Rehabilitative Services (HRS) Environmental Health Program Office. The hydrogeological component of this study of Florida soil types and groundwater was performed by the contracted Engineering firms, Ayres & Associates, and Kirkner & Associates. The investigation into potential virus pollution from septic tank effluents was conducted by the Epidemiology Research Center, HRS Office of Laboratory Services. This portion of the study included the collection of samples of groundwater from monitoring wells placed in the vicinity

of high density developments served by septic tanks, as well as samples from the septic tanks and their drainfields for virus determinations.

So that the groundwater analysis be meaningful, we must assure that viruses are entering the septic tanks during the study. Enteroviruses are the primary virus types assayed for in environmental monitoring for septic contamination. As will be discussed more fully in the literature review, these enteroviruses are most common in young, often asymptomatic children, who shed the viruses in their feces. It was therefore necessary that we study a population of small children in the communities where the groundwater monitoring is occurring, attempting to isolate enteroviruses from stools on a routine basis. These viruses may be transmitted via the fecal-oral route, as well as via the vehicle of contaminated water. When it was learned that one of the two communities to be sampled had a high water table with private onsite wells, whereas the other was serviced by an offsite community system, it was felt that a high potential for environmental exposure to potentially contaminated groundwater existed at the first site. Hence, an epidemiologic investigation to compare the two communities was conceived and added to the environmental investigations..

Literature Review

The Enteric Viruses: Virology

There are more than 100 different types of viruses in the enteric virus groups. These are viruses that enter the alimentary tract, reproduce in the tissue linings of the throat and intestine, and are excreted with the feces. This group includes the Picornaviridae, more commonly referred to as the Enteroviruses, the Reoviridae, and the Adenoviridae. The Enterovirus family includes Echo, Coxsackie, Polio, Hepatitis A, and others. These viruses contain a single stranded RNA genome in a protein capsid of 24 to 30 nm in size. The Reoviridae includes Reovirus (originally classified as Echovirus 10, when first isolated) and Rotavirus. The RNA of these viruses is double stranded, and the capsid size is 60 to 80 nm. Adenoviruses contain double stranded DNA, and are 70 to 90 nm in size. (Melnick, 1985) All of these viruses may be detected in water, as well as in the feces of infected individuals and in wastewaters (Nestor, 1984).

These viruses produce a broad array of symptomology in susceptible hosts: undifferentiated febrile illness, pharyngitis, upper respiratory infections, conjunctivitis, coryza, exanthems, pancreatitis, pleurodynia, myocarditis, orchitis, pneumonia, diarrhea, paralysis, aseptic meningitis, hepatitis, and encephalitis. Severe clinical illness is more frequently noted in neonates and young children. (Lennette & Schmidt, 1979) Possible causative links between Coxsackie B virus infection and juvenile-onset diabetes mellitus have been reported. (Gamble & Coleman, 1976) Infections are frequently asymptomatic or mild. The incubation period ranges from 1 day to 3 weeks. A short viremia may occur and lead to the involvement of organs such as the spinal cord or heart.

Presently, 68 distinct serotypes of Enteroviruses are recognized. (Menegus, 1985) There are no common group antigens. Isolation of virus from the feces or throat of a symptomatic individual does not confirm etiology, but when no other reasonable etiology can be ascertained, and the clinical and epidemiologic features are compatible, the isolate may be deemed probable cause (CDC, 1983). Shedding of enteroviruses in the throat is of much shorter duration than in the feces (1 to 2 weeks versus 4 to 6 weeks) and so isolation from a throat swab provides stronger temporal association with clinical illness (Chernesky, Ray & Smith, 1982). Current Estimates from the National Health Interview Survey (Vital & Health Statistics, 1985) record for syndromes which may be caused by enteric viruses 111.3 (all ages) and 206.9 (under 5 years) acute conditions per 100 persons per year, which resulted in 353.1 and 516.2, respectively, days restricted activity per year per 100 persons. Of course, not all the conditions are necessarily due to enteric viruses, but even if only 20% are, the amount of morbidity due to these agents is extensive.

The enteric viruses are shed in the stools of infected individuals, often in membrane associated "viral packets" (Williams, 1985). Virus concentrations may be greater than 1,000,000 infectious units per gram of feces (Nestor, 1984). Rotavirus, which may be shed in even higher concentrations, 1 billion particles per gram of feces (Farrah et al., 1978), is the most important cause of diarrhea requiring hospitalization of children under three years of age (Senturia, 1986). Adenoviruses have been related to both gastroenteritis (Isaacs et al., 1986) and respiratory syndromes (Cooney et al., 1972). Viral isolation in cell culture is the diagnostic method of choice for both Enteroviruses and Reoviruses (Menegus,

1985). Adenoviruses may be detected by electron microscopy (Isaacs et al., 1986) or by cell culture (Cooney, 1985). Rotavirus may be detected by electron microscopy (Senturia, 1986) or by a variety of new commercial enzyme immunoassay kits for rapid diagnosis (Brandt et al., 1987).

The Centers for disease control Enterovirus Surveillance Program is "a voluntary laboratory-based reporting system based on enterovirus isolations." (1981) Because these notifications are not required, and because virus studies are expensive and have a usually long time period to reporting, virologic studies are often not ordered. Moore et al. (1984) suggest that this results in a distortion of the distribution of clinical features associated with these agents, as specimens from only the most severely ill patients are submitted. This also results in a bias as to type prevalence, since only those virus serotypes which are capable of causing severe illness will thus be found. An "isolation" bias also exists, since the ease of isolation under the usual laboratory culture conditions varies among the different serotypes (Strikas, Anderson & Parker, 1986).

The Enteric Viruses: Epidemiology

Most reports of Enterovirus isolations are based on clinically ill populations. The Centers for Disease Control Surveillance (1981) reported a total of 17,130 enteroviral agents isolated from 16,978 patients during the 10 year period of 1970-1979. However, there were an average of only 26 states submitting reports each year. A strong seasonal pattern was seen: transmission during June to October was 6.5 times higher than for the rest of the year. Most isolates were from small children, 64% under 10 years of age, 29% under 1 year. The male to female ratio was 1.5:1 overall, but in the 20-29 year age group females predominated significantly. It is suggested that this was due to a greater exposure of females of that age group to young children. Moore et al. (1984) reports a 10 year hospital based study in Nassau County, New York, where specimens were collected from anyone suspected of having a viral illness. They found a similar seasonality, with major outbreaks occurring from June through September, and age distribution, with 92% of the isolations made from children under age 14. Both throat and rectal swabs were collected, but the positivity rate for rectal swabs was significantly higher. Most infections were asymptomatic or produced only mild clinical illness.

In Canada, Neumann (1986) reports Enteroviruses being associated with 6 to 8% of gastrointestinal illnesses examined, Rotaviruses with 50 to 55%. For cases of meningitis, however, 59% were associated with Echovirus isolations. Percentages due to specific virus types changed from year to year. Brandt et al. (1979) detected Rotavirus shedding in 39% of children hospitalized for gastroenteritis; highest virus presence was during January, when it was found in 71% of the patients. Their age prevalence study indicated that virtually all the children in the metropolitan area studied had been infected with Rotavirus by two years of age.

A temporal variation was also observed by Strikas et al. (1986) in their analysis of the CDC Enterovirus Surveillance data, 1970-1983. They consider the variation to partly be due to variation in virus activity, differences in associated clinical symptoms which would affect the likelihood of an isolation study being attempted, and differences in the ease of isolation of the various serotypes. There was also geographic variation in the temporal pattern, with more early (March to May) isolates being made in the South Atlantic, West South Central, Mountain and Pacific regions than in other parts of the country.

A twelve year study of Coxsackie B infections in Scotland (Bell & McCartney, 1984) reports most isolations were made from children with respiratory illness. Serological studies showed diagnostically significant titers in 33% of adult patients with myo-pericarditis. Antibody surveys of the normal population over a five year period demonstrated that 14% had had recent infections with one or more group B Coxsackie viruses (static titers of 256 were considered presumptive and ≥ 512 indicative of recent infection). Serology was also used in a study of Echovirus 30 infections in Japan (Matsumoto, Kobayashi, & Kimura, 1986). Sera collected before and after an epidemic were examined for neutralizing antibody to Echo 30. Five years prior to the epidemic no children under 15 years old had antibody, but two years later, 15% of children under 9 years had high antibody titers. During and after the epidemic, seropositivity in the children was high (50%). Based on antibody titers it was felt that infection occurred mainly in the young, as compared with adults.

An investigation into aseptic meningitis outbreaks occurring in high school football teams (Moore et al., 1983) also showed an August to October pattern. Attack rates in one outbreak were as high as 59% for players in contrast to 10% in the rest of the school. In at least two outbreaks, shared water cups were implicated as a possible transmission vehicle. The outbreaks were unrelated and associated with various enterovirus serotypes. Mixed serotype epidemics may occur where an outbreak of clinically similar illnesses was caused by multiple Enterovirus serotypes (Wenner et al., 1981).

A prospective study of neonates over their first month postpartum in Rochester, New York found the incidence of Enterovirus infection to be 12.8%, as determined by virus isolation from 75 out of 586 infants. The prevalence of virus excretion was 5.3% (116 positive out of 2178 cultures). Of those with positive cultures, 79% were asymptomatic. (Jenista, Powell, & Menegus, 1984) Modlin (1986), reports, however, that Enteroviruses are capable of causing severe disease and death when infection occurs within the first two weeks of life, and a later onset of infection may result in less severe disease and lowered mortality. He also found a seasonality of infection, with most infections occurring from June to November, and cyclic annual changes in the predominant strains circulating.

The occurrence of acute respiratory infections in children is frequently due to Enteroviruses. The incidence of these illnesses was found to be highest in infants in group day care situations, but declines as they age in the same setting (from about 10 down to 4 infections per year by age 5). The incidence is lower in home care situations only if the number of children involved is fewer than four to six. (Denny, Collier, & Henderson, 1986) In a study comparing a nursery where the children were permanent residents with a day care facility in Roumania, Zavate et al. (1986) isolated enteroviruses from the 13.5% of the babies in the "closed" community of the nursery, and 28.5% of those in the "open" day care community. The variety of serotypes isolated was also lower in the nursery. A seasonal variation in success of isolation was also seen, with more positives during the warmer months.

There have been a number of "virus watch" surveillance studies, where Enterovirus isolations were attempted from generally healthy populations.

Investigators from Tulane University performed serologic and stool examinations during the 1950's in Louisiana for Enterovirus analysis. Gelfand (1959) isolated 613 enteric viruses from 115 children over a three year period (1.8 per person per year). A consistent seasonal periodicity was observed; during summer and autumn over one third of the healthy children studied were excreting virus at any given time. Prevalence of serum antibody varied greatly with the virus type and with age. For example by age 9, 82% had antibody to Coxsackie A9, 36% to Coxsackie B, 6% to Echo 1, and 66% to Echo 7. More than 20% of the isolates made were not identifiable at the time of the study, thus those seroprevalence rates were not determined.

Henigst et al. (1961) in a continuation of this study also observed a multiplicity of agents infecting the community at any given time. In examining the incidence of Echo 7 infections over a three year period, virus isolation was made from less than 1% of the families being followed the first year, 12% the second, and 35% the third year. The mean duration of virus excretion by the children was 3 weeks. Virus titers were higher earlier in the infection than later. Clemmer et al. (1966) using isolation and serology found 51 incident infections with Coxsackie B3 virus in a population of 124 children (41%) over a 72 day (June through August, 1959) period of observation.

The Seattle Virus Watch was carried out from November, 1965 to September, 1969 (Hall, Cooney, & Fox, 1970; Fox et al., 1972; Cooney, Hall, & Fox, 1972). During an outbreak of aseptic meningitis associated with Echovirus 30, 24% of the total study population was infected. Within the 18 families experiencing infection, out of the total 64 families observed, 80% of the family members were positive. The study population consisted of middle class families with newborn infants. Out of 655 virus infections, 229 (35%) were due to Echo or Coxsackie viruses. For the nonpolio enteroviruses, isolation rates from fecal specimens ranged from 1 to 2.5% for children. The number of infections per person-year in index children of the study families averaged 0.50. Isolation attempts made in various cell culture types demonstrated differences in isolation sensitivity for some serotypes, which might have influenced isolation results.

A similar study was carried out in New York City from August, 1961 through March, 1965 (Spigland et al., 1966; Elveback et al., 1966; Kogon et al., 1969). This virus watch surveillance followed 178 families in metropolitan New York for over 10000 person-months. Seasonality of virus excretion was strong, the Echo and Coxsackie viruses being virtually absent from January thru July. Nineteen different non-polio Enteroviruses were isolated during the study, but only 4 to 6 were prevalent in any given period. For children 0 to 5 years of age, the Enterovirus isolation rate was only 2.4% overall. The authors compare this with Gelfand's Louisiana study (1959) where 10 to 12 viruses were often prevalent and an overall isolation rate of 14% was found. In the New York communities being monitored, the most commonly observed viruses were present at about the same times, even though the areas were far apart. A comparison of illness with viral excretion gave an estimate of overall enterovirus pathogenicity of 20.2%. Most infections were observed in young children (88% of the Coxsackie isolates were from those under 9 years of age), although adult family members were included in the study.

The Tecumseh, Michigan study of illness was carried out from 1965 through 1972 and 1976 through 1981 (Monto & Koopman, 1980; Monto, Koopman & Bryan, 1986). Common enteric illness usually lasted only two to three days, whereas more severe illnesses were found to last seven to ten days. Enteric illnesses were more severe when there were also respiratory symptoms present. Although these studies were aimed at respiratory agents, enteroviruses were isolated; 84% of the isolates were not associated with enteric symptoms.

Healthy children in each of six United States cities (Atlanta, GA, Miami, FL, Minneapolis, MN, Buffalo, NY, Seattle, WA, and San Francisco, CA) were the subjects of cross-sectional surveys carried out in 1960 to 1963 (Gelfand & Holguin, 1962; Gelfand et al., 1963; Froeschle, Feorino, & Gelfand, 1966). One hundred rectal swabs were collected in each city each month; different children participated in each sampling. The percentage of positive specimens decreased with increasing age. Because only one laboratory host system was used for viral isolation the authors suggest that the true rate of infection is actually higher than observed. Although the winter low, summer high seasonality was observed in isolations, the southern cities differed from the northern ones: some isolations were made all through the year and the seasonal rise began earlier in the south. During

the seasonal peaks, isolation rates reached 15 to 20% in the south, 10 to 15% in the north. Only a few Reoviruses were isolated, 63% in October to December. Overall, the isolation rate was 4.9% per year; for Atlanta, 7.2%, Miami, 7.6%, Minneapolis, 4.5%, Buffalo, 3.3%, Seattle, 3.7%, and San Francisco, 2.1%.

Serological studies of enterovirus antibody prevalence show positivity to vary with the serotype being tested. In a study of 34 adults, neutralizing antibody to the Coxsackie B viruses varied from 17.6% for CB6 to 82.4% for CB4, and titers ranged from 10 to 1280 (Torfsen, Reimer, & Keyserling, 1987). Because there are so many virus serotypes that could be tested for, most studies which include serology look for antibody to only a few of the prevalent ones. No group antigen is present to allow for screening of "Enterovirus antibody"; serum neutralization testing is type specific (Menegus, 1985) but Elisa tests do not correlate with neutralization assays using present technology (Torfsen, op. cit.).

Studies of antibody prevalence to seven "classical" enteroviruses in Israel (Morag et al., 1984; Margalith et al., 1986) utilized virus neutralization assays. Residents of agricultural kibbutzim were tested. At two to four years of age, prevalence of antibodies to Coxsackie and Echo viruses was 40 to 69%; at age 5 to 17 years, 85% had antibodies to 5 or more of the 7 enteroviruses assayed for. Volunteers, aged 18 to 34, from western Europe and North America, who came to one kibbutz for a two month stay were compared with residents. The visitors had 2.1 antibodies per person versus 4.7 for the residents when mean antibody prevalence to the seven Coxsackie and Echo viruses was measured. After the two month period, tests demonstrated 9.4% of the visitors had developed antibodies to at least one of the viruses studied. It is suggested that even though the kibbutzim are of a high socioeconomic level, environmental exposures, such as through common dining and nursery facilities, as well as the use of wastewater for sprinkler irrigation, may be of considerable significance.

Occurrence of Viruses in Water and Wastewater

Human enteric viruses can be detected in sewage effluents (wastewater), their occurrence reflecting those types which are circulating within the community. Thus, enterovirus numbers peak in summer and autumn (Krikelis et al., 1985). These viruses have been shown to survive sewage treatment processes which

included chlorination, and to enter natural waterways with the discharged effluent. Fujioka and Loh (1978), in Hawaii, isolated human enteroviruses from a shallow flowing stream three miles from the sewage discharge point. Nevertheless, public (community) sewage treatment has been recognized as an improvement over most on-site disposal systems. Anderman and Maritim (1986), reported a significant reduction in bacterial surface water contamination and health hazards in sewered versus unsewered areas in Oregon

Onsite-disposal systems, septic tanks, are simply underground chambers which allow the separation of sludges and floatable materials from wastewater. Some anaerobic decomposition of the sludge occurs, reducing its volume. The average retention time for single family tanks is 24 hours. The partially clarified liquid then flows to the drainfield. These septic tank effluents contain pathogenic viruses and bacteria. The bacteria are usually filtered out by the soil, but the viruses are not. Viruses are instead adsorbed to the soil particles due to colloidal charge interactions. Organic matter in the effluent may reduce the adsorptive capacity of the soil. (Canter & Knox, 1985; Hain & O'Brien, 1979) Those soils which perform best in drainfield percolation tests, loose sandy soils, also permit rapid passage of virus containing waters. For maximum virus adsorption, a slow percolation through a clay soil is best. This may, however, lead to lateral water flow and runoff of the effluent (Wellings, 1982). The density of septic tank placements may be such that it is greater than the natural capacity of the subsurface soil to receive the system effluents and allow for viral adsorption (Canter & Knox, 1985). Yates et al. (1986) using a highly simplified model, state that the probability of viruses reaching groundwater is quite small if the septic tank soil adsorption system is properly designed, installed, and maintained. They do, however, recognize that the appropriate requirements are more often not met, and report outbreaks of gastroenteritis related to septic tank effluents which had contaminated groundwater.

Septic tank leach fields were shown to be the source of viruses detected at down-gradient distances of 67.05m and from an aquifer depth of 18m (Vaughn, Landry, & Thomas, 1983). Viable enteric viruses have been isolated from properly functioning septic tanks and their drainfields, as well as groundwater influenced by septic tank effluent. Adsorption of virus to solids appears to increase its survival time. Subsequent elution by rain or floodwaters may carry the virus into the

groundwater. (Hain & O'Brien, 1979) Alhajjar et al. (1987) found that virus could spread into groundwater from properly functioning septic tanks. In their study using stools containing poliovirus introduced into the system via the household toilet, virus could be recovered in the tank effluents for over 109 days. The virus was also found in groundwater monitoring wells 6m from the drainfield. They suggest the long survival time and distances travelled were due to repeated adsorption to the aquifer sediments, followed by desorption under conditions of decreasing ionic strength.

In addition to ionic strength of the adsorbing milieu, soil pH, chemical composition, and the rate of infiltration influence viral movement through soil (Vaughn et al., 1978). Virus survival is also influenced by their occurrence as aggregates, or embedded in fecal solids of varying sizes, which protects them from environmental degradation and from chlorination during sewage treatment. Even though survival is longer with lower temperatures, the enteric viruses are reported to be quite heat stable. Virus movement through soil is greater during periods of saturated flow due to rapid infiltration; movement can easily occur through large pores in the soil (Lance & Gerba, 1984).

Survival times of enteric viruses in natural waters have been reported as high as 188 days (Melnick & Gerba, 1980). Laboratory studies by Mahnel, Ottis, & Herlyn (1977) demonstrated survival times of greater than 200 days for some enteric viruses in freshwater. In the soil, virus persistence varied from greater than 11 days in summer to 96 days in winter (Yeager & O'Brien, 1979). Temperature has been shown to be the most important factor in determining the rate of virus persistence in water (Yates, Gerba, & Kelley, 1985). The authors also found, however, that even at the same temperature, virus lasted longer in well water samples than in surface waters.

Enteric viruses have been isolated from both untreated river water and the treated tap water produced from it (Nestor & Costin, 1976; Payment, 1981). Vaughn et al. (1979) isolated enteroviruses from lake waters, creek waters, and marine embayments off Long Island, New York, and implicated seepage from septic tank systems as the likely source of pollution. Enteric viruses have been isolated from groundwater monitoring wells where sewage was used for cropland

irrigation (Goyal, Keswick, & Gerba, 1984). When treated sewage was held in a recharge basin 10.6m above the aquifer, viruses penetrated to the groundwater and travelled horizontally a distance of 45.7m (Vaughn et al., 1978). Wellings, Lewis, & Mountain (1974) detected virus in groundwater wells down-gradient from a sewage recharge site after heavy summer rains. Enteroviruses have been isolated from chlorinated drinking water produced from a 35 to 40 foot deep well which was located in an area served by septic tanks (Wellings, 1982).

These studies, and others, have demonstrated that enteroviruses applied to the land, either as incompletely treated sewage from public systems or as septic tank effluents can penetrate to the groundwater and travel long distances, remaining viable for extended periods of time. These viruses may be detected in both raw and chlorinated surface and groundwaters. Contaminations may be intermittent; a satisfactory monitoring approach which would justify a standard containment level is lacking (Geldreich, 1986). There is a concomitant need to examine large volumes of groundwaters in order to detect the presence of low numbers of viruses present. Thus, negative findings are often suspect and simply the result of not using the most sensitive techniques (Keswick & Gerba, 1980). Additionally, the high cost of virus assay mitigates against extensive sampling, producing false negatives.

Health Risks and Water-borne Outbreaks

Determining the etiologic agent causing an outbreak of waterborne illness is difficult. Symptoms are often not striking at first, so that some time passes before an outbreak is even recognized. By then it is often too late to isolate and identify the agent. During 1971-1978 an etiology was determined for only 45% of recognized waterborne disease outbreaks. The likelihood of an outbreak coming to the attention of public health authorities, and being investigated, varies from community to community. Types of investigations made often depend upon the interests of those individuals in charge, and the facilities available. Disease has been traced to both treated and untreated, community and private, water sources. A temporal distribution difference is seen when the different water sources are compared. Outbreaks related to untreated groundwater peaked during early summer; those related to untreated surface water peaked in late summer; those due to contaminated municipal systems did not show much variation through the year.

These latter outbreaks were primarily due to cross contamination and back siphonage in the distribution lines. Sewage, mainly from septic tanks, was held responsible for 41% of the outbreaks and 66% of the illnesses caused by contaminated untreated groundwater in the United States, 1971-1978. (CDC, 1983; Craun, 1981)

Although 65% of the 550 reported waterborne disease outbreaks from 1946 through 1977 can be attributed to probable viral etiology, Keswick and Gerba (1980), maintain that because of the difficulties of virus detection, this is only a fraction of the actual number of true viral etiology waterborne illnesses. Inadequately treated groundwater from an area contaminated by septic tanks was associated with an outbreak of viral gastroenteritis in South Dakota, as was surface water in a New Mexico outbreak, but no etiological agent was proven (Epidemiologic Notes, 1988). The need for an improved specific laboratory approach to identify responsible agents was recognized, and it has been suggested that new diagnostic technologies should reduce the number of future undiagnosed outbreaks.

Enteric viruses have been isolated from chlorinated potable water, which was free of coliform bacteria, during a community wide outbreak of gastroenteritis and Hepatitis A. (Hejkal et al., 1982) The authors suggested that contamination from septic tanks a few miles upgradient of the wells occurred via underground fault channels in the limestone aquifer. The well contamination was sporadic, and associated with heavy rainfall, making appropriate environmental viral assay difficult.

In Israel, where wastewater is extensively reused for irrigation purposes, health risks from this practice have been extensively investigated (Katzenelson, Buium, & Shuval, 1976; Fattal et al., 1986, 1987). A strong difference in morbidity was seen between those settlements that use partially treated sewage effluents for irrigation and those that do not; enteric disease incidence was two to four times higher in the exposed communities. A log-linear model was used to compare enteric disease rates with rates of non-enteric diseases, irrigation type, community, age group, etc. Stratified analysis separately by age and irrigation type confirmed the findings of twofold excess enteric disease rates in the summer

irrigation period for the 0 to 4 year age group exposed to effluent. When antibody to various enteroviruses was measured in similar communities, a significant excess to Echovirus 4 was found in the 0 to 5 year age group which was exposed to aerosols from sprinkler irrigation with partially treated wastewater from a nearby town. An epidemic of aseptic meningitis associated with Echovirus 4 had occurred in the town just prior to the study inception, and the pathogen was apparently transmitted to the kibbutz children via the wastewater used for irrigation. In each study the authors conclude that to minimize health risks, it would be a prudent public health policy to provide more effective wastewater treatment.

Low numbers of enteric viruses have been detected with increasing frequency in drinking waters. Payment and Trudel (1985), reporting on their work in Canada, feel the problem to be minimal. At the time of their isolations from finished water, there were no reported epidemics, although there was an increased incidence of gastrointestinal illness. They conclude that, since most enteroviruses are of low virulence (i.e., only a small percentage of those infected develop overt disease), even though highly infectious (a minimum infectious dose of less than 10 Tissue Culture Infectious Doses), there is no measurable health risk from exposure to the low viral concentrations they found in drinking water. Gerba and Haas (1986) also assessed the probability of infection, illness, and mortality for individuals who consume potable water containing various concentrations of enteric viruses. They suggest that there does exist a significant risk for both illness and mortality from exposure to low levels of enteric viruses. One viral Tissue Culture Infectious Dose per 1000 liters of water consumed was determined to result in a significant risk for enterovirus infection on an annual and lifetime basis; risk of disease and mortality were also considered significant. This risk is actually held to be an underestimate, since secondary and tertiary spread of the agent from the exposed individual is extensive.

Even when the virus penetrates the community by a common water source, most of those initially infected from the contaminated water do not develop disease. Nevertheless, these individuals serve as the sources for person to person spread. When virus is widely dispersed in the soil and water, many small foci of infection are initiated. Secondary spread from these foci magnifies the problem of source identification. As long as the virus concentration is low, cases of disease, especially if severe, will be sporadic, and thus difficult to trace back to the primary

infection. (Nestor, 1984) Thus, determination of the risks associated with the presence of enteric viruses in potable waters is difficult. The variety of syndromes presented by the large number of potential etiological agents, the high proportion of infection without observed illness, and the high rate of person to person secondary transmission all complicate an epidemiological approach to this problem. However, the technology to reduce the enteric virus load dumped into the environment with wastewater is readily available, and thus it would seem most prudent to maximize health potential by elimination of as much viral contamination as possible.

Environmental Study

Introduction

Water quality is a principle concern of Florida's planning and regulatory agencies. Sufficient data are at hand to indicate that human enteroviruses do percolate through soil and move with groundwater (Vaughn 1983; Wellings 1974).

Man's biological wastes have always been discharged onto and into the ground with little regard to the effect on the groundwater. Now that population density has increased the distance between discharges and public access has diminished, this dilemma presents skepticism that the past decreasing trend of enteric disease of viral etiology will continue (Craun 1986, 1988)

Individuals residing in the United States experience an enteroviral infection with greater than annual frequency (Sobsey 1983). The viral load per gram of discharged feces from infected individuals can be 10^6 - 10^{10} viral particles with shedding being up to thirty (30) days (Tyrrel and Kapikian 1982). Over one hundred (100) human enteric viruses have been identified, thus indicating a high potential for viral contamination of septic tank effluent discharged to a drainfield. These agents include adenoviruses, enteroviruses (coxsackie, polio and ECHO), hepatitis A virus, Norwalk virus, Norwalk-like agents, reoviruses and rotaviruses (Chitty and Davis 1972; Geldreich 1972; Hejkal 1983; Kowal 1985; Payment

1982). Hepatitis A, Norwalk virus, enteroviruses and reoviruses are known or suspected of being water transmissible.

In view of the previous studies it appears unfeasible that a drainfield will be sufficient to remove all enteric viruses exiting the septic tank, entirely inactivating them in the soil column before reaching ground water. Even so, it has been shown that slow removal of viruses within the drainfield doesn't insure permanent entrapment or inactivation (Fuhs and Taylor 1982; Wellings 1974; Melnick and Gerba 1980; Sobsey 1980).

This study addresses the issue of whether, under Florida climate and soil conditions, septic tank treatment of household biological wastes provides for adequate, safe treatment in close proximity to human habitation without constituting a public health problem related to human enteric viruses in the ground water being used.

Materials and Methods

Subdivision Groundwater Monitoring

Two (2) subdivisions were selected for this study. Lost Lake Subdivision is located in Polk county. The underlying soils are very well drained, fine and sandy with depths to groundwater being at least ten (10) feet. Mandarin Meadows Subdivision is located in St Johns county. The underlying soils are moderately to poorly drained, fine and sandy with depths to groundwater being less than three (3) to six (6) feet.

Water table elevations were determined for both subdivisions to ascertain the direction of groundwater flow. The monitoring wells were established downgradient of the flow.

Prior to specimen collection, at least 378 liters (100 gallons) was purged from the well. Specimens were pumped into a mobile laboratory, placed on-site,

to process 1512 liters (400 gallons) from each well. This effort was designed to capture virus through adsorption to a mixed esters of cellulose microporous media (APHA 1989).

Within the mobile laboratory, chemical proportioning pumps continuously adjusted the incoming groundwater to a 0.05M-MgCl₂ level. Additionally, the groundwater was adjusted to pH 3.5 through an inter connection between a pH controller and chemical feed pump injecting hydrochloric acid. The preconditioned specimen was subsequently passed through the filter media, pore size 450 micrometers secured in a 293 mm diameter filter holder, at a flow rate not exceeding 3.78 (1 gallon) per minute. The filter media was changed after each 378 liter aliquot to ensure adsorbing sites would be available. Elution of adsorbed virus was achieved on-site by forcing 500 mL. of 1% beef extract, pH 9.4, through the membrane. The eluant was held at 4°C until delivered within 24-48 hours to the base laboratory for additional concentration of the eluant. The method of Katznelson (Katznelson et al 1976) was used to further concentrate the eluant for subsequent inoculation onto susceptible cell culture.

Soil Sampling

Soil cores were collected directly below the infiltration system of two (2) homes in each subdivision. Specimens were collected at two (2) depths, the waste water infiltration surface, directly below the gravel, and two feet beneath it. They were immediately placed on wet ice and delivered to the laboratory within 24 hours of collection.

Additional processing involved suspending fifty (50) grams of the soil in fifty (50) mL. of 10% buffered, pH 7.0, beef extract and stirring the suspension for thirty (30) minutes. The soil was allowed to settle by gravity and the supernatant aspirated and subjected to sonication for three (3) minutes at 100 watts in an ice bath. The remaining particulates were removed by centrifugation and the specimen sterilized through a membrane filter, 450 micrometers average pore size, prior to being inoculated onto cell culture. Observation for a specific cytopathic effect extended over a fourteen day period.

Fecal enteroviral isolation attempts

Fecal specimens were obtained from children (0-13 years of age) of both subdivisions, not only during illness, but also, on a voluntary monthly basis. Collected feces, in a 1 ounce screw-capped bottle, were held by the householder in the freezer portion of a household refrigerator less than thirty (30) days until transported in the frozen state to the laboratory.

Processing in the laboratory involved preparing a 10% suspension of the feces in phosphate buffered saline using a mortar and pestle. The homogenate was subjected to two (2) separate centrifugations, 1200 and 3000xG to remove particulates and microorganisms. The resultant supernatant was treated with antibiotics and stored at -70°C until inoculated into two host systems, cell culture and newborn mice.

Examination of Septic Tank Effluent

Access to septic tank effluent (STE) was gained through a small basin (approximately 19 liters) which had been installed in the drain line on the effluent side of the septic tank. The STE in the basin was pumped into a stainless steel pressure vessel and preliminary processing achieved on-site. Sufficient $MgCl_2 \cdot 6H_2O$ was added to the specimen to render it 0.05M and the pH adjusted to 3.5 with N-HCl. Positive pressure was used to force the specimen through a mixed esters of cellulose membrane, average pore size 450 micrometers, which had been overlaid with thirty (30) grams of diatomaceous earth. The elevated nephelometer turbidity units of the specimen often necessitated the use of several membranes to complete filtration of the specimen due to clogging. Adsorbed virus was eluted by passing 500 mL of buffered beef extract, pH 9.4, slowly through the membrane. Both the eluant and membranes were returned to the laboratory for additional concentration by the method of Katznelson.

Virus Isolation and Identification in Cell Culture

Viral isolation attempts from prepared specimens were conducted by inoculation of Buffalo Green Monkey (BGM) kidney cell culture monolayers in 25 cm² flasks. The cultures were washed, inoculated, rocked for two hours at 36°C

two hours at 36°C to enhance viral adsorption, washed again and overlaid with a liquid maintenance medium. Inoculated cultures were incubated at 36°C and subjected to microscopic examination for fourteen (14) days post-inoculation. Cultures presenting a cytopathic effect were passaged to additional cell culture to confirm that the observation was of viral etiology.

Viral isolates were serotyped using immune sera directed against human enteroviruses. Serum neutralization tests using pooled sera and at least thirty-two (32) tissue culture infective doses of the unknown virus were incubated at 37°C for two (2) hours. The serum-virus mixtures were inoculated onto cell culture and observed for neutralization of the cytopathic effect of the virus. Each of the immune sera was common to one or more pools. Viral identity was determined by the neutralization pattern (Lim et al 1960)

Viral quantification

Viruses are intracellular parasites that cannot be directly quantitated in the field at the point of specimen collection. Therefore, methods were used to capture and concentrate viruses from large specimens taken in the field. Some of the known anticipated enteroviruses resist being cultured in vitro. The remainder that will replicate and provide visible evidence of their presence relinquish only an approximate estimate of their existence.

A virion in suspension, when inoculated onto susceptible cell culture penetrates a host cell, resulting in infection, viral replication, eventual death of the cell and subsequent release of infective virions. The newly replicated agent continues to infect susceptible cells resulting in a cytopathic effect (CPE) to all cells in the culture.

Measurement of the viral concentration in a specimen is usually an underestimate of the agents present due to aggregation of virions, the loss of virus during specimen manipulation through collection, concentration and isolation attempts, and because not all viruses cause a CPE. However, determination of the most probable number (MPN) of infectious units is, most certainly, a reliable, quantitative and conservative estimate of viral presence.

When estimating very small numbers of viruses in water, a MPN of infectious units can be estimated with undiluted specimens. Upon determining the number of positive and negative cultures inoculated with a specimen, an MPN can be computed from the relatively simple formula: (Chang, Shih,L. 1967)

$$\text{MPN} = -2.303 \log_{10}[(\text{number of negative cultures})/(\text{total cultures inoculated})]$$

Results and Discussion

Initial Drainfield Infiltration Soil Examinations

Soil sampling was performed at both Lost Lake Subdivision, Polk County, households 12 and 13, and Mandarin Meadows Subdivision, St. Johns County, households 22 and 24 during January, 1988. The effort was designed to detect the downward migration of human enteroviruses in the unsaturated sandy soil beneath the septic tank effluent (STE) drainfield.

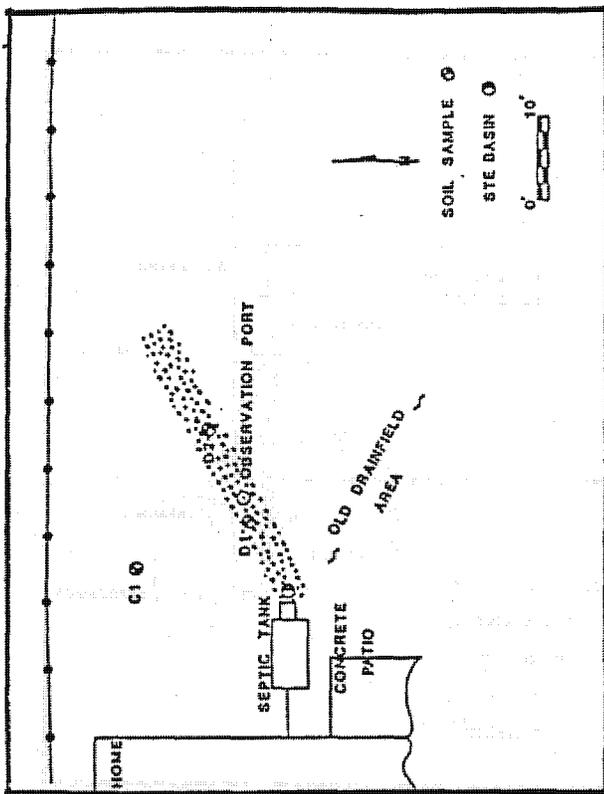
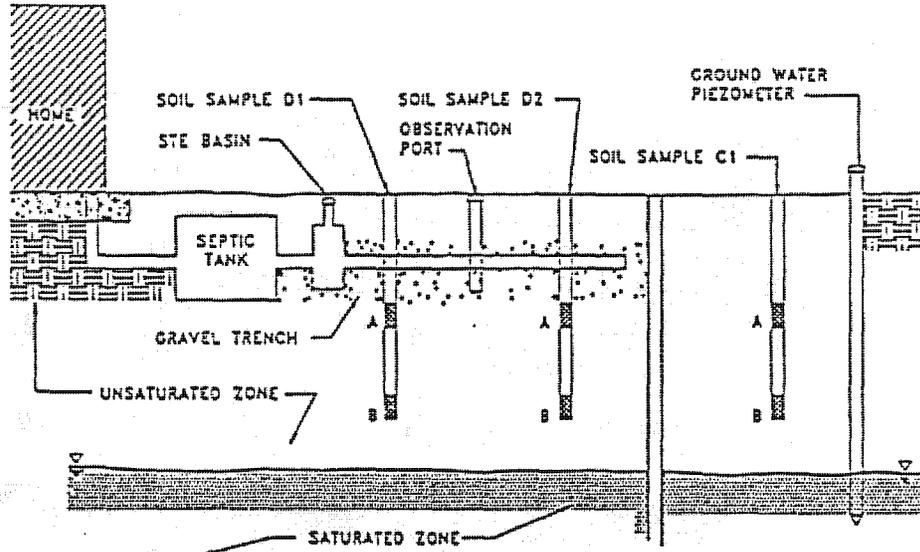
Two (2) locations at three (3) depths within each drainfield at Lost Lake Subdivision were sampled (Figure 1). The presence of high groundwater when sampling Mandarin Meadows Subdivision prevented getting an unsaturated specimen at the four (4) foot depth (Figure 2). The two (2) foot depth sampled is significant as it represents the minimum depth to groundwater for drainfields in Florida. Viruses detected at the two (2) and four (4) foot levels could indicate agents had reached groundwater.

One of the two (2) sampling locations along the STE drainfield was within five (5) feet and the other at 15-20 feet of the septic tank outlet. The presence of effluent at the sampling sites was verified through physical, chemical and biological parameters which were indicative of relatively recent exposure of the soil to waste water. These septic tank effluent contaminants were detected at the infiltrative surface plus two (2) and four (4) feet below in amounts that would be expected to occur due to waste water application. No human enteroviruses were detected in any of these fifty (50) gram specimens from either subdivision (Tables 1 & 2). Also, no virus was detected in control soil samples from each of the household

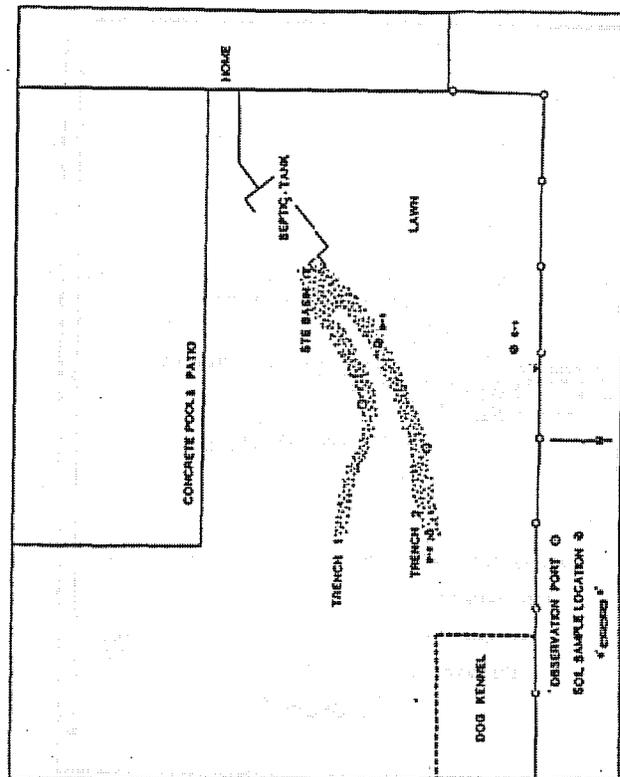
premises (yard), collected at depths corresponding to those at which STE drainfield specimens were obtained.

Viruses present in the soil at a given depth would be evidence of migration. If not detected, the agents might not have migrated due either to adsorption or inactivation in the zone between the drainfield surface and the sampling area. On the other hand, the agent may no longer have been viable or had migrated deeper, possibly into the ground water. Detection of a human enterovirus at the depths sampled would constitute evidence of migration, however lack of detection wouldn't necessarily indicate non migration.

Figure 1. Schematic of the relative subinfiltration system soil sampling locations and site layout for OSDS stations 12 and 13, Lost Lake community. (after Ayres, July 1989 Progress Report)

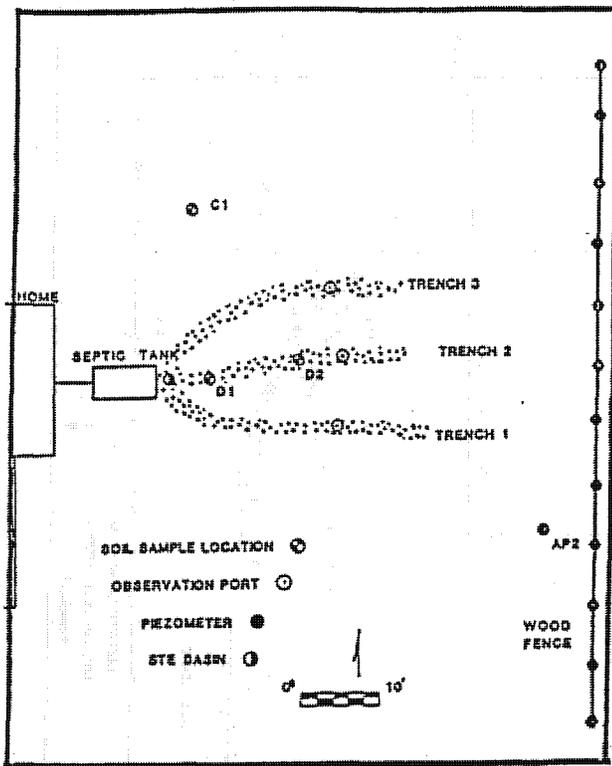
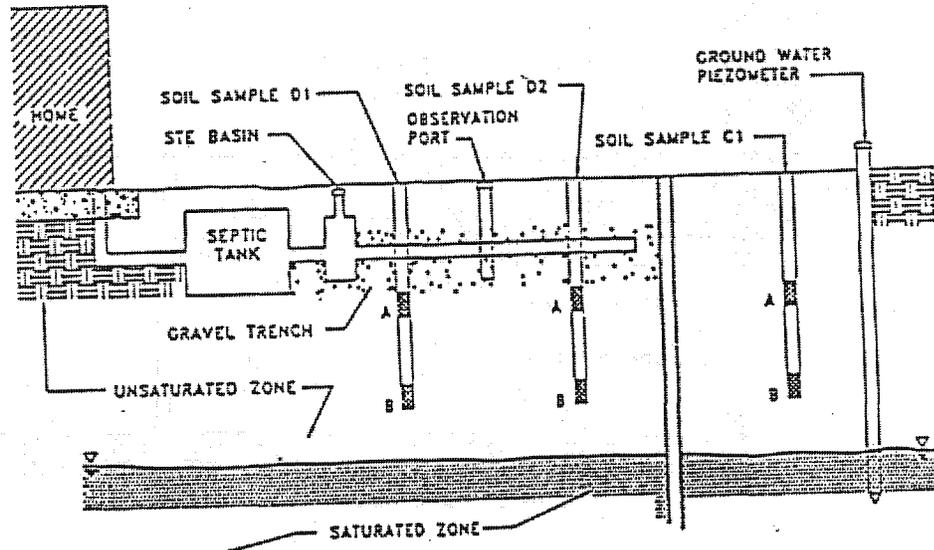


OSDS Site Plan Station 12

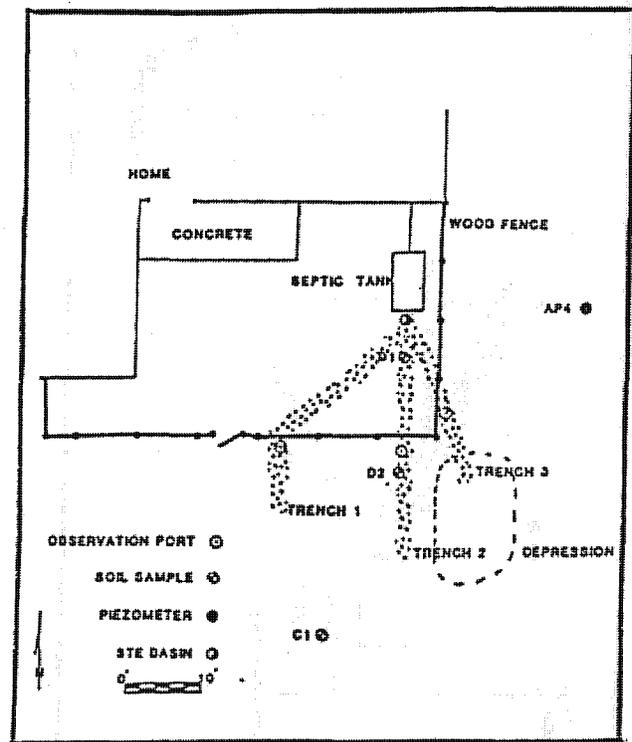


OSDS Site Plan Station 13

Figure 2. Schematic of the relative subinfiltration system soil sampling locations and site layout for OSDS stations 22 and 24, Mandarin Meadows community. (after Ayres, July 1989 Progress Report)



OSDS Site Plan Station 22



OSDS Site Plan Station 24

Table 1. Soil virology findings from Lost Lake subdivision, January 12, 13, 1988

Household #	Soil Area ^{*1}	Drainfield Site	Soil Depth	Findings MPN-IU ^{*2}
2	yard		A ^{*3}	0
2	yard		B	0
2	yard		C	0
2	drainfield	1	A	0
2	drainfield	1	B	0
2	drainfield	1	C	0
2	drainfield	2	A	0
2	drainfield	2	B	0
2	drainfield	2	C	0
3	yard		A	0
3	yard		B	0
3	yard		C	0
3	drainfield	1	A	0
3	drainfield	1	B	0
3	drainfield	1	C	0
3	drainfield	2	A	0
3	drainfield	2	B	0
3	drainfield	2	C	0

*1=50 g specimen

*2=Most Probable Number of Infectious units

*3 A=Immediately below drainfield gravel

B=2 feet below drainfield gravel

C=4 feet below drainfield gravel

Table 2. Soil virology findings from Mandarin Meadows subdivision, January 15, 1988

Household #	Soil Area*1	Drainfield Site	Soil Depth	Findings MPN-IU*2
2	yard		A*3	0
2	yard		B	0
2	drainfield	1	A	0
2	drainfield	1	B	0
2	drainfield	2	A	0
2	drainfield	2	B	0
4	yard		A	0
4	yard		B	0
4	drainfield	1	A	0
4	drainfield	1	B	0
4	drainfield	2	A	0
4	drainfield	2	B	0

*1=50 g specimen

*2=Most Probable Number of Infectious units

*3 A=Immediately below drainfield gravel

B=2 feet below drainfield gravel

Monthly examination of Ground Water from Monitor Wells

The application of septic tank effluent to soil demands consideration be given to viral survival, not only in the soil, but also the subsequent movement of the applied agents to groundwater. General considerations concerning viral survival in soil include adsorption rates, anaerobic environment, and temperature. Soils aren't homogeneous and attendant geography and climate will influence viral movement. Adsorption is reversible and doesn't necessarily involve inactivation of virus (Wellings et al 1974). Many households use private wells as a water source without adequate treatment prior to use. Thus, this water reuse of potentially viral contaminated water takes on a high degree of importance in water borne diseases.

Vaughn (1983) noted ground water entrainment of viruses following disposal of treated waste water on Long Island, New York. A prior study by

Vaughn (1978) indicated that two (2) of three waste water recharge areas had viruses appear in monitoring wells. Both studies indicate that groundwater may be mechanism for water transmission of human enteroviruses. The hydrogeological similarities between these Long Island studies and those of Florida have particular applicability to the study of these two subdivisions. Kiswich et al (1980) reviewed viruses isolated from drinking water wells and groundwater involving twenty-four (24) investigations. Viral agents identified belonged to the echovirus, coxsackievirus, poliovirus and rotavirus groups. The data indicate enteroviruses are able to travel through soil and reach groundwater.

Monitor wells were located after evaluation of data from subdivision test borings and temporary piezometers. Surveys of conductivity values of ground water showed the values decreased with distance from the residential septic drainfield systems. Therefore, wells were placed down gradient of an area of relatively high conductivity. The inferred direction of ground water flow for each of the subdivisions is depicted in Figures 3 and 4. Figures 5 and 6 show the relationship of those households whose septic tank effluent was monitored monthly, to the monitor wells in each subdivision.

Monitor well groundwater was examined monthly, fifteen times, February 1988 thru June 1989 in each of the subdivisions. The 1512 liter specimens failed to yield a viral agent on any occasion (Tables 3,4,5,6,7,8,9 and 10). The significance of these negative findings lie in the average velocity of groundwater flow in each subdivision. At Lost Lake the flow averaged from 0.01 to 0.02 feet per day or 3.65-7.3 feet per year, while at Mandarin Meadows the range was 0.03-0.05 feet per day. Even though enteroviruses were detected frequently in community fecal specimens and STE of study households, the low velocity of subdivision groundwater would preclude their arrival at the monitor wells even relatively late. Therefore, the potential for detection of virus in groundwater at the monitor wells in these subdivisions was limited to the potential of virus in STE from close-by households, less than 100 feet from the monitor wells.

{Tables 3-10: *1 Conductivity=U/mohs *2 NTU=Nephelometer Turbidity Units

*3 MPN-IU=Most Probable Number of Infectious Units}

Table 3. Laboratory findings on 1512 liter specimens of groundwater from Lost Lake subdivision, Monitor Well #2.

Collection Month	Conductivity *1	pH	NTU*2	MPN-IU*3
Feb 1988	148	4.7	1.17	0
Apr 1988	145	4.9	0.38	0
Jun 1988	172	5.0	0.46	0
Aug 1988	180	4.9	0.61	0
Sep 1988	227	4.6	0.42	0
Oct 1988	200	4.4	0.19	0
Nov 1988	190	4.3	0.35	0
Jan 1989	158	4.6	0.36	0
Jan 1989	166	4.8	0.23	0
Feb 1989	178	4.8	0.45	0
Mar 1989	163	4.5	0.39	0
Mar 1989	168	4.7	0.26	0
Apr 1989	191	4.8	0.38	0
May 1989	213	4.8	0.42	0
Jun 1989	235	4.7	0.53	0

Table 4. Laboratory findings on 1512 liter specimens of groundwater from Lost Lake subdivision, Monitor Well #3.

Collection Month	Conductivity *1	pH	NTU*2	MPN-IU*3
Feb 1988	78	4.5	0.87	0
Apr 1988	95	4.5	0.58	0
Jun 1988	117	4.5	0.66	0
Aug 1988	95	4.8	0.76	0
Sep 1988	127	4.9	0.49	0
Oct 1988	126	4.8	0.43	0
Nov 1988	126	4.8	0.46	0
Jan 1989	82	5.2	0.52	0
Jan 1989	89	5.1	0.69	0
Feb 1989	60	5.5	0.84	0
Mar 1989	62	5.2	0.93	0
Mar 1989	64	5.4	0.94	0
Apr 1989	119	4.6	0.88	0
May 1989	150	4.8	0.61	0
Jun 1989	130	4.6	0.88	0

Table 5. Laboratory findings on 1512 liter specimens of groundwater from Lost Lake subdivision, Monitor Well #4.

Collection Month	Conductivity *1	pH	NTU*2	MPN-IU*3
Feb 1988	127	4.9	1.91	0
Apr 1988	120	4.4	0.94	0
Jun 1988	157	4.8	0.65	0
Aug 1988	178	4.4	0.66	0
Sep 1988	137	4.6	1.88	0
Oct 1988	214	4.3	0.79	0
Nov 1988	187	4.0	0.58	0
Jan 1989	135	4.5	0.62	0
Jan 1989	126	4.8	0.51	0
Feb 1989	117	4.7	0.52	0
Mar 1989	105	4.5	0.40	0
Mar 1989	105	4.8	0.44	0
Apr 1989	102	5.0	1.26	0
May 1989	98	4.9	0.74	0
Jun 1989	113	4.6	0.48	0

Table 6. Laboratory findings on 1512 liter specimens of groundwater from Lost Lake subdivision, Monitor Well #5.

Collection Month	Conductivity *1	pH	NTU*2	MPN-IU*3
Feb 1988	58	4.8	2.11	0
Apr 1988	65	5.0	0.80	0
Jun 1988	70	4.6	1.74	0
Aug 1988	75	4.7	0.67	0
Sep 1988	82	4.6	1.88	0
Oct 1988	77	4.7	1.11	0
Nov 1988	82	4.8	1.37	0
Jan 1989	66	4.8	1.68	0
Jan 1989	65	4.8	1.07	0
Feb 1989	67	4.8	1.38	0
Mar 1989	85	4.7	1.39	0
Mar 1989	71	4.9	0.65	0
Apr 1989	90	4.9	0.69	0
May 1989	93	4.7	0.52	0
Jun 1989	69	4.2	0.53	0

Table 7. Laboratory findings on 1512 liter specimens of groundwater from Mandarin Meadows subdivision, Monitor Well #3.

Collection Month	Conductivity *1	pH	NTU*2	MPN-IU*3
Mar 1988	315	4.5	0.45	0
May 1988	294	4.2	0.72	0
Jul 1988	284	4.5	0.49	0
Sep 1988	400	4.8	4.00	0
Oct 1988	395	4.6	0.56	0
Nov 1988	298	4.6	1.37	0
Dec 1988	346	4.6	0.46	0
Jan 1989	325	4.5	0.22	0
Feb 1989	302	4.6	0.31	0
Feb 1989	305	4.6	0.27	0
Mar 1989	274	4.4	0.35	0
Apr 1989	286	4.5	0.45	0
May 1989	284	4.8	0.62	0
Jun 1989	256	4.6	0.46	0
Jul 1989	247	4.4	0.61	0

Table 8. Laboratory findings on 1512 liter specimens of groundwater from Mandarin Meadows subdivision, Monitor Well #4.

Collection Month	Conductivity *1	pH	NTU*2	MPN-IU*3
Mar 1988	663	5.6	1.13	0
May 1988	593	5.2	0.59	0
Jul 1988	633	5.8	0.76	0
Sep 1988	775	5.6	1.00	0
Oct 1988	818	5.8	1.02	0
Nov 1988	603	5.7	2.70	0
Dec 1988	525	5.9	0.55	0
Jan 1989	516	5.5	0.72	0
Feb 1989	438	5.5	0.89	0
Feb 1989	533	5.0	1.66	0
Mar 1989	485	5.7	0.80	0
Apr 1989	489	5.6	0.76	0
May 1989	338	5.5	1.06	0
Jun 1989	332	5.6	0.91	0
Jul 1989	302	5.5	0.43	0

Table 9. Laboratory findings on 1512 liter specimens of groundwater from Mandarin Meadows subdivision, Monitor Well #5.

Collection Month	Conductivity *1	pH	NTU*2	MPN-IU*3
Mar 1988	581	4.5	0.77	0
May 1988	635	4.6	0.24	0
Jul 1988	625	4.3	0.45	0
Sep 1988	583	4.3	5.13	0
Oct 1988	573	4.4	1.58	0
Nov 1988	593	4.5	2.80	0
Dec 1988	481	4.4	0.58	0
Jan 1989	365	4.5	0.28	0
Feb 1989	317	4.6	0.70	0
Feb 1989	328	4.2	0.34	0
Mar 1989	298	4.6	0.31	0
Apr 1989	310	4.8	0.21	0
May 1989	277	4.6	0.28	0
Jun 1989	270	4.6	0.44	0
Jul 1989	269	4.6	0.42	0

Table 10. Laboratory findings on 1512 liter specimens of groundwater from Mandarin Meadows subdivision, Monitor Well #6.

Collection Month	Conductivity *1	pH	NTU*2	MPN-IU*3
Mar 1988	538	5.6	2.73	0
May 1988	615	5.8	4.94	0
Jul 1988	583	5.7	3.10	0
Sep 1988	545	5.5	6.98	0
Oct 1988	593	5.6	2.55	0
Nov 1988	545	5.9	2.3	0
Dec 1988	516	5.8	0.89	0
Jan 1989	509	5.7	0.61	0
Feb 1989	531	5.8	0.48	0
Feb 1989	527	5.8	0.52	0
Mar 1989	520	6.0	0.45	0
Apr 1989	530	6.0	0.47	0
May 1989	526	6.0	0.58	0
Jun 1989	509	5.8	0.49	0
Jul 1989	535	6.0	0.54	0

Figure 3. Inferred direction of ground water flow in the surficial aquifer beneath the Polk County study site (after Ayres, July 1989 Progress Report).

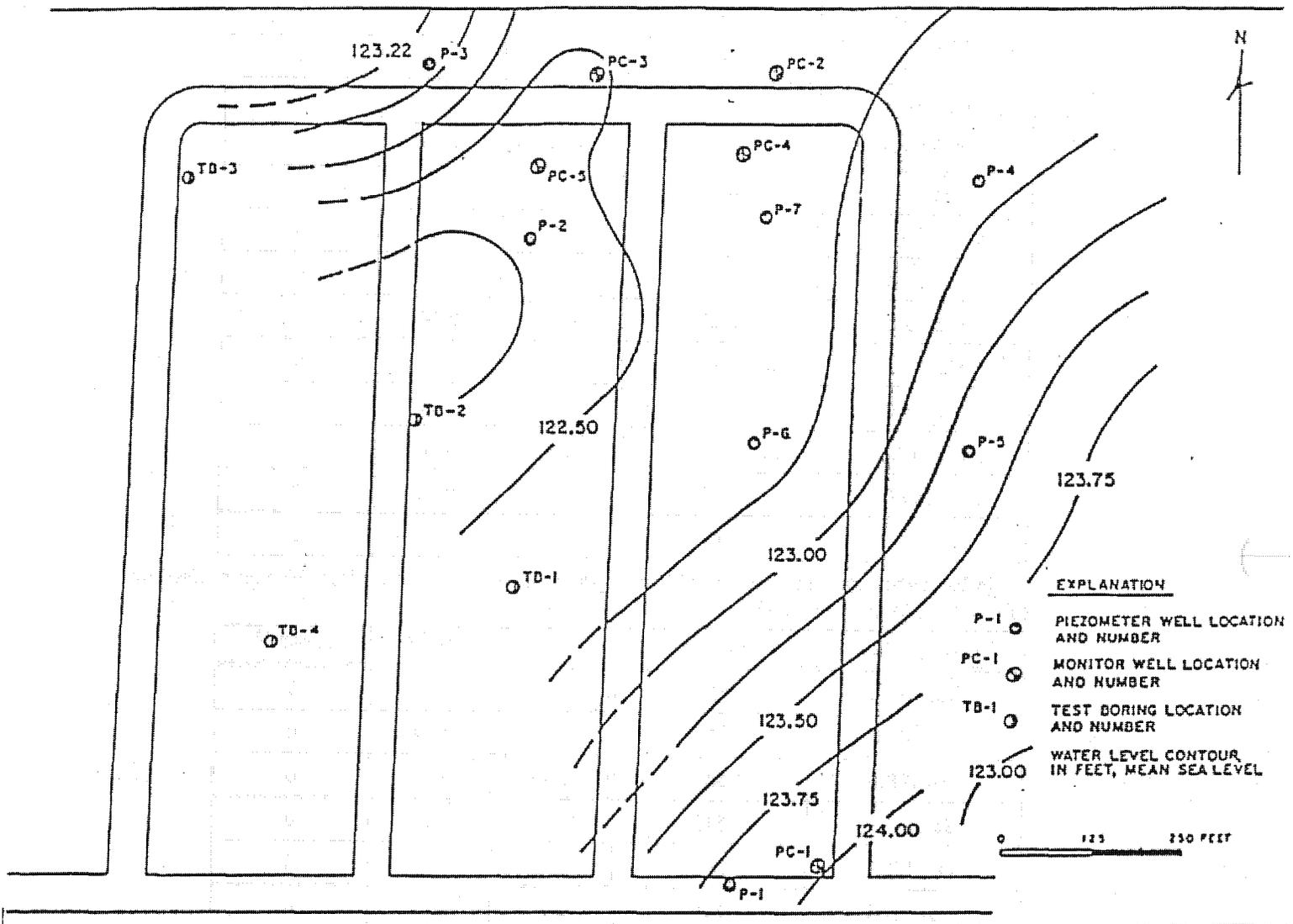


Figure 4. Inferred direction of ground water flow in the surficial aquifer beneath the St. Johns County study site (after Ayres, July 1989 Progress Report).

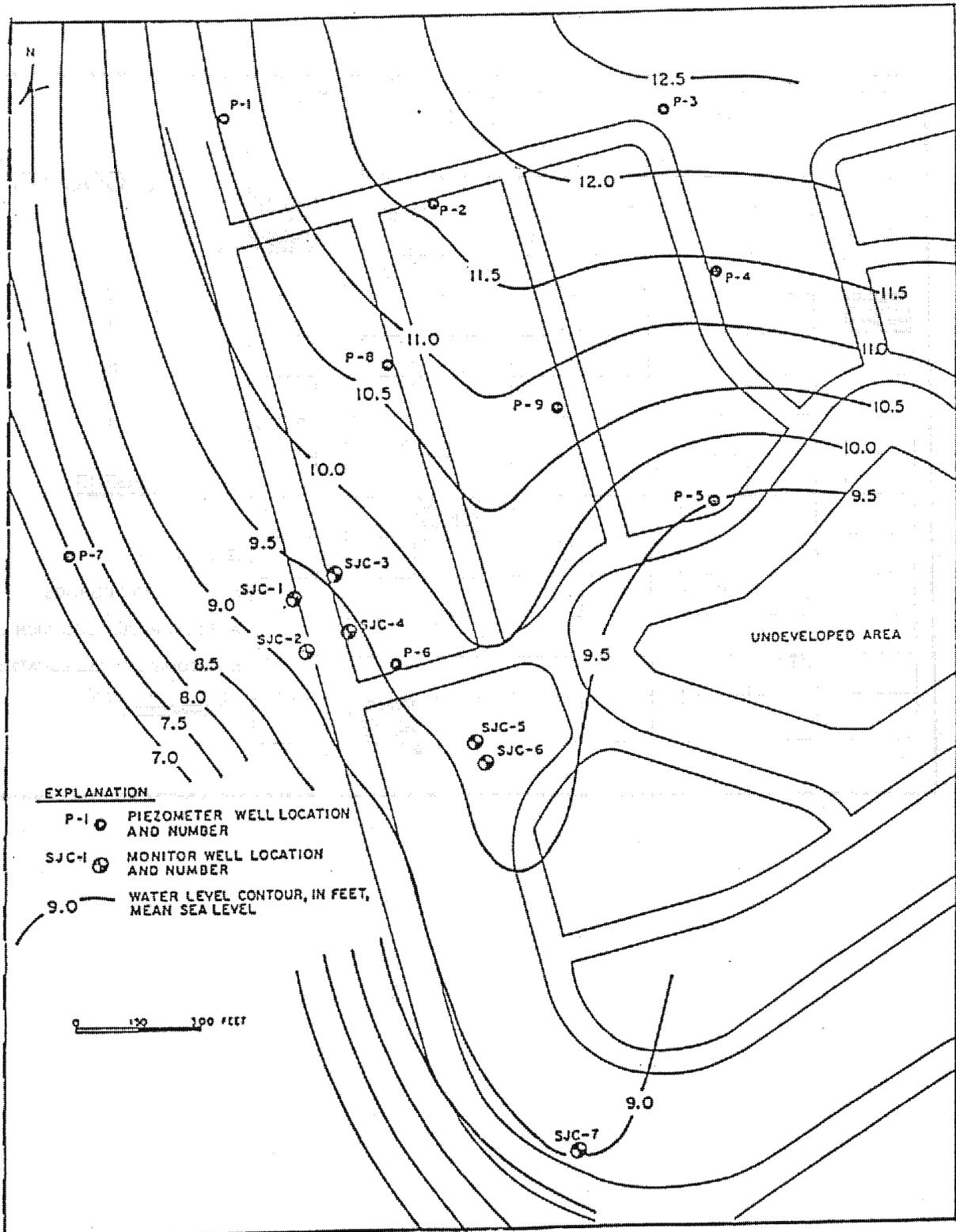


Figure 5. Map of Lost Lake subdivision showing locations of homes where individual OSDSs were monitored. (after Ayres, July 1989 Progress Report)

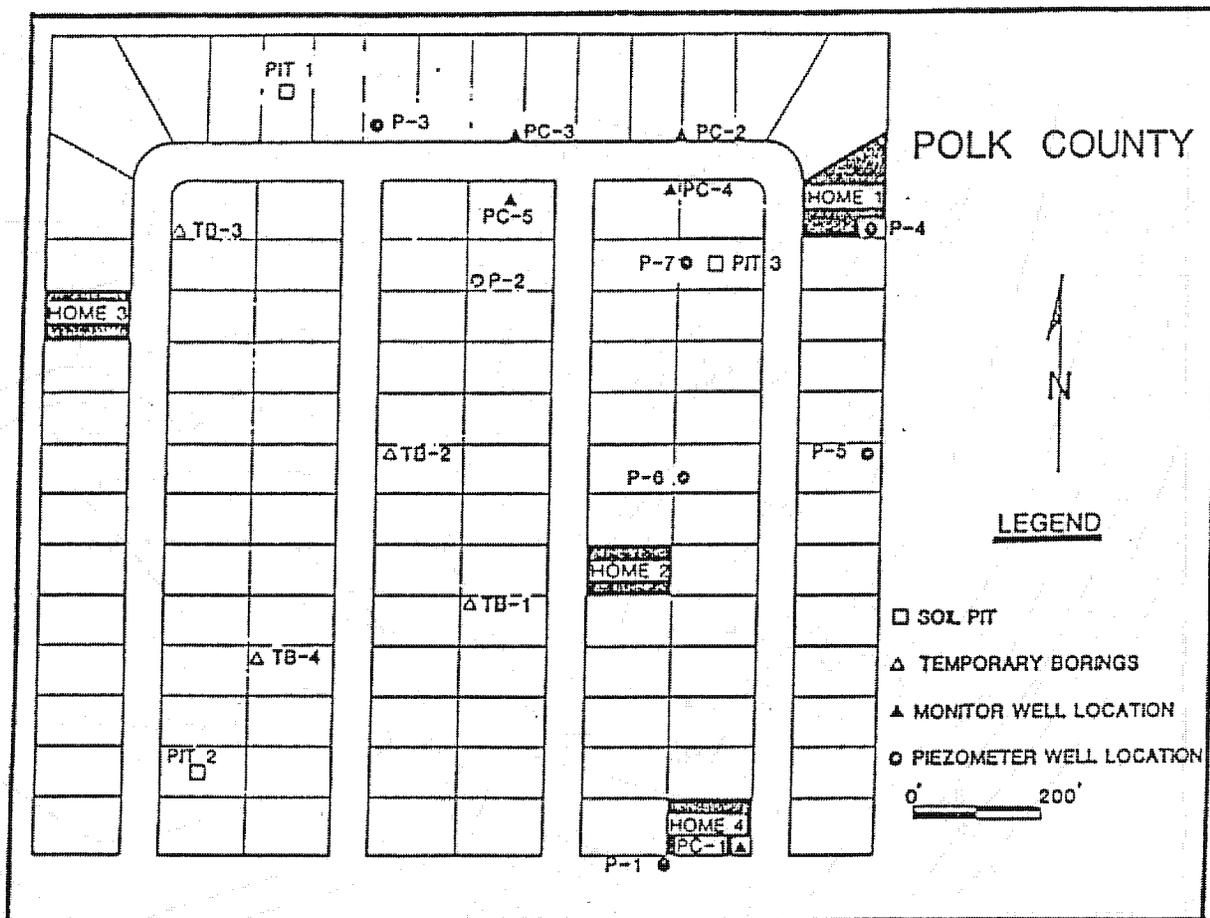
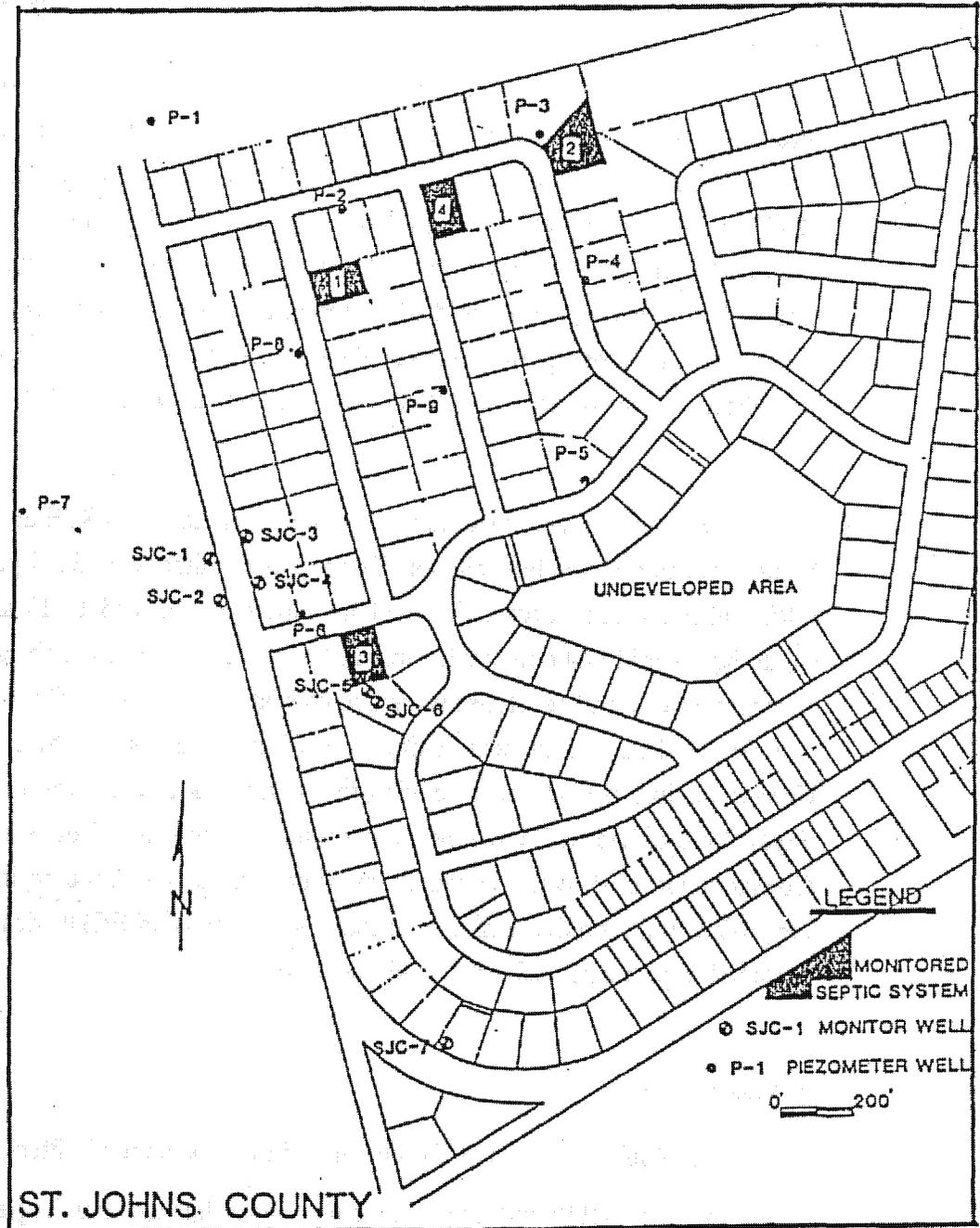


Figure 6. Map of Mandarin Meadows subdivision showing locations of homes where individual OSDSs were monitored. (after Ayres, July 1989 Progress Report)



Enterovirus Detected in Household Septic Tank Effluent

The effluent from four (4) Lost Lake Subdivision septic tanks was examined qualitatively and quantitatively for the presence of human enteroviruses, monthly. The volume in liters examined plus the most probable number of infectious units per liter (MPN-IU/L) of the viral serotypes detected is shown in tables 11,12,13 and 14. Household 11 had virus detected in STE on four (4) occasions. The five agents exiting household 12 were, not only more numerous, but also excreted over a longer time period; ECHOvirus 14 for at least 78 days, ECHOvirus 12 for at least 52 days, Coxsackievirus B2 for at least 30 days and reovirus for at least 137 days. Over the study period, seven (7) viruses were detected in STE from household 13. Among these Coxsackievirus A9 was noted over at least a 45 day period. Coxsackievirus B4, among seven viruses, from household 14 was detected in effluent over at least a 70 day period.

The same series of examinations were conducted on STE at Mandarin Meadows Subdivision households. The volume in liters examined plus the MPN-IU/L and the viral serotypes detected are shown in tables 15,16,17 and 18. Only a single household had detectable virus in STE over the length of the study. Six different enteroviral serotypes were detected at household 22; Coxsackievirus B4 was detected on two successive days following a clean-out of the septic tank, Coxsackievirus B5 was found exiting the septic tank over a period of at least 70 days, Coxsackievirus B3 was noted in effluent for at least 30 days, and Coxsackievirus A9 was isolated from effluent for at least 55 days in first increasing and then decreasing amounts. A reovirus and ECHOvirus 9 were detected in effluent, each on a single occasion.

Tables 11 - 18:

*1 Vol/L= Volume of Septic tank effluent examined in liters

*2 MPN-IU/L=Most probable number of infectious units per liter

Table 11. Laboratory findings on drainfield septic tank effluent at Lost Lake subdivision, household # 11.

Collection	Date	Vol/L *1	MPN-IU/L *2	Serotype
Apr 11	1988	3.8	0	
Aug 09	1988	8.0	0	
Sep 28	1988	15.1	0	
Nov 01	1988	15.1	0	
Nov 21	1988	11.3	0	
Jan 18	1989	11.3	0	
Feb 01	1989	15.1	0	
Feb 23	1989	15.1	0	
Mar 08	1989	11.3	0	
Mar 22	1989	3.8	0	
Apr 19	1989	11.3	0	
May 24	1989	9.5	0	
Jun 27	1989	18.9	0	
Jul 26	1989	9.5	0	
Aug 15	1989	11.3	0	
Sep 06	1989	15.1	0	
Sep 28	1989	7.6	0	
Oct 18	1989	22.6	0	
Nov 28	1989	22.6	0	
Dec 13	1989	18.9	0	
Jan 23	1990	7.6	0	
Feb 19	1990	3.8	0	
Mar 05	1990	3.8	0	
Apr 16	1990	3.8	0	
Apr 17	1990	3.8	1.07	Coxsackievirus B4
Apr 30	1990	3.8	0	
Jun 05	1990	household vacant		
Jul 03	1990	household vacant		
Aug 14	1990	household vacant		
Sep 27	1990	household vacant		
Oct 23	1990	household vacant		
Nov 15	1990	11.3	0.09	Coxsackievirus B1
Dec 12	1990	15.1	0.07	Poliovirus 1
Jan 16	1991	18.9	0	
Feb 18	1991	15.1	0	
Mar 13	1991	11.3	0	
Apr 03	1991	11.3	0	
May 15	1991	15.1	0.38	ECHOvirus 6

Table 12. Laboratory findings on drainfield septic tank effluent at Lost Lake subdivision, household # 12.

Collection	Date	Vol/L *1	MPN-IU/L *2	Serotype
Apr 11	1988	30.2	0	
Aug 09	1988	11.6	0	
Sep 28	1988	15.1	0	
Nov 01	1988	18.9	>5.03	ECHOvirus 14
Nov 21	1988	18.9	>3.62	ECHOvirus 14
Jan 18	1989	15.1	0.81	ECHOvirus 14
Feb 01	1989	15.1	>5.68	ECHOvirus 12
Feb 23	1989	15.1	0.21	ECHOvirus 12
Mar 08	1989	9.5	0	
Mar 22	1989	17.0	0.60	ECHOvirus 12
Apr 19	1989	15.1	0	
May 24	1989	18.9	0	
Jun 27	1989	11.3	0	
Jul 26	1989	15.1	0	
Aug 15	1989	15.1	0	
Sep 06	1989	18.9	0	
Sep 28	1989	11.3	0	
Oct 18	1989	18.9	0	
Nov 28	1989	37.8	0	
Dec 13	1989	30.2	0	
Jan 23	1990	32.9	0	
Feb 19	1990	18.9	0	
Mar 05	1990	15.1	0	
Apr 16	1990	24.3	0	
Apr 30	1990	26.4	0	
Jun 05	1990	11.3	>58.57	Coxsackievirus B2
Jun 06	1990	15.1	>11.32	Coxsackievirus B2
Jul 03	1990	11.3	>1.35	Coxsackievirus B2
Aug 14	1990	18.9	0	
Sep 27	1990	18.9	0	
Oct 23	1990	18.9	>6.34	Reovirus
Nov 15	1990	15.1	>8.20	Reovirus
Dec 12	1990	15.1	>7.93	Reovirus
Jan 16	1991	18.9	0	
Feb 18	1991	18.9	>5.46	Reovirus
Mar 13	1991	15.1	>5.75	Reovirus
Apr 03	1991	18.9	0	
May 15	1991	18.9	0.48	ECHOvirus 6

Table 13. Laboratory findings on drainfield septic tank effluent at Lost Lake subdivision, household # 13.

Collection	Date	Vol/L *1	MPN-IU/L *2	Serotype
Apr 11	1988	45.4	0	
Aug 09	1988	9.7	0	
Sep 28	1988	11.3	0	
Nov 01	1988	7.6	0.89	ECHOvirus 14, Polio 1 & 2
Nov 21	1988	15.1	0.07	ECHOvirus 14
Jan 18	1989	7.6	0	
Feb 01	1989	7.6	0.14	ECHOvirus 12
Feb 23	1989	11.2	0	
Mar 08	1989	3.8	0	
Mar 22	1989	7.6	0	
Apr 19	1989	5.7	0	
May 24	1989	7.6	0	
Jun 27	1989	15.1	0	
Jul 26	1989	7.6	0	
Aug 15	1989	11.2	0	
Sep 06	1989	7.6	0	
Sep 28	1989	5.7	0	
Oct 18	1989	12.5	0	
Nov 28	1989	30.2	0	
Dec 13	1989	15.1	0	
Jan 23	1990	26.5	0	
Feb 19	1990	11.2	0	
Mar 05	1990	11.2	0	
Apr 16	1990	7.6	2.9	Coxsackievirus A9
Apr 17	1990	3.8	0	
Apr 30	1990	7.6	0.78	Coxsackievirus A9
May 01	1990	11.2	0	
Jun 05	1990	15.1	0.60	Coxsackievirus A9
Jun 06	1990	18.9	0.30	Coxsackievirus A9
Jul 03	1990	11.2	0	
Aug 14	1990	11.2	0.36	Coxsackievirus B3
Sep 27	1990	15.1	0.14	ECHOvirus 22/23 complex
Oct 23	1990	11.2	0	
Nov 15	1990	household vacant		
Dec 12	1990	household vacant		
Jan 16	1991	household vacant		
Feb 18	1991	household vacant		
Mar 13	1991	household vacant		
Apr 03	1991	household vacant		
May 15	1991	household vacant		

Table 14. Laboratory findings on drainfield septic tank effluent at Lost Lake subdivision, household # 14.

Collection	Date	Vol/L *1	MPN-TU/L *2	Serotype
Apr 11	1988	11.3	0	
Aug 09	1988	13.5	0	
Sep 28	1988	11.3	0	
Nov 01	1988	11.3	>0.20	ECHOvirus 14
Nov 21	1988	11.3	>5.20	Polioviruses 1 & 2
Nov 29	1988	11.3	21.5	Polioviruses 1 & 2
Jan 18	1989	11.3	0	
Feb 01	1989	11.3	0	
Feb 23	1989	11.3	0	
Mar 08	1989	9.5	0	
Mar 22	1989	13.2	0.08	Poliovirus 3
Apr 19	1989	11.3	0.49	Polioviruses 1, 2, & 3
May 24	1989	7.6	0	
Jun 27	1989	15.1	0	
Jul 26	1989	15.1	0	
Aug 15	1989	11.3	0	
Sep 06	1989	18.9	0	
Sep 28	1989	11.1	0	
Oct 18	1989	37.8	0	
Nov 28	1989	22.7	0	
Dec 13	1989	22.7	0	
Jan 23	1990	35.0	0	
Feb 19	1990	14.2	>7.20	Coxsackievirus B4
Mar 05	1990	15.1	>12.2	Coxsackievirus B4
Mar 06	1990	15.1	>12.2	Coxsackievirus B4
Mar 28	1990	15.1	>12.4	Coxsackievirus B4
Apr 16	1990	15.1	1.45	Coxsackievirus B4
Apr 17	1990	15.1	0.32	Coxsackievirus B4
Apr 30	1990	11.3	0.09	Coxsackievirus B4
May 01	1990	11.3	0	
Jun 05	1990	22.6	0	
Jul 03	1990	11.3	0	
Aug 14	1990	11.3	0	
Sep 27	1990	15.1	>5.62	Poliovirus 3
Oct 23	1990	15.1	0	
Nov 15	1990	15.1	0	
Dec 12	1990	15.1	0	
Jan 16	1991	11.3	0	
Feb 18	1991	11.3	0	
Mar 13	1991	11.3	0	
Apr 03	1991	15.1	0	
May 15	1991	18.9	>3.79	ECHOvirus 6

Table 15. Laboratory findings on drainfield septic tank effluent at Mandarin Meadows subdivision, household # 21.

Collection	Date	Vol/L *1	MPN-IU/L *2	Serotype
Aug 23	1988	18.9	0	
Sep 14	1988	15.1	0	
Oct 05	1988	13.1	0	
Nov 16	1988	18.9	0	
Dec 14	1988	15.1	0	
Jan 25	1989	18.9	0	
Feb 15	1989	15.1	0	
Mar 01	1989	11.3	0	
Mar 28	1989	18.9	0	
Apr 11	1989	15.1	0	
May 16	1989	18.9	0	
Jun 06	1989	18.9	0	
Jul 11	1989	18.9	0	
Aug 08	1989	18.9	0	
Sep 20	1989	37.8	0	
Oct 03	1989	18.9	0	
Oct 31	1989	37.8	0	
Dec 05	1989	41.6	0	
Jan 09	1990	41.6	0	
Jan 30	1990	15.1	0	
Feb 05	1990	19.8	0	
Mar 12	1990	18.9	0	
Apr 02	1990	18.9	0	
May 09	1990	18.9	0	
Jun 19	1990	18.9	0	
Jul 16	1990	18.9	0	
Aug 29	1990	no specimen available		
Sep 25	1990	15.1	0	
Oct 10	1990	18.9	0	
Nov 27	1990	18.9	0	
Dec 10	1990	17.0	0	
Jan 07	1991	15.1	0	
Feb 04	1991	15.1	0	
Mar 04	1991	17.0	0	
Apr 08	1991	no specimen available		
May 30	1991	15.1	0	

Table 16. Laboratory findings on drainfield septic tank effluent at Mandarin Meadows subdivision, household # 22.

Collection	Date	Vol/L *1	MPN-TU/L *2	Serotype
Aug 23	1988	1.5 ^{*3}	10.3	Coxsackievirus B4
Aug 24	1988	15.1	0.22	Coxsackievirus B4
Sep 14	1988	15.1	0	
Oct 05	1988	18.9	0	
Nov 16	1988	18.9	0.23	Coxsackievirus B5
Dec 14	1988	15.1	>2.0	Coxsackievirus B5
Jan 25	1989	18.9	0.11	Coxsackievirus B5
Feb 15	1989	15.1	0	
Mar 01	1989	13.2	0	
Mar 28	1989	18.9	0	
Apr 11	1989	18.9	0	
May 16	1989	20.8	0	
Jun 06	1989	15.1	0	
Jul 11	1989	18.9	0	
Aug 08	1989	18.9	0	
Sep 20	1989	37.8	0	
Oct 03	1989	18.9	0	
Oct 31	1989	18.9	0	
Dec 05	1989	37.8	0	
Jan 08	1990	18.9	1.71	Coxsackievirus B3
Jan 09	1990	18.9	0.57	Coxsackievirus B3
Jan 30	1990	18.9	0.11	Coxsackievirus B3
Feb 05	1990	39.7	0.10	Coxsackievirus B3
Feb 06	1990	18.9	0	
Feb 26	1990	9.0	0	
Mar 12	1990	18.9	2.60	Coxsackievirus A9
Apr 02	1990	17.0	>7.29	Coxsackievirus A9
May 09	1990	17.0	0.19	Coxsackievirus A9
Jun 19	1990	18.9	>1.51	Reovirus
Jul 16	1990	18.9	0	
Aug 29	1990	30.2	0	
Sep 25	1990	18.9	0	
Oct 10	1990	18.9	0	
Nov 27	1990	18.9	0	
Dec 10	1990	17.0	0	
Jan 07	1991	15.1	0.68	ECHOvirus 9
Feb 04	1991	18.9	0	
Mar 04	1991	22.6	0	
Apr 10	1991	18.9	0	
May 30	1991	15.1	0	

*3= Sludge obtained during a pump out of the septic tank to a clean-out condition

Table 17. Laboratory findings on drainfield septic tank effluent at Mandarin Meadows subdivision, household # 23.

Collection	Date	Vol/L *1	MPN-IU/L *2	Serotype
Aug 23	1988	7.6	0	
Sep 14	1988	15.1	0	
Oct 05	1988	15.1	0	
Nov 16	1988	15.1	0	
Dec 14	1988	15.1	0	
Jan 25	1989	15.1	0	
Feb 15	1989	15.1	0	
Mar 01	1989	11.3	0	
Mar 28	1989	18.9	0	
Apr 11	1989	15.1	0	
May 16	1989	20.8	0	
Jun 06	1989	18.9	0	
Jul 11	1989	18.9	0	
Aug 08	1989	15.1	0	
Sep 20	1989	37.8	0	
Oct 03	1989	15.1	0	
Oct 31	1989	18.9	0	
Dec 05	1989	32.1	0	
Jan 09	1990	37.8	0	
Jan 30	1990	15.1	0	
Feb 05	1990	52.0	0	
Mar 12	1990	15.1	0	
Apr 02	1990	15.1	0	
May 09	1990	18.9	0	
Jun 19	1990	18.9	0	
Jul 16	1990	18.9	0	
Aug 29	1990	26.5		
Sep 25	1990	18.9	0	
Oct 10	1990	18.9	0	
Nov 27	1990	18.9	0	
Dec 10	1990	no specimen available	0	
Jan 07	1991	18.9	0	
Feb 04	1991	18.9	0	
Mar 04	1991	no specimen available	0	
May 30	1991	18.9	0	

Table 18. Laboratory findings on drainfield septic tank effluent at Mandarin Meadows subdivision, household # 24.

ollection	Date	Vo/L *1	MPN-IU/L *2	Serotype
Aug 23	1988	11.3	0	
Sep 14	1988	11.3	0	
Oct 05	1988	11.3	0	
Nov 16	1988	18.9	0	
Dec 14	1988	15.1	0	
Jan 25	1989	18.9	0	
Feb 15	1989	15.1	0	
Mar 01	1989	13.2	0	
Mar 28	1989	*4		

*4 This residence was lost to the study due to the drainfield failure and the owner's reluctance to continue participation.

Tables 19 and 20 summarize the enteroviral serotypes isolated by date and household from the STE within each subdivision. The frequent occurrence of polioviruses in households is the result of the immunization of some household member with the oral vaccine against poliomyelitis. Vaccinees often shed the immunizing agents for several weeks after immunization. The remaining agents and their distribution are a further indication that in Florida there is no seasonality to enteroviral infections as seen in the more northerly states of the United States. Financial constraints permitted only a portion of the more than 100 enteric viruses that may be found in infected human feces to be sought. These viruses include adenoviruses, enteroviruses (coxsackieviruses, polioviruses, and echoviruses), hepatitis A virus, Norwalk virus, Norwalk-like viruses, reoviruses and rotaviruses. All of these are known or suspected of possessing a potential for water transmission (Metcalf 1978).

Table 19. Enterovirus serotype isolated from septic tank effluent by collection date and Lost Lake household.

collection	date	11	12	13	14
Apr 11	1988				
Aug 09	1988				
Sep 28	1988				
Nov 01	1988		E14	E14, P1, P2	E14
Nov 21	1988		E14	E14	P1, P2
Nov 29	1988				P1, P2
Jan 18	1989		E14		
Feb 01	1989		E12	E12	
Feb 23	1989		E12		
Mar 08	1989				
Mar 22	1989		E12		P3
Apr 19	1989				P1, P2, P3
May 24	1989				
Jun 27	1989				
Jul 26	1989				
Aug 15	1989				
Sep 06	1989				
Sep 28	1989				
Oct 18	1989				
Nov 28	1989				
Dec 13	1989				
Jan 23	1990				
Feb 19	1990				CB4
Mar 05	1990				CB4
Mar 06	1990				CB4
Mar 28	1990				CB4
Apr 16	1990			CA9	CB4
Apr 17	1990	CB4			CB4
Apr 30	1990			CA9	CB4
May 01	1990				
Jun 05	1990		CB2	CA9	
Jun 06	1990		CB2	CA9	
Jul 03	1990		CB2		
Aug 14	1990			CB3	
Sep 27	1990			E22/23	P3
Oct 23	1990		Reo		
Nov 15	1990	CB1	Reo		
Dec 12	1990	P3	Reo		
Jan 16	1990				
Feb 18	1990		Reo		
Mar 13	1990		Reo		
Apr 03	1990				
May 15	1990	E6	E6		E6

Table 20. Enterovirus serotype isolated from septic tank effluent by collection date and Mandarin Meadows household.

collection	date	21	21	21	21
Aug 23	1988		CB4		
Aug 24	1988		CB4		
Sep 14	1988				
Oct 05	1988				
Nov 16	1988		CB5		
Dec 14	1988		CB5		
Jan 25	1989		CB5		
Feb 15	1989				
Mar 01	1989				
Mar 28	1989				
Apr 11	1989				
May 16	1989				
Jun 06	1989				
Jul 11	1989				
Aug 08	1989				
Sep 20	1989				
Oct 03	1989				
Oct 31	1989				
Dec 05	1989				
Jan 08	1990		CB3		
Jan 09	1990		CB3		
Jan 30	1990		CB3		
Feb 05	1990		CB3		
Feb 06	1990				
Feb 26	1990				
Mar 12	1990		CA9		
Apr 02	1990		CA9		
May 09	1990		CA9		
Jun 19	1990		Reo		
Jul 16	1990				
Aug 29	1990				
Sep 25	1990				
Oct 10	1990				
Nov 27	1990				
Dec 10	1990				
Jan 07	1991		E9		
Feb 04	1991				
Mar 04	1991				
Apr 10	1991				
May 20	1991				

CA=Coxsackievirus A; CB=Coxsackievirus B; E=ECHOvirus; Reo=Reovirus

Household viral infections and presence of the same infecting serotypes in septic tank effluent:

Fecal specimens were solicited from three (3) study households with children below 13 years of age in the Lost Lake Subdivision on a monthly basis and examined for the presence of enteroviruses. Table 21 indicates the date virus was isolated from householder's feces plus the quantity of the same viral serotype isolated from STE during a detection period. Three viruses were isolated from household 12: ECHOvirus 14, ECHOvirus 12, and Coxsackievirus B2. Each of these three (3) agents were also detected in septic tank effluent at levels varying from a specific MPN-IU/L to a condition wherein infectious units were too numerous to estimate. The length of time each viral agent was noted exiting the septic tank varied: ECHOvirus 14 at least 80 days, ECHOvirus 12 at least 20 days and Coxsackievirus B2 at least 30 days.

Table 21: Association of household viral excretion with duration of detection in septic tank effluent, Lost Lake subdivision household 12.

Date		Household excretion	MPN-IU/L in STE
Aug 10	1988	0	
Sep 28	1988		0
Nov 01	1988		>5.03 (E14)
Nov 14	1988		>3.62 (E14)
Dec 04	1988	E14	
Jan 18	1989		0.81 (E14)
Feb 01	1989		>5.68 (E12)
Feb 02	1989	E12	
Feb 23	1989		0.21 (E12)
Mar 03	1989	E12	
Mar 08	1989	0	0
Mar 10	1990	0	
Apr 30	1990		0
May 23	1990	CB2	
Jun 05	1990		>58.57 (CB2)
Jun 06	1990		>11.32 (CB2)
Jun 13	1990	CB2	
Jun 18	1990	CB2	
Jul 03	1990		>1.35 (CB2)
Aug 07	1990	0	
Aug 14	1990		0

MPN-IU/L=Most Probable Number of Infectious Units per liter Septic Tank Effluent

E14=ECHOvirus 14 E12=ECHOvirus 12 CB2=Coxsackievirus B2 Blank cell=no sample for that date

Another Lost Lake subdivision household, 14, (Table 22) also provided frequent fecal specimens. On four (4) occasions the identical enteroviral serotype was isolated from the householder's feces and temporally related to the same virus isolated from STE. Following oral modified live poliovirus immunization within the household, various poliovirus serotypes were found in septic tank effluent in decreasing amounts, November 21 and 29, 1988. Poliovirus in feces was again associated with detection in effluent over at least a thirty (30) day period, March 22 through April 19, 1988. Coxsackievirus B4's presence in both feces and septic tank effluent was noted on February 19, 1990. Initially, an increasing and then subsequently a decreasing MPN-IU/L of Coxsackievirus B4 was detected in effluent over at least a sixty (60) day period. Finally, a single poliovirus serotype in household feces paralleled the appearance of the same agent in septic tank effluent in greater than 5.62 poliovirus type 3 MPN-IU/L.

Table 22: Association of household viral excretion with duration of detection in septic tank effluent, Lost Lake subdivision household 14.

Date		Household excretion	MPN-IU/L in STE
Oct 10	1988	P1	
Nov 11	1988		>2.00 (E14)
Nov 12	1988	P1 & P2	
Nov 21	1988		>5.20 (P1 & P2)
Nov 29	1988		21.50 (P1 & P2)
Dec 03	1988	P1 & P2	
Dec 15	1988	P1 & P2	
Dec 24	1988	P1 & P2	
Jan 18	1989		0
Mar 06	1989	0	
Mar 20	1989	P3	
Mar 22	1989		0.08 (P3)
Mar 24	1989	P3	
Apr 01	1989	P3	
Apr 06	1989	P3	
Apr 18	1989	P1 & P3	
Apr 19	1989		0.49 (P1, P2, & P3)
May 15	1989	P3	
May 24	1989		0
Jun 05	1989	P3	
Jun 11	1989	0	
Jun 27	1989		0

Table 22: Association of household viral excretion with duration of detection in septic tank effluent, Lost Lake subdivision household 14. (continued)

Date		Household excretion	MPN-IU/L in STE
Feb 19	1990	CB4	>7.20 (CB4)
Mar 05	1990		>12.20 (CB4)
Mar 06	1990		>12.20 (CB4)
Mar 07	1990	CB4	
Mar 09	1990	CB4	
Mar 12	1990	CB4	
Mar 28	1990		>12.40 (CB4)
Apr 16	1990		1.45 (CB4)
Apr 17	1990		0.32 (CB4)
Apr 30	1990	0	
May 01	1990		0
Sep 11	1990	P3	
Sep 27	1990	P3	>5.62 (P3)
Oct 26	1990	0	
Oct 23	1990		0

MPN-IU/L=Most Probable Number of Infectious Units per liter Septic Tank Effluent

E14=ECHOvirus 14 P1, P2, P3= Polioviruses 1, 2, & 3 CB4=Coxsackievirus B4 Blank cell=no sample for that date

The close occurrence of the same virus in feces from household 13 and in the septic tank effluent was noted on a single occasion (Table 23).

Table 23. Association of household viral excretion with duration of detection in septic tank effluent, Lost Lake subdivision household 13.

Date		Household excretion	MPN-IU/L in STE
Aug 12	1988	0	
Sep 13	1988	P1	
Sep 28	1988		0
Nov 01	1988		0.89 (E14, P1 & P2)
Nov 03	1988	0	
Nov 21	1988		0.07 (E14)
Jan 18	1988		0

MPN-IU/L=Most Probable Number of Infectious Units per liter Septic Tank Effluent

E14=ECHOvirus 14 P1=Poliovirus 1 Blank cell=no sample for that date

Soil sampling following detection of virus concomitantly in feces and septic tank effluent.

Initially, examination of septic tank drainfield soil cores from households 12, 13, 22, and 24 had failed to detect the presence of virus (Tables 1 and 2). However, at the time of those collections, the occurrence of the last virus discharges to the soil were unknown. Additionally, factors such as pH, soil type, soil moisture and ionic changes all influence virus movement and survival in soil. Thus, if a discharge had occurred it might have migrated beyond the length of the soil core or have already been inactivated over time.

Coxsackievirus B4 was detected concomitantly in feces and septic tank effluent at household 14 on 2-19-90. Soil cores were collected on 3-28-90 beneath the septic tank drainfields along the two (2) trenches (Figure 7). A summary of all the sampling results relative to fecal virus and isolations from soil cores at various depths below the infiltration area plus the quantity of Coxsackievirus B4 in septic tank effluent is shown in Table 24. Coxsackievirus B4 was isolated from both trenches at the four (4) inch depth below the infiltration area only. The most probable number of infectious units per gram of soil was determined to be 0.015 for trench 1 and 0.014 for trench 2. At the time of the soil core collections the same viral serotype in STE was too high to estimate beyond 12 MPN-IU/L.

Coxsackievirus B3 was isolated from feces of household 22 on 12-31-89 and the same enteroviral serotype was detected in septic tank effluent on 1-08-90. Detection of this agent continued for at least thirty (30) days. Two (2) soil cores were collected at the site on 2-26-90 (Figure 8). The results of the soil core examinations are outlined in Table 25. No virus was isolated from any of the segments of either soil core. This failure to detect virus in the soil cores may be attributed to a number of factors, including the time span between the last detection of virus in STE and collection of the soil core, 21 days later. The sandy infiltration area may have a bearing also, in that viral strain and soil properties influence viral adsorption. Each soil type and the associated effluent allow transmigration at different rates. Sandy soil and organic constituents in soil are considered poor constituents for viral adsorption (Sobsey 1980) (Goyal and Gerba 1979).

Figure 7. OSDS site plan, station 14, Lost Lake, showing location of soil cores.
(after Ayres, July 1989 Progress Report)

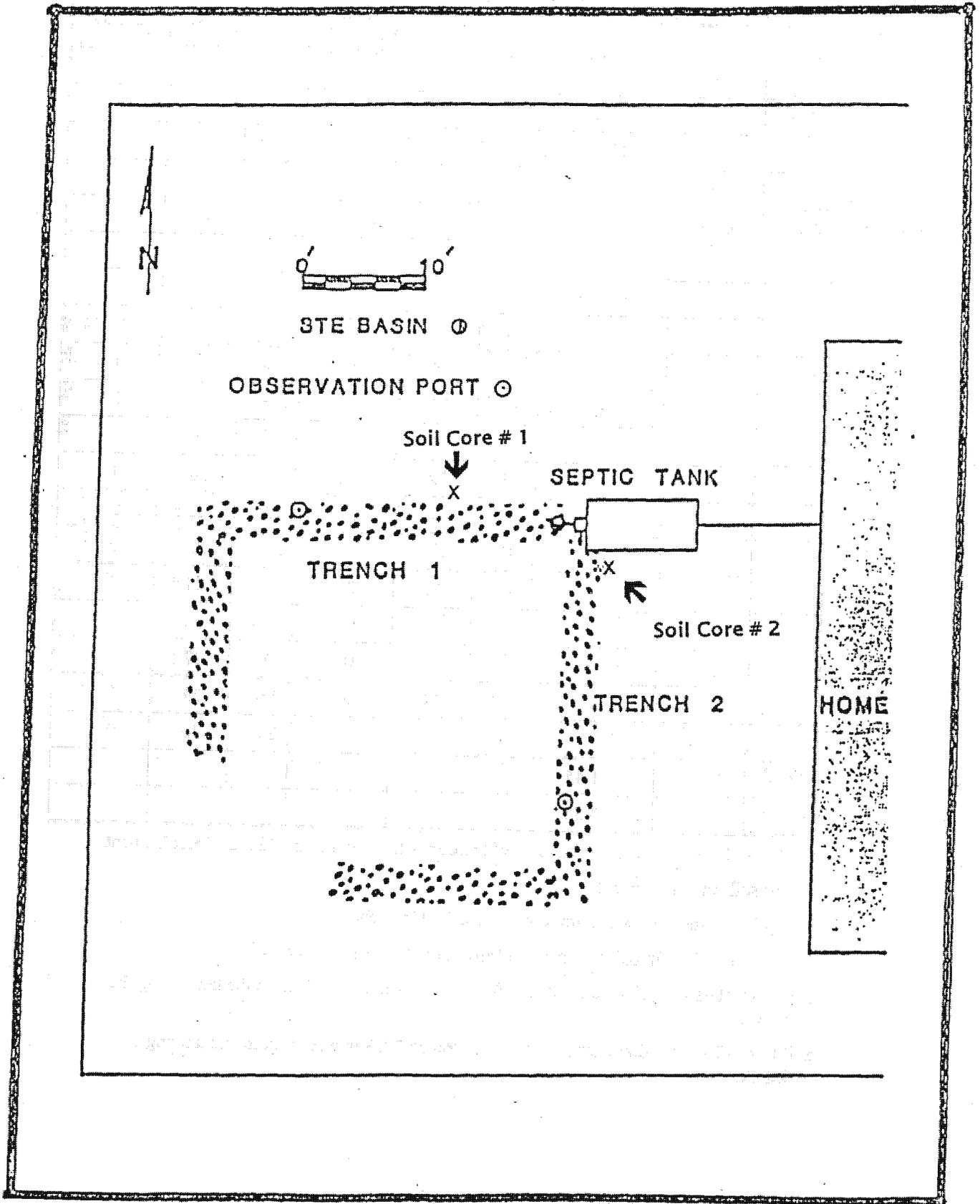


Table 24. Soil penetration of Coxsackievirus B4 after exiting septic tank 14, Lost Lake Subdivision

Date	Fecal virus	MPN-IU/L *1	Soil Core # 1			Soil Core # 2		
			wt *2	depth *3	MPN-IU *4	wt *2	depth *3	MPN-IU *4
02/19	CB4	>7.20						
03/05		>12.20						
03/06		>12.20						
03/07	CB4							
03/09	CB4							
03/12	CB4							
03/28		>12.4						
			64	4	0.015	71	4	0.014
			77	8	0	69	8	0
			87	12	0	73	12	0
			78	16	0	76	16	0
			88	20	0	78	20	0
			84	24	0	194	21.5	0
			73	28	0	230	23	0
			73	32	0	174	24.5	0
			76	36	0	216	26	0
			77	40	0	186	27.5	0
			103	44	0	158	29	0
						261	30.5	0
04/16		1.45						
04/17		0.32						

*1 MPN-IU/L=Most Probable Number of Infectious Units per liter of Septic Tank Effluent

*2 wt= soil weight in grams

*3 depth=distance below the drainfield gravel bed in inches

*4 MPN-IU=Most Probable Number of Infectious Units per gram of soil

Soil Core #1=1 inch diameter soil core from trench 1 taken 10 ft. from the tank along the drainfield.

Soil Core #2=1 inch diameter soil core from trench 2 taken 6 ft. from the tank along the drainfield.

Figure 8. OSDS site plan, station 22, Mandarin Meadows, showing location of soil cores. (after Ayres, July 1989 Progress Report)

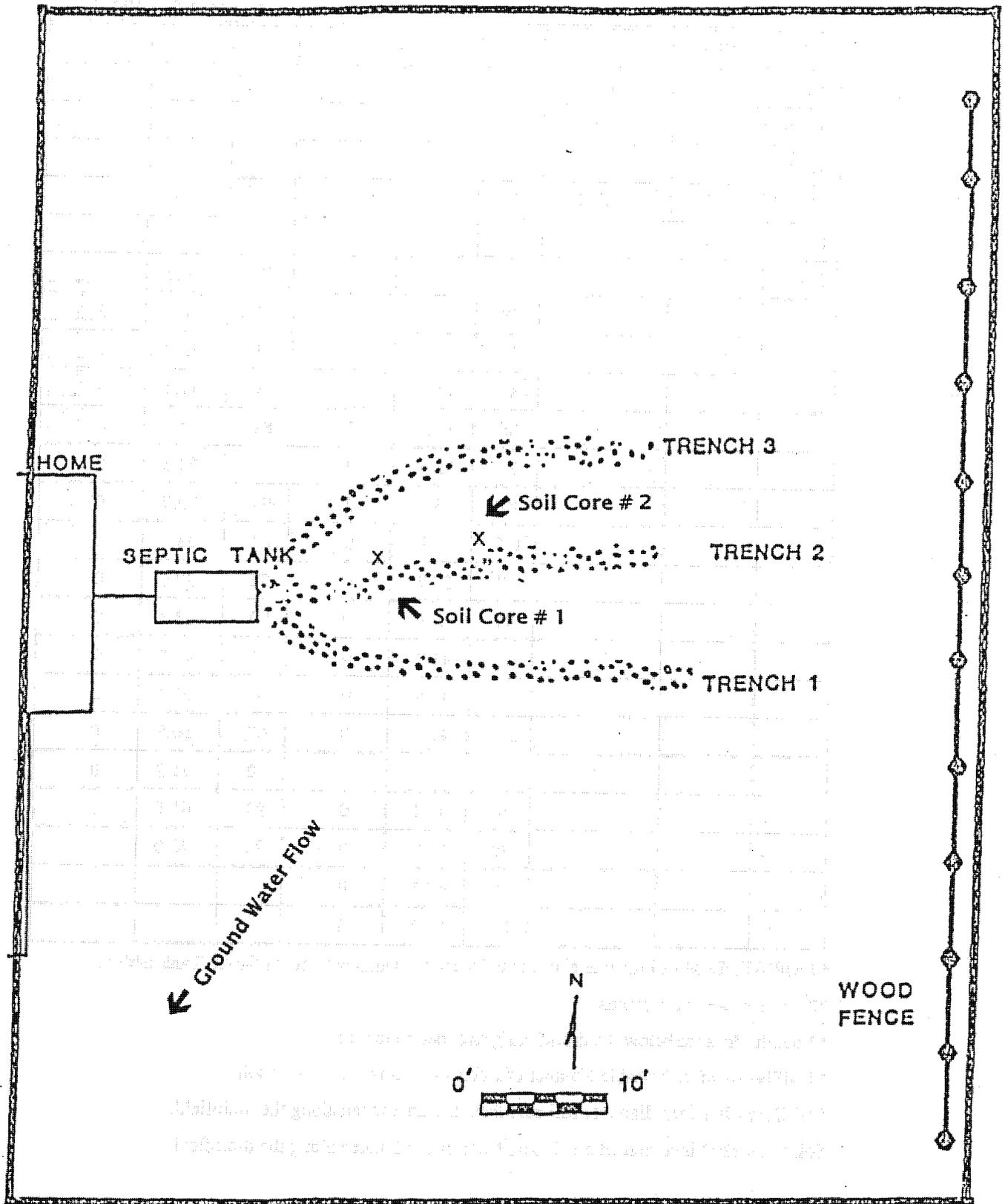


Table 25. Soil penetration of Coxsackievirus B3 after exiting septic tank 22, Mandarin Meadows subdivision.

Date	Fecal virus	MPN-IU/L *1	Soil Core # 1			Soil Core # 2		
			wt *2	depth *3	MPN-IU *4	wt *2	depth *3	MPN-IU *4
12/31	CB3							
01/08		1.71						
01/09		0.57						
01/30		0.11						
02/05		0.10						
02/06		0						
02/26		0	52	3.3	0	69	4.3	0
			58	6.5	0	87	8.6	0
			81	9.8	0	83	12.9	0
			81	13.0	0	89	17.1	0
			86	17.3	0	82	21.3	0
			79	21.6	0	77	25.5	0
			82	25.0	0	94	29.7	0
			103	29.9	0	63	33.9	0
			87	33.9	0	92	38.6	0
			84	37.9	0	90	43.3	0
			82	41.9	0	97	48.0	0
			94	45.9	0	141	52.4	0
			105	49.7	0	67	56.8	0
			79	53.6	0	90	61.2	0
			66	57.4	0	90	65.6	0
			80	61.2	0	95	70.0	0
			69	65.1	0			
			80	68.9	0			

*1 MPN-IU/L=Most Probable Number of Infectious Units per liter of Septic Tank Effluent

*2 wt= soil weight in grams

*3 depth=distance below the drainfield gravel bed in inches

*4 MPN-IU=Most Probable Number of Infectious Units per gram of soil

Soil Core #1=1 inch diameter soil core from trench 1 taken along the drainfield.

Soil Core #2=1 inch diameter soil core from trench 2 taken along the drainfield.

Relationship of the presence of an enterovirus in feces, septic tank effluent and groundwater.

An additional study seeking virus in ground water was provided through the installation of two (2) wells in close association with household 22's septic tank drainfield infiltration area on 2-26-90. The wells were located at locations designated W1 and W2 in Figure 9. Well 1 is downgradient of the central infiltration trench and well 2 was placed approximately ten (10) feet further down gradient. Neither of the viral serotypes, Coxsackieviruses B5 and B3, present in this STE previously were detected in 600 gallons of well water examined immediately following installation of either well (Table 26).

During March, however, Coxsackievirus A9 was present not only in householder feces, and septic tank effluent at 2.6 MPN.IU per liter, but also in 600 gallons of ground water from well 1 at 0.0024 MPN-IU/L. The depth to water in well 1 was 4'9". Twenty days (20) days later increasing amounts of Coxsackievirus A9 were present in septic tank effluent, >7.29 MPN.IU per liter, and the same agent was also present in ground water at 0.00047 MPN-IU/L. Overall, this virus was detectable in STE for at least sixty (60) days and in ground water for at least twenty (20) of those days. No virus was detectable in well 2, the furthest down gradient of the wells. The lack of detectable virus in well 2 can be attributed to the observation that the majority of septic tank effluent was diverted to the center trench. Thus, the low velocity of ground water flow, 0.03-0.05 feet per day, would have forestalled a viral plume being intercepted by well 2 which was 10 feet downgradient of well 1. These findings are similar to those of others (Vaughn et al 1983 and Wellings 1974) who have noted that some enteroviruses are able to travel through soil and enter ground water.

Figure 9. OSDS site plan, station 22, Mandarin Meadows, showing location of test wells. (after Ayres, July 1989 Progress Report)

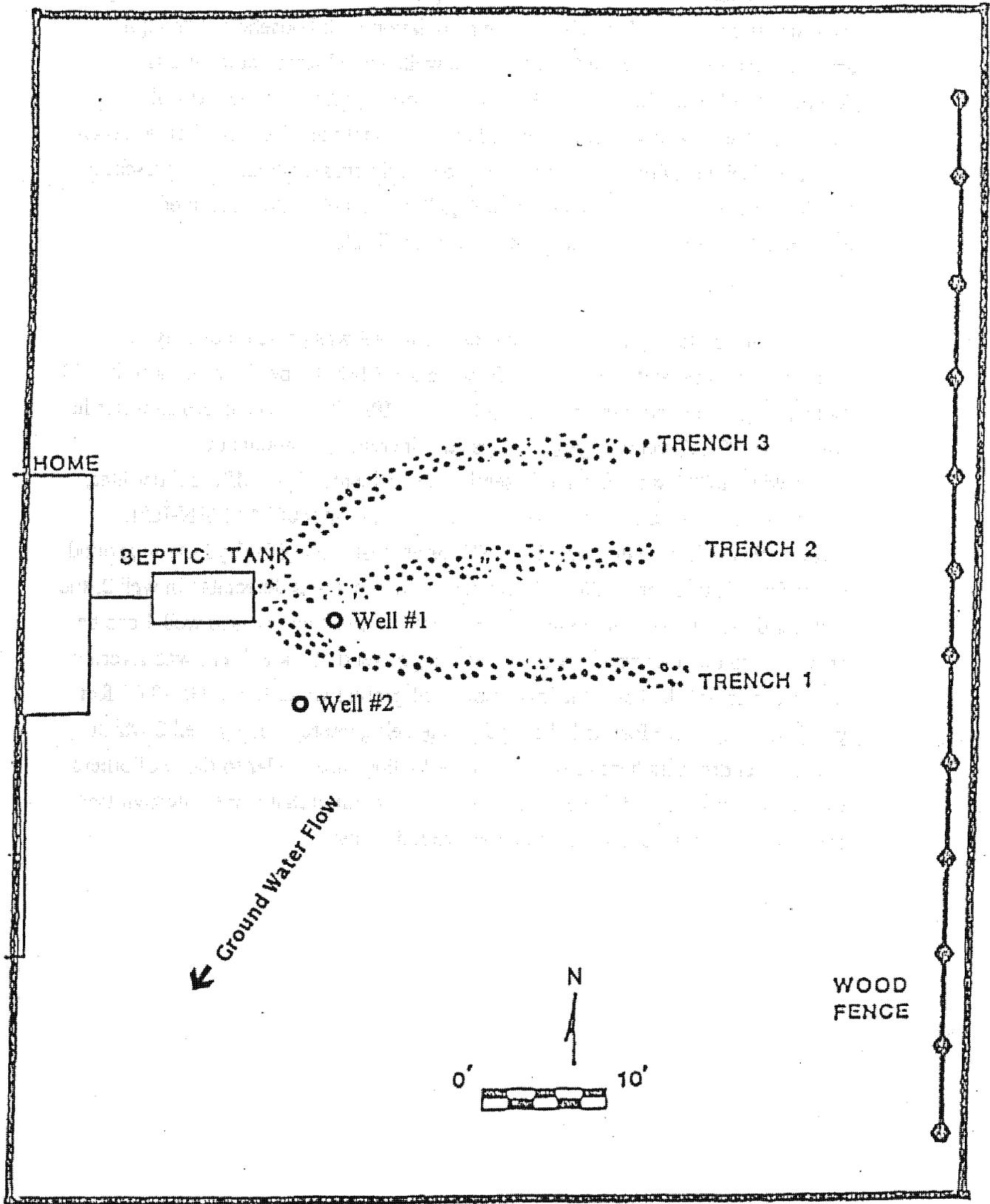


Table 26. Association of household viral excretion with duration of detection in septic tank effluent and groundwater, Mandarin Meadows subdivision site 22.

Date		Household excretion	MPN-IU/L in STE*1	MPN-IU/L in W1 groundwater*2
Oct 05	1988		0	
Nov 08	1988	0		
Nov 16	1988		0.23 (CB5)	
Dec 07	1988	CB5		
Dec 14	1988	0	>2.00 (CB5)	
Dec 21	1988	CB5		
Jan 16	1989	0		
Jan 25	1989		0.11 (CB5)	
Feb 15	1989		0	
Dec 02	1989	0		
Dec 05	1989		0	
Dec 31	1989	CB3		
Jan 08	1990		1.71 (CB3)	
Jan 09	1990		0.57 (CB3)	
Jan 30	1990		0.11 (CB3)	
Feb 05	1990		0.10 (CB3)	
Feb 06	1990		0	
Feb 28	1990	0		
Feb 26	1990		0	0
Feb 28	1990	0		
Mar 12	1990		2.60 (CA9)	0.0024 (CA9)
Mar 15	1990	CA9		
Apr 02	1990		>7.29 (CA9)	0.00047 (CA9)
May 03	1990	0		
May 09	1990		0.19 (CA9)	0
Jun 19	1990		0	0

*1 MPN-IU/L=Most Probable Number of Infectious Units per liter Septic Tank Effluent

*2 MPN-IU/L=Most Probable Number of Infectious Units per liter of groundwater from well W2

CB5=Coxsackievirus B5 CB3=Coxsackievirus B3 CA9=Coxsackievirus A9

Blank cell=no sample for that date

Conclusions

The data accrued show that human enteroviruses are present in OSDS effluent, adsorb to soil, can percolate through soil and reach ground water under the geographic, soil and climate conditions encountered in Florida. Due to many variables associated with monitoring under "real life conditions", data regarding the fate of viruses in the environment are difficult to obtain. Frequency of sampling is the most important aspect since viruses move rapidly and billions of virions can pass an area and be easily missed. Thus, it is difficult to speculate the length of time viruses remain viable in either soils or ground water.

Epidemiology Study

Experimental Design and Methods

Study Design

The investigation was a prospective cohort study design, with fecal specimens for virus isolation collected from young children residing in either of two communities. One of the two has both a high water table and private onsite "potable" water wells, thus the communities differ in potential for exposure to groundwater. Both communities are served by onsite wastewater disposal systems (home septic tanks). These communities were monitored concurrently for viruses in the environment by examination of selected septic tank effluents, drainfield soils, and groundwater study wells. All households in the communities were contacted, with the aim of enrolling all children under age 13 in the study. A questionnaire was used to assess exposure to groundwater as well as contact with other children. Fecal specimens were to be collected from each child enrolled every month for the study period, beginning in March, 1988, with additional specimens submitted whenever the child was ill. Specimens were assayed for enterovirus content upon receipt by the laboratory.

Study Sites and Populations

Sites for this study were selected by Ayres & Associates, an engineering consultant firm chosen to perform the non-viral portions of a major On Site

Disposal System (OSDS) study under the supervision of the Florida Department of Health and Rehabilitative Services. A survey of Florida soil types, and their ability to accept septic tank effluent was carried out. Representative areas in the State were evaluated for subdivisions that met set criteria. These criteria included: communities of 100 to 200 homes; housing density of 2 to 4 units per acre; relatively isolated subdivisions with no up-gradient sources of contamination in the immediate vicinity; installations under soils representative of the region; water table within 20 feet of the land surface and representative of the hydrologic area; installations made under the post 1983 code requirements. For the viral studies to be meaningful, it was also necessary that there be many small children residing in the communities. Thus, full season occupancy and three to five persons per home was preferred. In order to determine whether prospective communities met these last criteria, letters were sent to the homeowners in the communities; they explained the overall study purpose, and included a brief preliminary questionnaire concerning the number and ages of household residents and the household septic tank.

The two communities selected were Lost Lake, Polk County, and Mandarin Meadows, St. Johns County. The soils in both areas are mostly fine outwash sands. The St. Johns County area is approximately 1600 feet from the St. Johns River and is poorly drained. It has a high water table, from one to four feet below the surface. Homes in this community are served by private water wells and septic tanks on quarter to third acre sized lots. The Polk County site has well drained soil with the water table about 10 feet below the surface. This community is also composed of quarter acre lots with septic tanks, but is served by an offsite community water supply.

Although there are 200 homesites in Mandarin Meadows, and 70 in Lost Lake, not all sites had been built upon. At the project's inception, a listing by plot recorded 135 homes in Mandarin Meadows, 55 in Lost Lake. Visits to both sites by our investigators confirmed visually that both are similar as to exterior appearance of the homes. Of the 37 respondents to the engineers preliminary survey in Mandarin Meadows, 16 families reported children under 13 years of age, for a total number of 23 appropriately aged children. Similarly, in Lost Lake, 15 families reported a total of 25 children under 13 years out of 30 families

responding. Both communities show similar residence time distribution pattern for the responding families (Table 27)

Table 27. Mean family residence years in home.

Years in home	1-5	6-10	>10
Lost Lake	13	12	4
Mandarin	16	13	8

Hence, the preliminary analysis indicated that the two communities appear to differ primarily in their groundwater proximity, and thus exposure to viral agents which might be contaminating the groundwater from septic effluents.

Study Methods

- **Criteria for eligibility and exclusion**

Criteria for inclusion: Any household in the study area that had at least one child under 13 years of age, and agreed as follows to the collection and submission of stool specimens for virus studies from resident children under 13 years of age: A specimen is to be collected at a specific time each month and whenever the child has a gastrointestinal or respiratory illness. It is to be frozen until submitted to the public health nurse. A Specimen Identification form is to accompany the specimen. A family illness diary is to be kept.

Criteria for exclusion: Any household in the study area that did not have at least one resident child under age 13. Any household which refused to participate.

- **Selection bias**

There is a potential for selection bias, as only those residents who are concerned about health may agree to participate. Both communities had, however, been involved in the other, non-virus aspects of the general study for some time prior to the start of this phase. Thus, the idea of being part of such a project was not a completely new concept, and that may have encouraged participation. All residences with listed telephone numbers were contacted by telephone to solicit participation. The caller attempted to ascertain the contacts' reasons for refusal to participate, and recorded them in the telephone log. Records were reviewed to

determine if differential response rates occurred between the two communities; this did not appear to be a factor.

Additionally, to resolve the problem of bias due to non-response, telephone contacts were attempted repeatedly at differing hours of the day and early evening, as well as on weekends. Unlisted phone numbers exist in the study communities, and have been plotted on the subdivision map. The telephone caller asked the immediate neighbor of a home with an unlisted number whether appropriately aged children live next door. When the interviewer was in the neighborhood to administer the questionnaire to participating households, she also attempted to make contact with those families, and to enroll them in the study.

Preliminary site visits indicated that both study communities consist of middle class, single family dwellings. The communities appeared not to be highly mobile, based on length of residence data obtained from the site survey questionnaire conducted by Ayres and Associates. Thus, it was anticipated that loss to followup due to population instability should not be a major problem.

- **Participant recruitment**

Addresses in each subdivision were obtained from lot maps provided by the study Engineering firm. A letter introducing the study was sent to all community residents (Figure 10). A follow-up telephone call was made within two weeks of the letter mailing. The purpose of the call was to ascertain whether appropriate age children resided in the house, and, if so, to schedule an interview appointment with the parent or guardian. At the interview, our representative fully explained the purpose of the study and obtained an informed consent statement from the parent or guardian to participate in the study. Our representative (1) provided the participant with specimen collection kits and directions, (2) explained how to maintain the family illness diary, and its importance, (3) answered any questions by the participant, and (4) completed the questionnaire. Periodic contact was to be made with the participant families over the course of the study to encourage their continued interest and participation.

Figure 10. Letter introducing the virus study to community residents.



STATE OF FLORIDA
DEPARTMENT OF HEALTH AND REHABILITATIVE SERVICES

January 29, 1988

Dear Resident of Lost Lake Park:

Your community has been participating in a study of septic tank systems undertaken by the State of Florida. The purpose of this study is to determine the effects of septic tanks on your groundwater. Most of the drinking water in our state comes from groundwater, often the sole source for individual homes. With the rapid growth that Florida is experiencing, we must do all we can to assure that we do not pollute our drinking water through improper and unsafe use of septic tanks. This research project having been fully funded through impact fees, hopes to determine under what conditions are septic tanks proper and safe.

The engineering and geological portions of the study have been going on for about a year. Shallow monitoring wells have been placed in your neighborhood and are being sampled. Some residents are actively participating in the study by having their septic tanks sampled. We are now about to start the virus studies. You may soon see our Mobile Laboratory collecting groundwater samples in your area.

In order to determine whether viruses are draining from septic tanks, we have to know the extent any are going into the tanks. For this purpose we need the help of the residents of the study area subdivisions. The kinds of viruses we will be looking for cause upset stomachs, vomiting, diarrhea, colds, and "flu", most commonly in children under 13 years of age. These "enteroviruses" can survive in septic tanks and groundwater. We would like your help in determining which of these viruses are making your children ill through the next year.

In the next few weeks you will receive a telephone call about this study. The caller, a representative of the Florida Department of Health and Rehabilitative Services, will ask if there are any children age 13 or under living in your household. If there are, we would be most appreciative if you would consent to an interview and agree to help us with this study.

Remember, this will not cost you any money, nor will it in anyway affect your present septic tank permit. Your participation will be of great value in obtaining the best possible scientific results from this study, and will help influence the future of Florida's health.

If you have any questions please call or write us, Dr. Arthur Lewis or Dr. Lillian Stark at (813) 272-2316. Thank you for your time and attention.

Very truly yours,

E. Chariton Prather, M.D., M.P.H.
Acting Deputy Assistant Secretary for
Health and State Health Officer

slh

Epidemiology Research Center, 4000 West Buffalo Avenue, Tampa, Florida 33614

BOB MARTINEZ, GOVERNOR

GREGORY L. COLER, SECRETARY

- **The interview**

A personal interview was conducted with an adult from each consenting household recruited via telephone. The objectives of the interview were to: (1) explain the reasons for the study, (2) obtain informed consent to participate in the study, (3) administer the exposure questionnaire, (4) explain how, and when, stool specimens are to be collected, (5) provide the participant with specimen collection kits, (6) furnish the participant with a confidential demographic form. Figure 11 is the questionnaire used by the interviewer.

Of primary importance in the interview was the exposure questionnaire, which was designed to elicit information concerning possible exposures of the family members to groundwater. Even when a community water supply is available, some homes have private wells. These are primarily for yard irrigation. But if the tap water is not palatable, as is the case when heavily chlorinated, the private well may be used for drinking purposes. Thus, exposure to groundwater may occur within an area served by treated community water supply. Conversely, in an area served only by private onsite wells, exposure to raw groundwater may be reduced. For example, water softeners or iron filters may treat the well water; bottled or boiled water may be used for drinking. The questionnaire allowed us to verify actual groundwater contact.

A portion of the questionnaire was concerned with family structure and type of day care experienced by the children, providing an estimate of the number of non-family children routinely encountered by the subjects. Since the viruses being investigated are highly contagious, a large number and variety of potentially infectious contacts is a confounding variable. Information on day care should thus permit statistical control by stratification. A crude assessment of the childrens' medical history over the past year was included. If a child experienced many recent minor illnesses, he may not be susceptible to the currently circulating virus strains. On the other hand, severe illness might result in increased susceptibility. Polio vaccine is a live virus vaccine, and the virus may be isolated from an immunized child's feces for an extended period of time. Knowing when immunization was performed will aid in the laboratory analysis of the specimens.

FAMILY ID#

SEPTIC TANK STUDY FIELD QUESTIONNAIRE

Family Name _____ Person Interviewed _____ Date _____ Time _____

Street Address _____ How long have you lived at this address? _____ years _____ months

Subdivision _____ Telephone # (_____) _____

Residents Name	Household Position	Date of Birth	Sex	Daycare	Name/Location of School or Daycare
1. _____	(Father)	_____	M	_____	_____
2. _____	(Mother)	_____	F	_____	_____
3. _____					
4. _____					
5. _____					
6. _____					

Daycare: Does this child attend (1) nursery daycare, (2) daycare in a private home, (3) private school, (4) public school, (5) stay at home, (6) other (specify).

Name	A	B	C	D	E	F	G	H	I	J	K
3. _____											
4. _____											
5. _____											
6. _____											

(Y=Yes, N=No, D=Don't know)

- A. About how many time in the past year has this child had a minor, illness such as an upset stomach, diarrhea, or cold?
- B. Has this child had any serious illness in the past year? (Y/N/D)
- C. What type of serious illness? D. When did it occur? E. Was a physician consulted? (Y/N/D)
- F. Has this child been immunized with polio vaccine? (Y/N/D)
- G. Approximately when was he last given polio vaccine?
- H. Does this child drink tap water at home? (Y/N/D)
- I. Does this child drink tap water at school or daycare? (Y/N/D)
- J. Does this child play with water from the garden hose? (Y/N/D)
- K. Does this child ever play in rain puddles or water containing ditches? (Y/N/D)

Do you have a private well? Yes No Approximately how deep is it _____ ft. _____ don't know?
 Do you use it to water your garden? Yes No Do you use it for drinking water? Yes No
 Do you have any other source of drinking water? Yes No (Source) _____
 Do you boil water before using it for drinking? Yes No

Figure 11. OSDS epidemiology study questionnaire

Training sessions were held with the interviewers in order to review the purposes of the study and the protocols to be followed during the interview. The interviewer was instructed to avoid placing any emphasis on potential relationships between water contact and illness, so as not to alert the participant to possible behavioral modification of exposure risks.

- **Specimen collection**

Participant families agreed to provide the laboratory with a stool specimen from each child under age 13 years on or about the 5th of each month. Additionally, if a child was ill at any time during the month, another specimen was to be collected. They were instructed to store the specimen in their freezer until a representative of the laboratory collected them. Our courier picked-up the specimens each month, after contacting the participants by telephone. Specimens were transported to Tampa in an insulated cooler containing sufficient dry ice to prevent warming in transit. At the laboratory each specimen was given a sequential accession number by the clerical staff, as are all diagnostic specimens submitted to the laboratory. The accompanying form was removed from the specimen, and filed with the study records. This effectively blinded the laboratory technical staff to the source of the specimen. The specimens were stored at -70°C until processed for viral analysis.

Laboratory Procedures

- **Virus isolation**

Standard Laboratory protocols for the isolation of enteroviruses from stool specimens were followed. These procedures are routinely performed in our laboratory and the technicians involved are licensed, experienced and knowledgeable about the protocols. Specimens are only referred to by their sequential accession number in the laboratory.

Specimens were thawed at 36°C immediately prior to processing. A portion of the feces, approximately 1 gm if available, was triturated in a mortar and pestle with alundum, and suspended in 9 ml of phosphate buffered saline, pH 7.2. All reagents, mortars, pestles, glassware and pipets were sterile. To prevent any cross-contamination of specimens, separate sterile items are used for each

specimen. The 10 ml of stool suspension was centrifuged in an IEC PR2 centrifuge at 1200 X g for 15 minutes. The top 5 ml of the supernatant are transferred to a high speed centrifuge tube and spun in the IEC PR2 centrifuge for 1 hour at 3150 X g. The top 3.5 to 4 ml were carefully aspirated and transferred to a sterile test tube, containing 0.1 ml of antibiotic mixture (20,000 units penicillin, 20 mg streptomycin) and labeled with the specimen number. The specimen was aliquoted into portions for storage at -70°C until inoculation. A 0.2 ml portion was aliquoted into a tube of thioglycollate broth to confirm bacteria free status.

Processed specimens were assayed for enteroviruses following standard procedures; BGM (Buffalo Green Monkey Kidney) cell cultures and suckling mice were inoculated. We, as well as other laboratories, have isolated Echo, Polio, Coxsackie A & B, Adeno, and Reo viruses from stools using these host systems. For suckling mice inoculation, an 0.03 ml aliquot of specimen was inoculated with a syringe and needle intraperitoneally into each of 8 mice, 1 to 4 days old. The litter was maintained with their dam, and followed for 14 days. At signs of paralysis, severe morbidity, or death, the mice were harvested, and the musculature processed for passage into a second litter. If those mice show signs of illness or death, the virus containing homogenate was titrated, again using suckling mouse litters. A lethal dose of the viral suspension was thus determined, and the isolate then identified using Coxsackie A antiserum pools.

BGM cells were routinely subcultured each week. A newly confluent culture in a 25 cm² plastic tissue culture flask (Corning) was washed free of growth media (H-MEM/L15, 10% Newborn Calf Serum) with sterile phosphate buffered saline and inoculated with a 1 ml aliquot of the specimen. The flask was rocked at 36°C for two hours and cell maintenance media (8 ml of E-MEM, 5% Fetal Calf Serum) added. The culture was held at 36°C for 14 days, with periodic microscopic examination. If no cytopathic effect (CPE) is seen, the culture is frozen at -70°C for blind passage onto a fresh BGM culture in roller tubes. If after 14 days there is still no indication of CPE, the culture is discarded and reported as no virus isolated (NVI). If there is any questionable indication of CPE, another passage is made.

When sufficient CPE develops the culture is frozen and the virus isolate is identified by means of neutralization tests against pooled Enterovirus antisera. These tests are performed using BGM cell cultures grown in microtiter plates when large numbers of tests are required, or using tube cultures when only a few must be run at a given time. Both methods produce equivalent results. Briefly, the isolate is diluted to provide 500 to 1000 TCID₅₀ in 0.2 ml, and is mixed with an equal volume of antisera to Echo and Coxsackie A and B viruses in a series of pools. After a two hour incubation the serum-virus mixtures are inoculated into separate tissue cultures. The virus challenge is also titrated to assure a sufficient dose is present in the test. Cultures are examined microscopically for the occurrence of neutralization of the virus' lethal effect on the cells by the specific antiserum. Confirmation is made when required with single type antisera in another neutralization test. Isolates showing a CPE characteristic of Adenovirus were tested by complement fixation (CF) testing against adenogroup antisera.

- **Quality Assurance**

All those involved in participant recruitment and interviewing underwent training sessions: the purposes of the study and the protocols for telephone solicitation, interviewing, and questionnaire administration were thoroughly reviewed; the necessity for not influencing the participant in any way concerning water use habits was stressed; the confidentiality of all information was reinforced. The supervisor checked all records for completeness, legibility, and accuracy. Data entry was performed by one individual and independently checked by the supervisor. All records were kept in restricted access files.

Laboratory staff were all experienced in the handling and processing of human (diagnostic) specimens, and were licensed by the State of Florida, Department of Professional Regulation, HRS, at the Technician, Technologist, or Supervisor level. The Epidemiology Research Center Laboratory is also licensed under Medicare Requirements for the analysis of diagnostic specimens, and has been inspected and certified by both the State of Florida, HRS, and the United States Department of Health and Human Services. The Laboratory participates in the CAP quality assurance testing program for virus studies. The type of specimens to be analyzed in this study, and the laboratory methods involved have been routinely performed at ERC: over 1400 diagnostic specimens of various

types for virus isolation and identification were handled in the year prior to beginning this study.

When specimens arrived at the laboratory receiving department, they were logged in, their condition noted, and the accompanying identification form checked. An accession number was assigned, in normal sequence with all other specimens received for analysis. This number was the only identifier which the specimen had in the laboratory. Thus, the source of the specimen was not known to the technicians who processed them, nor to those who performed the examinations.

- **Definition of outcomes**

The isolation of Enterovirus, Reovirus, or Adenovirus from a stool specimen, and its identification as to serotype, was the primary outcome of the laboratory virus study. Each different serotype isolation was considered to be from a separate infection. Because of the length of the time between routine specimens, it was possible that the same virus might be reisolated on a second routine specimen, especially if it soon followed a specially collected "ill" specimen. Enteroviruses may be excreted for a few months by young children, depending on the virus type, as well as host factors. In one study, the overall mean duration of virus excretion was 0.76 months (Henigst et al., 1961). But virus titer, and thus ease of isolation, was much higher early in infection. Reinfection has been reported, but viral excretion in such cases is minimal (Gelfand, 1959). It is possible that the reported reinfections were in actuality due to infections by separate, but closely related strains of virus which were not recognized at the time of those studies. Nevertheless, when the same virus serotype was reisolated within a three month period of time it was considered a reinfection, or a continuation of the same infection. If, however, the same serotype was isolated on specimens taken four or more months apart, it was considered a new infection and as such, a separate event.

The primary outcome in this epidemiologic study was the cumulative incidence of enteric virus infection among children exposed and not exposed to groundwater contaminated by septic tank effluents. For each participant the number of infection events was the outcome of major interest. For each

community and family, not only the total number of infection events, but also the number of different serotypes occurring was of interest.

The participant families were asked to maintain an illness log, and when submitting specimens, record the occurrence of any symptoms. The relationship of illness to viral isolations was examined. Recognizing the general unreliability of such a record, it was hoped that the gross total level of community illness, i.e., the total number of episodes of possible Enterovirus causation among the two communities could be compared. Unfortunately, compliance with illness recording in the log was essentially nil, thus no such comparison could be made.

Data Management & Analysis

- **Sample size and power**

It was recognized that the power of this study to detect a statistically significant low increase in relative risk of enteric virus infection due to exposure to contaminated groundwater is low. The study sites were predetermined by other than epidemiologic constraints, and the numbers of available subjects are small. Power is related to the relative risk, incidence in the unexposed, and sample size (Schlesselman, 1974). Calculations demonstrated that if the incidence of infection in the unexposed was 8% or higher, an estimate not extremely different from that found in other studies, there was a not unreasonable chance of obtaining statistically significant results with the cumulative incidence outcome or with an incidence density approach. It would, of course, be of great benefit were the study populations larger, but even within the sample size limits imposed, meaningful results can be generated.

- **Data management and editing**

All completed questionnaires and coding cards were reviewed by the supervisor for completeness and consistency. Errors or omissions; were corrected; study participants recontacted if necessary. All records, forms, cards, etc., were maintained in separate files dedicated to this study. Access to these files was restricted. Computer data entry was performed at ERC, using IBM AT personal computers.

- **Data analysis**

Data analysis was performed on IBM type personnel computers using the following software: Lotus 123, Microstat, Epistat, and Kwikstat. Outcome variables, which include number of infections per individual during the study period, and the serotypes of those infections, were tabulated. Preliminary analysis involved descriptive statistics for each questionnaire variable, separately and in combination.

Exposure variables were analyzed in 2 X 2 tables with outcome variables. The cumulative incidence of infection (# of individuals infected in the study/# of individuals in the cohort) and the incidence density (# of infections among cohort members/total person months) in each population were calculated. Morgenstern, Kleinbaum and Kupper (1979) suggest that when each subject may develop multiple occurrences of the outcome in question, incidence density provides a better estimate of risk than does cumulative incidence measurements. Linear regression analysis was also employed.

- **Reporting of results to participants**

Participant households were notified when a virus was isolated and identified from a submitted specimen. The letter explained what virus was isolated and that these are common viruses, frequently not related to illness. The family will also receive, at the completion of the study a brief report providing an overview of the study results and significance.

Results and Discussion

Comparison of study site demographics

The two sites included in this study were remarkably similar, considering that they were not selected specifically for this epidemiology study. Table 28 presents an comparison of the hydrogeological features and demographics at each site. Because of the contagious nature of enteroviruses, possible confounding variables in this study included exposure to other children. Thus, multichild families and home reared versus day-care were variables of importance. The only notable differences between the two sites are the use of private home owner wells and shallow depth to groundwater at Mandarin Meadows .

Table 28. Comparison of the two study communities.

Characteristic	Lost Lake	Mandarin Meadows
Soil	Sand	Sand
Depth to groundwater	deep (25')	shallow (6')
Average lot size	0.26 acre	0.34 acre
Average annual rainfall	52"	54"
Total # of homesites	70	115
Median Residence time (years)	6-10	6-10
Ethnicity	White (1Asian)	White (1Asian)
Median education level	some college	some college
Median family income	\$30-40,000	\$30-40,000
# of families participating	28	15
Drinking water: bottled / tap water	6/23*1	5/10*2
# children age <5 years *3	35 (71.4%)*4	22 (71.0%)
# children age 5-10 years	9 (17.3%)	6 (19.4%)
# children age >10 years	5 (10.2%)	3 (9.7%)
# families with 1 child	11 (39.3%)	4 (26.7%)
# families with 2 children	12 (42.9%)	6(40.0%)
# families with 3 children	3 (10.7%)	5 (33.3%)
# families with 4 children	2 (7.1%)	0
Day care: stay at home	18 (31.0%)	10 (30.3%)
Day care: private home	3 (5.2%)	7 (21.2%)
Day care: nursery school	7 (12.1%)	4 (12.1%)
Day care: private school	7 (12.1%)	0
Day care: public school	23 (39.7%)	12 (36.4%)
#Children: male/female	35/27	11/21

*1: community water source

*2: private wells on site

*3: # submitting at least 1 specimen, age at entry

*4:(%)=percent of total reporting

Participation

Participation was less than hoped for at the start of the study. Not all children submitted specimens each month; some submitted multiple specimens some months, but not every month. Not all children in the family were represented at each collection. The families did not maintain the requested illness log, and the submittal sheets accompanying each specimen sometimes neglected to indicate whether the child was ill. Using available data, there was no relationship demonstrated between infection and illness. This was not surprising since enteroviruses are known to frequently cause only very mild illness. Additionally, because they are excreted for such long periods of time, the accompanying illness may have occurred up to a month prior to specimen collection, and thus have been forgotten. The same virus serotype was repeatedly isolated for up to a 30-35 day time span from children submitting specimens.

A potential for selection bias exists if the numbers of specimens per child were not the same for each community. The number of specimens submitted by children from whom a virus was isolated was significantly greater than the number of specimens submitted by children from whom no virus was isolated (NVI). Table 29 describes the frequency distribution of numbers of specimens submitted by the numbers by children in each community.

Table 29. Frequency distribution of numbers of specimens submitted per child at each study community

Number of specimens	Lost Lake	Mandarin	Z ^{*1}	p
1	2 (4.1%)*2	4 (12.9%)	-0.339	0.3672
2-10	20 (40.1%)	10 (32.3%)	0.416	0.3387
>10	27 (55.1%)	17 (54.8%)	0.019	0.4922

*1 Z-test for two proportions from independant groups

*2 number of children each submitting number of specimens (%)=%of total children

A t-test comparing the means of the number of specimens per child indicated no difference between the two communities (T=-0.8945, 78 d.f., p=0.1869). Table 30 presents a statistical analysis of the numbers of specimens submitted by the two categories of children in each community; the numbers of specimens submitted by children with at least one virus isolate do not differ by community, neither do the values for children from whom no virus was isolated.

Since there does not appear to be a differential in the selection (submission per child) between the communities, there is not a bias in the resulting risk ratio.

Table 30. Analysis of variance and multiple comparisons summary for the numbers of specimens submitted per child with at least one virus isolation (>1+) versus no virus isolated (NVI) at the two study communities.

Category	mean	standard deviation	number
Lost Lake, NVI	9.545	6.0924	22
Lost Lake, >1+	15.296	11.7402	27
Mandarin, NVI	4.125	4.2908	8
Mandarin, >1+	18.826	13.0894	23

Source	S.S.	d.f.	MS	F	appx. p
Total	10047.07	79			
Treatment	1785.78	3	595.26	5.48	0.002
Error	8261.29	76	108.70		

Newman-Kreuls Multiple Comparisons Summary: at the 0.05 level the means of any two groups underscored by the same line are not significantly different.

	Mandarin NVI	Lost Lake NVI	Lost Lake >1+	Mandarin >1+
population 1			_____	
population 2		_____		
population 3	_____			

Serotypes isolated

Twenty one enteric virus serotypes were isolated from 61 children infections comprising 46 family infections at the Lost Lake site; at Mandarin Meadows 21 serotypes were isolated from 68 children infections comprising 49 family infections. Table 31 presents the serotypes isolated, the number of children and the number of families in each community from which it was isolated. The same serotypes were not always isolated at both sites, and when they were, there was no temporal relationship. Up to three different serotypes were found to be circulating within each community during each month. Some serotypes were isolated sporadically over the entire 36 month study period, whereas others were only isolated once. Still others seemed to occur in "mini-epidemics"; for example,

from September, 1988, through May, 1989, Coxsackie B5 virus was isolated from eight children in six families in Mandarin Meadows.

Table 31. Serotypes of enteric viruses isolated from stool samples submitted by children from the OSDS study communities between June 1988 and May 1991.

Serotype	Lost Lake # children	Lost Lake # families	Mandarin # children	Mandarin # families
Coxsackievirus A2	4	3	3	2
Coxsackievirus A4	3	3	5	4
Coxsackievirus A5	2	2	6	4
Coxsackievirus A6	5	4	2	2
Coxsackievirus A8	4	3	3	1
Coxsackievirus A9	0	0	4	3
Coxsackievirus A10	1	1	0	0
Coxsackievirus B1	5	3	1	1
Coxsackievirus B2	4	2	3	3
Coxsackievirus B3	4	2	2	2
Coxsackievirus B4	6	4	4	2
Coxsackievirus B5	1	1	8	6
ECHOvirus 3	1	1	3	2
ECHOvirus 6	4	3	2	2
ECHOvirus 7	0	0	2	2
ECHOvirus 9	0	0	8	3
ECHOvirus 11	1	1	1	1
ECHOvirus 12	2	1	0	0
ECHOvirus 14	4	4	0	0
ECHOvirus 16	0	0	1	1
ECHOvirus 18	2	1	0	0
ECHOvirus 22	3	3	3	3
ECHOvirus 25	1	1	2	2
ECHOvirus 30	0	0	4	2
Adenovirus	2	2	0	0
unidentified	2	1	1	1

Incidence of Infection

Monthly infection rates varied greatly, most likely due to the small and unequal numbers of samples submitted each month. Figures 12 and 13 present the monthly incidence of infection (number of new infections divided by the number of children or families submitting). Children were included in the denominator, but not the numerator, if their specimen for that month provided a virus re-isolation. Multiple serotypes could be, and were, isolated from the same specimen, providing both a new, incident infection while still presenting virus from an old one. The rates indicated for individual children are very similar to those calculated on a per family basis, indicating that multiple child families did not have a major impact. It should be noted that there is no seasonality to infection, as is found in northern states. This implies an increased significance for enteroviruses as agents of disease throughout the year in the south, and a need for heightened concern about their transmission in such areas.

Summing each month's incidence rate as the study progressed, and plotting the cumulated values provides a clearer picture of viral infection rates in the two communities over the study period. (Figures 14 and 15). The smoothing effect of the larger numbers clearly indicates a higher incidence rate for Mandarin Meadows as compared with Lost Lake

The cumulative incidence of infection is the number of individuals infected during the study period divided by the number of individuals in the cohort. The tabulated data indicate the cumulative incidence of infection in the Mandarin Meadows community is higher than that in Lost Lake, and that this difference is approaching statistical significance ($p < 0.05$) for the total of all children, as well as that for age groups 1 and 2 only. Because age may be a confounder for enteric virus infections, the data were stratified by age, and analyzed separately using 2X2 tables to calculate the relative risk for infection. When this was done, however, the numbers in each strata were too small for valid analysis. This is especially noticeable for age group 3, where the numbers of children willing to provide specimens was very small, as was, the numbers of specimens each child in this group provided.

Figure 12. Monthly incident infections for children from the OSDS communities, June, 1988, through May, 1991.

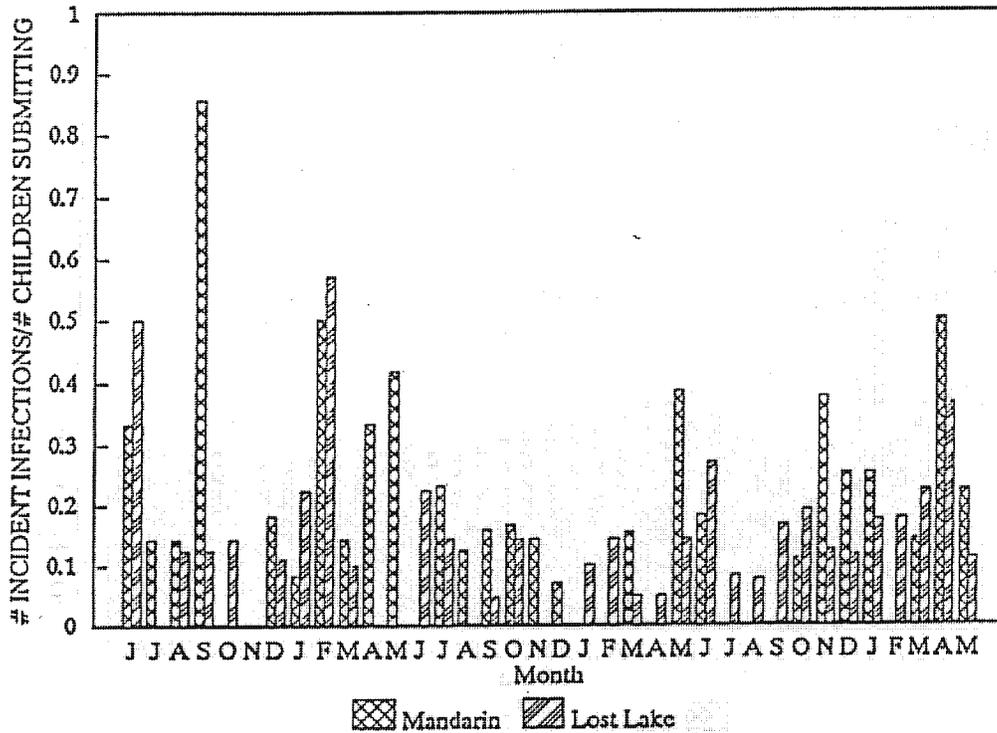


Figure 13. Monthly incident infections for families from the OSDS communities, June, 1988, through May, 1991.

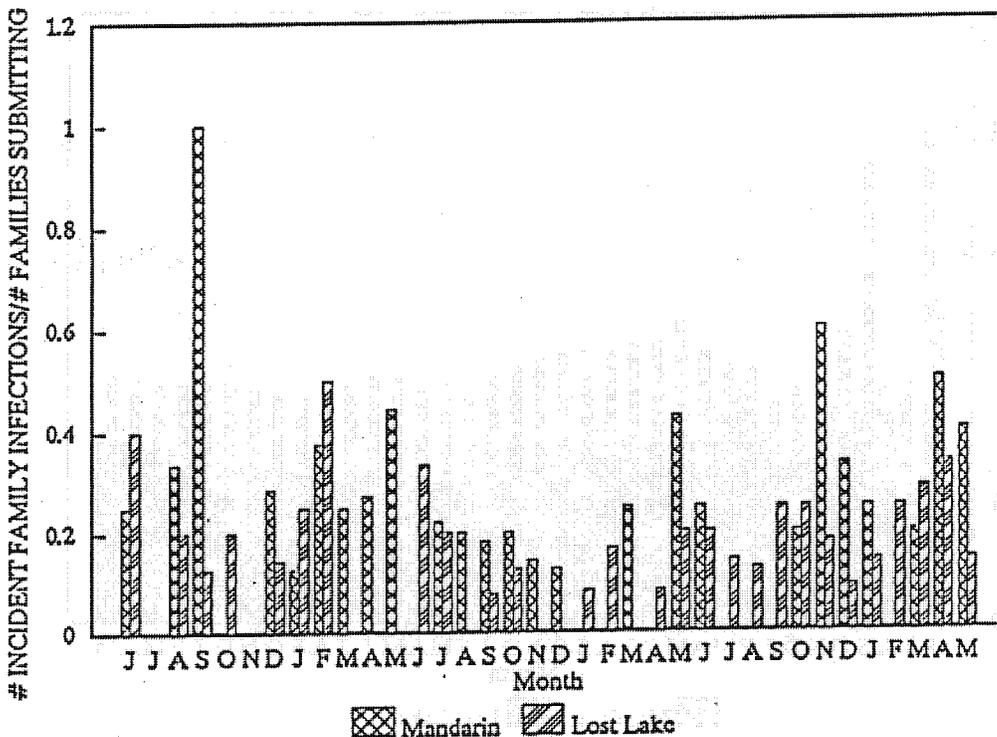


Figure 14. Cumulated monthly incident infections for children from the OSDS communities, June, 1988, through May, 1991.

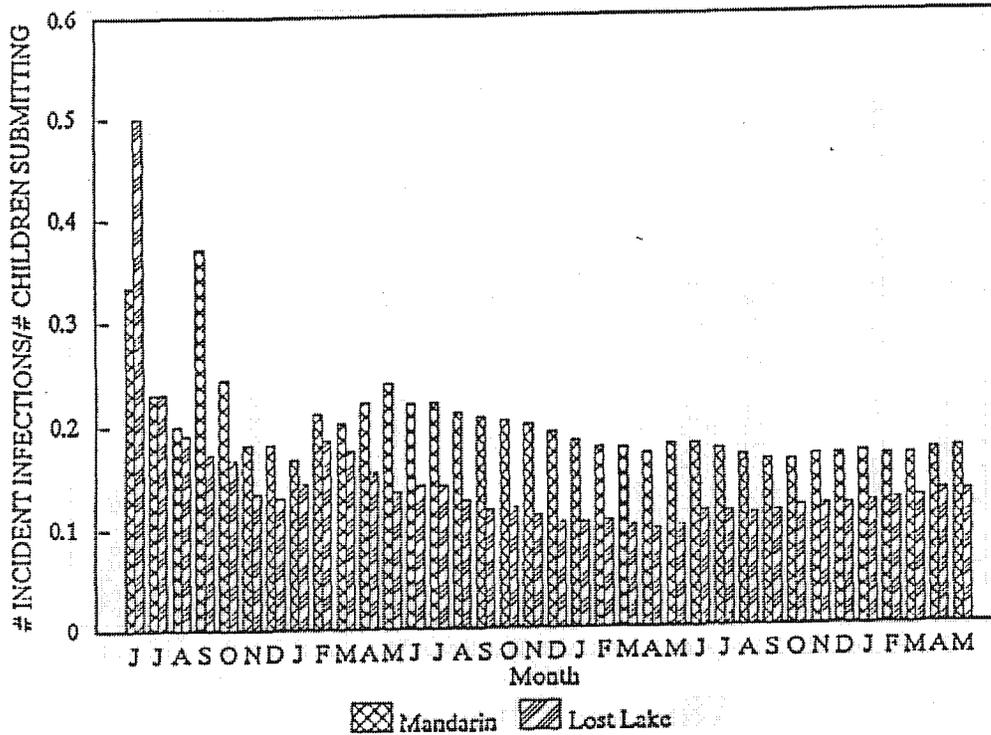


Figure 15. Cumulated monthly incident infections for families from the OSDS communities, June, 1988, through May, 1991.

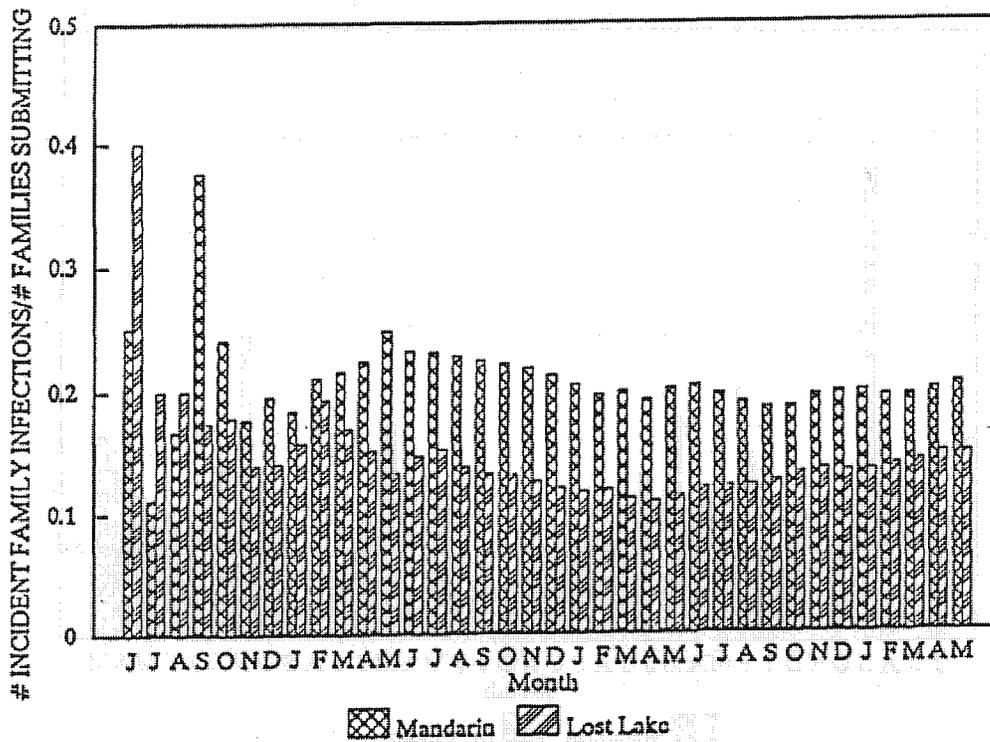


Table 32. Cumulative incidence of enteric virus infection for children from the OSDS study communities, June, 1988, through May, 1991.

Age group *1	Community	Virus + *2	NVI *3	C.I. *4	R.R. *5	p *6
1	Lost Lake	22	13	0.6286		
1	Mandarin	17	5	0.7727	1.2293	0.1994
2	Lost Lake	4	5	0.4444		
2	Mandarin	5	1	0.8333	1.877	0.1678
3	Lost Lake	1	4	0.2000		
3	Mandarin	1	2	0.3333	1.665	0.6429
all ages	Lost Lake	27	22	0.5510		
all ages	Mandarin	23	8	0.7419	1.346	0.0682
1 & 2	Lost Lake	26	18	0.5909		
1 & 2	Mandarin	22	6	0.7857	1.3296	0.0717

*1 Age group 1: < 5 years old; age group 2: 5 -10 years old; age group 3: > 10 years

*2 virus +: at least 1 enteric virus isolated *3 NVI: no virus isolated

*4 C.I.: Cumulative Incidence of infection

*5 R.R.: Relative Risk = C.I. exposed (Mandarin) / C.I. non-exposed (Lost Lake)

*6 p calculated with Fisher's exact test

The tabulated data indicate a cumulative incidence rate over the three year study of 74.2% at Mandarin Meadows and 55.1% at Lost Lake. At Mandarin, the average time from enrollment in the study to first virus isolation was 2.48 months, with a range from 0 (virus present in first submitted specimen) to 18 months. This is significantly lower than the Lost Lake value of 5.57 months from enrollment to first isolation (range, 0-24 months) by the t-test ($t=-2.22$, 46 df, $p=0.031$).

Another measure of infection incidence is incidence density, that is, the number of infections among cohort members divided by total person-months in the study. Morgenstern, Kleinbaum and Kupper (1979) suggest that when each subject may develop multiple occurrences of the outcome in question, incidence density provides a better estimate of risk than does cumulative incidence measurements. The incidence density of enteroviral infections (Table 33) was determined for each community, all ages combined, and stratified by age category. The incidence density ratio (rate ratio) for those exposed (Mandarin) versus those

not exposed (Lost Lake) was calculated, and the p-value for each table was calculated.

Table 33. Incidence density of enteric virus infection for children from the OSDS study communities, June, 1988, through May, 1991.

Age group *1	Community	# of infections*2	# of sample months *3	ID *4	IDR *5	p= *6
1	Lost Lake	42	278	0.1511		
1	Mandarin	54	237	0.2278	1.5079	0.0417
2	Lost Lake	18	157	0.1146		
2	Mandarin	13	128	0.1016	0.8862	0.4520
3	Lost Lake	2	33	0.0606		
3	Mandarin	1	30	0.0333	0.5501	0.7303
all ages	Lost Lake	62	468	0.1325		
all ages	Mandarin	68	395	0.1722	1.2993	0.0972

*1 Age group 1: < 5 years old; age group 2: 5 -10 years old; age group 3: > 10 years

*2 # of new non-polio infections during the study period

*3 the sum of the number of children submitting a specimen each month of the study period

*4 Incidence density: # of new infections / # of sample months

*5 Incidence density ratio: ID (Mandarin) / ID (Lost Lake)

*6 p calculated with Fisher's exact test

The tabulated data indicate a significant difference in the rate ratio only for age group 1, children less than 5 years of age. The numbers for age group 3 are too small to be meaningful. For age group 1, the observed rate translates to 2.7 enteroviral infections per year in the exposed community, as compared to 1.8 in the non-exposed for the youngest age group, and for the 5-10 year old group, 1.2 and 1.4 infections respectively.

Regression analysis

Univariate regression analysis was performed with the community (site) as the independent variable. The variables considered as the dependent variable were: being part of a multichild family (multichild), attending daycare vs. staying at home (daycare), total months in the study (totmonth), number of specimens submitted by

each child (specnum), total number of virus isolations made per child (totisol), number of incident infections per child (infect), age group at enrollment (startage), and age group at last submission (endage). Table 34 presents the results of this analysis. The number of new infections per child was the only significant one, indicating that the community was a valid predictor of the number of enteric virus infections.

Table 34. Univariate regression analysis with the community site as the independent variable for children participating in the OSDS epidemiology study, June, 1988 through May, 1991..

Dependent Variable	Regression Coefficient	Constant	Std. Error	T (DF=78)	p=	r=*1
multichild	0.0750	0.7959	0.0879	0.854	0.3958	0.0962
daycare	0.0244	0.6531	0.1099	0.222	0.8251	0.0251
totmonth	0.4595	17.3469	2.4279	0.189	0.8504	0.0214
specnum	2.3180	12.7143	2.5913	0.895	0.3738	0.1008
totisol	0.4845	2.6122	1.0453	0.464	0.6443	0.0524
infect	0.9282	1.2653	0.4504	2.061	0.0426	0.2273
startage	-0.00065	1.3878	0.1537	-0.0042	0.9966	-0.0005
endage	0.0020	1.8367	0.1578	0.013	0.9901	0.0014

*1 r=sample correlation coefficient

Stepwise multivariate regression analysis with the number of infections per child as the dependent variable was performed. The independent variables site, multichild (being part of a multichild family), daycare (attending daycare vs. staying at home), specnum (number of specimens submitted by each child), start age (age group at enrollment), and endage (age group at last submission) were considered as possible predictors of infection rate. The model is presented in Table 35. Total per child virus isolations was not included because the number of infections is a subset of that variable. Similarly, total months enrolled in the study was not included because specimen submissions frequently skipped months, and it was thus felt that numbers of specimens submitted was a better measure of degree of participation. In fact, numbers of specimens submitted was a very strong predictor of virus isolation. The age at leaving the study was also significant, albeit with a negative effect on virus isolation; the older children experienced fewer

virus infections. Age at entrance was not significant in this model. Neither was being part of a multichild family or attending daycare, as compared to being raised at home. Site was again a significant predictor ($p=0.0383$) of the number of infections experienced by the children in this study.

Table 35. Multivariate model for number of infections per child participating in the OSDS epidemiology study, June, 1988 through May, 1991.

Dependent Variable	Regression Coefficient	Std. Error	F (1,76)	p=	partial r^2
site	0.7057	0.3347	4.445	0.0383	0.0553
specnum	0.0966	0.0153	39.649	0.0000	0.3428
endage	-0.7388	0.2519	8.598	0.0444	0.1016

Constant 1.3938

Adjusted $R^2 = 0.4754$

$R^2 = 0.4953$

Multiple R = 0.7038

Analysis of variance table

Source	Sum of Squares	D.F.	Mean Square	F ratio	p=
Regression	156.8948	3	52.2983	24.864	2.58 E-11
Residual	159.8552	76	2.1034		
Total	316.7500	79			

Variables not in the equation

Variable	partial r^2	tolerance	F to enter	p=
multikid	0.0058	0.9871	0.441	0.5088
daycare	0.0230	0.8238	1.763	0.1882
startage	0.0024	0.4740	0.183	0.6697

Conclusions

The null hypothesis in this prospective design epidemiology study was that exposure to groundwater from unchlorinated wells on home lots with septic tanks does not increase risk of enteric virus infection. Both the cumulative incidence and incidence density of enteric virus infections were determined for each community. Risk and rate ratios calculated from the data were such as to deny the null hypothesis. The study was hampered by small sample size in numbers of children participating (49 in the non-exposed, 31 exposed), leading to risk ratios which were close to, but did not achieve statistical significance (0.068, all age). Nevertheless, there were significant differences found in the shorter time to first incident infection after enrollment, as well as a higher incidence density ratio (rate ratio) for the children less than 5 years of age in the exposed community. Regression analysis demonstrated that the community was a significant predictor of numbers of enteroviral infections experienced by young children, both in univariate and multivariate testing. Thus, the alternative to the null hypothesis, that there is a risk associated with exposure to groundwater from wells located on small lots (1/3 acre or less) with on-site sewage disposal systems, should be accepted.

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