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THE EVALUATION AND IMPACT OF COLIFORM BACTERIAL AND ENTERIC VIRUS POLLUTION IN SOUTHEASTERN NEW HAMPSHIRE

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FINAL REPORT

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INTRODUCTION

Nationally, the incidence of waterborne disease caused by microbially contaminated water is on the rise. In response, the United States Environmental Protection Agency has proposed recommended maximum contaminant goals (RMCG) of zero for enteric viruses in drinking water,(Federal Register, 1987), and new concentration and contact time standards for the disinfection of waste waters. Dramatic population growth has occurred in the southeastern section of New Hampshire in the last ten years. The rapid increase in population has strained many community wastewater treatment facilities and/or placed a heavy demand on on-site septic tank systems, leading to the introduction of fecal pollution into surface waters. Classical microbial indicators of water quality, total and fecal coliform bacteria, have shown to be inadequate indicators of enteric virus contamination. On several occasions, water which has met all bacterial standards has later been shown to be contaminated with enteric viruses (Margolin, 1987, Gerba, 1984).

In 1973, Metcalf et al., upon completion of their study entitled Enteric Pathogens in Estuary Waters and Shellfish, concluded that, "No reliable relationship was found between the fecal coliform index of water and the presence of enteric virus. Prediction of the viral hygienic quality of estuary water could not be made satisfactorily from a consideration of either coliform or fecal coliform counts. While viruses usually could be found in heavily polluted water, they were also found in water of very low fecal coliform density and a quality considered of approved status according to median coliform counts of 70 per 100 ml or less."

Source waters can become contaminated with fecal pollution in many ways. Rural communities which discharge sewage back into surface waters after only primary treatment or no treatment at all are a major source of surface water fecal contamination. In southeastern New

Hampshire, many of the publically-owned wastewater treatment plants (POTWs) are operating at near design capacity limits, and inadequately-treated wastewater discharges from these facilities are significant point sources of contamination throughout the Piscataqua River/Great Bay Estuary system. Other potentially important sources of microbial contaminants in New Hampshire are improperly functioning septic drainage fields, compost fields, landfills, and improper sludge disposal (Gerba, 1984; Alhajjar et al., 1988; Yates and Yates, 1988).

In New Hampshire the accepted microbiological criterion for evaluating water quality is the MPN (most probable number) multiple tube fermentation test for coliform bacteria. This test has come under increasing criticism because of the length of time required to run it and, more importantly from a public health viewpoint, the tenuous relationship between numbers of total coliforms and human pathogens, especially viruses. This is of critical importance because the overwhelming majority of diseases attributed to contaminated water are probably caused by viruses (Grimes, 1987; Yates and Yates, 1988). Fecal coliforms have been a preferred indicator group for surface waters since 1976 (USEPA, 1976), while enterococci are presently the preferred indicator for both estuarine and fresh surface waters (Dufour and Ballantine, 1986). However, direct detection methods for specific viral and bacterial pathogens are being developed and some techniques that have emerged may become standard methods if they can be made to be faster, more accurate, and more cost-effective than traditional indices.

Virus detection in water requires passage of 400-1000 liters of water being sampled through a filter to which the viruses adsorb. Viruses are eluted and then concentrated, after which the sample is ready to be assayed for the presence of enteric viruses, (Sobsey and Glass, 1981). Some of the several techniques that are used for detecting viruses provide the necessary sensitivity, but are slow to provide results. Other assay systems are more rapid, but lack the needed sensitivity required to detect low levels of virus.

Animal cell culture is the most widely used technique for the detection of enteric viruses in environmental samples (Melnick et al., 1980). This procedure uses cell lines of human or simian origin, which are grown in vitro with a minimal essential media, supplemented with fetal bovine serum. The cell line can either be primary, such a primary African monkey kidney cells or continuous, such as HeLa cells or Buffalo Green Monkey (BGM) cells. Animal cell culture is sensitive to viral infection only when the correct cell line is chosen (Schmidt et al., 1978). With over 100 different enteric viruses known to exist, there is presently no one cell line, either primary or continuous, which permits viral replication with equal efficiency (Bitton, 1980). Some viruses such as HAV can grow in animal cell culture without producing CPE, permitting the viral replication to continue undetected in cell culture. Other viruses such as Norwalk virus have not yet been grown in cell culture. Another problem associated with animal cell culture is the slow growth rate of certain viruses, such as adenovirus. To ensure enough time for virus replication, tissue culture must be held and maintained from 3 days to 6 weeks.

Other, more rapid, clinical tests used in laboratories, such as: radioimmuno assay (RIA); fluorescent labeled antibody; enzyme-linked-immunosorbent assay (ELISA) are based on the formation of immune complexes and require the use of at least one antibody. They also lack the needed sensitivity to detect low levels of virus in a contaminated sample.

Gene probes are small strands of labeled nucleic acids (either DNA or RNA) that will hybridize to their complementary strand. Recombinant DNA technology now makes it possible to produce large quantities of a probe inexpensively and with relative ease. Once the probe is produced, isolated, and labeled, it can be used for the detection of viruses in a dot blot assay.

Gene probes have been used for the detection of enteric viruses. Margolin et. al., (1988) reported using a cDNA probe for the detection of poliovirus in contaminated groundwater in Southern Arizona. Jiang et al., (1986) reported using a hepatitis A virus cDNA probe for the

detection of the virus in estuarine samples. Flores et al., (1982) used ssRNA transcripts of rotavirus that were labeled with ^{32}P GTP during transcription or ^{125}I as probes for the detection of rotavirus in stool samples and other biological materials.

This manuscript reports on the incidence of fecal-borne viral and bacterial contamination found in specified sampling sites along the Oyster River, in New Hampshire. Emphasis was given to sampling around the outfall of the city wastewater treatment facility, located on the tidal portion of the Oyster River and to a sampling site located immediately above the dam, which is not under tidal influence. In addition, this study evaluates the use of gene probes, compared to tissue culture, for the detection of poliovirus from the Oyster River.

METHODS

Sampling: Water samples collected from five sites on the Oyster River in Durham, New Hampshire were monitored for enteric viral and bacterial contaminants. The sites included both tidal and non-tidal sites along the river from just above the tidal dam to near the mouth of the river. The non-tidal site was 1) at Mill Pond (MP) just above the dam, and the rest of the sites going downstream were 2) the Town Landing (TL) located between the dam and the POTW; 3) the POTW effluent outfall (SO); 4) Johnson Creek (JC) located just downstream from the outfall, where minimal mixing has occurred; and, 5) at Painted Rock (PR), where an extensive oyster bed is located and which is further downstream, closer to the mouth of the Oyster River. The latter site is critical for assessing the impact of contaminants associated with tidal flow into the river, dilution of contaminants emanating from the POTW, and the impact of pollution on oyster contamination. A sixth site, Jackson's Landing (JL), was included during the winter because it was the only site on the tidal portion of the river that was both accessible and safe to sample. Sampling was conducted from boats at the PR and JC sites, from the shoreline at the TL and MP sites, from the middle of the river through the ice at MP and JL during the winter, and directly from the effluent pipe at the Durham wastewater treatment facility.

Virus detection in water required passage of approximately 88 gallons of water being sampled through a filter to which the viruses adsorb. Prior to filtration, water was collected in 44 gallon plastic garbage cans and the pH was lowered to 3.5 by the addition of 1 N HCl. To this was added 1 M $AlCl_3$ to yield a final molarity of 0.0001 M. Adsorbed viruses were eluted from the filter using 800 ml of 3% beef extract, pH 9.5. Beef extract filter eluent was then further concentrated to a final volume of 30 ml by organic flocculation (Katzenelson et al., 1976). In this procedure, the pH of the beef extract filter eluent is lowered to 3.5 with 1 N HCl. The sample is mixed at

room temperature for 30 minutes to create a flocculent and the flocculent was collected by centrifugation. Collected floc was resuspended in 0.1 M Na_2HPO_4. pH 9.5 for 10 minutes. The pH of the sample was returned to 7.0 and any particulates were removed by centrifugation.

To remove bacteria without loss of virus, the sample was filtered through a 0.45 um and 0.2 um filter that was first pretreated with 3% beef extract, pH 9.0. Once this was complete, the sample was aliquoted and assayed for the presence of enteric viruses.

Virus Detection by cell culture: Water samples were screened for the presence of virus using the cell culture technique of Sobsey, 1976. In this procedure, 1 ml of the concentrated water sample was inoculated on to a 25 cm^2 confluent monolayer of Buffalo Green Monkey Kidney cells which were then observed for the next fourteen days. Cytopathic effects indicated the presence of viruses. Since hepatitis A virus does not produce CPE in tissue culture, this virus was detected only by gene probes.

Samples which demonstrated CPE were confirmed positive for virus by passing suspected monolayer supernatants to confluent monolayers of BGM cells. Cells were then observed for 14 days for the presence of CPE.

Virus Detection by Gene Probes: Two cDNA probes were used in this study. The first cDNA probe was made from poliovirus type 1 and contains the entire genome minus the first 114 base pairs from the 3' end. The second probe was made from hepatitis A virus and is a 4.5 Kb section of the 5' end of the viral genome. Viral inserts were obtained by preparing 1 L preps of E. coli which had previously been transformed with our viral probe inserted into the Pst 1 site of the plasmid pBR322. Plasmid/probe cDNA was isolated on a cesium chloride/ethidium bromide ultracentrifugation gradient. To recover viral inserts for labeling and probe production, the cDNA

was digested with the restriction enzyme Pst 1 and then electrophoresed in a 1% agarose gel. For the poliovirus probe, two bands representing an 1100 and 1600 base pair piece of the poliovirus cDNA were visualized in the gel by ethidium bromide staining. For the HAV probe, one band, representing a 4.5 Kb section of the Hepatitis A virus cDNA was obtained. Bands were cut from the gel and the cDNA was eluted by phenol/chloroform extractions. The eluted cDNA was labeled with $^{3}2P$ dCTP using the random priming method to a specific activity greater than 1.0 X 10^{9} cpm/ug DNA

Methods previously described by Margolin et. al., (1986) for the detection of virus by gene probes were used in this study. In this procedure, concentrated water samples were treated with an RNase inhibitor (proteinase K, 0.1 ml/ ml of sample) to prevent RNA degradation and then heated to 65° C for 30 to 60 minutes to liberate viral nucleic acid, (Richardson et. al., 1987). Samples were spotted on to a hybridization membrane (Gene Screen Plus, New England Nuclear, Boston, MA) using a Biorad vacuum manifold dot blot apparatus (Bio Rad, Richmond, CA). Hybridization membranes were baked for two hours in a oven at 80° C.

Prehybridization and hybridizations were done according to the methods described in the Gene Screen Plus (New England Nuclear, Boston, MA) product information sheet. Prehybridization occured at 42° C with constant agitation for two hours. Hybridization occured at the same temperature but proceeded for 24 to 36 hours.

Post hybridization, membranes were washed twice to remove any non-specific binding of the probe and mismatched base pairing of the probe. The first wash was in 1X Sodium Chloride/Sodium Citrate (SSC), 1% sodium dodecyl sulphate (SDS) for five minutes at room temperature. The second wash was in 1 X SSC, 0.1% SDS at 52° C for 30 minutes.

Results were visualized by 24-36 hour autoradiographies done at -70° C using intensifying screens (Lighting Plus, Dupont, Willmington Delaware). The presence of viral nucleic acid was

determined by a dark area on the x-ray sensitive film.

Bacteriological analyses were conducted on duplicate water samples collected from 15-30 cm below the surface in sterile one liter plastic bottles. Water samples were immediately refrigerated, transported to Jackson Estuarine Laboratory (JEL) within 2 hours of collection, and processed for analysis within 24 hours. Total and fecal coliforms were detected using multiple tube fermentation, MPN tests as described in Standard Methods (APHA, 1985). Serial, decimal dilutions were made in buffered peptone water, added to a series of 5-tube sets of lauryl tryptose (LT) broth in Durham tubes, and incubated for 24-48 hours at 35°C. Tubes positive for gas production were used to inoculate Brillian Green Bile 2% broth and EC broth tubes, which were incubated for 24-48 hours at 35°C and for 24 hours at 44.5°C, respectively. Gas positive tubes were considered positive total coliform (TC) and fecal coliform (FC) tests, and were confirmed by conducting gram stains and standard biochemical tests on isolates from positive tubes. Samples collected after December, 1989 were also analyzed for E. coli (Ec) by adding methylumbelliferyl-ß-D-glucuronide (MUG) to EC broth prior to incubation as for FC, as described by Rippey et al. (1987). Gas positive and/or turbid EC tubes were exposed to UV light, and tubes fluorescing under were considered positive for Ec. Concentrations of TC, FC, and Ec were determined using standard MPN tables and sample dilution factors.

Enterococci were detected using a membrane filtration method (U.S. E.P.A., 1984). Duplicate water samples of $\leq$ 100 ml were filtered through sterile, 47 mm diameter, 0.45 µm pore size, membrane filters (Gelman), the filters were transferred to mE agar plates, and the plates incubated at 41°C. After 48 hours incubation at 41°C, filters were transferred to EIA (esculin iron agar) agar plates and incubated 20 minutes at 41°C. Positive enterococci colonies were considered those that were pink to reddish-brown and that caused a black or reddish-brown precipitate on the underside of the filter. Colonies were confirmed using gram staining and standard biochemical

tests. Positive colonies were counted and enterococci concentrations were determined by multiplying by appropriate dilution factors.

RESULTS

The results of the bacteriological analysis of the water samples are summarized in Table 1. All indicator groups of bacteria were detected in all except one sample (FC at MP) of river water collected from MP, TL, JC, and PR. Samples where one or more indicators were not detected were more common for the SO site, especially in the effluent itself, indicative of the effectiveness of the chlorination of the effluent for killing indicator bacteria. Total coliforms are used in New Hampshire as the bacteriological index of water quality, and the levels of total coliforms at all sites are consistent with the State data (Flanders, 1990) indicating that the Oyster River does not meet Class B criteria or standards for allowing shellfish harvesting.

Levels of the bacterial indicators differed between sample sites (Table 1). For example, geometric means of levels (per 100 ml) of enterococci were 28 at TL, 12 at MP and JC, 8 at PR and the river at the SO outfall, and 3 in the SO effluent. The variability in numbers of the different indicators over the sampling period (summer, 1989 to summer, 1990) was usually large enough to not be able to accept the observed differences between sites as statistically significant, except for fecal coliforms at TL and PR and enterococci at TL and PR. These two sites represent the two most separated tidal sites. Levels of all indicators were greater at TL, which is more densely populated and shallow, than at PR, where cleaner tidal water was more likely to dilute contaminents because PR is closer to the mouth of the river and the river is wider and much deeper.

Concentrations of the other indicators were consistent with total coliform data and the resultant water quality classifications. Correlation tests between levels of different indicators were conducted on logarithmically transformed data. Except for the relationship between total coliforms and enterococci at MP, correlations were generally not significant between the gram positive

enterococci and the other, gram negative indicator groups (Table 2). There was always a significant correlation between the related indicators FC and Ec correlation coefficients for TC and FC levels at TL and PR were statistically significant, while TC and Ec correlation coefficents were only significant at TL. The low numbers of bacterial contaminants in the SO effluent and in the river near the outfall indicate that the SO was not a significant source of pollutants to the Oyster River.

The concentrations of the different bacterial indicators varied over a wide range of concentrations and showed no significant trends with time, as shown in Figure 1 for the summer and early fall samples from 1989. Some of the peak concentrations corresponded with rainfall events and may have been caused by runoff. However, high concentrations at other times did not correspond to rainfall events and some rainfall events did not show corresponding higher indicator concentrations. Figure 3 shows the effects of tidal stage on enterococci levels at PR and TL. The levels at PR for the two dates shown exhibited the type of trend that would be expected if the main source of pollutants was upstream near TL and MP, i.e., highest levels at low tide that decrease as the tide comes in, are lowest at high tide, and becomer higher as the tide goes back out. The levels at TL for the two dates shown did not give consistent trends.

Detection of poliovirus by gene probes was in agreement with tissue culture results for 93% of the samples (Table 2). It must be noted however, that 50 samples (83%) were negative for the presence of enteric viruses by either method. Of a total 60 samples, 9 were positive for virus by gene probes and 6 were positive by tissue culture (Table 1). There were 3 gene probe positive samples that were tissue cultue negative and 1 tissue culture positive sample that was gene probe negative. This sample could not be confirmed for the presence of virus, however. Agreement between gene probes and tissue culture for only positive samples was much less, 5/10 or 50%.

Comparisons of bacterial indicator concentrations with virus detection incidence were made for samples where both types of analyses were conducted. The relationship between incidence of

virus detection and concentration of bacterial indicators revealed no clear trend. Examination of data for all samples where viruses were detected revealed average total coliform concentrations that were near the middle of the range of TC levels for all samples (Table 5). The percent samples positive for viruses was 20-22% for MP, TL, and PR sites, 10% for SO, and 0% for JC. However, viruses were detected at more than one site only on one sampling date (September 18, 1989; TL and PR). The samples collected for PR, JC, and TL included samples from different tidal stages on the same dates, and viruses were only detected more than once on a given date one time (September 18, 1989; TL). Despite the similarity in incidence of viruses at MP, TL, and PR based on total number of samples (20-22%), the frequency for detection of viruses on given sample dates differed between sample sites. Viruses were detected more frequently at TL and PR (40%) than at MP (22%).

DISCUSSION

The Oyster River in Durham, New Hampshire, was an excellent site for this 1989-90 study. Bacteriological indicator bacteria were detected consistently at the different sites, and the observed concentrations varied over a wide range. The source(s) of the contaminants are probably a number of typical nonpoint sources and not any distinct point sources, as the Durham municipal wastewater treatment facility was not a significant source of bacterial contaminants. Possible nonpoint sources include inefficient or failing on-site sewage disposal systems both near the southern shoreline of the tidal portion of the river as well as upstream in the freshwater portion, because the municipal sewage system does not serve residences on the south side of the Oyster River. It is highly likely that the elevated levels of contaminants observed at the Town Landing sample site are caused by on-site systems on the south side of the river amongst the high density of residences close to the tidal dam.

Enterococci are presently the recommended bacterial indicator for fecal contamination in both freshwater and marine and estuarine waters in the US (U.S. E.P.A., 1986). The lack of correlation with the other three indicators was not surprising, and brings into question the use of total coliforms in New Hampshire as the standard indicator. However, trends observed for enterococci levels at different sites were not consistent with virus detection. The most striking inconsistency was the observation that viruses were detected as frequently at Painted Rock as they were at Town Landing, despite the significantly lower levels of enterococci observed at Painted Rock compared to Town Landing. This may be an indication of the ability for viruses to persist longer than enterococci can survive and as likely to be detected downstream from the major sources of contamination as they would be detected near the source(s). These results provide added evidence to the growing body of data that suggests that bacterial indicators are poor indices of viral contamination.

Poliovirus was chosen as the model virus for evaluation of the gene probe assay for several reasons. Due to the current vaccination program in the United States, poliovirus is the most ubiquitous enteric virus found in the environment and hence, is an ideal choice for the evaluation of a new assay. In addition, poliovirus is well characterized and can readily be detected in tissue culture.

Nine samples were positive for virus by gene probe and six were positive by cell culture. Three samples that were gene probe positive were tissue culture negative and one tissue culture positive sample was gene probe negative. Previous work by Dr. Margolin and other investigators using gene probes have reported higher incidences of gene probe positive samples upon comparison to tissue culture. There are several possible reasons for this. Gene probes detect the genome of poliovirus and do not differentiate between infectious virus and non-infectious virus. This could account for the higher number of gene probe samples, since tissue culture only detects infectious

virus. This is an important consideration when evaluating treated water, such as the samples obtained from the sewage outfall. However, our gene probe and tissue culture results only indicated one positive SO sample, which would suggest that the SO is not the primary source of virus pollution. Since more positive samples were obtained from Mill Pond, which is not under tidal influence, and Town Landing, this may imply that viral contamination is originating from private disposal of inadaquately treated or untreated sewage in that area. This may be occuring from private septic systems lining the banks of the river or from disposal of sewage from boats in the area.

Another reason why the gene probe assay yields more positive results compared to tissue culture may be due to the very nature of the assay. Viruses are coloidal in nature, and hence the volume or thickness of the water layer resting on the monolayer of cells will affect the sensitivity of the assay. Theoretically, because of the colloidal nature of viruses, a sample innoculum size which produces a water layer on the cells greater than one virus in diameter increases the potential for the virus not to come in contact with the cells. As the thickness of the sample water layer increases, the probability of a virus infecting a cell decreases and correspondingly so does the sensitivity of the assay for virus detection. It is impossible to use a sample inoculum size which would yield a sample water layer of one virus thick because of the amount of cells and cost incurred when trying to assay a 10-15 ml sample. Upon comparison to gene probes, the entire sample to be assayed is passed through a hybridization membrane which binds the nucleic acid. This may account for the greater frequency of positive results.

The tissue culture positive/gene probe negative sample may be due to an enteric virus other than poliovirus. The gene probe used in this study was for the detection of poliovirus. The probe does cross react to some degree with echo and coxsackie viruses, but with a sensitivity of virus detection much below the concentration of viruses found in a contaminated sample.

It is also important to note that when the water sample is aliquoted for assay after concentration, viruses, which tend to clump, do not assume a true Possion distribution. It is then theoretically possible that one aliquot contained viruses, while the other aliquot did not. This can be seen within each assay; for example, seldom do all five tissue culture flasks, used in the assay, demonstrate cytopathic effects. Quite often, only 1, 2 or 3 flasks will be positive while the others are negative.

Results of our study indicate that gene probes can be used to detect viruses from the Oyster River. This tool provides an alternative method to the traditional tissue culture assay, which is cost and time prohibitive for routine monitoring of water. In addition, on one occasion at the SO, gene probes and tissue culture assays were positive for virus while total coliform levels were at 13, which is below the SO discharge permit level. This is only one example of the inadequacy of total coliforms as predictors of viral pollution. It should be noted however, on most occasions where virus was detected, by either assay, total coliform levels were high. Previous to the development of the gene probe assay, reliance on indicator organisms was the only practical method for assessing the overall sanitary quality of a body of water. Now however, the gene probe assay permits the direct detection of pathogens without the use of indicator organisms.

Table 1. Distribution of bacterial indicators at 5 sites on the Oyster River, NH: 1989-1990

Indicator	Mill Pond	Town Landing	Sewage Outfall River	Sewage Outfall Effluent	Johnson Creek	Painted Rock
Total coliforms						
Log mean	1480	1430	118	8	570	770
Standard deviation	6	5	41	7	4	4
Maximum	24,000	16,000	9000	130	3000	5000
Minimum	20	49	0	0	20	40
No. of samples	17	15	9	6	9	17
% positive	100	100	67	50	100	100
Fecal coliforms						
Log mean	160	240	28	1	71	68
Standard deviation	7	7	9	2	2	3
Maximum	9000	8100	230	3	300	500
Minimum	0	1	0	0	9	19
No. of samples	15	16	8	8	9	19
% positive	93	100	75	25	100	100
Escherichia coli						
Log mean	180	220	ND	2	ND	63
Standard deviation	9	18	ND	1	ND	4
Maximum	2500	8100	ND	3	ND	500
Minimum	27	1	ND	0	ND	11
No. of samples	4	7	ND	5	ND	8
% positive	100	100	ND	60	ND	100
Enterococci						
Log mean	12	28	8	3	12	
Standard deviation	4	4	5	6	5	5
Maximum	66	450	60	105	81	63
Minimum	1	5	0	0	1	1
No. of samples	13	12	8	6	9	12
% positive	100	100	88	33	100	100

Table 2. Correlation coefficients between fecal indicators at three sites in the Oyster River: 1989-90.

	Fecal coliforms	Escherichia coli	Enterococci
Mill Pond			
Total coliforms	0.46	0.22	0.72**
Fecal coliforms	0.95 *	0.16	
Town Landing			
Total coliforms	0.73**	0.84 *	0.14
Fecal coliforms	1.0**	0.44	
Painted Rock			
Total coliforms	0.59**	0.53 *	0.01
Fecal coliforms	0.84**	0.37	

* Significant at the 95% confidence level.
** Significant at the 99% confidence level.

Table 3. Detection of viruses by gene probes and tissue culture as compared to total coliforms based on sample site location

Location	Sample Volume (gal)	Tide	Date	Total Coliforms	Gene Probe	Tissue Culture
Durham Waste Water Treatment Plant (SO)	88	---	8/8	350	Negative	Negative
Durham Town Landing (TL)	40	---	8/11	2400	Negative	Negative
TL	88	L	8/11	---	Negative	Negative
Mill Pond (MP)	100	___	8/16	2200	Positive	Positive
SO	80	---	8/22	400	Negative	Negative
TL	80	L	8/23	500	Negative	Negative
MP	80	---	8/23	2700	Positive	Negative
Johnson Creek (JC)	60	L	8/24	2400	Lost	Sample
Painted Rock (PR)	60	L	8/24	950	Negative	Negative
MP	60	---	8/31	1300	Negative	Negative
SO	60	---	8/31	0	Negative	Negative
PR	80	---	8/31	110	Negative	Negative
JC	80	L	9/18	800	Negative	Negative

TL	66	L	9/18	5000	Positive	Negative
TL	80	Mid Low (ML)	9/18	---	Negative	Negative
TL	80	H	9/18	0	Negative	Negative
TL	80	Mid High	9/18	1700	Negative	Negative
PR	80	L	9/18	260	Negative	Negative
PR	80	ML	9/18	230	Positive	Negative
PR	80	H	9/18	130	Negative	Negative
PR	80	MH	9/18	140	Negative	Negative
MP	80	---	9/25	1700	Negative	Negative
SO	80	---	9/25	130	Negative	Negative
JC	80	L	10/5	800	Negative	Negative
PR	40	L	10/5	1300	Negative	Positive/no confirmation
TL	88	H	10/8	5000	Negative	Negative
SO	80	---	10/10	0	Negative	Negative
MP	80	---	10/10	130	Negative	Negative
TL	88	L	10/16	500	Negative	Negative
TL	125	ML	10/16	500	Negative	Negative

TL	88	H	10/16	300	Positive	Negative
TL	88	MH	10/16	240	Negative	Negative
PR	88	L	10/25	500	Negative	Negative
PR	88	H	10/25	900	Negative	Negative
SO	88	---	11/1	0	Negative	Negative
SO	88	---	1/5/90	0	Negative	Negative
TL	88	L	1/17	9100	Negative	Negative
Jackson Lab (JL)	88	L	1/18	9000	Negative	Negative
JL	88	H	1/18	6000	Negative	Negative
SO	88	---	2.14	13	Positive	Positive
MP	88	---	2/22	12,500	Negative	Negative
TL	88	ML	3/15	2800	Negative	Negative
TL	88	L	3/15	8500	Negative	Negative
TL	88	MH	3/15	850	Negative	Negative
TL	88	H	3/15	1600	Positive	Positive
MP	88	---	4/10	---	Negative	Negative
TL	88	H	4/24	9500	Negative	Negative

TL	88	L	4/24	170	Negative	Negative
TL	88	MH	4/24	5700	Negative	Negative
TL	88	ML	4/24	---	Negative	Negative
SO	88	---	4/26	1	Negative	Negative
SO	88	---	7/2	9	Negative	Negative
MP	88	L	7/25	120	Negative	Negative
TL	88	L	7/25	1600	Negative	Negative
PR	88	L	7/31	130	Negative	Negative
TL	88	H	7/31	---	Negative	Negative
MP	88	---	8/24	4750	Negative	Negative
SO	88	---	9/12	2	Negative	Negative
TL	88	L	9/12	300	Positive	Positive
TL	88	H	9/12	1600	Negative	Negative

Table 4. Comparison of gene probe results and tissue culture for the detection of enteric viruses

Number of samples	60	
Samples positive by gene probes	9	15%
Samples positive by tissue culture	6	10%
Samples positive by tissue culture and gene probes	5	8.3%
Samples positive by gene probes but negative by tissue culture	4	6.6%
Samples positive by tissue culture but negative by gene probes	1	1.16%
Agreement between tissue culture and gene probes for positive samples	5/10	50%
Agreement between tissue culture and gene probes for all samples	56/60	93%

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