Florida Keys Microbial Water Quality Update Report

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Introduction

The following report is an update on a research project supported by Dr. Joan B. Rose. This study was an extension of several research grants, which were supported by funds from the United States Environmental Protection Agency (USEPA, contact William Kruckzenski, Marathon, FL office).

The following is a copy of an abstract from an article that was published in 1999 and summarizes the results of the first portion of the USEPA funded project. (Griffin et al. 1999, Applied and Environmental Microbiology, Vol.65. No.9:4118-4125).

"In order to access the microbial water quality in canal waters throughout the Florida Keys, a survey was conducted to determine the concentration of microbial fecal indicators and presence of human pathogenic microorganisms. Nineteen sites, including 17 canal sites and two nearshore water sites, were assayed for total coliforms, fecal coliforms, *Escherichia coli, Clostridium perfringens*, enterococci, coliphages, F^+ specific RNA coliphages, *Giardia lamblia*, *Cryptosporidium parvum*, and human enteric viruses (Polio, Coxsackie A, Coxsackie B, echoviruses, Hepatitis A viruses, Norwalk viruses and Small Round-Structured Viruses). Coliforms ranged from <1 to 1410, *E.coli* <1 to 130, *Clostridium spp*. <1 to 520 and enterococci from <1 to 800 colony forming units/100ml of sample. Two sites were positive for coliphage but no F+ specific phage were identified. The sites were ranked according to microbial water quality and compared to various water quality standards and guidelines. Seventy-nine percent of the sites were positive for the presence of enteroviruses by reverse transcriptase-polymerase chain reaction (Polio, Coxsackie A, Coxsackie B and echoviruses). Sixty-three percent of the sites were positive for the presence of Hepatitis A viruses. Ten percent of the sites were positive for the presence of Norwalk viruses. Ninety-five percent of the sites were positive for at least one of the virus groups. These results indicate the canals and nearshore waters throughout the Florida Keys are being impacted by human fecal material carrying human enteric viruses through current wastewater treatment strategies such as septic tanks. Exposure to canal waters through recreation and work may be contributing to human health risks."

After the above results were released to the USEPA, additional funds were made available to determine if the original samples contained live/viable viruses (viruses which can initiate an infection). This was dome because the original detection assay used in the initial part of the study (RT-PCR) could only determine if viruses were present, not if they could cause infection. Using an assay known as cell culture which allows detection of viable viruses, it was determined that these samples did not contain viable viruses.

There was concern that the high water temperatures (on average ~30°C. High water temperatures such as those observed have been identified as one of the top physical factors effecting virus survival) observed in the first study may have inactivated the viruses and that when water temperatures were cooler, one could possibly identify live viruses in these type of sample sites.

In order to determine if viable viruses could be detected in cooler water temperatures in the Florida Keys, a total of seven sites were screened. Using the same collection protocols as used in the first study, water column samples were collected and eluted in the field. Samples were immediately shipped to the University of Florida (UF) for cell culture analysis. Table 1 contains information about sample sites, water temperatures and sites, which were positive by cell culture. It should be stated that these samples (cell culture extracts) will also be screened by RT-PCR (by USF researchers) after the cell culture aliquots are received from UF. This additional screening will allow

the identification of viable viruses that can not be detected by cell culture alone (the are certain enteroviruses which can infect and replicate cells used in the cell culture assay without causing cell lysis or changes in cell morphology. Cell lysis or changes in cell morphology is the means in which the researcher determines if viable viruses are present in cell culture assays).

Table 1. Site identification and viral cell culture analysis results.

Site	Water temp. (Celsius)	Viruses detected by cell culture*
Keys 3. Capt. Cove. L. Matecumbe	Dnt	Yes
Keys 6. Sexton Cove. Key Largo	25.0	Yes
Keys 7. Jolly Roger Pk. Key Largo	27.0	No
Keys 16. Ocean Reef Resort. Key Largo	24.5	No
Keys 20. Rest Beach, Key West	24.0	No
Keys 21. Eden Pines, Big Pine Key	26.0	No
Marathon Government Center – Gulf side cove area	28.0	Yes

Sites identified by Keys # correspond to sites used in the initial study.
Dnt = did not take (thermometer broke).
* = all sites pending RT-PCR results.

All sites were also screened for fecal coliforms, enterococci, coliphage and *Clostridium spp*.. No sites were in violation of the indicator standards or suggested standards. Table 2 contains that information.

Table 2. Indicator data expressed as numbers of colony forming units (CFU's) per 100ml of water.

Site	Fecal coliforms	Enterococci.	*Clostridium spp.*	Coliphage
Keys 3	4	4	0	0
Keys 6	260	22	30	0
Keys 7	25	5	0	0
Keys 16	3	0	0	0
Keys 20	2	0	1	0
Keys 21	15	0	0	10
Marathon	0.5	2.5	0	0

Results were similar to the first study in which indicator information suggested acceptable water quality and the human virus data suggested just the opposite. As with the initial study, the current virus data demonstrates a direct link between human sewage and canal and nearshore water quality contamination. Cell culture analysis alone has demonstrated that 43% of the sites tested positive for live infectious viruses. More sites may be identified as positive via RT-PCR analysis of cell culture aliquots. If viruses are detected by RT-PCR we will clone the amplified gene and sequence it to determine exactly what type of virus it is using a Genbank-Blast search (polio vaccine versus pathogenic enterovirus). We have sequence data from a couple of sites around Key West and the Fort Jefferson mote in which specific pathogenic enteroviruses and a Sabin strain of Polio virus has been identified via genetic cloning and sequence evaluation (results not released at this time).

A history of nutrient and tracer study research involving the use of septic tanks and shallow injection wells in the Florida Keys, in addition to this series of human pathogen research has demonstrated that the current waste disposal systems (septic tanks and shallow injection wells) are directly impacted water quality in the region. These systems pose a linked threat to both human and environmental health.