Diversity and Distribution of Pathogenic Viruses in the Florida Keys

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Final Report

Summary:

The presence of pathogenic viruses in coastal marine environments has the potential to be an important tool in evaluating water quality and human health risks. Millions of viruses are excreted in fecal matter and current methods of sewage treatment do not always remove these pathogens. Furthermore, studies have shown that current bacterial indicators of fecal pollution do not accurately reflect the extent of fecal pollution or the associated health risks. Enteroviruses have been used to identify fecal pollution in the environment; however, other viruses transmitted via the fecal-oral route could indicate fecal pollution.

The purpose of this research is to develop a baseline understanding of the diversity of viruses found in the Florida Keys and to assess their presence in the marine environment. Viruses were concentrated from raw sewage inflow collected from the Key West Wastewater Treatment Plant using centrifugal filter units. Viruses were also concentrated from four marine environments ranging in their exposure to human influence using tangential flow filtration, density-dependent centrifugation, and PEG precipitation. PCR and RT-PCR were used to detect the following viral groups: *Adenoviridae, Herpesviridae, Papillomaviridae, Reoviridae, Picobirnavirus, Caliciviridae, Paramyxoviridae, Tobamoviridae,* and *Hepadnaviridae*. All viral families were identified by comparing the sequences of positive PCR products from purified raw sewage and environmental viral samples to those found in GenBank. Adenoviruses, Noroviruses, Picobirnaviruses, and Tobamoviruses were detected in Florida Keys raw sewage. However, none of these viruses were detected in any of the seawater samples examined. This suggests that at the time sampled, these environments were not significantly impacted by fecal pollution. More detailed sampling is needed in order to assess the impact of fecal pollution on the coastal environment of the Florida Keys.

To further understand the viral types present in sewage, raw sewage samples from across the United States were collected and assayed for the presence of these viral groups. Adenoviruses were found in 92% of the sewage samples, suggesting that these viruses are good markers of fecal pollution. Caliciviruses were found in 58% of sewage samples, suggesting that these viruses underestimate fecal pollution. Interestingly, Picobirnaviruses and Tobamoviruses (Pepper Mild Mottle Virus) were found in 100% of the sewage samples, suggesting that these viruses are promising new indicators of fecal pollution. In particular, Pepper Mild Mottle Virus may be a human-specific indicator, which will enable source tracking of fecal pollution in coastal waters.

Specific Objectives

This project had two specific objectives. First, to use PCR amplification, cloning, and sequencing of conserved genes to examine the diversity and distribution of major groups of pathogenic viruses in sewage, seawater, sediment, and coral mucus samples from the Florida Keys (Objective 1). Second, pepper mild mottle virus (PMMoV), a Tobamovirus commonly found in human feces, was tested for its potential to serve as a novel indicator virus (Objective 2).

Objective 1: Determine the presence and diversity of 8 major groups of pathogenic viruses in raw sewage and marine samples from the Florida Keys.

Viral Family	Virus	Type
Paramyxoviridae	Morbillivirus	ssRNA (- strand)
Caliciviridae	Norwalk- & Sappro-like Caliciviruses	ssRNA (+ strand)
Tobamoviridae	Pepper Mild Mottle Virus	ssRNA (+ strand)
Reoviridae	Mammal Reovirus	dsRNA
	Rotavirus, Group A	
Unclassified	Human Picobirnavirus	dsRNA
Papillomaviridae	Human Papillomaviruses	dsDNA
Adenoviridae	Human Adenovirus, A - F	dsDNA
Herpesviridae	Human Herpesviruses	dsDNA

The presence of the above viral families was analyzed in raw sewage samples from the Florida Keys, and collected from 10 other coastal states around the United States (listed below).

Date	Location
11/5/2007	Alabama
10/18/2007	California
11/14/2007	Connecticut
11/30/2007	Florida
12/8/2006	Florida Keys
11/12/2007	Louisiana
11/5/2007	Maine
11/24/2007	Maryland
11/13/2007	New Jersey
11/13/2007	North Carolina
11/13/2007	Oregon
11/8/2007	Washington

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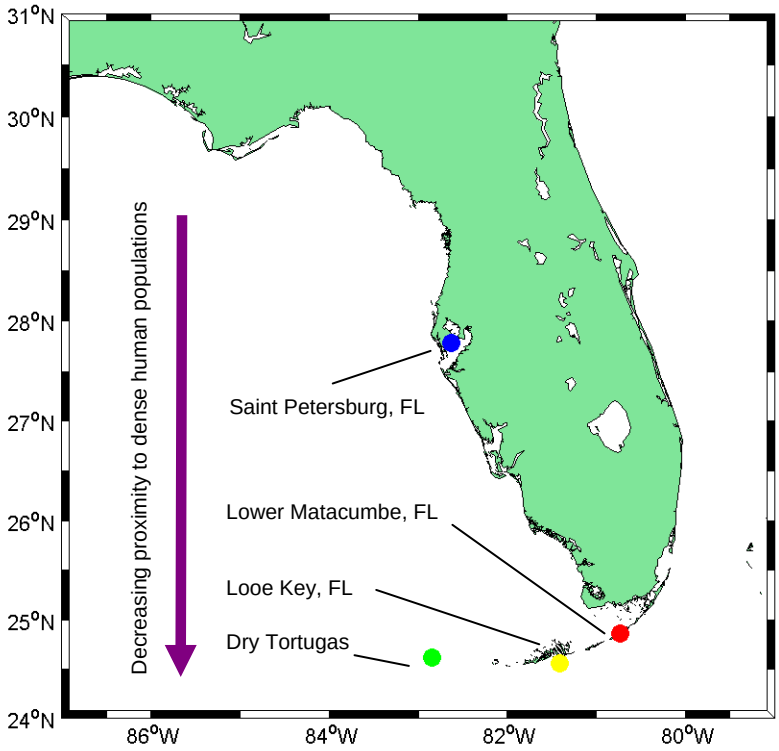
The second part of this objective was to determine if these viral groups were also found in the marine environment. To accomplish this, marine samples ranging in their exposure to human influence were examined using the same PCR assays (Figure 1). Seawater, sediment, and coral mucus samples were examined for the presence of each of the viral groups. All environmental samples tested negative for these viral families, even after large-scale viral concentration (e.g., 200 liters of water) and sequence-independent

DNA amplification. Previous studies in the Florida Keys have shown that fecal indicators are not present at all times, and the level of fecal pollution can be dependent on many factors such as tide and temperature. Future work will need to examine more environmental samples with the newly refined list of viruses (Adenoviruses, Caliciviruses, Picobirnaviruses, Tobamoviruses).

Figure 1. Sites of environmental sampling in Florida. The colored dots represent the four areas sampled: North Shore Beach (blue), Lower Matacumbe canal (red), Looe Key (yellow), and offshore the Dry Tortugas (green).



Objective 2: Evaluate the potential of Pepper Mild Mottle Virus (PMMoV) to serve as a water quality indicator.

Recently, it has been demonstrated that plant viruses are abundant in the feces of healthy humans (Zhang et al. 2006). The most common virus was pepper mild mottle virus (PMMoV), a rod-shaped single-stranded RNA virus belonging to the *Tobamoviridae* family. PMMoV was detected in the feces of over 65% of healthy individuals by quantitative RT-PCR. In addition, PMMoV was extremely abundant, with concentrations up to 10^9 virions per gram dry weight of feces. A number of pepper-based foods also tested positive for PMMoV, suggesting a dietary origin for the PMMoV found in human feces.

The purpose of this objective was to determine if PMMoV would serve as a good indicator of water quality. To accomplish this goal, raw sewage samples from across the United States were examined for the presence of PMMoV. PMMoV was found in 100%

of the sewage samples, at high concentrations (10^3 - 10^5 copies per ml sewage). Viruses belonging to the *Tobamoviridae* family are known to be extraordinarily stable, and these viruses could still be detected in the final treated effluent from 92% of the samples. This suggests that PMMoV would be an ultra-conservative tracer for following fecal contamination or discharge of treated effluent. PMMoV was not found in any unpolluted seawater or sediment samples, and is not expected to replicate in the marine environment.

Summary of characteristics that make PMMoV a good candidate indicator virus

1. Found in high numbers in human fecal samples
2. More resistant to natural and artificial inactivation than the pathogen
3. Rapid, sensitive, specific assay exists
4. Not known to be pathogenic in mammals
5. Absent in unpolluted marine systems, and not expected to grow in the environment
6. Likely allows for source-differentiation

The ability to identify the source of pollution is extremely important for water-quality management. In humans, the presence of PMMoV in fecal samples has been linked to foods consumed in their diet. The diets of humans are significantly different from other organisms, suggesting that PMMoV may be useful for source tracking. As part of this proposal, we have tested numerous fecal samples from horses, cows, and birds for the presence of PMMoV. These samples have all been negative for PMMoV, supporting the use of this virus as a human-specific indicator.

References:

Zhang, T, M Breitbart, WH Lee, J-Q Run, CL Wei, SWL Soh, ML Hibberd, E Liu, F Rohwer, Y Ruan (2006) RNA viral community in human feces: Prevalence of plant pathogenic viruses. PLoS Biology. 4: e3.

Resulting Presentations and Publications:

This work has contributed to the training of one Masters student (Erin Symonds) and one PhD student (Karyna Rosario). The work described here constitutes the majority of Erin Symonds' Masters thesis. She will be defending this work in June 2008. Two publications (one on each of the specific objectives) are currently being prepared, and should be submitted in Summer 2008.

<u>This work has resulted in the following presentations:</u>

Breitbart, M. "Viruses in sewage and reclaimed water – implications for human and environmental health". Invited seminar. University of South Florida ERIC symposium; Tampa, FL. 4/08.

Symonds, E, K. Rosario, M. Breitbart. "Viruses found in sewage and their potential to indicate fecal pollution in coastal waters". Oral presentation by E. Symonds. USF College of Marine Science Graduate Student Symposium; Saint Petersburg, FL. 4/08.

Symonds, E. and M. Breitbart. "Viruses found in sewage and their potential to indicate fecal pollution in coastal waters". Oral presentation by E. Symonds. Florida Branch of the American Society for Microbiology Annual Meeting; St. Petersburg, FL. 10/07.

Rosario, K. and M. Breitbart. "Viruses in reclaimed water". Oral presentation by K. Rosario. Florida Branch of the American Society for Microbiology Annual Meeting; St. Petersburg, FL. 10/07. K. Rosario was awarded 3rd place best graduate student presentation.

Breitbart, M. "Viruses in human feces". Invited guest lecture for Plant Virology Course; University of Florida Department of Plant Pathology; Gainesville, FL. 9/07.

Symonds, E, M Breitbart. "Diversity and distribution of pathogenic viruses in the Florida Keys". Oral presentation by E. Symonds. University of South Florida College of Marine Science Graduate Student Symposium; Saint Petersburg, FL. 10/06

Breitbart, M. "Plant viruses in human feces". Invited guest lecture for Plant Virology Course; University of Florida Department of Plant Pathology; Gainesville, FL. 9/06.