REPORT

Title: Sources and Risks of Waterborne Pathogens in the El Paso del Norte Region

Project Number: 2009TX322B

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Abstract:

Vigilant water quality monitoring is very important for compliance with government standards and in the interest of the public. The Rio Grande river is the natural boundary between the U.S. and Mexico and is the only source of surface water for the El Paso, Texas and Ciudad Juarez, Mexico area known as the El Paso del Norte region. Agriculture, farming, domestic activities, and effluent from local wastewater treatment plants increase the contamination potential of water supplies along the region. Monitoring of selected sites along the Rio Grande river has showed the occurrence of *Cryptosporidium* and *Giardia*. Higher pathogen levels were observed during the non-irrigation season when the river flow is dominated by wastewater effluents. This indicates that there could be an increased risk of using the river water as a source of drinking water during the winter if it is not properly treated. Therefore, the objective of this research is to determine the sources and risks of contamination in the Rio Grande river and assess the potential impact to human health. These efforts will aid our understanding of effective treatment of wastewater and drinking water. With this increased understanding, we will be able to make recommendations for wastewater and drinking water treatment, and aid in developing cost effective treatment strategies.

Problem and Research Objectives:

The Rio Grande River is the primary surface water resource for the Paso del Norte region. It is heavily utilized for agriculture and as a drinking water supply, but has been poorly characterized for its microbiological quality. Winter return flows during the non-irrigation season (typically November through April) contain significantly higher levels of pathogens due to agricultural return flows and wastewater treatment plant effluents. It is assumed that higher pathogen levels in winter return flow water leads to increased health risk in utilizing the winter return flows for drinking water. The objectives of this research are to: *1) define the sources and risks of contamination in the Rio Grande river; 2) determine the infectivity of* Cryptosporidium *in wastewater effluents; and, 3) perform a microbial risk assessment for Rio Grande river winter return flows as a source of drinking water.*

Materials/Methodology:

To assess the sources and magnitude of human and animal fecal pollution sources impacting the Rio Grande river during the summer irrigation and winter return flow seasons, *Bacteroidales* quantitative PCR (qPCR) is being utilized to identify key points in the watershed of possible contamination. Importantly, the impact of Bacteroidales fecal pollution markers present in wastewater effluents on estimates of pollution is being investigated. The potential risk to public health from wastewater effluents is being determined by analyzing the infectivity of *Cryptosporidium* oocysts using a cell culture method. Levels of infectious *Cryptosporidium* are being determined using human ileocecal adenocarcinoma cells (HCT-8 cells) labeled with an indirect antibody procedure and examined with epifluorescence microscopy. For both *Cryptosporidium* and *Giardia*, total levels of (oo)cysts are being determined using standard microscopy, and genotype analysis is being performed. Finally, data generated from this study will be incorporated into a risk assessment of the Rio Grande river.

Principal Findings:

The *Bacteroidales* PCR method is a culture-independent molecular method which targets genetic markers of *Bacteroides* and *Prevotella* spp. fecal bacteria that are specific to humans, ruminants (including cattle and deer) and pigs (including feral hogs) (Bernhard and Field 2000; Dick, Bernhard et al. 2005). There is also a general *Bacteroidales* marker (GenBac) that can be used as a general indicator of fecal pollution.

For this project, most of the activity involved method development focusing on *Bacteroidales* qPCR. Previous work in our lab revealed that Rio Grande river water samples were positive for the GenBac marker using *Bacteroidales* conventional PCR. This indicated that there is a presence of fecal contamination at all sampling sites. It was also noted that several samples tested positive for the human and hog markers. However, conventional PCR is only a presence/absence test of fecal pollution and is not quantitative. In order to estimate the relative abundance of host-specific *Bacteroidales* we are now using qPCR.

1. *A novel* Bacteroidales *quantitative PCR (qPCR) was applied to determine relative abundance of human and animal fecal pollution*

   In theory, the GenBac marker detects the majority of the *Bacteroidales* in the samples, including those detected with the host-specific markers. Using river water samples from a concurrent project, GenBac standard curves were developed using 10^0, 10^{-1}, and 10^{-2} dilutions of each water sample DNA. Since the actual copy number of GenBac target sequences in each sample was unknown, arbitrary values of 1,000, 100, and 10 were assigned to the dilutions, respectively. The hog, human and ruminant host-specific markers were quantified using the GenBac standard curve for each water sample and results are expressed as semi-quantitative marker abundance as determined by quantitative PCR (qPCR). This attempted to make the marker quantitation data for different water samples comparable by accounting for sample-to-

sample variation in *Bacteroidales* DNA concentration and any effects of PCR inhibitors on quantitation. This approach makes it possible to compare the relative abundance of each marker between stations or at the same station over time. The developed approach is being used to identify key locations in the Rio Grande River that may be contributing to fecal contamination.

2. *Human marker persistence through wastewater treatment*

   As stated previously, analysis of Rio Grande river samples indicated the presence of the human and hog *Bacteroidales* marker. There are very few hogs in this region, and based on analysis of wastewater samples, our results suggest that human sewage presents a low level of hog marker cross-reactivity. More importantly, we have found that the human and hog *Bacteroidales* markers were present in chlorine and UV disinfected wastewater effluents. This is an important observation because it shows that *Bacteroidales* bacteria can persist through the wastewater treatment process. The presence of the human *Bacteroidales* marker in treated effluents could impair our ability to differentiate between river water influenced by properly treated wastewater and untreated, raw sewage. However, it is still unclear if the *Bacteroidales* bacteria found in the treated effluents are viable or non-viable. In order to address this issue, we are collecting effluents from wastewater treatment plants that utilize chlorine disinfection only or chlorine and UV disinfection. We can then characterize the human marker using the current qPCR method which detects both viable and nonviable *Bacteroidales*. A new approach using propidium monoazide (PMA) and qPCR to differentiate viable from non-viable *Bacteroidales* will be applied. Final results are anticipated by the end of summer 2010.

3. Cryptosporidium *genotyping training*

   I received training and participated in a technology transfer workshop on a *Cryptosporidium* genotyping method that includes the use of forensic DNA sample purification techniques combined with a single round of multiplex PCR targeting the *Cryptosporidium* genes for 18S ribosomal RNA (18S rDNA) and heat shock protein 70 (hsp70). This genotyping method is being applied to characterize the total (viable and non-viable) and infectious *Cryptosporidium* spp. present in wastewater effluents.

4. *Microbial risk assessment for Rio Grande river winter return flows as a source of drinking water.*

   A substantial amount of data from our lab was provided to Dr. Kristina Mena, U.T. Houston School of Public Health, El Paso Regional Campus, and will be incorporated into a quantitative microbial risk assessment for *Cryptosporidium* in the Rio Grande. Additional results from this project will be incorporated into the risk model in the future.

Significance:

This research will provide data necessary for understanding the sources of pathogen contamination in the Rio Grande river. Determining the infectivity of *Cryptosporidium* and levels of *Giardia* in wastewater effluents and their impact on river water quality will help identify the sources and health risks associated with using the Rio Grande river as a source of drinking water. In conclusion, the developed risk assessment will include important data generated by this project to properly address *Cryptosporidium* and *Giardia* risks and in the implementation of effective water treatment. Results will be broadly disseminated among stakeholders to effectively address surface water treatment and appropriate management of water resources.

REFERENCES

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