Introduction

- Hepatitis A virus (HAV) is a non-envelope, RNA virus that is transmitted through the fecal-oral route by direct contact or ingestion of contaminated food or water.
- Understanding and detecting human exposure to waterborne pathogens is a growing global health challenge.
- According to the CDC, HAV surveillance can assist in detecting and providing data to control outbreaks, identifying contacts of case-patients who require postexposure prophylaxis, characterizing changes in the epidemiology of infected populations and risk factors, and guiding vaccination policies and other prevention efforts.
- Existing tools to determine the burden of disease HAV and other waterborne pathogens are expensive and invasive requiring serological assays.
- This study aims to exploit the use of biomarkers of exposure in population surveillance studies by using a bead-based multiplex immunoassay to assess the prevalence of salivary IgG antibodies against HAV and subsequent incident of infections (immuno-conversions) and immuno-prevalence in visitors to Boquerón Beach in Puerto Rico.
- This non-invasive, rapid, cost-effective tool for examining pathogenic exposure, prevalence and incidence can provide critical information for medical practitioners and policy makers in improving community health.

Methods

- Boquerón Beach, Puerto Rico was selected as the study location because it had the potential for fecal contamination from a discharging wastewater treatment plant and two smaller package plants.
- Water sampling and testing, epidemiological surveys, and collection of saliva samples were performed.
- After Institutional Review Board approval, informed consent and initial saliva samples (S1) were obtained from participants. Saliva samples were then self-collected on days 10 and 45 after the initial sample (S2 and S3).
- Samples were shipped on ice, stored at -80°C until processed, then evaluated using the protocol outlined in the development and application of a microsphere-based salivary antibody multiplex immunoassay.
- HAV antigen coupling was confirmed by exposing anti-HAV polyclonal antibodies to the antigen-coupled beads with uncoupled beads serving as assay control.
- Saliva samples were exposed to the antigen-coupled beads which were measured on a Luminex 200™ and reported in Median Fluorescence Intensity (MFI) units.
- MFI results were used to estimate immunoprevalence and immunoconversions for the targeted pathogens.

Results

- Figure 1. Summary of Results. (A) Map of the United States showing Boquerón Beach, Puerto Rico (red star). (B) Coupling confirmation of duplex HAV antigen and uncoupled control beads using goat-anti-HAV polyclonal antibodies. (C) Scatterplot of anti-HAV responses measured in median fluorescence intensity (MFI) units for all saliva samples analyzed (n = 5,438). Positive samples (MFI ≥ cutoff) are shown in red (n = 849). (D) MFI response curves of the 20 individuals who immuno-converted. (E) Immunopositivity heatmap for study participants who returned all three samples (n = 1,399). Red lines denote immuno-positive samples (MFI > cutoff).

Conclusion

- This study utilized a bead-based salivary antibody immunoassay in a multiplex format to determine the immuno-conversions and immuno-prevalence of HAV in visitors to Boquerón Beach in Puerto Rico.
- Results revealed an immuno-prevalence rate of 16.17% for HAV with 1.43% of the cohort immuno-converting to HAV.
- Among those who immuno-converted, 10% reported chronic gastrointestinal symptoms although none experienced diarrhea.
- This rapid salivary antibody immunoassay is a time-saving, inexpensive, and non-invasive tool requiring small sample volumes that has the potential to measure the prevalence and incidence rates of infection in communities for multiple pathogenic organisms simultaneously.
- The speed and efficiency of this assay is highlighted by that fact that while an ELISA would take 5 days and ~$9,000 to analyze 29 analytes, this multiplex assay, which can accommodate 50-100 analytes, takes 45 minutes and costs ~$3,700.
- Previous work has demonstrated the utility of the approach in monitoring epidemiological trends in public health including the detection of asymptomatic infections.
- The assay provides information on exposure susceptibility and can facilitate risk assessments for potential future outbreaks.
- Such information can be used by policy makers, health practitioners, and risk assessors in mitigating the health and the financial burden posed by exposure to existing and emerging pathogens.

Acknowledgements

This project was supported by the United States Environmental Protection Agency (EPA), the Institute for Science and Education and Department of Energy. A special thanks to all the members of the research team at the EPA.

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