

## Introduction

- In 2021, over 1.7 billion people used a drinking water source contaminated with feces.
- It is estimated that 1 million people die each year from diarrhea caused by unsafe water and lack of sanitation or hygiene and diarrheal diseases account for 1 in 9 child deaths worldwide.
- Assessing exposures to environmentally transmitted pathogens is essential to mitigating the burden of disease globally.
- A prominent area of concern is the association of fecal contamination and the risk of gastrointestinal illnesses.
- This study aimed to determine the prevalence and incidence of infection among riverine beachgoers by measuring salivary immunoglobulin G (IgG) antibodies against six waterborne pathogens.

## Methods

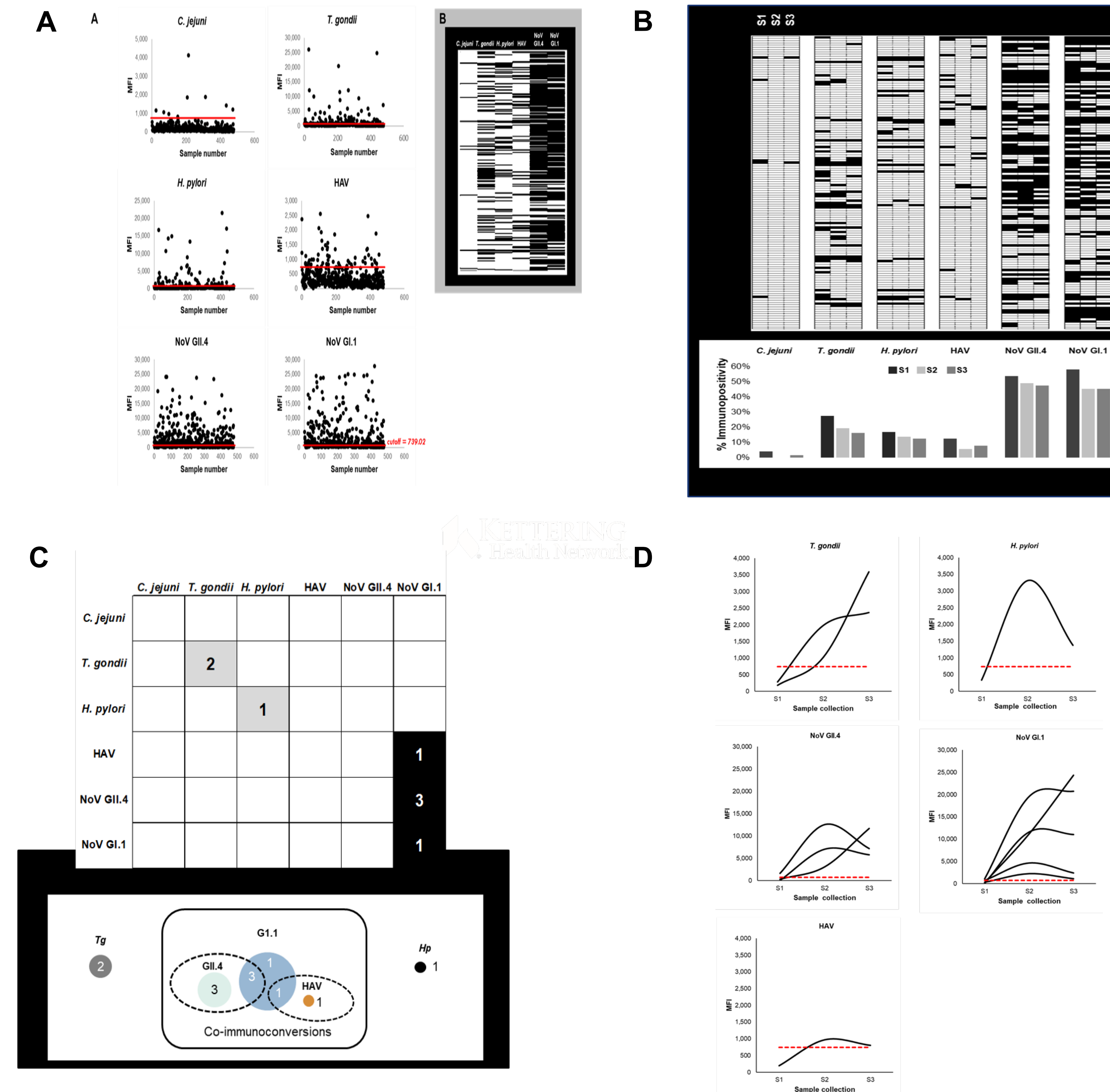
- Buffalo Shores Beach in Buffalo, Iowa was selected as the study location because it is located downstream of multiple wastewater treatment plants (WWTPs) that are believed to contribute to water contamination and waterborne infections in individuals recreating there, and the beach is well-populated in the summer months.
- This study used a multiplex Luminex platform to assess salivary antibody responses to antigens (Ag)
- Water samples and initial saliva samples (S1) were obtained at the study site.
- Participants were instructed to ship samples collected on days 10 and 40 (S2 and S3) stored at -80°C and evaluated using the microsphere-based salivary antibody multiplex immunoassay.
- Samples were exposed to antigen-coupled microspheres which were measured on a Luminex 200™ and reported in Median Fluorescence Intensity (MFI) units.
- MFI results were used to estimate immunoprevalence, co-immunopositivity and immunoconversions for the targeted pathogens.

## Location



**Figure 1:** Maps of (A) the United States displaying the study area on the eastern border of Iowa. (B) The study area (lower left in dark blue) is along the Mississippi River downstream from Davenport, Iowa, and multiple wastewater treatment plants (WWTP). (C) A snapshot of the Buffalo Shores Beach recreational area.

## Results



**Figure 2.** Summary of Results. (A) Median Fluorescence Intensity units (MFI) scatterplot showing all S1 samples with the cutoff (red line: 739.02 MFI) distinguishing positive (above) and negative (below) samples. (B) Exposure status of the individuals who provided a saliva sample for each collection period (S1–S3). Upper panel: black lines represent the samples positive (MFI ≥ cutoff) for the targeted pathogens. Lower panel: % immunopositivity for the targeted pathogens. (C) Number of immunoconversions and co-immunoconversions to the pathogens under study. Note Tg: *T. gondii*; GI.1: norovirus GI.1; GII.4: norovirus GII.4; HAV: hepatitis A virus and Hp: *H. pylori*. (D). MFI curves for the pathogens under study. Plots showing IgG in MFI from S1 to S3 for immunoconversions. Red dashed line = cutoff (739.02 MFI).

## Conclusion

- This study utilized a bead-based salivary antibody multiplex immunoassay to determine antibody prevalence to select waterborne pathogens among a group of beachgoers in Buffalo Shores Beach, IA.
- Results revealed that nearly 80% of beachgoers were exposed to at least one of the targeted pathogens.
- While there were exposures to norovirus GI.1 (59.41%), norovirus GII.4 (58.79%) and *Toxoplasma gondii* (22.80%), over half (56.28%) of beachgoers showed previous exposure to multiple pathogens.
- Analysis of serially collected samples resulted with 6.11% immuno-converted to one or more pathogens, largely to noroviruses (GI.1: 3.82% and GII.4: 2.29%) and *T. gondii* (1.53%).
- 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 utilization of this antibody multiplex allowed us to investigate the exposure, prevalence, and incidence of infection of six waterborne pathogens among riverine beachgoers downstream from WWTPs.
- Analyzing the initial sample allowed us to determine these pathogens as etiological agents to previous infection and the prevalence within the subject population.
- The assay facilitates the development of risk assessments for potential future outbreaks useful for policymakers, health-practitioners, and risk-assessors in mitigating the health and financial burden posed by exposure to existing and emerging pathogens.

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