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Recreational swimmers' exposure to *Vibrio vulnificus* and *Vibrio parahaemolyticus* in the Chesapeake Bay, Maryland, USA

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ABSTRACT

Vibrio vulnificus and *Vibrio parahaemolyticus* are ubiquitous in the marine–estuarine environment, but the magnitude of human non-ingestion exposure to these waterborne pathogens is largely unknown. We evaluated the magnitude of dermal exposure to *V. vulnificus* and *V. parahaemolyticus* among swimmers recreating in *Vibrio*-populated waters by conducting swim studies at four swimming locations in the Chesapeake Bay in 2009 and 2011. Volunteers (n = 31) swam for set time periods, and surface water (n = 25) and handwash (n = 250) samples were collected. Samples were analyzed for *Vibrio* concentrations using quantitative PCR. Linear and logistic regressions were used to evaluate factors associated with recreational exposures. Mean surface water *V. vulnificus* and *V. parahaemolyticus* concentrations were 1128 CFU mL^{-1} (95% confidence interval (CI): 665.6, 1591.4) and 18 CFU mL^{-1} (95% CI: 9.8, 26.1), respectively, across all sampling locations. Mean *Vibrio* concentrations in handwash samples (*V. vulnificus*, 180 CFU cm^{-2} (95% CI: 136.6, 222.5); *V. parahaemolyticus*, 3 CFU cm^{-2} (95% CI: 2.4, 3.7)) were significantly associated with *Vibrio* concentrations in surface water (*V. vulnificus*, $p < 0.01$; *V. parahaemolyticus*, $p < 0.01$), but not with salinity or temperature (*V. vulnificus*, $p = 0.52$, $p = 0.17$; *V. parahaemolyticus*, $p = 0.82$, $p = 0.06$). Handwashing reduced *V. vulnificus* and *V. parahaemolyticus* on subjects' hands by approximately one log (93.9%, 89.4%, respectively). It can be concluded that when Chesapeake Bay surface waters are characterized by elevated concentrations of *Vibrio*, swimmers and individuals working in those waters could experience significant dermal exposures to *V. vulnificus* and *V. parahaemolyticus*, increasing their risk of infection.

1. Introduction

Vibrio vulnificus and *Vibrio parahaemolyticus* are normal functioning members of natural bacterioplankton communities in estuarine and marine waters that are routinely used for swimming and other recreational activities. These microorganisms can also cause mild to severe infections, including wound infections, gastroenteritis, and septicemias, among individuals who are exposed to contaminated waters (Dziuban et al., 2006; Hlavsa et al., 2011; Yoder et al., 2008). In the Chesapeake Bay region, the Centers for Disease Control and Prevention (CDC) reported 65 illnesses associated with *Vibrio* spp. infections in 2011 (CDC, 2013a). Foodborne Diseases Active Surveillance Network (FoodNet) data showed a 43% increase (CI: 16%–76%) in the incidence of *Vibrio* infections at ten U.S. sites in 2012 compared with 2006–2008 (CDC, 2013b).

Specifically, there are approximately 93 serious (requiring hospitalization) cases of *V. vulnificus* reported in the United States annually (Scallan et al., 2011). A study of non-foodborne *Vibrio* infections (NFVIs) from 1997 to 2006, before Vibriosis became a nationally notifiable disease, reported that *V. vulnificus* was responsible for 35% of all NFVIs and 78% of NFVI deaths in the United States (Dechet et al., 2008). For immunocompromised individuals infected with *V. vulnificus*, there is an estimated 50% mortality rate (Oliver, 2005). In contrast, *V. parahaemolyticus* infections

Abbreviations: ANOVA, analysis of variance; ATCC, American Tissue Culture Collection; CDC, Centers for Disease Control and Prevention; CFU, colony forming unit; CI, confidence interval; Ct, cycle threshold; DNA, deoxyribonucleic acid; FAO, Food and Agricultural Organization; FDA, Food and Drug Administration; HIV, human immunodeficiency virus; ID_{50}, median infective dose; NFVI, non-foodborne *Vibrio* infection; PBS, phosphate buffered saline; PCR, polymerase chain reaction; qPCR, quantitative polymerase chain reaction; TBSA, total body surface area; *tdh*, thermostable direct hemolysin; *trh*, thermostable related hemolysin; *vcgC*, virulence correlated gene, clinical; WHO, World Health Organization; YSI, Yellow Springs Instruments.

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are not as severe as those caused by *V. vulnificus*, rarely progressing to septicemias (5%). However, the percentage of *V. parahaemolyticus* manifesting as wound infections (34%) is comparable to that of *V. vulnificus* (45%), and the percentage of *V. parahaemolyticus* infections manifesting as gastroenteritis (59%) is significantly higher than that of *V. vulnificus* (5%) (Dechet et al., 2008).

Routes of exposure to *V. vulnificus* and *V. parahaemolyticus* include ingestion of contaminated seafood, dermal contact with contaminated estuarine/marine water and, in the case of *V. vulnificus*, dermal contact with contaminated fish (CDC, 2013c; CDC, 2013d). While the non-ingestion infectious dose is largely unknown for both *V. vulnificus* and *V. parahaemolyticus* (FDA, 2012), risk assessments from the U.S. Food and Drug Administration suggest that the ingestion infectious dose, producing a 50% probability of illness for *V. parahaemolyticus*, is approximately 10^6 to 10^8 CFU g^{-1} (FDA, 2005). Risk of illness modeled by the Food and Agricultural Organization of the World Health Organization (FAO/WHO) approximated an ingestion infectious dose of 10^3 to 10^7 CFU g^{-1} oyster tissue for *V. vulnificus* (WHO, 2005). Meanwhile, the use of sub-cutaneous *V. vulnificus* inoculums in murine models has demonstrated a non-ingestion infectious dose of 1000 CFU, with an ID_{50} of approximately 10 CFU for iron-dextran treated mice (Thiaville et al., 2011). Therefore, it is conceivable that the non-ingestion human infectious dose, encountered from direct contact between an open wound and *Vibrio*-populated media (e.g., water, surfaces, seafood products), may equate to a fraction of the estimated ingestion infectious dose.

While non-ingestion, dermal exposures to *Vibrio* are likely important with regard to public health—potentially contributing to increasing rates of illness and deaths associated with these microorganisms—very little is known about the magnitude of dermal exposure to these environmental pathogens in recreational settings. Therefore, we investigated the magnitude of non-ingestion, dermal exposures to *V. vulnificus* and *V. parahaemolyticus* among swimmers in select locations of the Chesapeake Bay by testing the prevalence of these microorganisms in handwash samples. Using the handwash data, we also quantified total body dermal exposures that could result from swimming in *Vibrio*-contaminated surface water. Finally, we assessed the efficacy of handwashing to remove *Vibrio* species from the skin surface following dermal exposure, and evaluated surface water conditions that favor the transmission of these pathogens to humans. To our knowledge, these are the first data of their kind.

2. Materials and methods

2.1. Swimming sites

Recreational beaches on four different rivers in the Chesapeake Bay were chosen for our swimming sites: Choptank River, Chester River, Tred Avon River and Chesapeake mid-Bay (Sandy Point State Park) (Fig. 1). These sites were selected based on differing salinities and geographic locations to ensure a range of surface water *Vibrio* spp. concentrations in order to test associations between *Vibrio* spp. concentrations in water and dermal exposures among swimmers. Swims were conducted approximately 1–2 h post high tide to standardize tidal cycle across swims and best attempts were made to schedule each swim during midday hours, although sampling in the Chester River was completed slightly later in the midafternoon.

2.2. Institutional review board

This study was reviewed and approved by the University of Maryland Institutional Review Board (Protocol: 11-0442).

2.3. Study population

The study population was a convenience sample of individuals recruited from a local academic institution. The initial 2009 swim (Sandy Point State Park) included 19 participants, and subsequent 2011 swims (Choptank River, Tred Avon River, and Chester River) included four participants for each swim, based upon a sample size calculation performed using the 2009 data. Specifically, sample size was calculated for a desired power of 0.90, preferred detection level of 25 CFU and an alpha of 0.05, using standard deviation calculations from the 2009 swim study handwash samples: 4.89 CFU mL^{-1} (between swim), 10.5 CFU mL^{-1} (between swimmer) (*V. vulnificus*); and 3.31 CFU mL^{-1} (between swim), 4.4 CFU mL^{-1} (between swimmer) (*V. parahaemolyticus*). It was determined that three swims were needed

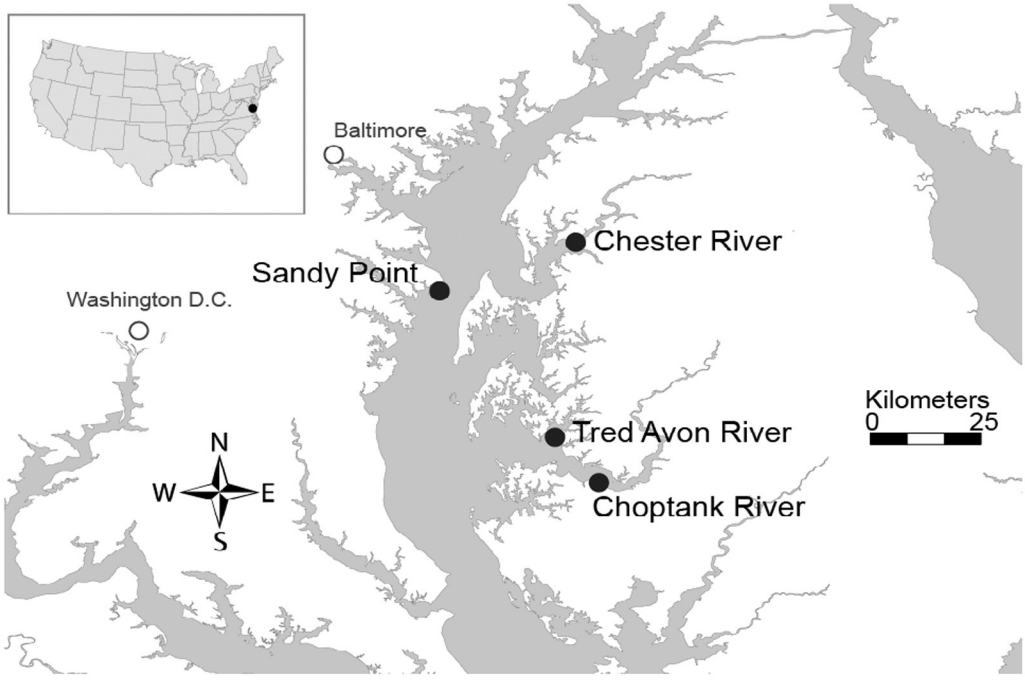

Fig. 1. Map of swimming sites in the Chesapeake Bay that were included in this study. From: Tracey Saxby, Kate Boicourt, Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/imagelibrary/displayimage-127-5815.html).