Are fecal indicator bacteria appropriate measures of recreational water risks in the tropics: A cohort study of beach goers in Brazil?

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ABSTRACT

Regulating recreational water exposure to pathogens within the tropics is a major public health and economic concern. Although numerous epidemiological studies estimating the risk to recreational marine water exposure have been conducted since the 1950s, few studies have been done in the tropics. Furthermore, many have suggested that the use of fecal indicator bacteria for monitoring recreational water quality in temperate regions is not appropriate in the tropics. We analyzed a large cohort study of five beaches in Sao Paulo, Brazil, conducted during consecutive weekends in the summer of 1999 that estimated risk to water, sand, and food exposures. Enterococci and *E. coli* concentrations were measured each day of the study. Elevated risks were estimated for both swimming (OR = 1.36 95%CI: 1.05-1.58) and sand contact (OR = 1.29 95% CI 1.05-1.58). A 1 log increase in enterococci concentration was associated with an 11% increase in risk (OR=1.11 95% CI: 1.04-1.19). For *E. coli* a 1-log increase in concentration was associated with 19% increase in risk (OR=1.19 95% CI: 1.14-1.28). Most countries with beaches in the tropics are lower or middle income countries (LMIC) and rely on tourism as a major source of income. We present data that suggests fecal indicator bacteria such as enterococci are an appropriate indicator of risk in tropical urban settings where contamination is coming from predominantly human sources. Additional studies in tropical settings could help inform and refine guidelines for safe use of recreational waters.

Keywords: Diarrheal disease, recreational waters, Brazil, tropics

INTRODUCTION

Recreational activities in coastal waters are a major economic driver worldwide, especially in lower and middle income countries (LMIC) (Ghermandi and Nunes 2013). Increasingly, these waters have been documented to be contaminated by human fecal contamination through numerous sources including: sewage outflow, urban runoff, and other point and non-point sources (Abdelzaher et al. 2010, Korajkic et al. 2011, McQuaig et al. 2012, Wong et al. 2009). Associations between these contaminated recreational water exposures and disease have been documented since 1950s (Prüss 1998, Wade et al. 2003). As a consequence, water quality criteria were developed (United States Environmental Protection Agency 2012, WHO 2003) and applied around the world based on epidemiologic studies conducted in the United States (Cabelli et al. 1982, Dufour 1984) and United Kingdom (Kay et al. 1994). It is notable that LMIC countries are underrepresented in the scope of these global recreational water quality and epidemiological studies. Nonetheless they rely on World Health Organization (WHO) water quality criteria with relatively little information known about their local applicability (Henrickson et al. 2001). Exceptions include studies performed in beaches from Egypt in 1983 and South Africa in 1995 (Prüss 1998). In addition, it has been suggested that in tropical environments, fecal indicators such as enterococci may persist and regrow in soils and waters and as a result may not be appropriate indicators for such locations (Fujioka 2001). Epidemiological studies of water quality and swimming associated illness in tropical environments were recently identified as a major data need (Boehm et al. 2009). Based on this lack of representation and questions regarding applicability of widely used fecal indicator bacteria in many parts of the world including Latin America, the Environmental Agency of São Paulo State - Brazil (CETESB)

undertook a prospective cohort study on the disease risks associated with swimming in five beaches along the São Paulo coast.

Due to the expense of measuring pathogens, as well as the vast number of pathogens that may be present in the marine waters, surrogate indicators (organisms that inhabit the intestinal tract of humans) are generally measured. In the 1950s, Stevenson (1953) first reported that incidence of illness occurred more frequently in swimmers than in non-swimmers at freshwater beaches and that data was used by the National Technical Advisory Committee (NTAC 1968) to develop guidelines suggesting the use of fecal coliforms for measuring recreational water quality. These guidelines provided the basis for using fecal coliforms to characterize water quality at marine and freshwater beaches throughout the United States for the next 30 years. Later, enterococci for marine waters and *E. coli* for fresh water replaced the coliform criteria based on epidemiology studies conducted in the 1970s and 1980s (Cabelli et al. 1982, Dufour and Strickland 1979), which suggested these were superior indicators to fecal coliforms. However few other countries monitor for enterococci (Henrickson et al. 2001). The WHO “Guidelines for safe recreational water environments”, developed a decade ago (WHO 2003), are based on enterococci and provide guidance for countries worldwide on marine water quality. However, to this day most South American countries continue to employ thermotolerant coliforms or *E. coli* criteria for marine recreational water quality. Brazil is an exception, as they established a federal law in 2000 that stipulated recreational water quality criteria for marine water using enterococci (Brazil 2000). These criteria were based on the WHO guidelines draft (WHO 1998) and a pilot epidemiological study conducted at Sao Paulo Coast in 1987 that demonstrated enterococci levels in bathing water were more strongly correlated to health effects than fecal coliforms (data

not published). Even with these federal standards, most Brazilian states currently test waters for thermotolerant coliforms or *E. coli*.

To inform locally-specific risks associated with water and sand exposure, we describe a large cohort study performed at five urban beaches near São Paulo, Brazil. The objectives of this study were to provide locally-specific data to: 1) investigate the rates of gastrointestinal (GI) illness for swimmers and non-swimmers, for those with and without sand contact, and for swimmers exposed to waters with different enterococci concentrations; 2) determine health risks for different age groups following sand and water exposure; and 3) use water quality and epidemiological data to evaluate whether a dose-response relationship exists for swimmers using the fecal indicator bacteria enterococci.

MATERIALS AND METHODS

Study sites: São Paulo State is located in the southeastern region and represents 10% of the Brazilian coast, with 292 beaches. There are 15 coastal counties with a population of 2 million inhabitants that can double during the summer months. This study was conducted in five marine beaches (Enseada, Pitangueiras, Asturias, Aparecida and Ocian) located in four coastal municipalities of São Paulo State (Figure 1). These beaches were selected based on: 1) representing a range of bathing water quality; 2) high bather density; and 3) presence of families with children at the beach. At the time of this study, the major municipalities in the study regions (e.g. Santos, Guarujá and Praia Grande) had sewer systems that provided preliminary treatment and chlorination before discharging effluent approximately 4 km offshore. However, many of the outer lying communities had unimproved sanitary systems, partial population connections, or

were occupied by slums (Table S1), leading to contamination of streams that flow into the ocean and nearby beaches. Although conditions have improved somewhat, these beaches continue to be influenced by untreated sewage.

Epidemiological study design: Visitors to each of the five study beaches were enrolled into a prospective cohort study over five consecutive weekends (Saturdays and Sundays) during the summer period from January 9th to February 6th 1999 utilizing eleven trained interviewers per beach. Families were approached on the beach and provided with information on the study. Anyone who reported contact with other types of water, such as another beach, lake, or river in the previous seven days was excluded from the study. Verbal consent was obtained from all eligible individuals who agreed to participate. Following consent, participants were interviewed in parallel with microbiological water quality assessment (see below). An adult member (> 18 years of age) answered questions for the other household members. A follow-up questionnaire was conducted by telephone 7-10 days later to assess whether the participants had developed any gastrointestinal (GI) or other potentially related symptoms. Questionnaires, protocols, consent procedures, and study materials were reviewed by the School of Public Health at the University of Sao Paulo. In Brazil, the National Committee of Ethics in Research was created in October 1996; however, the first operational manual was not published until 2002. IRB approval to conduct secondary data analysis for this study was obtained from the University of Michigan and the University of Arizona.

Beach questionnaires: Beach questionnaires were administered upon enrollment to gather demographic data and anticipated exposure information, such as contact with water and sand.

Because participants were approached while on the beach, they were asked, “did you enter or do you intend to enter the water today”. The potential for non-differential exposure misclassification, because some participants only reported their intention to swim, could result in a bias towards the null (see discussion section for further details). They were then asked if they usually swallow water or usually submerge their head. Sand contact was defined based on whether a participant usually sits or lies down directly on the sand. Beyond level of exposure to water (i.e. enter water only, usually submerge head, or usually swallow water) and sand contact, other variables known to contribute to GI illness were also collected such as (a) consumption of food sold on the beach (juices, drinks, snacks, ice-cream, etc.); and (b) regular consumption of seafood while at the coast.

Follow-up questionnaires: Individuals were contacted by telephone within 7-10 days of their initial beach interview to determine the onset of any symptoms that occurred following the beach visit, such as stomach ache, diarrhea, nausea, vomiting, fever, runny nose, earache, and eye irritation. Participants were also questioned about contact with other bathing water (e.g. pools, lakes, river, or ocean) during the past week. People who could not be reached by telephone were lost to follow-up.

Water sampling and microbiological analysis: Marine water samples were collected on the day of the beach interviews around 11:00 am utilizing the standard sampling site the Environmental Agency of Sao Paulo State (CETESB) uses for routine beach quality assessment (CETESB 2015). Two additional stations were sampled 300 meters to the right and to the left of the standard one. Volumes of 500mL were taken in sterile wide mouth plastic bottles just below

the surface where water was 1 m in depth, in accordance with standard methods and the Brazilian regulation (Brazil 2000). Samples were kept chilled during transportation and analyzed within 8 hours. Samples were taken at the same time of day at each beach and were tested for *E. coli* and enterococci.

Bacterial analyses were performed by membrane filtration technique. Sample volumes of 1, 5, 25, and 70 ml were assayed in order to produce an acceptable number of bacterial colonies following approved standard laboratory methods. Analyses were not performed in duplicates. *E. coli* was assayed on mTEC agar with the addition of indoxyl beta D-glucoside (1979) and enterococci were enumerated on mE and EIA agar as described by Levin et al (1975). Bacterial results were reported as colony forming units (CFU)/100ml. Multiple dilutions were tested, resulting in no Too Numerous to Count (TNTC) samples. One sample, processed for 70 ml, was below the detection limit and was reported as 1 CFU/100ml.

Statistical analyses: Chi-squared tests were used to determine statistical differences in demographic data between participants who completed the initial beach interview and individuals who completed the follow-up interview. As we were interested in assessing whether children < 10 years were at higher risk, participants were divided into two age groups: < 10 yrs and >10 years. These age groups were chosen to account for differences in behavior on the beach and potential host susceptibility factors (e.g., differences in immune status). Classifying children as < 10 years is also consistent with prior definitions in the literature (Wade et al. 2008). Initial analyses were conducted separating teenagers and young adults (11-19 yrs) from adults (20+),

but there were few differences noted between these groups, so they were collapsed for all reported analyses and models.

Symptom data was summarized by age group, both as counts and cumulative incidence per 1000 cases. Since our focus was gastrointestinal illness, we did not include runny nose, earache, and conjunctivitis in our data summary or analyses. Our primary outcome variable, referred to as NGI (NEEAR-GI Illness, from the US EPA NEEAR studies), is a composite defined as anyone experiencing the following symptoms: diarrhea (three or more loose stools in a 24 hour period); vomiting; or nausea with stomachache. This definition is very similar to the definition adopted by the USEPA in 2012 (one criterion used by the USEPA, nausea or stomachache that was disabling, was not used in our definition as we did not collect that information) (EPA 2012). Prior definitions required fever to co-occur with diarrhea, stomachache or nausea. NGI was developed to include viral infections that can occur with mild or no fever (Wade et al. 2010).

Water exposure and water quality were considered the primary exposure variables. For GI illnesses, questions related to ingestion, the exposure of concern, were phrased ‘do you usually submerge your head’ or ‘do you usually swallow water’. A swimmer was defined as those that reported yes to entering, or intending to enter the water that day. Those who reported not entering the water that day (or not planning to) were defined as non-swimmers. Highly exposed swimmers were defined as those who reported both entry into the water and usually swallowing water – they were also compared to non-swimmers for the purposes of the logistic regression models. Models also took into account other possible routes of pathogen exposure including contact with sand and consumption of food prepared on the beach. Estimated exposures were

assessed by comparing swimmers to non-swimmers, as well as those who reported yes to either sand exposure or food consumption compared to those who reported no to either sand exposure or food consumption.

Three separate analyses were conducted. The first considered the general risks associated with water exposure (highly exposed swimmers versus non-swimmers), without considering water quality. The second and third analyses included water quality measures to determine the relationship between NGI and either enterococci or E.coli levels. All models took into account other possible routes of pathogen exposure including contact with sand and consumption of food prepared on the beach. Additional variables such as age, gender, beach, and contact with either untreated or pool water in the week between the beach and telephone interviews were added to the model to determine if confounding was present. Only significant covariates were retained in the final models. This was assessed by standard model comparison methods at a significance value of $\alpha=0.05$. We assessed the variables of sand exposure and individual beach location as potential effect modifiers of swimming exposure through both stratification and by including an interaction term. Interaction terms were kept in the model if the p-value was less than 0.1.

Daily exposures for swimmers were assigned the log of the daily geometric mean (mean of the log₁₀ CFU/ 100 ml) of the fecal indicator bacteria concentrations measured at three spatially representative points across each beach. Although this sampling strategy is sparser than the sampling practices currently applied during recreational water epidemiological studies (Wade et al. 2010), the three locations and three time points were selected to coincide with the majority of the swimming activity and are thought to have captured exposure for the majority of the

swimmers. Any misclassification associated with exposure is likely to be non-differential with respect to the outcome and therefore associations will be biased towards the null. Non-swimmers were assigned a value of zero for the log of the enterococci geometric mean (geometric mean=1 CFU/ 100ml). Results are reported as odds ratios (OR) or adjusted odds ratios (aOR).

RESULTS

A total of 6,343 families or groups of people were interviewed, corresponding to a total of 23,237 individuals (Table 1). Of these, 16,637 (72%) people were successfully contacted and followed up 7-10 days later by telephone. A comparison of the total initial cohort and the group who completed the follow up questionnaire suggests little difference between them (Table 1). Of the final cohort, 14,010 (84%) were swimmers and were included in the analyses, with 5,552 (40%) of those considered to be highly exposed swimmers.

A total of 1,408 (8.5%) subjects reported illness (NGI) within 7-10 days of the beach exposure. For all participants NGI incidence rates varied by age: 131 per 1000 for children (≤ 10 yrs) and 51.5 per 1,000 for teenagers and adults (>11 yrs.) (Table 2). Rate of NGI also varied across beaches, ranging from a low of 60.4 (Beach 1) to a high of 129.2 (Beach 5) per 1,000 individuals. These differences by beach were preserved when the data are stratified by age (Figure 2 and Table 2). The most common GI symptoms reported were diarrhea and vomiting (Table 2).

Water quality also varied by beach. The geometric mean ranged from 42 (Beach 2) to 234 (Beach 4) CFU/100 ml for *E. coli* and 16 (Beach 2) to 64 (Beach 5) CFU/100 ml for enterococci

(Table 3 and Figure 3). Corresponding to greater pollution, the beaches with higher contamination during the study weekends were also those beaches with higher rates of noncompliance with water quality criteria throughout the year, except for Beach 3. Noncompliance is usually greater in the summer months (January and February) because of the increase of population and rainfall during that period. Noncompliance was defined as conditions when more than 20% of measurements within a 5 week period were greater than 1,000 thermotolerant coliforms/100ml as it was the indicator used by CETESB's regular surveillance at that time (Figure 3). The high levels of enterococci in Beach 5 corresponded with the high levels of reported NGI (Figure 2).

Associations between NGI and water, sand and food exposures: All exposures investigated (water, sand, and food) were significantly associated with NGI (Table 4). Swimming was associated with NGI (aOR=1.24; 95% CI 1.04-1.48) and the effect was more pronounced among highly exposed swimmers (aOR=1.5; 95% CI 1.23-1.84). The highest odds of illness were found for highly exposed swimmers at Beaches 2 (OR=3.75 95% CI 2.31-6.07) and 4 (OR=3.87; 95% CI 2.40-6.25). Odds of illness from sand contact was also elevated for both age groups for combined beach data, but when stratified by beach, significant associations were only observed for children under 11, particularly at beaches 1, 2, and 5. Participants with both water and sand contact had a higher risk than those with either water or sand contact alone; i.e., for highly exposed swimmers without sand contact aOR = 1.41; (95% CI 1.02-1.94, n= 3030) and for those only exposed to sand (aOR=1.32; 95% CI 0.89-1.96, n= 2484). Sand contact was only significantly associated with NGI in children (OR=1.54; 95% CI 1.26-262 1.88) and significant

associations between food consumption on the beach and NGI were only identified for adults (OR=1.41; 95% CI 1.18-1.71).

After adjusting for potential confounders, highly exposed swimmers (OR=1.36; 95% CI 1.05 – 1.58), contact with sand (OR=1.29; 95% CI 1.05 – 1.58), and eating food prepared at the beach (OR=1.16; 95% CI 1.00 – 1.35) were all independently associated with elevated risk of NGI (Table 5 Model 1). These elevated risks were largely driven by children (highly exposed swimmers: OR=1.41; 95% CI 1.6 - 1.9 and contact with sand: OR=1.43; 95% CI 1.1 - 1.9). The older age group (> 10yrs) had non-significant associations. An interaction term used to test effect modification between highly exposed swimming and sand contact was not statistically significant. However a stratified analysis did suggest a significant interaction with respect to the beach visited. In comparison to beach 1 (reference group) highly exposed swimmers at beaches 2 (OR=1.47; 95% CI 1.12-1.91) and 5 (OR=2.65; 95% CI 2.06-3.41) were at highest risk (beaches 3 and 4 were not statistically significant).

Associations between water quality and NGI: Among swimmers only, and without adjustment for covariates, there was a dose-dependent association between the incidence of NGI and enterococci geometric mean measures (Figure 4) and NGI and *E. coli* geometric mean measures (Figure 5). After adjusting for covariates, a 10-fold increase in enterococci was associated with an 11% increase in the odds of NGI among highly exposed swimmers (Table 5-Model 2). Results for *E. coli* also showed a dose-response relationship with an increase of 19% in the risk of disease for every 1 log increase (Table 5-Model 3). Risks for sand exposure and food consumption were similar in all three multivariate models. The exposure-response association

appeared to be most pronounced among children age 0-10 (OR=1.16; 95% CI 1.05-1.28 for enterococci and OR=1.23; 95% CI 1.12-1.35 for *E. coli*). A counterfactual analysis was performed for non-swimmers by using the fecal indicator bacteria geometric means at each beach-day to test the effect of water quality on this unexposed subgroup. No associations were identified between water quality at the beach and non-swimmer NGI cases. The full logistic regression models for *E. coli* and enterococci for NGI risk among non-swimmers are provided in Supplementary Information Tables S2 and S3, respectively.

DISCUSSION

Results from this large cohort study performed at five urban beaches in a tropical climate near Sao Paulo city, Brazil, demonstrate elevated illnesses among those in contact with water and sand, as well as a significant exposure-response association between enterococci and *E. coli* levels and NGI among swimmers. As most of these sites were located in urban areas with insufficient sewage collection, they received regular discharges of partially treated or untreated wastewater from the nearby communities. It has been well documented that fecal indicator bacteria are good predictors of GI risk due to exposures of recreational waters when the predominant source of contamination is human, such as a wastewater runoff (Prüss 1998, Wade et al. 2003). This association is attributed to the pathogenic microorganisms that generally co-occur with human fecal contamination (e.g., norovirus; *Cryptosporidium*), most of which cause gastrointestinal symptoms. A study carried out at beaches with nonpoint sources in California, where human sources were not dominant, showed that traditional fecal indicators were not associated with health risks (Colford et al. 2007).

Most recreational epidemiological studies have been conducted in temperate climates (Prüss 1998). The few studies performed in tropical environments have demonstrated fecal indicator organisms, such as *E. coli* and enterococci, were ambiguous and potentially regrowing in the environment (Bordalo et al. 2002, Carrillo et al. 1985, Shibata et al. 2004). These differing population dynamics have prompted many to suggest that the bacterial indicators found to be good health indicators in temperate regions for recreational water exposure are not appropriate in the tropics. Numerous reports and papers have suggested that traditional fecal indicator organisms such as enterococci and *E.coli* are unsuitable markers of water quality and swimming associated illness in tropical environments (Boehm et al. 2009, Fujioka 2001). A recent study at a tropical beach site conducted by the US EPA in Puerto Rico, with relatively low observed levels of fecal contamination, found no evidence of increased risk associated with swimming, and no significant associations between NGI and enterococci densities (EPA 2009). In contrast, the findings of the present study suggest that enterococci may be in fact an effective marine indicator of swimming associated NGI even in tropical environments, at sites impacted by wastewater. Although the suggestion that FIB are not good indicators of fecal contamination in tropical settings may be correct when contamination sources largely consist of treated wastewater, our findings suggest that this may not be true when the sources of contamination comes from untreated or minimally treated wastewater. Since these conditions found in Brazil is similar to most tropical settings that reside in lower and middle income countries (LMIC), this study may have more relevance to developing guidelines and policy for the tropics than those conducted in a developed country setting.

We observed an elevated risk for GI illness among swimmers compared to non-swimmers, a finding consistently observed at beach sites impacted by fecal contamination (Prüss 1998, Wade et al. 2003). In addition, we observed an 11% increase in the risk of GI illness per 10-fold increase in enterococci exposure (aOR=1.11; 95% CI 1.04-1.19). This association is similar in magnitude to that observed with culturable enterococci at temperate marine sites (Wade et al. 2010) and temperate freshwater sites using a molecular measure of *Enterococcus* (Wade et al. 2008). We also found that a 10-fold increase in *E. coli* concentrations was associated with elevated risk, which is in contrast to findings in the prior temperate setting studies. Our observation that *E. coli*, which is not stable in marine waters, was also associated with illness may also be explained by the fact that untreated sewage was discharged close to coastal waters. Under these conditions, *E. coli* could remain viable and therefore could be a reliable indicator of sewage contamination in marine waters.

Despite the large sample size and overall retention there are some caveats worth mentioning in regards to exposure assessment. As with most recreational water studies, except for randomized controlled trials conducted in the UK (Kay et al. 1994), water quality was not matched in time and space with each swimmer; rather the concentration of enterococci in the water was measured in three locations once per day. In addition, misclassification of exposure can occur if someone reported the intention of certain behavior that did not take place that day. This is because the questions related to whether they swam, submerged their head or swallowed water were based on interviews during their stay at the beach, and some answers could be based on their intentions. There is no indication, however, that the misclassification is differential between those that reported NGI compared to those did not report NGI. Non-differential misclassification would

bias any association towards the null, thus the unbiased association would be higher than what we reported. Further evidence that the bias did not impact our conclusions is that we do not see increased risk of NGI in those that claimed to not enter the water during the epidemiological study. This suggests those that say they did not intend to swim were by in large correctly classified and the risk for people who reported both water entry and usually swallowing water (or submerging their head) was higher than people who reported only entering the water, which likely indicates a higher level of exposure. Additionally, our data suggests that the large majority of beachgoers (84%) actually enter the water in summertime (Table 1), which is consistent with a prior survey carried out in São Paulo coast (CETESB 2015).

In addition, in this study, there was some evidence of a stronger association between water quality and GI illness among children consistent with one previous study (Wade et al. 2008). This enhanced effect could be due to poorer hygiene among children, more prolonged swimming exposures, and the increased likelihood of swallowing water.

Estimated contact with sand and consumption of food on the beach were also found to be independent risk factors for NGI. Sand contact has previously been shown to be associated with NGI risk (Heaney et al. 2012) and sand can harbor microbes including fecal indicator organisms and pathogens (Goodwin and Pobuda 2009, Heaney et al. 2012, Mika et al. 2009, Sabino et al. 2011, Shibata and Solo-Gabriele 2012, Yamahara et al. 2012). Especially in the tropical environment, warm and moist conditions may be favorable for the persistence and growth of microbes. Sand contamination (or poor microbiological quality) was reported in São Paulo beaches in studies carried on 1997-1998 and 2009 (Pinto et al. 2012, Sato et al. 2005). A sand

focused risk assessment provided some guidelines on acceptable risk, but was limited to temperate regions (Shibata and Solo-Gabriele 2012). Food consumption at the beach may be a risk factor for gastrointestinal illness at the beach sites, especially if it is eaten after water or sand contact and without hand-washing, or if food has been stored without proper refrigeration.

CONCLUSIONS

In summary we found an elevation of health risk among swimmers, especially highly exposed ones, when compared with non-swimmers. In addition, children (age < 10) showed higher risk of illness when compared to the teenager and adult group. Besides the water, sand was an important route of exposure for GI illness at the study beaches.

The beaches that more often classified as unsafe in routine recreational water surveillance performed by CETESB also demonstrated higher reported rates of illness among swimmers. Thus, a higher percentage of noncompliance, based on thermotolerant coliform measurements, was consistent with an increased risk of illness. This demonstrates the effectiveness of water quality monitoring programs for the protection of human health.

Data presented here suggests that fecal indicator bacteria such as enterococci and *E. coli* may be appropriate for assessing water quality under conditions in which fecal contamination to recreational waters comes predominately from human sources, those same conditions that apply in temperate regions.

423 The large sample size and overall retention are important strengths to the study. Additionally, the
424 design is comparable to other studies conducted in other parts of the world and therefore the data
425 can also be compared. Importantly, there are no other published studies on recreational water
426 risks in Latin America and few in tropical climates.

427

428 Studies in a broader range of tropical settings are needed to better inform guidelines for
429 minimizing risks associated with recreational water use. These studies will help fill a void in the
430 literature and will provide important information for tropical regions, the location of a huge
431 proportion of recreational activity worldwide.

Table 1: Demographic information of those initially enrolled at the beach and those successfully followed up 7 – 10 days later.

	Beach Interview	Follow-up Interview
	Initial Cohort	Final Cohort
Total	23237	16637
Age		
0-10	9835 (42%)	6928 (42%)
11+	13512 (58%)	9709 (58%)
Gender		
Male	9926 (43%)	7113 (43%)
Female	13310 (57%)	9524 (57%)
Income		
Less than 650	4285 (18%)	2906 (17%)
651-1950	7830 (34%)	5637 (34%)
1951-3900	6459 (28%)	4866 (29%)
Greater than 3901	4662 (20%)	3228 (19%)
Beach		
Beach 1 (Bertioga - Enseada)	3215 (14%)	2466 (15%)
Beach 2 (Guarujá - Pitangueiras)	6309 (27%)	4436 (27%)
Beach 3 (Guarujá - Astúrias)	3611 (16%)	2637 (16%)
Beach 4 (Santos - Aparecida)	4338 (19%)	2757 (17%)
Beach 5 (Praia Grande – Ocian)	5763 (25%)	4341 (26%)
Swimmers	19536 (84%)	14010 (84%)
Highly exposed swimmers	7778 (33%)	5552 (33%)
Length of time in water		
Short	9416 (41%)	6757 (41%)
Medium	7282 (31%)	5275 (32%)
Long	5031 (2%)	3496 (21%)
Didn't enter water,		
Never do	938 (4%)	687 (4%)
Sick that day*	72 (0%)	37 (0%)
Other	2237 (10%)	1584 (10%)
Sand contact	11943 (51%)	8493 (51%)
Food contact		
Ate food sold at the beach	11909 (51%)	8374 (50%)
Regularly eat seafood at the coast	8062 (35%)	5761 (35%)
Entered a pool in previous week	6212 (27%)	4394 (26%)

*No symptoms provided

Table 2: Cumulative incidence per 1000 (CI) of symptoms by beach and age. NGI (NEEAR-GI Illness) = Vomiting or Diarrhea or Stomach Ache + Nausea

Symptom	Beach 1		Beach 2		Beach 3		Beach 4		Beach 5		Pooled Beach Data	
	n = 2466		n = 4436		n = 2637		n = 2757		n = 4341		n = 16637	
Age	Individuals	CI	Individuals	CI	Individuals	CI	Individuals	CI	Individuals	CI	Individuals	CI
Follow-up												
0-10	996		1839		1078		1184		1831		6928	
11+	1470		2597		1559		1573		2510		9709	
ALL	2466		4436		2637		2757		4341		16637	
NGI												
0-10	90	90.4	233	126.7	111	103.0	108	91.2	366	199.9	908	131.1
11+	59	40.1	121	46.6	72	46.2	53	33.7	195	77.7	500	51.5
ALL	149	60.4	354	79.8	183	69.4	161	58.4	561	129.2	1408	84.6
Diarrhea												
0-10	60	60.2	179	97.3	79	73.3	91	76.9	290	158.4	699	100.9
11+	50	34.0	97	37.4	58	37.2	48	30.5	166	66.1	419	43.2
ALL	110	44.6	276	62.2	137	52.0	139	50.4	456	105.0	1118	67.2
Nausea												
0-10	32	32.1	84	45.7	33	30.6	23	19.4	117	63.9	289	41.7
11+	19	12.9	53	20.4	34	21.8	23	14.6	76	30.3	205	21.1
ALL	51	20.7	137	30.9	67	25.4	46	16.7	193	44.5	494	29.7
Vomiting												
0-10	63	63.3	154	83.7	81	75.1	69	58.3	260	142.0	627	90.5
11+	27	18.4	67	25.8	42	26.9	27	17.2	108	43.0	271	27.9
ALL	90	36.5	221	49.8	123	46.6	96	34.8	368	84.8	898	54.0
Fever												
0-10	35	35.1	94	51.1	58	53.8	51	43.1	122	66.6	360	52.0
11+	9	6.1	28	10.8	12	7.7	15	9.5	36	14.3	100	10.3
ALL	44	17.8	122	27.5	70	26.5	66	23.9	158	36.4	460	27.6

Table 3 – Enterococci and *E. coli* measurements (CFU/100 ml) at each beach during the study period. Each beach had 30 measurements corresponding to 3 daily samples across 5 consecutive weekends (10 days). Noncompliance is based on CETESB's regular surveillance

Beach	<i>Enterococci</i>					<i>E. coli</i>					% Non-Compliance Jan-Feb 1999*	% Non-Compliance 1999*
	Geometric Mean	Range		Percentile		Geometric Mean	Range		Percentile			
		Min	Max	25th	75th		Min	Max	25th	75th		
Enseada (Beach 1)	19.3	2	500	8	35	58.4	2	2200	17	159	50	15
Pitangueiras (Beach 2)	16.2	1	460	7	30	41.8	5	540	17	101	50	17
Astúrias (Beach 3)	39.3	4	384	4.5	87	76.9	9	460	42	149	59	6
Aparecida (Beach 4)	26.0	1	236	10	82	233.6	6	7100	60	920	73	33
Ocian (Beach 5)	64.0	11	700	32	112	177.6	5	5700	56	565	82	29

Table 4: Univariate and age adjusted odds ratios (OR/aOR) of NGI due to exposure, by beach and age group and aggregated by beach and by age. A swimmer is defined as someone who said they were going to enter the water, a highly exposed swimmer also said that they usually swallowed water (the reference group for both of these estimated exposures were non-swimmers). Non-swimmers were those participants exposed to pathogens through non-water sources, such as sand.

Exposure	Beach 1 (OR)	Beach 2	Beach 3	Beach 4	Beach 5	All Beaches OR/aOR^
Swimmer	0.93	2.27*	1.55	2.34*	1.28	1.59*/1.24*
0-10	1.64	2.12*	1.27	1.53	0.98	1.34*
11+	0.51*	1.66	1.22	2.20*	1.05	1.22
Highly exposed swimmer	1.23	3.75*	2.17*	3.87*	2.10*	2.46*/1.50*
0-10	1.93	2.47*	1.34	1.93*	1.17	1.55*
11+	0.40*	2.65**	1.60	3.82**	1.84**	1.79*
Sand contact (vs. no sand contact)	1.97*	2.55*	1.75*	2.22*	2.13*	2.13**/1.33*
0-10	2.52*	2.36*	0.88	1.53	1.74*	1.54**
11+	1.00	1.24	1.31	1.22	1.21	1.23*
Ate food prepared at beach	1.81**	1.26*	1.03	0.97	0.93	1.13*/1.21*
0-10	1.72*	1.38*	0.93	0.92	0.91	1.10
11+	2.22**	1.35	1.42	1.18	1.25	1.41**
Untreated Water Contact†	1.74*	1.32*	1.73*	2.13*	1.58*	1.51*/1.52*
0-10	2.44*	1.33	1.98*	1.94*	1.76*	1.58*
11+	0.87	1.22	1.44	2.38**	1.51*	1.37*
Pool Water Contact†	0.93	0.91	0.76*	0.55*	0.79*	0.79*/0.97*
0-10	0.76	0.83	0.69*	0.57*	0.87	0.75*
11+	1.28	0.89	0.79	0.47*	0.53*	0.75*

*p-value< 0.05

^aOR is adjusted for age as a continuous variable

†Water contact occurred in the 7-10 days between the beach and telephone follow-up interviews

Table 5: Multivariate logistic regression models for swimming and water quality. Model 1: Adjusted odds ratios for risk of illness in highly exposed swimmers. Models 2 and 3: Odds of an increased level of enterococci or *E. coli*, respectively, given the adjustment of other known risk factors. For these models, exposed persons were assigned the mean value recorded during their exposure periods. Non-swimmers were assigned an exposure value of 0.1.

	Model 1		Model 2 (Enterococci)		Model 3 (<i>E. coli</i>)	
Dependent Risk Factors	Odds Ratio	95% CI	Odds Ratio	95% CI	Odds Ratio	95% CI
All Participants						
Highly Exposed Swimmers	1.36	1.05-1.58	-----	-----	-----	-----
Geometric Mean of Enterococci Concentrations	-----	-----	1.11	1.04-1.19	-----	-----
Geometric Mean of <i>E. coli</i> Concentrations	-----	-----	-----	-----	1.19	1.11-1.28
Age	0.97	0.96-0.98	.97	.97-.98	.97	.97-.98
Male	-----	-----	1.14	1.01-1.27	1.13	1.01-1.28
Contact with Sand	1.29	1.05-1.58	1.34	1.16-1.53	1.32	1.15-1.52
Ate food prepared on the beach	1.16	1.00-1.35	1.14	1.01-1.28	1.13	1.01-1.27
Untreated water contact in previous week†	1.72	1.46-2.03	1.62	1.43-1.84	1.58	1.39-1.79
Pool water contact in previous week†	0.79	0.68-0.93	.84	.75-.96	.84	.75-.96
Beach						
Beach 1	Reference	Reference	Reference	Reference	Reference	Reference
Beach 2	1.47	1.12-1.91	1.37	1.11-1.69	1.40	1.13-1.72
Beach 3	1.15 ^{NS}	.86-1.56	1.19NS	.94-1.51	1.21NS	.96-1.52
Beach 4	.94 ^{NS}	.69-1.28	.93NS	.73-1.18	.89NS	.69-1.26
Beach 5	2.65	2.06-3.41	2.26	1.85-2.76	2.23	1.83-2.73
Age 0-10						
Highly Exposed Swimmers	1.41	1.6-1.88	-----	-----	-----	-----
Geometric Mean of Enterococci Concentrations	-----	-----	1.16	1.05-1.28	-----	-----
Geometric Mean of <i>E. coli</i> Concentrations	-----	-----	-----	-----	1.23	1.12-1.35
Age	-----	-----	.97	.95-.998	.97	.95-.99
Contact with Sand	1.43	1.10-1.85	1.63	1.33-2.01	1.61	1.30-1.98
Untreated water contact in previous week†	1.80	1.49-2.16	1.73	1.48-2.03	1.68	1.43-1.97

Pool water contact in previous week†	0.78	0.65-0.93	.82	.71-.96	.83	.71-.96
Beach						
Beach 1	Reference	Reference	Reference	Reference	Reference	Reference
Beach 2	1.59	1.17-2.17	1.58	1.21-2.07	1.62	1.24-2.13
Beach 3	1.18 ^{NS}	.84-1.67	1.21 ^{NS}	.89-1.64	1.24	.91-1.68
Beach 4	1.02 ^{NS}	.71-1.47	1.01 ^{NS}	.74-1.37	.94	.69-1.29
Beach 5	2.91	2.17-3.91	2.72	2.10-3.54	2.69	2.07-3.49
Age 11+						
Highly Exposed Swimmers	1.21 ^{NS}	.86-1.69	-----	-----	-----	-----
Geometric Mean of Enterococci Concentrations	-----	-----	1.08 ^{NS}	.98-1.19	-----	-----
Geometric Mean of <i>E. coli</i> Concentrations	-----	-----	-----	-----	1.17	1.05-1.29
Age	.97	.96-.99	.98	.97-.99	.98	.97-.99
Untreated water contact in previous week†	1.50	1.08-2.10	1.46	1.19-1.80	1.43	1.17-1.77
Ate food prepared on the beach	1.44	1.05-1.97	1.36	1.12-1.65	1.35	1.11-1.64
Beach						
Beach 1	Reference	Reference	Reference	Reference	Reference	Reference
Beach 2	1.10 ^{NS}	.64-1.88	1.04 ^{NS}	.75-1.45	1.06 ^{NS}	.76-1.48
Beach 3	1.12 ^{NS}	.63-2.01	1.08 ^{NS}	.75-1.56	1.09 ^{NS}	.76-1.57
Beach 4	0.72 ^{NS}	.39-1.32	.79 ^{NS}	.53-1.16	.76 ^{NS}	.52-1.13
Beach 5	2.24	1.40-3.58	1.75	1.28-2.39	1.73	1.26-2.36

† Water contact reported by participants in the time between beach interview and telephone interview

^{NS}Non-significant variable – kept in final model

CI – Confidence interval

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Figure captions

Figure 1: Map of beach locations and respective municipalities

Figure 2: Illness incidence of NGI among swimmers reported by age and beach along with geometric mean of enterococci concentrations

Figure 3: Enterococci, *E. coli* concentrations reported by beach (and corresponding municipality), along with percent noncompliant (black diamond). All data collected in January and February 1999 during the study period. Noncompliance measured weekly in the regular monitoring and was defined as conditions when more than 20% of measurements within a 5 week period were greater than 1000 thermotolerant coliforms./100mL

Figure 4: NGI incidence among swimmers as a function of *Enterococci* concentrations across all beaches. Each point is a geometric mean of 3 water samples taken on a given day at a given beach ($n = 5 \text{ beaches} \times 10 \text{ days} = 50$). Least squares linear fit: $\text{NGI} = 39 \times \text{Log}_{10}(\text{Ent}) + 26$; $R^2 = 0.16$)

Figure 5: NGI incidence among swimmers as a function of *E. coli* concentrations across all beaches. Each point is a geometric mean of 3 water samples taken on a given day at a given beach ($n = 5 \text{ beaches} \times 10 \text{ days} = 50$). Least squares linear fit: $\text{NGI} = 29 \times \text{Log}_{10}(\text{Ecoli}) + 26$; $R^2 = 0.15$)

Figure 1

figures

Figure 2

Figure 3

Figure 4

Figure 5

Graphical Abstract