Turkiye Parazitol Derg 2024;48(2):82-8
DOI: 10.4274/tpd.galenos.2024.69733

Microbial Health Risks of *Cryptosporidium parvum* and *Giardia lamblia* in Tropical Coastal Water in Araromi, Nigeria

Nijerya, Araromi'deki Tropik Kıyı Sularında Cryptosporidium parvum ve Giardia lamblia'nın Mikrobiyal Sağlık Riskleri

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Cite this article as: Ogundare OT, Olalemi A, Triumphant E-O. Microbial Health Risks of *Cryptosporidium parvum* and *Giardia lamblia* in Tropical Coastal Water in Araromi, Nigeria. Turkiye Parazitol Derg. 2024;48(2):82-8.

ABSTRACT

Objective: *Giardia* and *Cryptosporidium* are enteric protozoa that can cause a variety of gastrointestinal diseases, especially in vulnerable people like children, the elderly, and those with impaired immune systems. In order to ascertain the microbiological quality of the recreational water from Araromi Beach in Ilaje Local Government Area, Ondo State, Nigeria. This risk assessment is of great significance to human health protection against waterborne diseases. The aim of this study was to determine the microbial quality of recreational water from Araromi Beach in Ilaje Local Government Area, Ondo State, Nigeria.

Methods: Microscopic examination of *Cryptosporidium* and *Giardia* oocysts were done.

Results: Results revealed maximum occurrence of *Cryptosporidium parvum* (20 oocysts/100 mL) of water sample in the month of April and maximum occurrence of *Giardia lamblia* (300 cysts/100 mL) of water sample in the month of June. Additionally, according to Kolmogorov-Smirnov tests for normalcy Ho =0.05, *Giardia lamblia* and *Cryptosporidium parvum* were not regularly distributed in the water samples collected from the beach throughout the study period. The average likelihood of contracting *Giardia lamblia* and *Cryptosporidium parvum* infections after consuming 100 mL of beach water was 0.96 and 0.35, respectively. The risks of infection associated with *Cryptosporidium parvum* was lower than those associated with *Giardia lamblia* in water from the beach, but were both above the acceptable risk limit of 10-4.

Conclusion: The results of this study indicate that *Giardia* and *Cryptosporidium* may represent serious health hazards to people who engage in aquatic activities. Adopting a comprehensive strategy that includes regular inspections, enhanced detection techniques, and the prevention of aquatic environment pollution may provide clean and safe recreational water for all, thereby safeguarding the public's health.

Keywords: Enteric protozoa in coastal water, protozoa, water quality assessment, beach water quality assessment, *Cryptosporidium parvum* and *Giardia lamblia*

ÖZ

Amaç: *Giardia* ve *Cryptosporidium*, özellikle çocuklar, yaşlılar ve bağışıklık sistemi zayıf olanlar gibi hassas kişilerde çeşitli gastrointestinal hastalıklara neden olabilen enterik protozoalardır. Nijerya'nın Ondo Eyaleti, Ilaje Yerel Yönetim Bölgesi'ndeki Araromi Plajı'ndan gelen rekreasyonel suyun mikrobiyolojik kalitesini belirlemek için. Bu risk değerlendirmesi, su kaynaklı hastalıklara karşı insan sağlığının korunması açısından büyük önem taşımaktadır.

Yöntemler: *Cryptosporidium* ve *Giardia* ookistlerinin mikroskobik incelemesi yapıldı.

Bulgular: Sonuçlar, su örneğinde *Cryptosporidium parvum*'un (20 ookist/100 mL) maksimum oluşumunun Nisan ayında, maksimum *Giardia lamblia*'nın (300 kist/100 mL) ise Haziran ayında oluştuğunu ortaya çıkardı. Ayrıca Kolmogorov-Smirnov normallik testlerine göre Ho =0,05, *Giardia lamblia* ve *Cryptosporidium parvum*'un çalışma dönemi boyunca plajdan toplanan su örneklerinde düzenli olarak dağılmadığı görüldü. 100 mL plaj suyu tükettikten sonra *Giardia lamblia* ve *Cryptosporidium parvum* enfeksiyonlarına yakalanma ortalama olasılığı sırasıyla 0,96 ve 0,35 idi. *Cryptosporidium parvum* ile ilişkili enfeksiyon riskleri, plajdaki suda *Giardia lamblia* ile ilişkili olanlardan daha düşüktü, ancak her ikisi de kabul edilebilir risk sınırı olan 10-4'ün üzerindeydi.

Sonuç: Bu çalışmanın sonuçları *Giardia* ve *Cryptosporidium*'un suda yaşayan insanlar için ciddi sağlık tehlikeleri oluşturabileceğini göstermektedir. Düzenli denetimleri, gelişmiş tespit tekniklerini ve su ortamı kirliliğinin önlenmesini içeren kapsamlı bir stratejinin benimsenmesi, herkes için temiz ve güvenli dinlenme suyu sağlayabilir ve böylece halk sağlığının korunmasını sağlayabilir.

Anahtar Kelimeler: Kıyı suyunda enterik protozoa, protozoa, su kalitesi değerlendirmesi, plaj suyu kalitesi değerlendirmesi, *Cryptosporidium parvum* ve *Giardia lamblia*

Received/Geliş Tarihi: 14.09.2023 **Accepted/Kabul Tarihi:** 25.05.2024

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Türkiye PARAZİTOLOJİ Dergisi

INTRODUCTION

Water bodies have acquired significant amounts of pollution from a number of sources due to the development of human populations, commercial, and recreational activities. Recreational water refers to rivers, lakes and coastal waters that are used for recreational purposes. Humans use recreational water for all manner of activities, including swimming, surfing, white water sports, diving, boating and fishing (1). Consuming or being exposed to contaminated water can result in the transfer of a number of pathogens, such as enteric bacteria, viruses, and parasites, which can cause serious illnesses and pose a serious threat to global public health (2). Other activities are enhanced by being close to water, such as hiking, nature viewing, and hunting waterfowl. However, recreational waterways can become contaminated with faecal pathogens from human sewage and animal manure. These pathogens can cause gastrointestinal illnesses (causing diarrhoea and vomiting), respiratory diseases, eyes, ear, nose and throat infections.

Protozoa are diverse group of eukaryotic, typically unicellular microorganisms. The majority of protozoa are free-living organisms that can reside in fresh water and pose no risk to human health.

However, some protozoa are pathogenic to humans. These protozoa fall into two functional groups: Enteric protozoa and free-living protozoa. Human infections caused by free-living protozoa are generally the result of contact during recreational bathing (or domestic uses of water other than drinking). Enteric protozoa, on the other hand, have been associated with several drinking water-related outbreaks, and drinking water serves as a significant route of transmission for these organisms. Enteric protozoa are common parasites in the gut of humans and other mammals. The enteric protozoa that are most often associated with waterborne disease in the world are *Giardia* and *Cryptosporidium*. These protozoa are commonly found in surface waters which include recreational waters. Some strains are highly pathogenic and can survive for long periods of time in the environment and are highly resistant to chlorine-based disinfection (3). Protozoan parasites like *Cryptosporidium* spp. and *Giardia* spp. are the most frequently identified cause of diarrheal outbreaks in middle-income and low-income countries (4). The right to access water, which is essential for the survival of living beings, has been acknowledged as a universal human right (5). A sizable portion of epidemics worldwide are caused by waterborne protozoa. According to a recently released comprehensive assessment, from 1953 to 2019 at least one waterborne parasite was documented in 86.7% of eastern African nations (6). Protozoa pollution can lead to waterborne epidemics, which is a serious problem. Despite the application of disinfectants, *Giardia* and *Cryptosporidium* species, which can endure in aquatic environment (7). Unfortunately, many people around the world lack access to clean water that is free of diseases and toxins. Due to its intimate ties to human health, this issue is a substantial public health concern, even for high-income nations (8).

Cryptosporidium spp. is a leading global cause of waterborne disease, with many reported outbreaks related to main water supplies (9). They are known as the main protozoa parasite reported from waterborne outbreaks (10-12). A most recent study reported that, from 251 waterborne outbreaks with parasitic agents *Cryptosporidium* was identified among 198 outbreaks (13). The enteric parasite *Cryptosporidium*, along with *Norovirus*, *Giardia*, *Campylobacter* and *Rotavirus* are among the most frequent causes of waterborne disease (14). Humans can contract *Cryptosporidium* by drinking untreated water from a lake or river that is contaminated, through the faecal-oral route by coming into contact with infected people or animals directly (person-to-person transmission), swallowing recreational water (water in swimming pools, waterparks, fountains, lakes, rivers) contaminated with cryptosporidiosis (15). Oocysts of *Cryptosporidium* and cysts of *Giardia* occur in the aquatic environment throughout the world. They have been found in most surface waters, where their concentration is related to the level of faecal pollution or human use of the water. In fact, it is well known that the prevalence of parasitic protozoan infection is high in low-income countries, this is as a result of their low economic status and poor sanitation. Effects can range from minor illnesses to potentially fatal diseases. Children, the elderly, and people with compromised immune systems are most at risk. *Giardia lamblia* is the second most reported protozoa from watery outbreaks worldwide similar to *Cryptosporidium* spp., (16), the main reason for the high prevalence of *G. lamblia* in waterborne outbreaks is the capability of this protozoan to remain viable during water treatment processes (17,18). However, outbreaks of *G. lamblia* and *Cryptosporidium* spp. are recorded far more frequently in high-income countries than in low-income countries, which may be related to the adoption of more advanced detection methods. According to World Health Organisation, proper monitoring and adjustment of disinfection processes are necessary to ensure adequate control of *Cryptosporidium* and *Giardia* (19).

The aim of this study was to determine the microbial quality of recreational water from Araromi Beach in Ilaje Local Government Area, Ondo State, Nigeria. The objectives were to determine the load of enteric protozoa (*C. parvum* and *G. lamblia*) in water samples from Araromi beach, examine the risk posed by the protozoa on humans and carry out quantitative microbial risk assessment of the pathogen in the water samples.

METHODS

Description of the study area and collection of water samples Araromi Beach is located in Ilaje local government area of Ondo State in the south-western region of Nigeria. There is a popular night market close to the beach where trading starts at night. The beach is situated on the same beach line from Lagos and about 290 km on the shoreline to Lagos. The study area lies between longitudes 2° 24E. 5° 1E of the Greenwich meridian and latitude 5° 51 N. 6° 42 N of the equator (Figure 1). Over the years the beach has been known for its space for relaxation or engagement in activities especially beach ball and picnic etc. It is also used as a major source of fishing activities. It has been recorded over the years that people visit the beach more often during the festive seasons. Water samples were collected from the representative monitoring points on the beach monthly over a period of 12 months (i.e., December 2021 to November 2022). On each sampling occasion, about 10 litres of water was collected using sterile plastic bottles and transported in a cool box with ice packs to the Microbiology Laboratory at the Federal University of Technology, Akure.

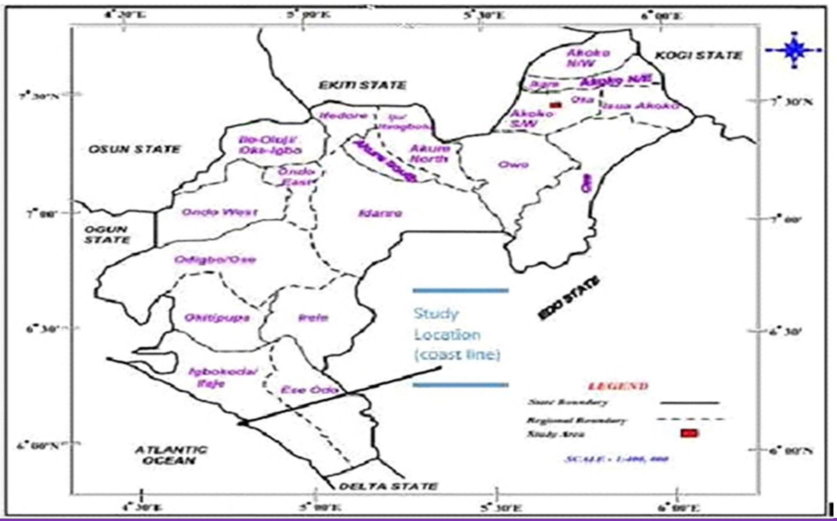

Figure 1. A map showing the location of the beach in Araromi, Ilaje Local Government Area, Ondo State, Nigeria

Enumeration of *Cryptosporidium parvum* in Water Samples from the Beach

Cryptosporidium oocysts were concentrated from the water samples (100 mL) in triplicates by centrifuging at 1000 rpm for 15 minutes. Pellet was suspended in normal saline and placed on a microscopic slide, and then stained using modified Ziehl Neelsen method. The oocysts were identified using shape, size and color, and thereafter counted and expressed as the total number of *Cryptosporidium* per 100 mL.

Enumeration of *Giardia lamblia* in Water Samples from the Beach

Giardia cystic forms were concentrated from the water samples (100 mL) in triplicates, centrifuging at 1000 rpm for 15 min. The centrifugation's pellet product was suspended in sterile saline, put on a microscopic slide and stained using a modified iodine, thereafter counted and expressed as the total number of *Giardia* per 100 mL.

Distribution Pattern of Enteric Protozoa in Water Samples From the Beach

The distribution pattern of *Cryptosporidium parvum* and *Giardia lamblia* in water from the beach were determined using Skewness and kurtosis, Kolmogorov-Smirnov tests for normality.

Microbial Risk Assessment

The risk of infection with *C. parvum* and *G. lamblia* in water from the beach during swimming activities was estimated. *Cryptosporidium parvum* causes watery diarrhea including symptoms such as nausea, vomiting, abdominal cramps and fever. In immune-compromised individuals, infectious dose as low as 1-5 oocysts may result into an infection, whereas about 132 oocysts are capable of causing infection in healthy humans. *Giardia lamblia* causes diarrhea in humans with accompanying symptoms such as weight loss, nausea, vomiting, abdominal pain and fever. Infectious dose a low as 10 cysts may elicit an infection.

The exponential model (Equation 1) was adopted to determine the probability of infection (Table 1) associated with exposure to *Cryptosporidium parvum* and *Giardia lamblia* in water from the beach. The annual probability of infection (Equation 2) was also determined. For exposure assessment, the ingestion of 1 mL, 10 mL and 100 mL was assumed as the volume of water consumed during recreational activities in order to determine the human health risks.

$$Pi = 1 - \exp\ (-rN) \quad (1)$$

$$P_A = 1 - (1 - Pi)^{365} \quad (2)$$

Where: Pi = Probability of infection; r = Parameters defining the dose-response curve (determined by the infectivity the organism); and N = exposure (colony forming unit); P_A = Annual probability of infection.

Statistical Analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 20 software and Microsoft Excel.

RESULTS

Detection of *Cryptosporidium parvum* and *Giardia lamblia* in Water Samples from the Beach

The minimum occurrence of *Cryptosporidium parvum* was observed to be 1.1 oocysts/100 mL of water sample in the month of March. On the other hand, the maximum occurrence of *Cryptosporidium parvum* in the water sample was observed to be 20 oocysts/100 mL of water sample in the month of April. Similarly, the minimum occurrence of *Giardia lamblia* was observed to be 73 cysts/100 mL of water sample for the month of July On the other hand, the maximum occurrence of *Giardia lamblia* in the water sample was observed to be 300 cysts/100 mL of water sample in the month of June (Figure 2).

Table 1. Dose-response model adopted to evaluate microbial health risks from exposures to enteric protozoa in water samples from the beach

Pathogens	Model	Parameters	Reference
Cryptosporidium parvum	Exponential	$r=5.72\times10^{-2}$	(19)
Giardia lamblia	Exponential	$r=1.99\times10^{-2}$	

Distribution Pattern of Enteric Protozoa in Water Samples from the Beach

Cryptosporidium parvum and *Giardia lamblia* following Kolmogorov-Smirnov tests for normality H_o =0.05 were not normally distributed in the water samples from the beach over the period of study (Table 2).

Probabilities of Infection

The mean probability of *Cryptosporidium parvum* infection from ingestion of 1 mL of water from the beach was 0.0035, whereas ingestion of 10 mL of water from the beach revealed mean probability of infection of 0.035. The mean probability of *Cryptosporidium parvum* infection from ingestion of 100 mL of water from the beach was 0.35. The mean probability of *Giardia lamblia* infection from ingestion of 1 mL of water from the beach was 0.0096, whereas ingestion of 10 mL of water from the beach revealed mean probability of infection of 0.096. The mean probability of *Giardia lamblia* infection from ingestion of 100 mL of water from the beach was 0.96. The risks of infection associated with *Cryptosporidium parvum* was lower than those associated with *Giardia lamblia* in water from the beach, but were both above the acceptable risk limit of 10^{-4} (Figure 3).

The mean annual probabilities of infection as a result of contact or ingestion of water from the beach were estimated. The mean annual probability of *Cryptosporidium parvum* infection due to ingestion of 1 mL of water from the beach was 0.72; 10 mL of water from the beach was 1.0; and 100 mL of water from the beach was 1.0. The mean annual probability of *Giardia lamblia* infection due to ingestion of 1 mL of water from the beach was 0.97; 10 mL of water from the beach was 1.0; and 100 mL of water from the beach was 1.0. The mean annual probabilities of infection due to *Giardia lamblia* was than those due to *Cryptosporidium parvum* (Figure 4).

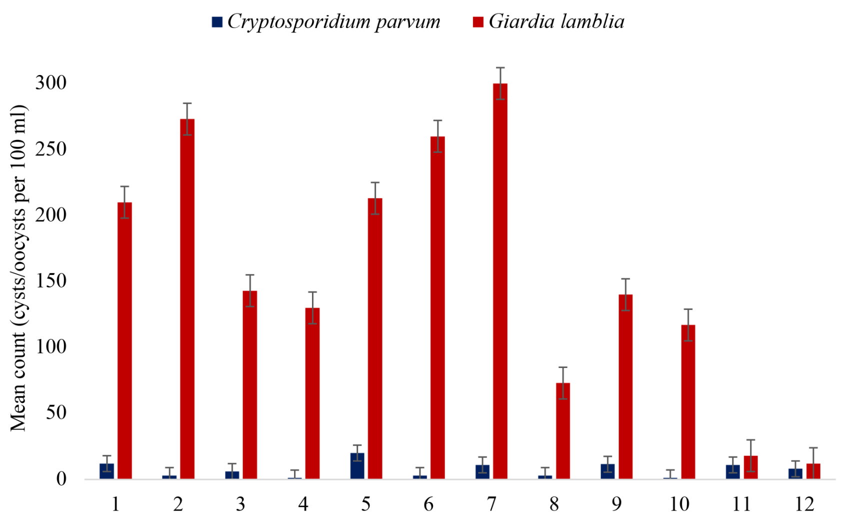

Figure 2. Mean count of *Cryptosporidium parvum* and *Giardia lamblia* in water samples from the beach (1 – Dec'21; 2 – Jan'22; 3 – Feb'22; 4 – Mar'22; 5 – Apr'22; 6 – May'22; 7 – Jun'22; 8 – Jul'22; 9 – Aug'22; 10 – Sep'22; 11 – Oct'22; 12 – Nov'22)

Table 2. Skewness and kurtosis, Kolmogorov-Smirnov tests for normality in water samples from the beach

Microorganisms	Skewness	Kurtosis	Kolmogorov-Smirnov statistic	Normal distribution
Cryptosporidium parvum	-0.502	-0.920	0.776	No
Giardia lamblia	-1.442	1.215	0.985	No

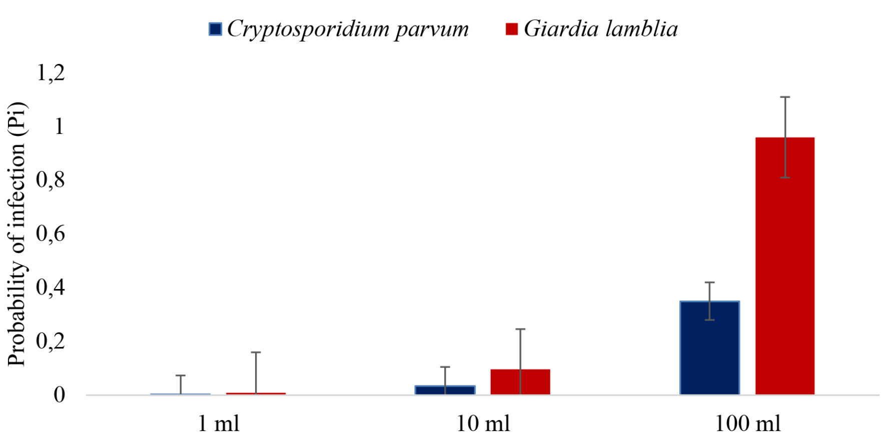

Figure 3. Probability of infection (*Pi*) associated with exposure to pathogens in water from the beach

DISCUSSION

The concentration of enteric protozoa in recreational water from Araromi Beach in Ilaje Local Government Area, Ondo State, Nigeria was determined in this study. The human population in the area depends on the water for fishing, agriculture, and other household and recreational activities in addition to increasing beach activity. *Giardia* and *Cryptosporidium* are important etiological agents of waterborne illnesses primarily because of their high levels of environmental persistence and infectivity. Using conventional water treatment procedures, it is challenging to get rid of them from water supplies. Their small size and resistance to chlorine, commonly used for disinfection, enable them to persist and remain infectious even after standard treatment processes. This persistence has resulted in several waterborne disease outbreaks, underscoring the need for improved detection and control measures. As a result of this it is important to investigate the presence of *Cryptosporidium parvum* and *Giardia lamblia* in recreational waters to estimate the infection risk associated with swimming and other recreational activities.

Cryptosporidium species have been described, infective to a wide range of hosts, including humans, domestic and wild mammals, birds, reptiles, amphibians and fishes (20). These parasites spread between hosts via the faecal-oral pathway, either directly through contact with an infected person or indirectly by transmission in faecally contaminated water or, less frequently, food (21). Water serves as an important channel for the spread of *Cryptosporidium* oocysts throughout the ecosystem. Different water resources, including drinking, recreational, groundwater, and wastewater, are commonly polluted with this parasite (22,23). The distribution of *Cryptosporidium parvum* in the water sample increased regularly with month (April, November), then average increases in the month (December, June, August, October) with little or no increases in the month (January, February, March, May, July, September). This observation might be the effect of more people being near and in the water between the months of April and November.

One of the most frequent global causes of water and food borne illnesses is *Giardia* infection (giardiasis). In this study, the distribution of *Giardia lamblia* was sporadic, as there was an

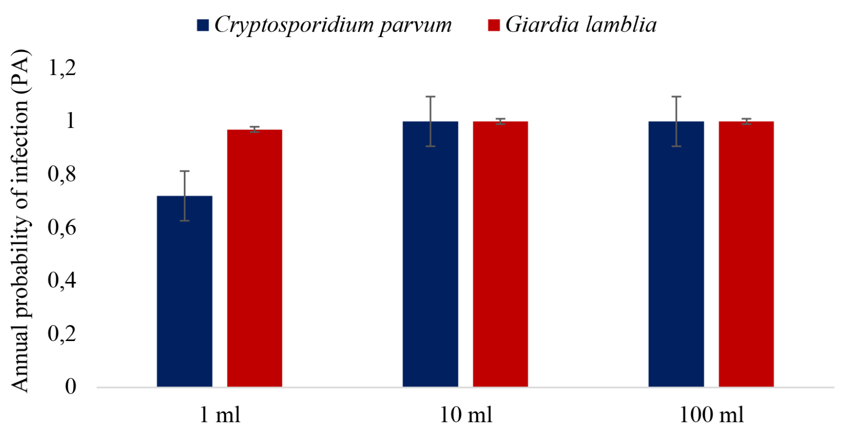

Figure 4. Annual probability of infection (P_A) associated with exposure to pathogens in water from the beach
In children, the mean probability of infection from contact or ingestion of 37 mL of water from the beach due to *Cryptosporidium parvum* was 0.13 and *Giardia lamblia* was 0.36. The risks of infection associated with exposure of children to the enteric protozoa in water from the beach were lower for *Cryptosporidium parvum* than *Giardia lamblia.* In adults, the mean probability of infection from contact or ingestion of 16 mL of water from the beach due to *Cryptosporidium parvum* was 0.056 and *Giardia lamblia* was 0.15. Again, the risks of infection associated with exposure of adults to the enteric protozoa in water from the beach were were lower for *Cryptosporidium parvum* than *Giardia lamblia* (Figure 5).

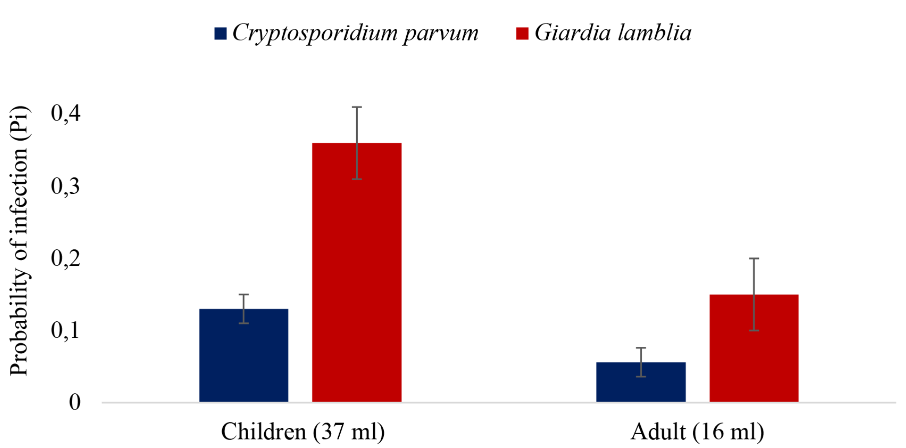

Figure 5. The probability of infection associated with exposure of children and adults to *Crptosporidium parvum* and *Giardia lamblia* in the water from the beach

increase in the counts observed in the months of January, May and June. This could be as a result of the increase in human populations that visited the beach. *Giardia lamblia* was more prevalent in this study than *Cryptosporidium parvum*, and its prevalence exceeded the acceptable risk level of 10^{-4} set by the World Health Organization (24).

Giardia lamblia was irregular in its spread, with increases in counts seen in the months of January, May, and June. This could be as a result of the increase in human populations that visited the beach. In this study, *Giardia lamblia* prevalence was much greater than *Cryptosporidium parvum* prevalence. The amount of *Cryptosporidium parvum* in the water sample increased consistently in the months April and November, followed by average monthly increases in December, June, August, October and then minimal or no monthly increases in January, February, March, May, July and September.

QMRA was conducted to assess health risks associated with the beach water in the presence of the pathogens. Two pathogenic microorganisms were chosen, *Cryptosporidium parvum* and *Giardia lamblia*. These organisms are known to cause the most common water-related gastrointestinal illnesses caused by protozoa. Cryptosporidiosis is caused by the intestinal parasite *Cryptosporidium*. Cryptosporidiosis is an acute illness characterised by diarrhoea and abdominal pain, whereas Giardiasis is caused by the protozoan parasite *Giardia lambia*. The main symptoms are diarrhoea and cramps. These gastrointestinal illnesses can be contracted in various ways, including contact with contaminated water, farm animals, sick animals, faecal matter, other symptomatic people, and eating contaminated food. Therefore, the presence of *Cryptosporidium parvum* and *Giardia lamblia* in water at Araromi Beach may pose significant risk to public health. The estimated risks of infection with *Cryptosporidium parvum* were highest in the month of April and lowest in the month of March and September. The estimated risks of infection with *Giardia lamblia* were highest in the month of June and lowest in the month of November.

CONCLUSION

The findings of this study demonstrated the presence of *Cryptosporidium parvum* and *Giardia lamblia* in water at Araromi beach. The presence of these enteric protozoa in the water samples suggest faecal pollution of the water and may pose significant health risks to humans using the water for recreational activities. The estimated risks of *Cryptosporidium parvum* and *Giardia lamblia* infections which could be as a result of human exposure to the beach were highest in April and June respectively. Adopting a comprehensive approach that involves routine monitoring with improved detection methods and prevention of pollution of aquatic environment may offer clean and safe recreational water for all, thereby protecting public health.

* Ethics

Ethics Committee Approval: N/A.

Informed Consent: N/A.

* Authorship Contributions

Surgical and Medical Practices: O.T.O., A.O., E-O.T., Concept: O.T.O., A.O., E-O.T., Design: O.T.O., A.O., E-O.T., Data Collection or Processing: O.T.O., A.O., E-O.T., Analysis or Interpretation: O.T.O., A.O., E-O.T., Literature Search: O.T.O., A.O., E-O.T., Writing: O.T.O., A.O., E-O.T.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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