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ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

HUMAN-PATHOGENIC PARASITES AND BACTERIA IN STAGNANT-WATER DURING EL NIÑO-COSTERO PHENOMENON IN PERU

BACTERIAS Y PARÁSITOS PATÓGENOS HUMANOS AISLADOS DE AGUAS ESTANCADAS DURANTE EL FENÓMENO EL NIÑO-COSTERO EN PERÚ

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ABSTRACT

Several countries are extremely susceptible to climate change and the El Niño phenomenon affecting more than two million people in Ecuador, Colombia, and Peru. We aimed to evaluate the microbiological quality of stagnant waters in Lambayeque region, Peru during the lockdown caused by El Niño phenomenon. We conducted a cross-sectional study performed in the four Lambayeque districts: (downtown Chiclayo, Mocupe, San José, and Pimentel), Perú. Two simultaneous samples were taken from each evaluation district and were transported to Laboratory for entire microbiological analysis. We isolated the human-pathogenic parasite (*Trichuris trichiura* (Linnaeus, 1771) and *Entamoeba histolytica* Schaudinn, 1903), and bacteria (*Staphylococcus aureus* Rosenbach 1884 and *Salmonella typhi* (Schroeter, 1886)) that showed patterns of resistance to conventional first-line antimicrobials (penicillin, nalidixic acid, nitrofurantoin, and chloramphenicol). Likewise, we showed evidence of microorganisms related to the sampling site (district) and with a degree of affectation by the phenomenon. Our result suggests that the stagnant waters of four districts of Lambayeque presented Human-pathogenic parasites and bacteria of high-medical importance related to the sudden changes in the climate through El Niño.

Keywords: Climate change – Diarrhea – El Nino-Southern Oscillation – Floods – Water-borne disease

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RESUMEN

Varios países son extremadamente susceptibles al cambio climático y el fenómeno El Niño-costero afectó a más de dos millones de personas en Ecuador, Colombia y Perú. Nuestro objetivo fue evaluar la calidad microbiológica de las aguas estancadas en la región de Lambayeque, Perú, durante el estado de emergencia causado por el fenómeno El Niño-costero mediante un estudio transversal realizado en cuatro distritos de Lambayeque: (Centro de Chiclayo, Mocupe, San José y Pimentel), Perú. Se tomaron dos muestras simultáneas de cada distrito de evaluación, y se trasladaron a Lima para su análisis microbiológico completo. Aislamos parásitos patógenos humanos (*Trichuris trichiura* (Linnaeus, 1771) y *Entamoeba histolytica* Schaudinn, 1903) y bacterias (*Staphylococcus aureus* Rosenbach, 1884 y *Salmonella typhi* (Schroeter, 1886)) que presentaban patrones de resistencia a los antimicrobianos convencionales de primera línea (penicilina, ácido nalidíxico, nitrofurantoína y cloranfenicol). Asimismo, mostramos evidencia de microorganismos relacionados con el sitio de muestreo (distrito) y con el grado de afectación por el fenómeno. Nuestro resultado sugiere que las aguas estancadas de cuatro distritos de Lambayeque presentaron parásitos patógenos humanos y bacterias de alta importancia médica por los cambios repentinos en el clima a través del fenómeno El Niño-costero.

Palabras clave: Enfermedades transmitidas por el agua – Diarrea – Cambio climático –Inundaciones – Fenómeno de El Niño

INTRODUCTION

Several countries are extremely susceptible to climate change, a phenomenon that not only has been causing irretrievable changes in our biodiversity but is changing the patterns of diseases affecting human health (Metcalf *et al*., 2017). The greatest consequences for infectious diseases caused by climate change are the rapid changes in their transmission nowadays (Greenspan *et al*., 2017).

Asia-Pacific and Africa countries are affected by El Niño phenomenon that causes extreme events (persistent heavy rain, floods, etc.) which results in a large number of victims, poverty, inequality, and diseases (Glantz, 2001). In 2017, Peru was affected by intense rains, floods, and high-daily dry temperatures (100.4±4 ºF), which has shaped an unusual phenomenon called El Niño-costero, similar to El Niño phenomenon but located in the coasts of Ecuador, Colombia, and Peru (Chinchay, 2017).

This phenomenon affected more than two million people and had a large socioeconomic impact in Peru, principally in the northern coastal region (Lambayeque region). In Chiclayo, capital of the Lambayeque region, >28000 people were affected by the El Niño-costero phenomenon (ENCP) with losses of infrastructure, agriculture, social and health problems (mainly respiratory diseases and vector and water-borne disease)(WHO, 2016).

As this phenomenon caused a stagnation of water, stagnant waters were used as a source of human consumption for several months. The health consequences of the stagnation of water have not been evaluated during ENCP, but in several previous studies the potential risk of these for human health is underlined (Chen *et al*., 2013; Aghajani *et al*., 2016).

We evaluate the microbiological quality of stagnant waters in Lambayeque region, during the ENCP, focused on the isolation of microorganisms of medical importance that represent a risk for the communities exposed to these stagnant waters.

MATERIAL AND METHODS

Geographic location

This descriptive study was carried out in four main districts of Lambayeque region (27 meters-above-sea-level,) northwest of Peru (Figure 1). Chiclayo had ~843 445 inhabitants and is divided into eight urban districts.

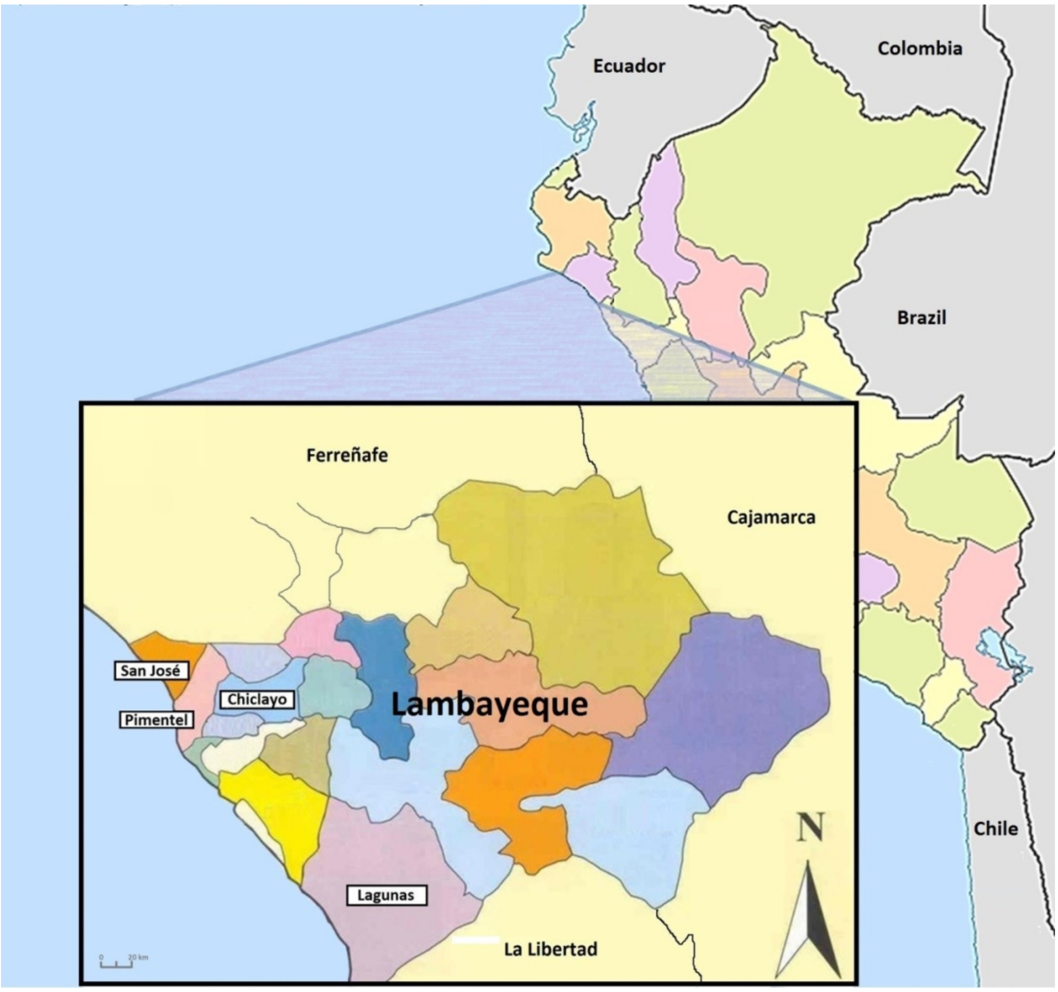

Figure 1. Geographic distribution of the districts evaluated in the Lambayeque region, Peru.

DISCUSIÓN

The Chiclayo's downtown has a hospital (Hospital Regional Docente Las Mercedes -Level II), 14 health centers and six health stands, all belonging to the Ministry of Health of Peru. Moreover, the city has a Hospital Level I (Naylamp Hospital) and a Level IV (Almanzor Aguinaga Asenjo Hospital) belonging to Social Security. The ENCP affected this region between January to May 2017, with February being the most affected (an approximate of 5 363 collapsed houses, and 4 595 families affected).

Samples

The sampling was performed in the four Lambayque's districts: two zones of Mocupe district (north of Chiclayo, semi-urban district of Lagunas, >10 thousand inhabitants), the metropolitan area of Chiclayo (downtown, >550 thousand inhabitants), San José district (west of Chiclayo, >15 thousand inhabitants), and Pimentel district (west of Chiclayo, >35 thousand inhabitants). These districts were the most affected by the phenomena keeping the waters stagnant for ≥4 weeks. We have collected randomly 4±1 ml of samples of stagnant water from these districts during the afternoon, after the rain, with protection barriers.

Two simultaneous samples were taken from each evaluation area in sterile plastic bottles of 10 ml. The samples were stored under refrigeration (2 ±

1ºC) for ≤5 hours until transport (~8 hours) to the Hospital Nacional Docente Madre Niño San Bartolome (HONADOMANI SB) in Lima. To avoid errors in this phase, part of the sample was transported with the BBL Culture Swab™ Plus collection and transport system (BD, Le Pont de Claix, France).

Microbiological analysis

All the samples were processed once they arrived at HONADOMANI SB. The isolation were performed following bacteriological methods in the stool-culture area in 5% blood-sheep agar with membrane filter grid GN-6 Metricel® 0,45µm, 47mm (PALL, NY, USA); in Karmali agar, McConkey agar, Salmonella-Shigella agar, Thiosulfate-Citrate-Bile-Sucrose agar, Sorbitol-MacConkey agar, and Mannitol salt agar (all from Merck, Darmstadt, Germany). Likewise, we used cetrimide agar (Britania, CABA, Argentina) and Saboraund-dextrose agar (Oxoid, Hampshire, England).

For identification of bacteria the biochemical analysis was performed with the system Vitek®2 Compact (BioMérieux, Marcy-l'Étoile, France), and antibiogram was by disc diffusion with Breakpoints from CLSI M100S (CLSI, 2016). The sample was referred to the parasitology area for identification of human-pathogenic parasites following a previous concentration methods (Zarlenga & Trout, 2004), included bright-field microscopy and rapid test (rbiopharm, Darmstadt, Germany) for most frequently parasite as amoebas and helminths. Also, we conducted a determination of Rotavirus (Rota-Strip, Coris Bioconcept, Gembloux, Belgium). The microbial enumeration techniques employed in this research were the Most Probable Number (MPN) techniques following the previous protocol (Leuta, 2015). Opportunistic bacteria of non-fecal origin were not evaluated.

Statistical analysis

The MPN was estimated by the number of positive tubes for coliforms in each dilution. The Spearman correlation and T-student non-paired test analysis was performed to show the difference between the samples analyzed and isolations considering a *p*-value <0.05 was considered statistically significant. The statistical analysis was done by IBM SPSS v21.0 (Armonk, USA).

Ethical aspects

This study was approved by the Research Committee of the University of Chiclayo (Officio Nº102-2017). In addition, this study was approved by the HONADOMANI SB Ethics and Research Committee (Exp. Nº 16913-16).

RESULTS

Pimentel's district was the most affected and Chiclayo was the least affected. Ten samples [four (40%) samples from Mocupe district, and two samples (20%) from San José, Chiclayo, and Pimentel each] were included in this study. Of the 20 analyzes (10 with the transport-medium system and ten directly from water samples), in 6 (30%) and 2 (10%) were isolated Enterobacterias (*Salmonella typhi* (Schroeter, 1886) and *Plesiomona shigelloides* (Bader, 1954) Habs & Schubert, 1962), and *Staphylococcus aureus* Rosenbach, 1884 (both ≥100 000 UFC). *Escherichia coli* (Escherich, 1885) were not isolated and rotavirus was not evident in stagnant water samples. Differences were found between the sampling sites (districts), and isolates of pathogenic bacteria (t=-2.70, p = 0.001).

We reported *S. aureus* beta-lactam (penicillin) resistant and sensitivity to methicillin (SAMS), also *S. typhi* were resistant to nitrofurans (nitrofurantoin), anfenicoles (chloramphenicol), and quinolones (nalidixic acid).

The highest faecal coliform count was 5.1×10^6 microorganisms/100 ml obtained in Pimentel district, and the average faecal coliform count was $4.4 \pm 0,7 \times 10^6$ microorganisms/100mL in all the sampling sites. The MPN counts ranged between 4.9×10^4 microorganisms/100mL (lowest) recorded at Chiclayo district and 1.9×10^8 microorganisms/100mL (highest) detected at Pimentel district. Both faecal coliform count and MNP exceeded the acceptable limits.

As for parasitological findings, only in the Pimentel district was found eggs of *Trichuris trichiura* (Linnaeus, 1771) (human whipworm) and two trophozoites of the protozoa *Entamoeba histolytica* Schaudinn, 1903 (Figure 2).

Figure 2. Typical views of the district areas evaluated in Lambayeque, Peru, and the parasitological finds in Pimentel district. A. San Jose district under stagnant water, B. water stagnation conditions for two weeks in Chiclayo downtown near one of the health centers (left), C. Many of the streets of the Pimentel district are unpaved and the heavy rains and floods caused by El Niño-costero phenomenon resulted in stagnation of water on stilts. Even in this image you can see a group of children swimming in these waters (white narrow). D. the nematode *Trichuris trichiura* (40x) found in the samples of Pimentel district.

The Pimentel district showed the greatest bacterial and parasitic contamination (50%). A significant positive correlation was found between the parasitological findings and the place of origin of the samples (rho=0.52, p = 0.002). No difference was found in the microbiological analysis between samples (t= -0.38, p = 0.74), nor between the findings in the transport-medium system and from water samples (t= -2.95, p = 0.001).

DISCUSIÓN

We demonstrated for the first time the isolation of pathogenic microorganisms of high medical importance that showed patterns of resistance to conventional first-line antimicrobials. Our results also showed evidence of parasites related to the sampling site (district) and the level of effects by the ENCP at Lambayeque, Peru.

Climate change is not only changing the dynamics of infections, but it is also threatening public and planetary health. From these changes, economic opportunities arise but also natural phenomena that bring with them changes in the parasite-host relationship. These changes have been accentuated on the coast at the marine, protozoic and bacterial level (Bradley *et al*., 2005; Byers *et al*., 2020). The findings found in this study are consistent with previous studies that place human pathogens as emergent related to climate change phenomena (Ryan *et al*., 2019; El-Sayed & Kamel, 2020). It is necessary in Peru, a country with a high prevalence of infectious diseases and vulnerable to climate change, that continuous monitoring of emergencies and re-emergencies of infectious diseases associated with climatic phenomena be developed in order to prevent and mitigate their consequences for health and environmental (Ogden, 2018).

The ENCP caused that most of water (potable and free) contains impurities and organic remains, polluting the large numbers of water reserves, and shortages and/or limiting access to clean water for human consumption in the affected districts of Lambayeque. This leads the phenomena-affected communities to use and consume water from other sources (such as stagnant water) under different rudimentary water-treatment systems. Greater faecal coliform (5.1×10^6 microorganisms/100 mL) and the MPN (1.9×10^8 microorganisms/100mL) were indicative of the low quality of stagnant water and the high risk to which these communities are exposed. Our findings agree with previous reports on MPN in waters in developing countries with high frequency of climatic phenomena (Emiliani, 2004; Venegas-Pérez *et al*., 2016).

Faecal contamination of these stagnant waters may be responsible for health-associated problems, like the acute diarrheal disease (220 425 cases) mainly in children (Silva & Hernández, 2017). Our data agree and showed coliforms, Enterobacterias (*S. typhi* cause of Typhoid fever, *P. shigelloides* related to diarrheal and gastroenteritis) and parasites (*T. trichiura* and *E. hystolitica* cause of persists diarrhea) highly dangerous for human health.

The adaptation of these pathogens in stagnant waters composes a risk mainly for children (Figure 2). Several evaluations have shown microbiological contamination in drinking water worldwide, highlighting high frequency and risk in low-income countries (Ashbolt 2004; van der Wielen & van der Kooij, 2013). Other findings only in the district of Pimentel evidenced protozoa and helminths on these stagnant waters. Under the precarious conditions caused by the ENCP the regional level four major hospitals were declared in red alert (Vega, 2017), and the other health centers showed flaws in basic services (due to flooding, access obstruction (Figure 2), lack of personnel, etc.). This deficiency of solidity in the health system has been affected during the current COVID-19 pandemic, under this scenario; new prevention strategies must be included in a framework of sustainability (Ottersen & Engebretsen, 2020).

The results of this study should be interpreted with the following limitations: i) Due to the limitations of access to the sampling areas, no more sampling areas were analyzed and our results may be restricted to the analyzed areas, ii) due to the 2017 Peruvian lockdown we did not perform molecular analyzes to confirm the genes associated with bacterial resistance, iii) the present study was developed with conventional bacteriological methods, therefore no sequencing of the analyzed samples was used, iv) finally, we did not monitor the areas analyzed, in order to understand the behavior and changes in pathogenic microorganisms.

In conclusion, this study suggests that the stagnant waters of four districts of Lambayeque presented Human-pathogenic parasites and bacteria of high-medical importance that places people at high risk of infection and development of acute diarrheal disease.

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