

Research Paper

Detection of Intestinal Pathogens in River, Shore, and Drinking Water in Lima, Peru

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Published: 2017.01.17

Abstract

Water quality management is an ongoing struggle for many locations worldwide. Current testing of water supplies can be time-consuming, expensive, and lack sensitivity. This study describes an alternative, easy-to-use, and inexpensive method to water sampling and testing at remote locations. This method was employed to detect a number of intestinal pathogens in various locations of Lima, Peru. A total of 34 PCR primer pairs were tested for specificity and high-yield amplification for 12 different pathogens using known DNA templates. Select primers for each pathogen were then tested for minimum detection limits of DNA. Water samples were collected from 22 locations. PCR was used to detect the presence of a pathogen, virulence factors, or differentiate between pathogenic species. In 22 water samples, cholera toxin gene was detected in 4.5% of samples, *C. perfringens* DNA was detected in 50% of samples, *E. histolytica* DNA was detected in 54.5% of samples, *Giardia intestinalis* DNA was detected in 4.5% of samples, *Leptospira* spp. DNA was detected in 29% of samples, and *T. gondii* DNA was detected in 31.8% of samples. DNA from three pathogens, *C. perfringens*, *E. histolytica*, and *T. gondii*, were found in residential samples, which accounted for 10 out of 22 samples.

Key words: water sampling, water-borne pathogen detection, polymerase chain reaction (PCR), gel electrophoresis.

Introduction

The ability of waterborne pathogens to infect a vast population over a relatively short period of time can have profound effects in both developed and developing countries, as demonstrated by past and current epidemics. The World Health Organization asserts about 22% (2.4 million) of 10.8 million deaths in children aged less than five years were caused by diarrheal diseases developed after being exposed to contaminated water sources.[1] The pathogens evaluated in this work are major contributors to diarrheal diseases in developing countries that lack quality drinking water and proper sanitation of sewer systems.

The densely populated areas, limited potable water access, and substandard water sanitation infrastructure of Lima, Peru have contributed to historic epidemics. The 1990s cholera outbreak that claimed the lives of millions of South Americans was attributed to one of the most highly publicized waterborne pathogen outbreaks originating in Peru. A number of waterborne pathogens, including cholera, continue to plague the population of Lima and other underdeveloped areas in Peru. Previous studies in impoverished areas of Peru discovered early childhood infection by gastrointestinal pathogens *Giardia intestinalis* and *Cryptosporidium* species are

widespread.[2,3,4,5,6] Additionally, other studies have shown the presence of *Leptospira* species[7], *V. cholera* and *Aeromonas*[8,9], *Cyclospora cayetanesis*[10], and *E. coli*[11] in a variety of water samples around Peru. Other waterborne pathogens that are common in developing countries, yet have not been identified in Peru waters supplies, include *Entamoeba histolytica*[12], *Clostridium perfringens*[13,14], and *Toxoplasma gondii*[15,16]. Water sanitation and quality has been a concern of previous researchers, yet no study has evaluated both source and delivered water until now.

Past and present day struggles with waterborne pathogens in this region suggest water quality management is substandard or inconsistent. Highly sensitive testing platforms that are cost-effective and produce accurate results in rapid turnaround time can improve diagnostic capacity and relevance. Currently, testing of water samples are conducted by expensive, time-consuming, less sensitive experiments such as immunofluorescence assays and culturing pathogens. Polymerase chain reaction (PCR) is a highly sensitive alternative experiment that detects and amplifies DNA of interest in a shorter period of time. By targeting and replicating DNA, PCR can identify the presence of an organism; and, in some instances, detect specific genes carried by known to lead to disease in humans.[17, 18]

Previous investigators have developed PCR primers to detect the presence of various organisms of interest, due to the importance of gastrointestinal

disease identification in medical and food safety industries.[17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27] In this study, we develop a universal protocol derived from published PCR protocols and optimized to decrease material and equipment cost and increase throughput and sensitivity. The creation of such a toolbox is designed to facilitate robust, efficient water monitoring in areas or countries that demonstrate need. Following the characterization of a standardized protocol, we applied our toolbox to water samples from environmental and residential water samples from Lima, Peru to identify the presence or absence of various pathogenic species.

Materials and Methods

Water Sample Acquisition

Unlike previous water sampling studies using culture or microscopy methods, relatively few pieces of field and lab equipment were used for this study (Supplemental Table 1). Locations for sampling were determined by satellite imaging along the Rimac River (Figure 1). Peruvian water samples were collected from the ocean shore and 11 locations from the Rimac River, along with 10 residential locations. To collect waters, 1 liter water bailers connected to a 50m retractable line were employed, followed by transferring the bailer volume to 1.5 liter containers. GPS coordinates were recorded at each location by record book, on the collection container, and in the GPS system itself.

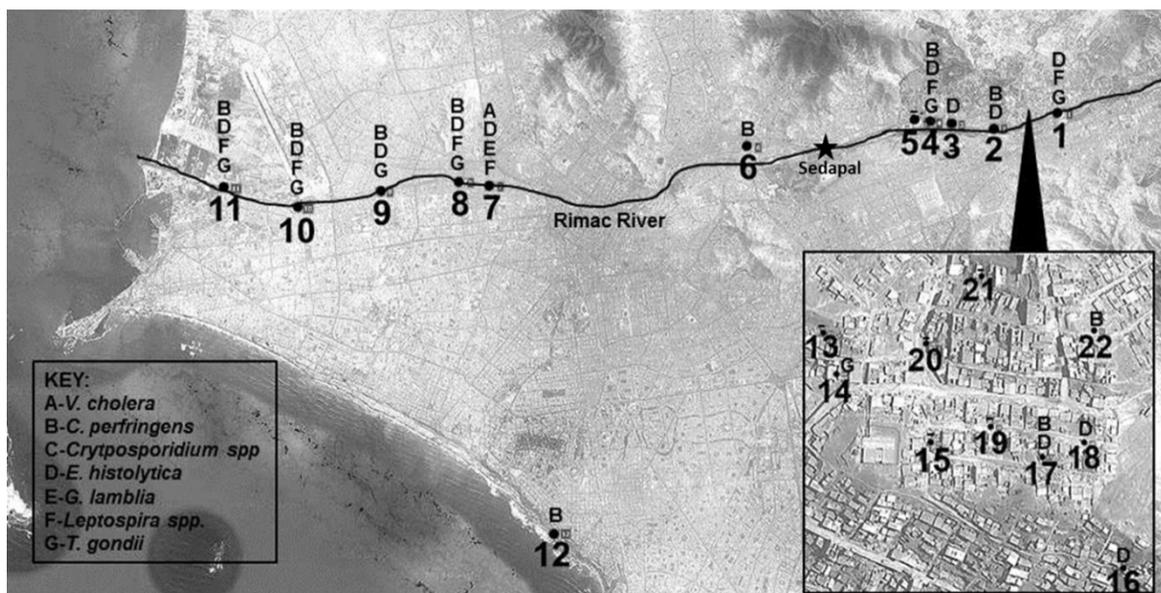


Figure 1: Sampling locations and results. This map shows water sampling locations: along the Rimac River which flows westward (1-11), at a beach site (12) and from residential sink taps in region of Ate (13-21). The key identifies each pathogen found at the different sampling locations. The Sedapal drinking water production facility location is noted along the Rimac River, which causes the volume of water in the river to drop significantly due to water capture. As the river continues following west to the Pacific Ocean, wastewater is the primary source of the river's volume.

Water Sample Filtering and Storage

For each sample, 1 liter (as measured by the Steriflip unit) was processed within 24 hours from time of collection. To process the water samples, each sample was filtered using a 25 μ m polyester filter to remove debris. The flow-through was concentrated onto a 0.22 μ m membrane (the Stericup's filter) using a handheld vacuum, leaving behind organisms between 25 μ m and 0.22 μ m in size (thus excluding viral pathogens). After flow-through, the membrane was simultaneously fixed and disinfected by spraying approximately 0.5mL of 70% ethanol on each membrane and followed by air drying. This last step was an addition to previously published methods, and facilitates disinfection of viable pathogens for safe storage and transport, while also preserving nucleic acids. Filters were then removed with a clean, ethanol-rinsed knife and stored in sterile 50mL Falcon tubes for transport at room temperature.

Positive Controls

To provide template DNA for primer pair characterization in PCR assays, genomic DNA was purchased from ATCC as follows: *L. interrogans* serovar Copenhageni ATCC-BAA-1198D-5, *G. intestinalis* Portland-1 ATCC 30888D, *V. cholerae* MO45 ATCC 51394D-5, *V. parahaemolyticus* EB101 Y ATCC 17802D-5, *C. parvum* Iowa strain ATCC PRA-67D. Additional genomic DNA was isolated from ATCC cultures provided by BEI resources as follows: *E. histolytica* NR 178, *C. hominis* NR 2520, *C. parvum* NR 2519, *C. perfringens* wal-14572 HM 310D, *Leptospira interrogans* NR 19896, *G. lamblia* Egypt NR 9231, *V. cholera* El-tor 34 NR 150, *V. cholera* O1 NR 147. *Toxoplasma gondii* DNA was isolated from a maintained laboratory culture of RH Δ HXGPRT Δ UPRT. DNA isolated from cultured organisms were rapidly thawed from -80°C. The organisms were then centrifuged at 10,000g for 5 minutes, passing through a Qiagen shredder column before DNA isolation using Promega genomic DNA wizard kit per manufacturer instructions. DNA concentration and quality was determined via Nanodrop 2000c. 1ng/ μ L aliquots of stock concentration DNA were created to minimize contamination risk.

Primer optimization

With known template DNA, 34 primer pairs were screened for amplification specificity and robustness. To identify the best primer pair for each organism, PCR reactions were set up in a 25 μ L volume as follows: 1X DreamTaq Buffer, 10mM dNTPS, 10 μ M each forward and reverse primers, 1ng

of known DNA (purchased or isolated from sources listed above), 1.25 units of DreamTaq polymerase, and up to 25 μ L with nuclease-free water. Gradient PCR was used to determine optimal annealing temperatures, and optimized amplification protocols are listed in Table 1. Gel electrophoresis of PCR products was completed using 3% agarose gels containing ethidium bromide with TAE running buffer. Following gel electrophoresis of all runs, the protocol that resulted in a single amplified fragment and/or the stronger band presence was selected for the individual primer pair to be used in sensitivity assays (Table 1).

Sensitivity testing

In order to determine sensitivity of primer pairs using the optimized amplification protocol, isolated DNA for each organism was serially diluted from 100pg/ μ L to 1 ag/ μ L using 10-fold dilutions. PCR reactions were set up in 25 μ L volumes, with DNA concentrations resulting in a range of 1ng/25 μ L to 10ag/25 μ L. Negative controls were also performed to control for band formation without template DNA and also amplification of template DNA without primers. All PCR products were run on 3% agarose gels containing ethidium bromide with TAE buffer. Initial detection limits were determined to be the concentration at which the presence of a band was not clearly visible.

To determine the quantity of genomic copies present in each sample following detection limit assays (Table 2), previously determined genome sizes were utilized in the calculation of the genome mass using the average mass of a base pair as 650 Daltons. Genome sizes were used as follows: *V. cholerae* 4.03MB[28], *C. perfringens* 3.031MB[29], *C. hominis* 9.2MB[30], *E. histolytica* 20MB[31], *G. lamblia* 11.7MB[32], *L. interrogans* 4.659MB[33,34], *T. gondii* 63MB[35]. To further evaluate the sensitivity of these primers in the presence of environmental DNA, local lake water ("environmental background DNA") was collected and combined with positive control samples to simulate the condition of mixed DNA samples from collection points- see Figure 2.

Pathogen Detection

Upon returning to the laboratory (approximately 1 month after acquisition & fixation for all samples), filters were soaked in 2.5mL TE Buffer pH7.4 for 24hr. After soaking, the filters were scraped with a cell scraper, and the suspension was passed through a QiaShredder column. The filter was then cut into <5mmx5mm pieces and resuspended in 2mL TE buffer. The volume, including filter pieces, was

vortexed vigorously then spun down at 2000rpm for 10 minutes. The supernatant was passed through a QiaShredder column and combined with the previous column flow-through. All flow-through was spun a final time at 13000rpm. The supernatant was removed, leaving a pellet behind for processing using a Promega Wizard DNA isolation kit according to the manufacturer protocol.

Isolated DNA concentration and quality was confirmed using a Nanodrop 2000c. Supplemental Table 2 shows the sample location and the quantity and quality DNA isolated from each Peruvian water sample. After determining concentration and purity of the isolated DNA, samples were stored at -20°C until further use. PCR reactions were subsequently run on each DNA sample, screening for the presence or absence of an organism or pathogen-related gene by specific PCR primers. Thermo Scientific DreamTaq PCR kit was used for all reactions in a total volume of 25µL. Cycling parameters were run as follows: (1) 90s at 95°C, (2) 40 cycles of 30s at 95°C, 30s at indicated annealing temperature listed in the figure legends, 60s at 72°C, followed by (3) 10 min 72°C and (4) 4°C. Both positive and negative controls were run in conjunction with the water sample DNA. Positive controls were run with organisms or DNA supplied by ATCC; and two negative controls were run to identify non-specific amplification: 1) reactions lacking any DNA template and 2) reactions lacking primer pairs. Results from the water sample PCRs were run against the positive and negative controls on 3% agarose gels containing ethidium bromide with TAE running buffer. Bands were visualized on a UV box to determine any amplification (Figure 2).

Results

Primer Pair Selection and Sensitivity

Following initial test amplifications, minimum detection limit assays were performed on 34 primer pairs to determine the sensitivity of the primer pair to a known target. After showing the visualization of bands decreasing as DNA concentration decreased, select primers were further tested to amplify target DNA in the presence of foreign DNA. The primer pairs that most consistently resulted in robust amplification of intended products are shown in table 1. In order to quantify our results, the minimum detection limits in units of genomic copies are shown in table 2. No non-specific amplification was shown in the PCRs containing foreign DNA, suggesting high specificity of the selected primers was sustained.

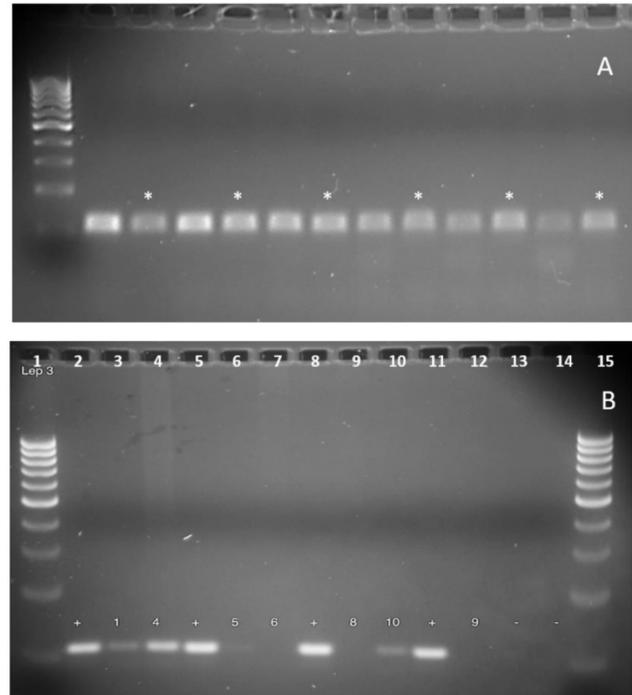


Figure 2: Example gel of *Leptospira* PCR sensitivity and detection. (A) The gel depicts the sensitivity and specificity of the *Leptospira* 3 primer pair (F)-TAGTGAACGGGATTAGATAC and (R)-GGTCTACTTAATCCGTTAGG, described previously as primers 16S-P1 and 16S-P2.²⁶ The primer pair predicted amplicon length is 110bp. *Leptospira* DNA from ATCC (*Leptospira interrogans* serovar Copenhageni Fiocruz L1-130) was diluted in a series of 10-fold dilutions in sets of two, from 1ng (lanes 2 and 3) to 1ag (lane 12 and 13), by using 1X TE buffer. One sample of each set, marked by *, contained DNA isolated from a local lake in Omaha, Nebraska not known to contain *Leptospira*, spiked with 1ng of lab cultured isolated *Leptospira* DNA. (B) The gel depicts PCR results used to determine presence of *Leptospira* DNA from collected water samples, using the same *Leptospira* primer pair as above. '+' indicates the positive control (known DNA obtained from ATCC), '-' indicates negative control (primer pair only, no template DNA), and an annealing temperature of 48.5°C was used for the PCR cycle. Lanes 1 and 15 contain Fisher exACTgene 100bp ladder. The numbers correspond to samples numbers in Table 2. Presence of *Leptospira* was identified in samples 1, 4, 5, and 10.

Water Sample Testing

The Peru water samples, 22 total, were screened using 12 different pathogen-detecting assays. Table 3 shows all 22 samples and the pathogen presence or absence. In the 22 samples test: cholera toxin was detected in 4.5% of samples, *C. perfringens* was detected in 50% of samples, *E. histolytica* was detected in 54.5% of samples, *Giardia intestinalis* was detected in 4.5% of samples, *Leptospira* spp. was detected in 29% of samples, and *T. gondii* was detected in 31.8% of samples. Most of the pathogens found were detected in the samples from the Rimac River. Only *C. perfringens*, *E. histolytica*, and *T. gondii* were found in the residential samples, suggesting that water treated by the Sedapal drinking water facility is mostly efficient at removing waterborne pathogens. Figure 2 depicts an example agarose gel.

Table 1: Primer pair table. Primer pairs with the listed purpose of “Presence / Absence” are designed to detect the presence of listed genus or species by amplifying highly conserved genomic sequences. Primer pairs Chol 14 and Chol 18 detect the presence of the Cholera serovars O139 and O1 respectively, which are known to cause human infection through the presence of the cholera toxin gene (ctxA). The primer pair Chol 18 detects the cholera toxin gene, which is responsible for the diarrheal symptoms characteristic of the cholera infection. The Crp 14 primer pair can detect the two species of *Cryptosporidium* that cause the most cryptosporidiosis disease in humans, *C. parvum* and *C. hominis*. The primer pair can also differentiate between the species by an additional restriction enzyme digestion step with BsiEI on the PCR product (no fragmented bands-*C. parvum*; two fragments-*C. hominis*).[22] Other *Cryptosporidium* species have also been reported to cause cryptosporidiosis in immune competent and compromised individuals, which include *C. felis*, *C. melagridis*, *C. canis*, and *C. muris*. [6] The “Presence / Absence” primer pair Crp 10 detects the presence of these additional *Cryptosporidium* species. The Lep 11 primer pair functions to detect the *Leptospira*-specific virulence factor LipL32, found exclusively in pathogenic leptospires; this allows for differentiation of pathogenic and nonpathogenic *Leptospira* species.[42]

Primer ID	Organsim	Sequence (5' to 3')	Amplicon Length	Purpose	Annealing Temperature	Reference
Chol 4	<i>V. cholerae</i>	TTAAGCSTTTTCRCITGAGAATG (F) AGTCACTTAACCATACAACCCG (R)	300bp	Presence/ Absence	55°C	19
Chol 14	<i>V. cholerae</i>	AGCCTCTTTATTACGGGTGG (F) GTCAAACCCGATCGTAAAGG (R)	449bp	O139 Serovar	55°C	20
Chol 15	<i>V. cholerae</i>	GTTCACITGAACAGATGGG (F) GGTCATCTGTAAGTACAAC (R)	192bp	O1 Serovar	48.5°C	20
Chol 18	<i>V. cholerae</i>	GGTCTTATGCCAGAGGACAG (F) GTTGGGTGCAGTGGCTATAAC (R)	219bp	Cholera toxin ctxA	48.5°C	17
Crp 10	<i>Cryptosporidium</i>	TCGGTACAGCATCAGGTTCA (F) GTTTTGCTCCCCAGTTTT (R)	368bp	Presence/ Absence	48.5°C	21
Crp 14	<i>Cryptosporidium</i>	CAACCCAGAAGTTGAGGTT (F) CTAGTATGCTTCAGACCATGAG (R)	171/183bp	<i>C. parvum</i> vs. <i>C. hominis</i>	48.5°C	22
Clo P 1	<i>C. perfringens</i>	AAAGATGGCATCATCATTCAAC (F) TACCGTCATTATCTTCCCCAAA (R)	276bp	Presence/ Absence	48.5°C	23
Ent 1	<i>E. histolytica</i>	ATTGTCTGGGCATCCTAACTCA (F) GCGGACGGCTCATTATAACA (R)	172bp	Presence/ Absence	55°C	24
Gia 4	<i>G. lamblia</i>	CAGTACACCTCYGCTCTCGG (F) GTTTCTTGCACATCTCC (R)	410bp	Presence/ Absence	48.5°C	25
Lep 3	<i>Leptospira</i>	TAGTGAACGGGATTAGATC (F) GGTCTACTAATCCGTTAGG (R)	110bp	Presence/ Absence	48.5°C	26
Lep 11	<i>Leptospira</i>	CGCTGAAATGGGAGTTCGTATGATT (F) CCAACAGATGCAACGAAAGATCCTTT (R)	423bp	Virulence factor LipL32	48.5°C	18
Tox 2	<i>T. gondii</i>	AGAGACACCGAATGCGATCT (F) CCCTCTCTCCACTCTTCAATTCT (R)	529bp	Presence/ Absence	55°C	27

(F)- forward primer; (R)- reverse primer. Cycling parameters: (1) 90s at 95°C, (2) 40 cycles of 30s at 95°C, 30s at indicated annealing temperature, 60s at 72°C, followed by (3) 10 min 72°C and (4) 4°C.

Table 2: Primer pair minimum detection limits. Primer sensitivity listed by the minimum quantity of genomic copies required for detection of the specified DNA fragment. Bacteria sampled here are generally considered to maintain a single genome copy (*V. cholerae*, *C. perfringens*, and *Leptospira*), whereas the sampled parasites vary in genome count (typically 2-4 copies). Tests were performed using 10-fold dilutions of template DNA with a starting concentration of 1ng per reaction and were measured either from DNA isolated from lab cultured organisms, or intact organisms spiked into an environmental sample consisting of Nebraska lake water (which was negative for all tested organisms). Minimum tested template concentrations varied by organism between 10 fg to 10 ag per reaction. The concentration of nonspecific DNA, isolated from an environmental sample, was maintained at 1ng per reaction. No bands were present in negative controls.

Primer ID	Organsim	Purpose	Minimum detection limit (Genomic copies) Lab cultured sample	Minimum detection limit (Genomic copies) Environmental sample-spiked
Chol 4	<i>V. cholerae</i>	Presence/ Absence	230	230
Chol 14	<i>V. cholerae</i>	O139 Serovar	2300	2300
Chol 15	<i>V. cholerae</i>	O1 Serovar	230	230
Chol 18	<i>V. cholerae</i>	Cholera toxin ctxA	23	2300
Crp 10	<i>Cryptosporidium</i>	Presence/ Absence	3	3
Crp 14	<i>Cryptosporidium</i>	<i>C. parvum</i> vs. <i>C. hominis</i>	1	1
Clo P 1	<i>C. perfringens</i>	Presence/ Absence	101	101
Ent 1	<i>E. histolytica</i>	Presence/ Absence	5	5

Gia 4	<i>G. lamblia</i>	Presence/Absence	79	79
Lep 3	<i>Leptospira</i>	Presence/Absence	199	199
Lep 11	<i>Leptospira</i>	Virulence factor LipL32	199	19
Tox 2	<i>T. gondii</i>	Presence/Absence	14	14

Table 3: Results from sampling locations. Table 3 demonstrates the detection results from the water sampling of 22 locations in Lima, Peru. Sample IDs reflect the corresponding locations in Figure 1. “+” indicates detection of presence, specific species or virulence factor of pathogens, whereas “-“ indicates no presence found. In the 22 water samples tested, cholera toxin was detected in 4.5% of samples, *C. perfringens* was detected in 50% of samples, *E. histolytica* was detected in 54.5% of samples, *G. intestinalis* was detected in 4.5% of samples, *Leptospira* spp. was detected in 29% of samples, and *T. gondii* was detected in 31.8% of samples. “**” indicates insufficient remaining sample volume for retesting.

Sample ID	Chol 4 (+/-)	Chol 14 (O1 serovar +/-)	Chol 15 (O139 serovar +/-)	Chol 18 (Cholera toxin ctxA +/-)	Clo P 1 (+/-)	Crp 10 (+/-)	Crp 14 (C. parvum/C. hominis)	Ent 1 (+/-)	Gia 4 (+/-)	Lep 3 (+/-)	Lep 11 (Virulence factor LipL32)	Tox 2 (+/-)
River 1	-	-	-	-	-	-	-	+	-	+	-	+
River 2	-	-	-	-	+	-	-	+	-	**	-	-
River 3	-	-	**	-	-	-	-	+	-	-	-	-
River 4	-	-	-	-	+	-	-	+	-	+	-	+
River 5	-	-	-	-	-	-	-	+	-	-	-	-
River 6	-	-	-	-	+	-	-	-	-	-	-	-
River 7	-	-	-	+	-	-	-	+	-	+	-	-
River 8	-	-	-	-	+	-	-	+	-	+	-	+
River 9	-	-	-	-	+	-	-	+	-	-	-	+
River 10	-	-	-	-	+	-	-	+	-	+	-	+
River 11	-	-	-	-	+	-	-	+	-	+	-	+
Ocean 12	-	-	-	-	+	-	-	-	-	-	-	-
Residential 13	-	-	-	-	-	-	-	-	-	-	-	-
Residential 14	-	-	-	-	-	-	-	-	-	-	-	+
Residential 15	-	-	-	-	-	-	-	-	-	-	-	-
Residential 16	-	-	-	-	-	-	-	+	-	-	-	-
Residential 17	-	-	-	-	+	-	-	+	-	-	-	-
Residential 18	-	-	-	-	-	-	-	+	-	-	-	-
Residential 19	-	-	-	-	-	-	-	-	-	-	-	-
Residential 20	-	-	-	-	-	-	-	-	-	-	-	-
Residential 21	-	-	-	-	-	-	-	-	-	-	-	-
Residential 22	-	-	-	-	+	-	-	-	-	-	-	-
Total	0/22	0/22	0/21	1/22	11/22	0/22	0/22	12/22	1/22	6/21	0/22	7/22

Discussion

Some current detection methods for waterborne pathogens, i.e. culture and microscopy, are manageable, yet not feasible to be completed in a timely, cost-efficient manner. PCR can be an effective alternative that has improved sensitivity and can be done in a short period of time for less money. Though there can be drawbacks to PCR, such as non-specific amplification or detection of nonviable organisms, our study shows the ability of DNA isolation protocol and PCR assay to survey environmental and residential water samples in the presence of background DNA.

Using previously established primer pairs, PCR assays were run to determine an optimized set of primers and amplification protocols by screening PCR for robust amplification using known DNA sources.

After the best primer pairs were identified, the sensitivity of each pair was tested using 10-fold dilutions of known template DNA in the presence of environmental background DNA. In 11 of 12 PCR assays, no detectable inhibition was found. The Chol 18 primer pair, which detect the presence of cholera toxin ctxA, decreased sensitivity 100-fold in the presence of environmental background DNA.[36]

Previous studies describe the prevalence of disease cause by *Leptospira*, *Cryptosporidium*, *Cholera*, and *Giardia* species in Peru[2,3,4,5,6,7,9,25,37,38]; however, few studies have focused on the quality of water available to those with and without access to potable water connections. Sample 11, the westernmost sample collected from the Rimac River and closer to the mountain water source, contained six out of the seven pathogens discussed in this study: *V. cholerae*, *Giardia intestinalis*, *Clostridium perfringens*,

Entamoeba histolytica, *Leptospira* spp., and *Toxoplasma gondii* (Table 3). In contrast, sample 12, collected in the Miraflores district near the Pacific Ocean, only had the detectable presence of *C. perfringens*- a limited result likely due to dilution.

Residential samples collected in the Ate region of Lima, Peru showed low frequency of contamination from pathogenic organisms. This finding can be attributed to the chlorination of water at the drinking water facility, Sedapal. Samples collected at residential locations 13, 15, 19, 20, and 21 did not contain detectable quantities of DNA. Samples 16, 17, and 18 were determined to contain *C. perfringens*; and sample 14 tested positive for *T. gondii*. The low number of detectable pathogens suggests that contamination is highly localized. This confirms previous publications that cite examples of localized contamination due to a number of factors such as biofilm, non-continuous pressure, and leaks. [39, 40, 41]

Conclusion

The importance of a highly sensitive, cost-effective, timely test kit for waterborne pathogens is paramount for screening a number of water supplies in both developed and undeveloped countries. While current testing, i.e. culture methods and microscopy, are useful, this study describes the DNA isolation and PCR methods that can be utilized to test a large number of water samples with quick turnaround and accuracy. The methods discussed in this study led to an identification of several pathogenic organisms in Peruvian waters along the Rimac River and in the Ate region of Lima.

Future studies and testing of water supplies can be expanded to include a number of waterborne pathogens, including *Cyclospora* species, pathogenic *E. coli*, *Salmonella typhi*, and Hepatitis A and E viruses. Additionally, sampling volumes can be increased to reflect an average daily intake of water in the areas of water sampling. By increasing the volume of samples taken, a more accurate picture of pathogenic species can be seen. The seriousness of waterborne pathogen contamination in worldwide water supplies cannot be understated. A total of 780 million people do not have access to clean water, and exposure to contaminated water sources can result in death.⁴² By utilizing a simple water testing method that is cheap, highly sensitive, and timely, drastic improvements can be made to water quality worldwide.

Supplementary Material

Supplementary tables.

<http://www.jgenomics.com/v05p0004s1.pdf>

Acknowledgments

This work was supported by the NIH National Institute for General Medical Science (NIGMS) (P20GM103427). Further support was provided by The University of Nebraska DNA Sequencing Core, which receives support NIH COBRE P30GM110768 as well as The Fred & Pamela Buffett Cancer Center Support Grant P30CA036727. The GRACA and FUSE programs of the University of Nebraska at Omaha additionally supported this work.

Competing Interests

The authors have declared that no competing interest exists.

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