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PAULA LADEIA BARROS

EVALUATION OF HUMAN FECAL CONTAMINATION IN IRRIGATION WATER USED IN URBAN GARDENS OF SALVADOR/BA

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Trabalho de Conclusão de Curso apresentado à Escola Bahiana de Medicina e Saúde Pública, como parte dos requisitos para obtenção do título de Bacharel em Biomedicina.

Orientadora: Prof. Dr. Lúcio Macedo Barbosa.

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Esta monografia foi julgada adequada à obtenção do grau de Bacharel em Biomedicina e aprovada em sua forma final pelo Curso de Biomedicina da Escola Bahiana de Medicina e Saúde Pública.

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1. Artigo Científico

Title: Evaluation of human fecal contamination in irrigation water used in urban gardens of Salvador/BA.

Evaluation of human fecal contamination in irrigation water used in urban gardens of Salvador/BA

Running Title

Human fecal contamination in urban gardens of Salvador/BA

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Abstract

In Brazil, the main problem regarding natural water bodies is the incorrect disposal of domestic sewage directly into rivers. This constant degradation of water bodies combined with lack of basic sanitation compromises the environment health, facilitating waterborne diseases. This problem can become more sensitive when contaminated water is used for irrigation of agricultural crops. Due this problem, water needs to be analyzed and is necessary to identificate the source of the problem. To protect consumers from contaminated water, CONAMA's resolution was created (Resolução Nº 357/2005) to identificate the water that are within thee defined standard. Even though, the constant control of these waters is very complicated due to the size of Salvador and quantity of points distributed in all city. This article aims to identify and describe the contaminated urban gardens and identify the possible source of contamination, focusing on specific human bacteria. We performed coliform analysis for identificate urban gardens with fecal contamination and, to identify more specific fecal sources was used Microbial Source Tracking (MST), with *Human bacteroides* and *Lachnospira* primers. Using bacterial counts to assess water contamination, 43.8% of the evaluated urban gardens were not suitable for human use and/or irrigation. PCR results indicated 31.25% of the sites with *Human bacteroides* positive samples and 6.3% with *Lachnospira* positive samples. So, water is being compromised due to the disposal of decomposed organic material or faeces, even with CONAMA's resolution. Those waters should be monitored and shouldn't be contaminated, considering the importance they represent to the community.

Key words: MST; urban gardens; water; coliforms; microbiological parameters.

Introduction

Water is essential for life and is a resource used in different ways by all living being on the planet. Humans use water in the biological, industrial, agricultural ways, common use and as energy source[1]. However, despite its recognized importance, we live, in a general way, a great paradox that is the constant degradation of water bodies.

In Brazil, the main problem regarding natural water bodies is the incorrect disposal of domestic sewage directly into rivers[1,2]. This misuse contributes for water quality degradation that compromises the environmental health[3]. It releases many harmful microorganisms, such as bacteria, viruses and parasites, into the environment, facilitating among other things, the spread of waterborne diseases[2]. This problem can become more sensitive when contaminated water is used for irrigation of agricultural crops. In large metropolises, such as Salvador, urban gardens are becoming more frequent due the intense process of migration from rural area. However, due to the increasing population density of cities, lack of basic sanitation and pollution have become more present, contaminating water, soil and air[4].

Whereas the problem of basic sanitation, there is a need for constant water analysis for its safe use and consumption. Considering this situation, the Conselho Nacional do Meio Ambiente (CONAMA – National Council for the Environment) regulates the water quality for use and irrigation (Resolução Nº 357/2005)[5]. In this ordinance, thermotolerant (*Escherichia coli* - *E. coli*) and total coliforms are used as standard for microbiological analysis of water, however, those parameters are still nonspecific for identifying the microbial source. So, for more specific identification, more specific testing is required, as Microbial Source Tracking (MST). This

technique consists of identifying bacteria of interest through the PCR, making the study more specific due to the choice of bacteria to be researched.

The need for constant analysis of water quality, especially in urban gardens, is evident, considering that is a problem of basic sanitation and public health. Therefore, the present study aims to assess the water quality, in the microbiological aspects, used for irrigation in the urban gardens of Salvador, through the description of the amount of total coliforms and *E. coli* and the more specific identification of the microbial source through the research of bacteria of the gastrointestinal tract of the genus *Bacteroides* and *Lachnospira* that have a greater specificity for humans.

Materials and Methods

Urban Garden Identification in Salvador

Salvador's urban gardens were identified and cataloged in a doctoral thesis in 2013[6].The total of 48 was fully described regarding local infrastructure and other features related to their residents and local workers[6]. For this study, we re-identified all the urban gardens due to the long period of the initial description. All sites were re-visited, and permission was solicitated to perform the water evaluation. All locations were georeferenced with Nomad GPS unit Model 65220-11 (Trimble Navigation Ltd., Sunnyvale, CA) for graphical presentation on maps made with QGIS 3.6 Noosa (QGIS Development Team (2019). QGIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org) and subdivided on Salvador´s Sanitary District (SD) and neighborhoods.

Of the initial 48 urban gardens in 2013, 32 were deactivated and 16 remained actives in 2019 (Figure 1), that received a matching code for identification (ID) (Table 1). The activated

urban gardens were accounted for as follows: Pau da Lima (A), district São Rafael/São Marcos with 4 urban gardens; Cabula/Beiru (B), district Narandiba with 3 gardens, Pernambués, with 1 garden and Saramandaia, with 5 gardens; São Caetano/Valéria (C), district Valéria with 1 garden and Subúrbio Ferroviário (D), district Pirajá with 2 gardens.

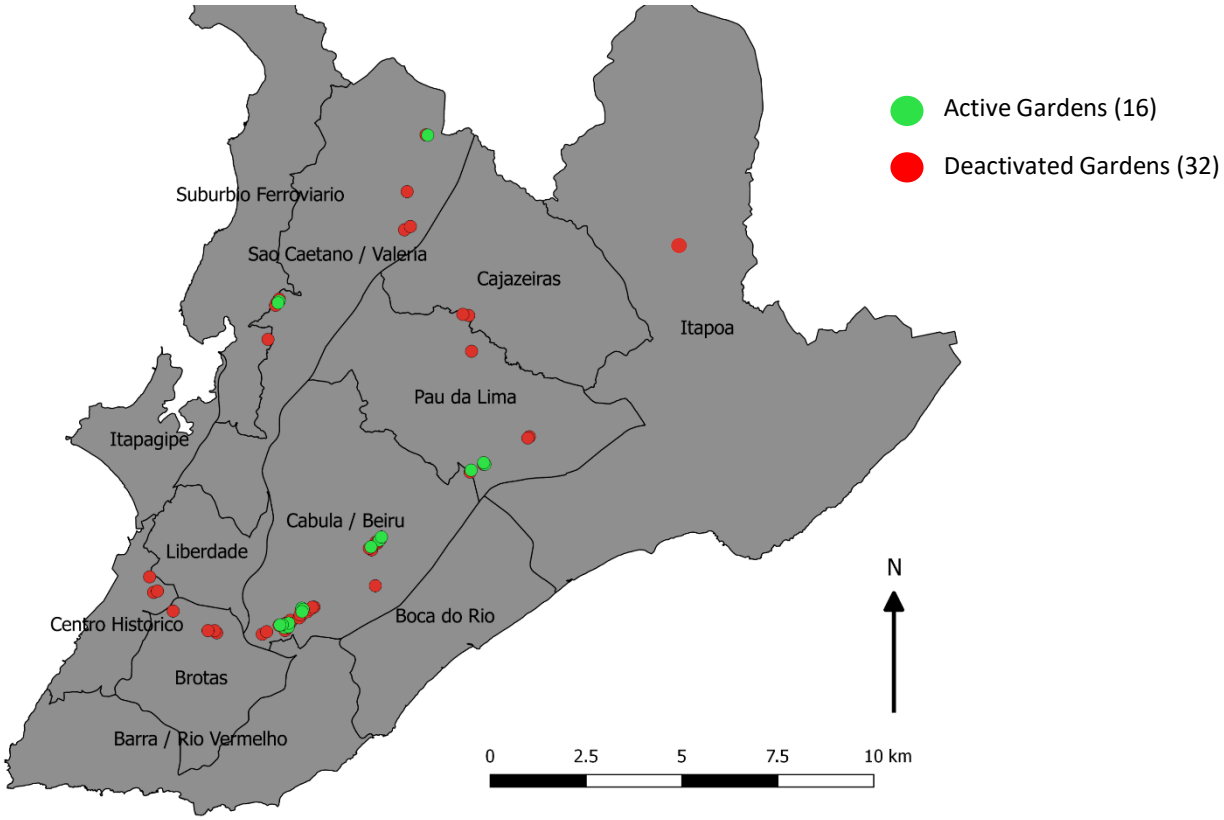

Figure 1 – Geographical distribution of the active/deactivated urban gardens in Salvador – Bahia in 2019.

Water collection

Water collection was performed in the mornings in February, the low raining season of Salvador, BA, in the same week in order to avoid possible interferences, like rain, due the possibility of sample dilution (Figure 2)[7]. It was collected on every point indicated by the workers as used for irrigation. On the districts São Rafael/São Marcos (A2), Pernambués (B4),

Saramandaia (B5), Valéria (C1) and Pirajá (D1), irrigation was performed following the waterbed that presented different characteristics, requiring collection of different water points in order to be better representative. A total of 500 mL was collected using clean and sterile collector. Water was kept in thermal boxes at 4º C, using chemical ice, until microbiological analysis that occurred within three hours of the collection.

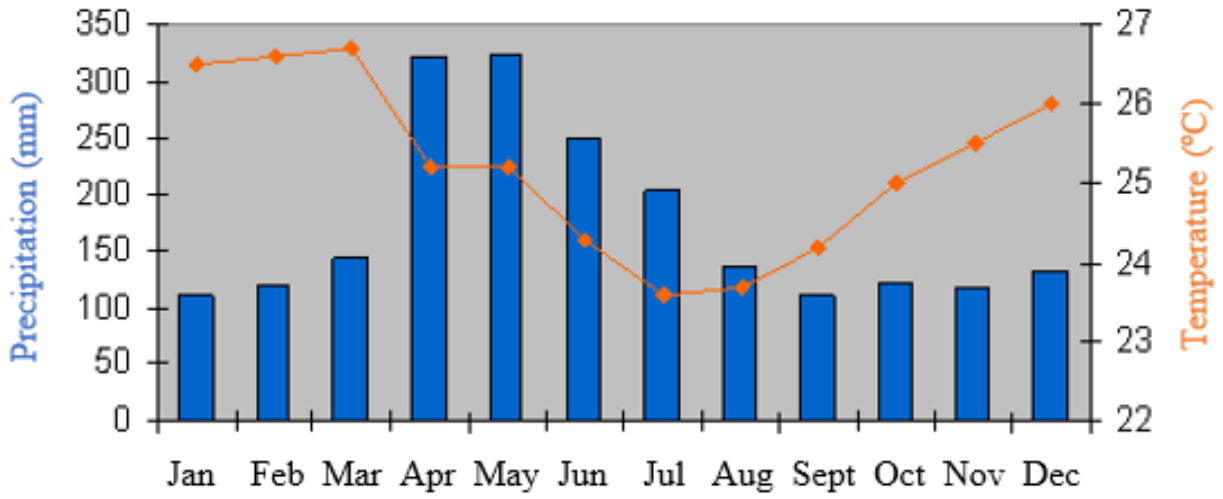

Figure 2 – Precipitation and Temperature Climatology, 2018. Information acquired on the Instituto Nacional de Pesquisas Espaciais (INPE – National Institute of Space Research)'s website.

Coliform analysis

Total coliforms and *E. coli* quantification was performed using Coliscan Easygel® kit (Microbiology Laboratories, Goshen, IN, EUA), following the manufacturer's guidelines. Colony forming units (CFU) counts was performed manually and in duplicate. The limit of the count established in this study was 500 CFU/mL. The water was classified according to the CONAMA's resolution (Resolução Nº 357/2005) to fresh water, class 1. This resolution states that a limit of

200 *E. coli* per 100mL (2/mL) should not be exceeded, 80% or more of at least 6 samples, but don't states a limit to total coliforms.

Microbial Source Tracking (MST) – Bacterial rDNA analyzes

Collected water samples were filtered through cellulose membranes 47 mm in diameter with 0.22 μm pores (EMD Millipore Corporation, Billerica, MA). Membranes were completely dried and stored at -20 °C until DNA isolation. Frozen membranes were broken into small fragments and subjected to the phenol-chloroform procedure for DNA extraction. Isolated DNA were diluted in proportion to 1:1 with deionized H_2O, to diluted PCR inhibitors.

Bacterial presence was evaluated by conventional Polymerase Chain Reaction (PCR) using primers targeting 16S rRNA gene. Primers sequences for human source bacteria of the order Bacteroidales were F: 5' ATCATGAGTTCACATGTCCG 3'; and R: 5' CAATCGGAGTTCTTCGTG 3' (*Human Bacteroides*); and for *Lachnospira* were F: 5' TTCGCAAGAATGAAACTCAAAG 3'; and R: 5' AAGGAAAGATCCGGTTAAGGATC 3'. PCR amplification were performed with 25 μL for final volume using 2 μL of each primer, 12,5 μL of TopTaq DNA Polymerase, 2,5 μL of CoralLoad Concentrate (TopTaq® Master Mix Kit (250) (Qiagen, Hilden, Germany) and 5 μL of DNA. Each test was performed in duplicate. The cycles followed an already standardized conformation: 94 ºC for 3 minutes, 30 cycles of 94 ºC (30 seconds), 53 ºC (30 seconds), 72 ºC (1 minute), and finally 72 ºC for 10 minutes.

Results and Discussion

Due to the problems of precarious basic sanitation and financial difficulties, city authorities are in constant labor to improve districts´ infrastructure and land invasion. Thus, majority of urban gardens were deactivated. We chose to evaluate irrigation water considering that contamination of

this resource would make possible to pathogens infect humans through the cultivated inputs[8]. To preserve the health of residents, and consumers, it is extremely important to control the quality of water in the urban gardens.

Using bacterial counts to assess water contamination, 43.8% (7/16) of the evaluated urban gardens were not suitable for human use and/or irrigation (Table 1)[5]. When the urban gardens were extensive, waterbeds accompanied their entire length and their onset were less contaminated. Presence of *E. coli* in water samples may indicate contamination by other intestinal pathogens[9].

However, the use of this parameter alone is usually not enough to associate with disease due to non-specificity of the markers, since these are present in all homeothermic beings[10].

Using more specific markers can help make better association of fecal contamination with waterborne diseases in the water bodies. Applying MST make possible to determine the fecal sources of contamination in water through identification of bacteria through molecular analysis[11]. In this study, we used two primers to identify two different genus, *Bacteroides* and *Lachnospira*. These bacteria were chosen due to their characteristics. They are specific anaerobics, therefore, do not multiply in the environment, indicating recent contamination if found and presents a high specificity for humans[12].

Inappropriate use and neglect with the water contributes to the spread of waterborne diseases such as diarrhea, hepatitis and schistosomiasis, for example. These diseases can be transmitted either by direct ingestion of water or indirectly, through ingestion of contaminated food. Thus, one of the simplest and most effective prophylactic measure is caution with food before consumption, as knowing from where those foods were produced and under what conditions. Such

149 care must be constant and should take place from obtaining the water to the consumption of the
150 products, including the irrigation of these products, especially in agricultural areas.

Table 1. Coliforms and PCR's results of each sample

Sanitary District	Districts	ID	E. coli (CFU/mL)	Total Coliforms (CFU/mL)	*Human bacteroides*	*Lachnospira*
Pau da Lima	São Rafael / São Marcos	A1	0	0	-	-
		A2.1 *	1	> 500	-	-
		A2.2 *	12	28	+	-
		A2.3 *	0	135	+	-
		A3	0	0	-	-
		A4	0	0	-	-
Cabula / Beiru	Narandiba	B1	0	0	-	-
		B2 *	2	> 500	-	-
		B3	0	0	-	-
	Pernambués	B4.1 *	0	60	-	-
		B4.2 *	2	222	+	-
		B4.3 *	2	116	+	-
	Saramandaia	B5.1 *	0	> 500	-	-
		B5.2 *	0	17	-	-
		B6	0	0	-	-
		B7	0	0	-	-
		B8	0	0	-	-
		B9	0	0	-	-
São Caetano / Valéria	Valéria	C1.1 *	0	3	-	-
		C1.2 *	0	50	+	-
		C1.3 *	0	113	+	-
Subúrbio Ferroviário	Pirajá	D1.1 *	0	> 500	+	+
		D1.2 *	5	> 500	+	+
		D2 *	0	69	+	-

TABLE 1. All active gardens were organized to their Sanitary District and districts. All samples marked with "*" was above CONAMA's standard (Portaria Nº 357/2005).

151 To analyze in a more representative way the urban gardens with more than one point of
152 collection, the results were counted separately but analyzed together for those ones (A2, B4, B5,

C1 and D1). All positive samples for coliforms were above standards of CONAMA's resolution (Table 1). In addition, of all 15 positive samples, only 6 sites (A2.1, A2.2, B2, B4.2, B4.3 and D1.2) were *E. coli* positive. Total coliforms denote presence of organic material due to the presence of non-pathogenic bacteria and *E. coli* presence implies fecal contamination of homeothermic animals.

PCR results indicated 31.25% (5/16) of the sites with *Human bacteroides* positive samples and 6.3% (1/16) with *Lachnospira* positive samples (Table 1). As expected, all these locations were also positive for total coliforms. Whereas that *Human bacteroides* and *Lachnospira* belongs to human microbiota, is possible to infer that gardens located in São Rafael / São Marcos (A2), Pernambués (B4), Valéria (C1) and Pirajá (D1 and D2) present greater risk for the human health, due to the higher risk of transmitting human pathogens.

The analysis of irrigation water was already studied in other occasions in Brazil, showing that there is contamination by total coliforms and *E. coli* that contaminate the vegetables of urban gardens, contributing to the spread of diseases[13,14,15]. Despite the studies proving water contamination, either by fecal matter or organic material in decomposition, there were no changes in the Brazilian legislation that governs this follow-up to be monitored, especially in the matter of defining a standard for total coliforms.

Conclusion

Fecal contamination was found in irrigation water used in the urban gardens in Salvador, due mainly lack of basic sanitation. Water is being compromised due to the disposal of decomposed organic material or faeces. We described all contaminated water by presence of total coliforms being above CONAMA's standards, making it unfit for irrigation. Samples that were

positive to total coliforms and *E. coli* and for any or both PCR primers means that are being deposited recent human feces requiring further attention from the urban garden owners and the authorities. Otherwise, positive samples for total coliforms and *E. coli* and negative for both PCR primers indicates that are feces of warm-blooded animals, whether human or not. However, this also requires attention from legal authorities due to the potential for transmission of other types of pathogens.

Although the Brazilian legislation doesn't have a standard for total coliforms in irrigation waters or vegetables, the monitoring of these microorganisms is necessary, considering that they are potential deteriorators belonging to the group of bioindicators of food hygiene. Each country has an own standard to follow, adapting as much as is needed to express the reality of the country. Even though, isn't all resolutions that create a standard to total coliform. In general, they're focus on *E. coli* analyses. Still, some countries like South Korea are being more restrict and considerate to total coliform not being detected as a standard[16].

It is possible to conclude that some districts need a local sanitary restructuring and treatment of the water to be used. Residents and local workers of the urban gardens in Salvador/BA should be aware of the risks they are being exposed to, and how to minimize them. In addition, with CONAMA's resolution establishing standards for use, waters should be monitored and shouldn't be contaminated. Still, is necessary considerate a definition of standard to total coliforms, for more complete analysis and considering the importance they represent to the community.

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Disclosures

There were no conflicts of interest in this article.

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2. Proposta de submissão

2.1 Revista:

The American Journal of Tropical Medicine and Hygiene é uma revista publicada mensalmente pela American Society of Tropical Medicine and Hygiene. Está entre as revistas de medicina tropical mais conceituadas do mundo, com fator de impacto de 2,564 (2017).
A revista publica artigos científicos originais e as últimas descobertas científicas, com ênfase em população, ciências clínicas e laboratoriais e aplicação de tecnologia nas áreas de medicina tropical, parasitologia, imunologia, doenças infecciosas, epidemiologia, biologia básica e molecular, virologia e medicina internacional.

2.2 Regras para submissão:

Author Instructions

Contents:

- Cover letter and signatures
- Authorship
- Manuscript types
- Submission process
- Manuscript formatting
- Additional information

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All manuscripts should be accompanied by a cover letter with the following information:

1. The title of the paper.
2. A brief description of the significance of the paper to the readers of the AJTMH.
3. A statement confirming that the material is original, has not already been published, and has not and will not be submitted for publication elsewhere as long as it is under consideration by the AJTMH. This does not include preprints posted on bioRxiv.

4. Written disclosure of any relationships or support which might be perceived as constituting a conflict of interest.
5. A completed Copyright and Disclosure form from each author. Ideally, the corresponding author will collect this form from all authors and submit them to the journal at the time of initial submission. The ICMJE Copyright and Disclosure form can be found here. These forms can be uploaded at the time of submission; if the corresponding author does not have all forms at the time of submission, the forms can be uploaded or sent to the journal office at a later date. Any author forms can be designated as "for internal use" upon upload.

Authorship

There is no limit to the number of authors that may be listed (except for Images in Clinical Tropical Medicine articles; see below). Only two individuals may be listed as first authors.

All authors must meet the standards described in the ICMJE authorship guidelines: 1) Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; 2) Drafting the work or revising it critically for important intellectual content; 3) Final approval of the version to be published; and 4) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Please read Section II.A.2 of the ICMJE recommendations for more information on authorship guidelines.

In some cases, for papers with a large number of authors participating in a working group, the group may be cited as author, and all individual authors listed in a footnote.

Manuscript types

Original research reports. These form the large majority of papers published by the AJTMH and consist of reports of novel research. There is no word limit or limit to the number of references, but efforts should be made to keep manuscripts as succinct as possible. Full reports should include separate sections entitled Abstract, Introduction, Materials and Methods, Results, and Discussion; the last two sections can be combined. The abstract should not include sub-headings and should contain no more than 250 words. The following sections should be included after the text: Acknowledgments, Financial Support, and Disclosures regarding real or perceived conflicts of interest. Manuscripts should end with a listing of all authors' current addresses, including affiliation, city, country, and email address, followed by References (see Reference section below).

Short reports. This format can be used for submission of important preliminary observations, technique modifications, or data that do not warrant publication as a full paper. Short reports

should contain no sub-headings, and be no more than 1500 words in length, with no more than 150 words in the abstract, 3 tables and/or figures, and 20 references.

Reviews. The AJTMH will consider reviews on relevant topics in tropical medicine, global health, and related areas. Typically reviews will be submitted by leading authorities in a field. We encourage mini-reviews, providing concise reviews of focused topics in no more than 2000 words, but larger reviews will also be considered. Mini-reviews are an opportunity to examine a specific aspect of a disease in great depth to shed light on new perspectives or highlight key areas of interest. They may also be about diseases or fields that are poorly understood. Reviews may be solicited by the editors, but for unsolicited reviews, prospective authors are asked to send a presubmission letter to the Journal explaining why the topic is important and relevant to the readership. Reviews require a short abstract.

Perspectives. These are short articles (up to 1500 words) on timely topics that offer both a focused review of the subject and a balanced presentation of issues that may include key recent changes or areas of controversy. A short abstract is required. Perspectives may offer the opinions of authoritative experts on timely topics or personal accounts from those with compelling tropical medicine experiences. As with reviews, some Perspectives may be solicited, and for unsolicited manuscripts a presubmission letter should be sent to the managing editor for consideration of the proposed topic before submission. Please be sure to explain why the topic is important and relevant to the readership.

Case reports. Short reports of no more than 1500 words can describe a single case or small case series. These must present novel information about a tropical medicine problem of broad interest. Case reports should include an abstract offering a succinct description of the area, a Case Report section including only clinical information that is relevant to the manuscript, and a brief Discussion.

Images in Clinical Tropical Medicine. Short reports (typically up to 200 words, but up to 400 words if complex descriptions are needed, with 5 references and no abstract) including images that demonstrate particularly informative, striking, or unusual presentations of tropical disease are welcome. Manuscripts that offer visual immediacy and clinical relevance will be prioritized. Images will be published in black and white in the print version of the Journal (author will have the option to pay for color) and in color online. Images articles should have no more than 3 authors.

Stories From the Field. These essays are descriptive personal stories based on authors' experiences practicing clinical tropical medicine, performing tropical medicine research, taking part in international health education, and/or delivering global health services. We are interested in compelling stories that relate interesting experiences, express opinions, challenge conventional viewpoints, highlight historical perspectives, share sustainable solutions, or

encourage innovation. All articles should inform our readers about the world, people, health, hygiene, disease, and the myriad of issues that impact tropical medicine and global health. Essays should be titled but have no section headings. The maximum length is 1500 words. The maximum number of authors is 3. If patients are discussed, they should be de-identified. Preapproval is not required before submission, but potential authors are welcome to request feedback on suggested topics before submission. Submissions from authors from LMICs are encouraged.

Book Reviews. These are occasionally solicited by the editors. They should have no sub-headings and be no more than 1000 words in length.

Letters to the Editor. Letters are uncommonly published and should only be responses to recently published articles in the AJTMH. If letters are deemed worthy of publication, they will typically be sent to the authors of the published paper for a response.

Submission process

Prepare your manuscript using a word processing program and save it as a .doc file using Microsoft Word. For items that accompany the text (letters, figures, copyright forms, etc.), you may upload the following file types: .xls, .ppt, .gif, .pdf, .jpg, .eps, .png, and .tif. However, for the manuscript text, do NOT upload .pdf files, but rather use the Word "Save As" option to save your text as a .doc file. Reviewers will see a PDF containing all files you uploaded except for those files you have marked as “Not for Review.” Other file types such as LaTeX files and QuickTime movies can be uploaded. Videos are best uploaded in mp4 format.

Manuscript formatting

Spacing. The text should be in 11 or 12 point type, fully double-spaced, leaving a margin of 1 inch on all sides. Continuous line numbers (NOT restarting with each page) should be included throughout the manuscript and pages should be numbered consecutively.

Title page. This should include, in the following sequence, the title, a list of all authors, and author institutions, identified by superscripts in Arabic numerals. The corresponding author should be denoted by an asterisk, with address, e-mail, and phone number in a footnote. Also include a list of up to 5 key words and the word counts for the abstract and for the text (not including the abstract, figures, or references). The title page should also list the number of figures, tables, and other pertinent information such as supplementary materials.

Title. The manuscript title should be as succinct as possible. Titles should generally not include abbreviations. A running head, for use as a header, should also be provided; the running head should be not longer than 60 characters (including spaces).

Style. American spelling should be used. Indent the first sentence of each paragraph. Use only one space between sentences. For presentation of a series of terms, a serial comma (e.g. “red, white, and blue”) should be used. For italics, italicize the words and phrases in your text, but do not underline. Italicize genus and species. For words that were originally foreign, but are now standard English (e.g. i.e., e.g., in vitro, in vivo), italics are not necessary. For complex sentences, parentheses should enclose brackets. Punctuation should follow the parentheses. Superscripts, including reference numbers, should directly follow punctuation marks. Numbers in text should be in Arabic format, except for one. Insert a space between a number and a unit of measure and both before and after the < symbol, > symbol, and = symbol; no space is needed between a number and the % sign.

Abbreviations. Abbreviations are commonly overused, compromising the clarity of manuscripts. Authors are advised to keep abbreviations to a minimum, using them when they are clearer than long terms (e.g. PCR, DNA), but avoiding them when possible when they are non-standard and idiosyncratic. Abbreviations should conform to the AMA Style Manual. Terms should be spelled out with first usage in both the abstract and text, with the abbreviation following in parentheses. After this first usage, the abbreviation must be used consistently. Plurals of abbreviations do not require apostrophes.

Drug names. Proprietary names of drugs may not appear in the title but may be used in conjunction with the generic name when the drug is first mentioned in the abstract, and again when first mentioned in the text. Thereafter, use only the generic name.

Names of organisms. Genus and species should be italicized. After the first usage the genus should be abbreviated with a single letter (e.g. E. coli). For different species within a genus, the genus should be spelled out with the first usage of each. Adjectives referring to organisms (e.g. plasmodial, falciparum malaria) are not italicized. Viral nomenclature should be based on the International Committee on Taxonomy of Viruses (ICTV; see the AMA Style Manual. A complete listing of ICTV recognized viral species can be found here.

Figures. Figures should be numbered in Arabic numerals and cited in the text. It should be noted that a fee is required for color illustrations in print, but authors can choose black & white in print, but color online at no charge. All figures should contain a brief legend.

Figure specifications. Monochrome (1-bit) images/ line art: The preferred resolution for this type of image is between 1000 and 1200 ppi. Examples include charts and graphs made of solid black and white, with no gray values. Combination halftones: The preferred resolution for this type of image is between 600 and 900 ppi. Examples include color or grayscale figures containing halftone and line art elements. Halftones: The suggested minimum resolution for this type of image is 300 ppi. Examples include color or grayscale figures containing pictures only, with no text or thin lines. We prefer PDF files for line art and tiff files for images. Please

do not embed figure files in word documents, as this decreases their resolution. Figures should be submitted as a separate file or files.

Tables. Tables should be serially numbered in Arabic numerals and cited in the text. Each table should be placed on a separate page at the appropriate point in the text or at the end of the manuscript.

Co-Author Contact Information. Co-author contact information must be included in the manuscript before the references. Include their proper name (check spelling), institution, and email address. Co-author contact information must also be entered into the system upon submission.

Additional sections. The following sections should be included after the text: Acknowledgments, Financial Support, and Disclosures regarding real or perceived conflicts of interest. Manuscripts should end with a listing of all authors' current addresses, including affiliation, city, country, and email address, followed by References.

References. References must use standard AJTMH formatting; please refer to prior issues of the Journal and the information below to assure correct formatting. References should be cited by consecutive numbers in the text. The numbers should appear in superscripts that appear after closing punctuation. All authors should be listed, unless a referenced manuscript has more than 10 authors; in that case, please list only the lead author then "et al". Abbreviate journal names as in PubMed, with journal name and volume number in italics. References should be from peer-reviewed publications. Unpublished sources, including abstracts, conference proceedings, dissertations, and manuscripts not yet accepted for publication, should be cited in parentheses in the text as unpublished data or a personal communication (e.g. Kazura, J., personal communication).

Examples of references:

Durbin AP, Whitehead SS, 2013. The dengue human challenge model: has the time come to accept this challenge? J Infect Dis 207: 697–699.

Muirhead-Thomson RC, 1953. Mosquito Behavior in Relation to Malaria Transmission and Control in the Tropics. London, UK: Edward Arnold and Company.

White GW, 2007. Terminology of insect repellents. Debboun M, Frances SP, Strickman D, eds. Insect Repellents. Boca Rotan, FL: CRC Press, 31–46.

GAVI, 2013. Cholera Vaccine Investment Strategy. Available at: http://www.gavialliance.org/about/strategy/vaccine-investment-strategy/. Accessed March 11, 2014.’

3. Anexos

3.1 Cover Letter:

Evaluation of human fecal contamination in irrigation water used in urban gardens of Salvador/BA

Water is essential for life and is a resource used in different ways by all living being on the planet. However, this natural resource is in constant degradation throughout the world, leading to different sorts of issues. In Brazil, majority source for waterbodies contamination are domestic sewage being discharged without proper treatment, which increases the chances for infection of waterborne diseases. In this paper we describe how contaminated is the water used irrigation in urban gardens in the fourth largest city of Brazil, by classical methods, analysis of total coliforms and *Escherichia coli*, and by molecular biology, where we can track the microbial source (PCR). Nearly half of the urban gardens were above regulated standards. We were also able to identify human bacteria, which makes water treatment more urgent in the evaluated sites.

We state that all information produced data sent to the American Journal of Tropical Medicine and Hygiene is original and has not be submitted for publication elsewhere. We declare no conflict of interest in this study.

3.2 Formulário para divulgação de potencial conflito de interesse: