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Risk Assessment and Human Health Implications of *Escherichia coli* and Hepatitis A Virus in River Owena, Osun State, Nigeria

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ABSTRACT

The purpose of this study was to quantify the amounts of hepatitis A virus (HAV) and *Escherichia coli* (*E. coli*) found in water samples obtained from the River Owena. About five (5) litre of water were randomly obtained in a sterile plastic container for duration of ten weeks. The culture-based approach (eosine methylene blue and membrane lauryl sulphate agar) was used to count the number of *E. coli*. The 16S rRNA (Ribosomal ribonucleic acid) gene of the virus was amplified to yielded quantifiable data regarding the presence of the HAV in water samples. Quantitative microbial risk assessment (QMRA) was used to determine the risk of consuming water from the river that contained *E. coli* and the HAV. Findings demonstrated that throughout the course of ten weeks, the bacterial load ranged from 1.8 to 2.6 x 10^4 (CFU/100 ml) while the viral load varied from 0.71 to 2.4 x 10^6 copies per milliliter. It has been established by the pollution impact research that River Owena was contaminated. By assessing the bacterial and viral counts, it has shown that the water samples obtained at the river site contained trace of fecal contamination and this could result into possible risk of gastrointestinal sickness for unsuspecting consumers.

Keywords: River Owena, Fecal Indicator Organism, Quantitative Microbial Risk Assessment; Hepatitis A Virus Load.

Introduction

Water is one of the most important and abundant compound in the ecosystem. All living organisms on the earth require water for their survival and growth. Oftentimes, water is prone to pollution because of increased human population, industrialization, use of fertilizers in the agriculture and other anthropogenic activities (Basavaraja *et al.,* 2011). The availability of good quality water is an indispensable factor in human health protection from waterborne diseases. The increased use of metal-based fertilizer in agricultural revolution of the government may result in continued rise in concentration of metal pollution in fresh water reservoir due to the water run-off. Also, fecal pollution of environmental waters from both point and non-point or diffuse sources has led to the death of millions of people (Adefemi & Awokunmi, 2010). Industrial development results in the generation of industrial effluents, and if untreated results in water, sediment and soil pollution (Fakayode, 2005).

A region's surface water quality is largely influenced by human activity (overuse of water resources, discharge of industrial and municipal wastes, and variations in temperature, precipitation, and soil erosion) as well as environmental factors (Twine *et al*., 2005). Among them, the constant polluting source is the discharge of industrial and urban sewage, therefore improving the quality of the water depends heavily on the efficient management of sewage discharge (Younis *et al*., 2015; Gashi *et al*., 2016).

Surface water runoff is a seasonal occurrence mostly influenced by the basin's climate (Mustapha *et al*., 2012). This quality, defined in terms of physical, chemical and biological compositions, is governed by both natural (precipitation, watershed geology, topography, climate) and anthropogenic (point and non point sources like urban and industrial activities, other domestic activities, agricultural runoff) factors (Cabral, 2010).

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In addition to assessment of quality of the aquatic systems, identification of the factors controlling their behavioral properties is increasingly becoming inherent part of the water quality management. Water pollution occurs when organic or inorganic materials are released into water sources (Ekiye & Luv, 2010). Nigeria is the most populous country in Africa with a population of over 200 million people. The country is endowed with generous resources of water bodies. The span of water bodies within the country is estimated at 900 km^2. This water provides resources for fishery, transportation, irrigation, recreation and domestic use. Different regulations put in place to protect the marine environment and other water bodies in Nigeria have not been effective in controlling the indiscriminate dumping of effluent into open water bodies. These effluents contain substances that range from chlorides, phosphates, oil and grease, nitrates, heavy metals, among others (Begum, 2009). The deterioration of water quality as a result of indiscriminate discharge of industrial effluents and municipal sewage is well documented in literature, but the contamination of Owena river in Osun State is mainly attributed to domestic, agricultural, industrial and other anthropogenic activities around the river (Medema *et al*., 2003). Fecal pollution is a primary health concern in the environment, in water, in food. The use of index microorganisms (whose presence points to be possible occurrence of a similar pathogenic organism) and indicator microorganisms (whose presence represents a failure affecting the final product) to assess the microbiological quality of waters or food and has been practiced for many years (Archana *et al*., 2012). Classic microbiological indicators such as fecal coliforms, *E. coli*, and enterococci are commonly analyzed to evaluate the level of fecal contamination. The presence of *E. coli* in drinking water has been associated with digestive disorders, such as diarrheal sickness (Hunter, 2003). HAV is a single stranded, positive-sense, linear, non-enveloped ribonucleic acid (RNA) enterovirus (Howard *et al*., 2006). Hepatitis A virus is a member of the family Picornaviridae and is the common cause of gastroenteritis and acute viral hepatitis worldwide (La Rosa *et al*., 2012). One of the new biological contaminants causing the occasional global outbreak of watery sickness is enteric viruses (Upfold et al., 2021; WHO, 2017).

Research indicates that approximately 1.5 million individuals are yearly infected with HAV; however, this number may be overestimated because the virus often presents with no symptoms and there is little epidemiologic data available (Lemon *et al*., 2018; Soller, 2006).

Microbial risk assessment (MRA) is a process that evaluates the probability of adverse human health effects upon exposure to a medium in which pathogens are present (Soller & Eisenberg, 2008). The estimation of risk or illness can be achieved directly using epidemiologic data or by indirect estimates, which employ exposure data as input to numerical models to compute estimates of illnesses (WHO, 2001). The process entails four steps: hazard identification, exposure assessment, dose response assessment and risk characterization (Toze, *et al*., 2010). Despite limitations such as difficulty in characterization of exposure due to uncertainty and variability, limited availability of dose-response relations and subjectivity in the selection of model and parameters (Soller & Eisenberg, 2008; WHO, 2001).

It is increasingly acknowledged that human disorders like encephalitis, meningitis, hepatitis, and gastroenteritis may be caused by the waterborne transmission of human enteric viruses (Tang, *et al*., 2020; DuPont *et al*., 1971). Since human exposure to waterborne enteric viruses can result in related disorders, this research showed presence of HAV and *E. coli* in River Owen Osun State, as well as public health risk associated for domestic use.

Materials and Methods

Study area

River Owena in Owena, Osun State, Nigeria, served as the research area. The chosen river was used due to its closeness to vicinity where human and farm animal fecal pollution (cattle, goats, sheep, dogs, etc.) as well as other anthropogenic activities occurred in and around the river. The coordinates for the Owena River in Ondo State are approximately 7°00' - 7°30'N and 5°00' - 5°30'E.

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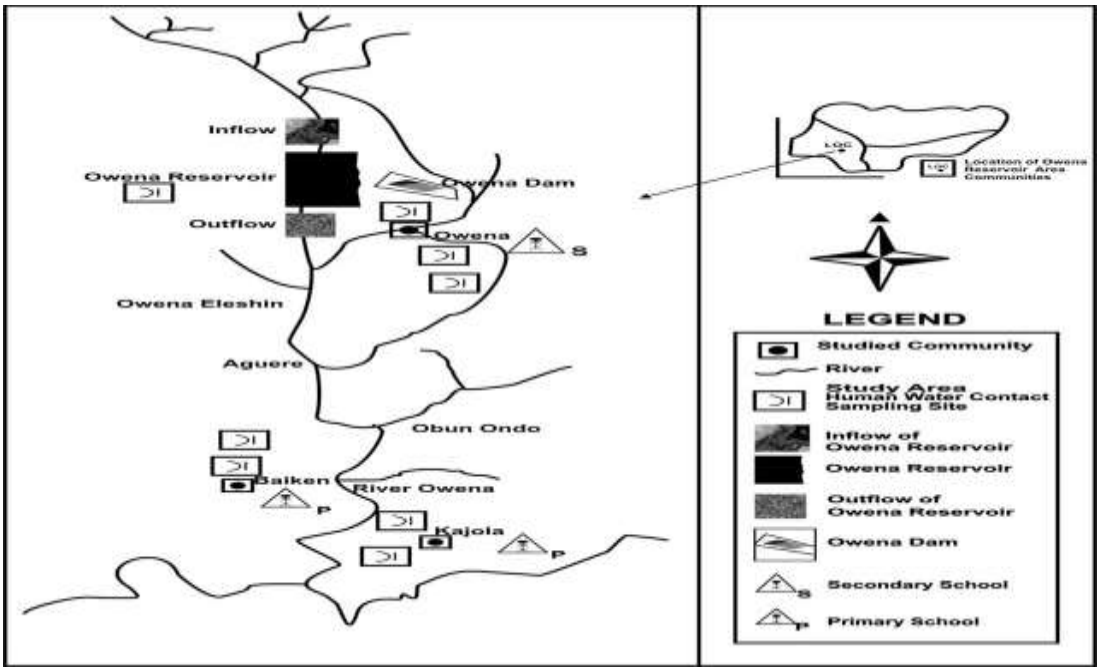

Figure 1: Nigerian locality map displaying the River Owena

Study design

This was a ten weeks course to quantify the amount of *E. coli* through a culture-based method and detect the presence of HAV using Reverse transcription polymerase chain reaction (RT-PCR). The goal of this monitoring project was to assess surface water quality.

Collection of samples

Sterile sampling (universal bottles) was used to gather the five-liter water samples. Weekly sampling operations were conducted on Wednesdays. This was done for 10 weeks in a row, started on 3rd July, 2024 and ended on 4th September, 2024. Within an hour, every sample was transported in a cold package to the Department of Microbiology, Federal University of Technology, Akure, Nigeria for further processing.

Quantification of *E. coli* in River Owena water samples

Following the culture-based approach for the examination of waste water, *E. coli* was analyzed by membrane filtration as soon as the sample was collected. The vacuum pump set-up aid to facilitate easy water filtering. First, peptone water was added to the membrane filter.

Next, a measured volume of the water sample (0.1 and 1 ml) was filtered via 0.45 µm nitrocellulose acetate membrane filters (NAMF). The filtrates were then put on the membrane lauryl sulphate agar (MLSA) and eosine methylene blue agar (EMBA) that had been made as selective media. Bacterial colonies were counted and expressed as colony forming units (CFU) per milliliter of the water sample after the plates were incubated at 37 °C for 18 to 24 hours. Plates containing 18 to 26 colonies that had a green metallic sheen were used for the counts, which were reported as the total count colonies per 100 milliliters (CFU/100ml).

HAV molecular detection and quantification in River Owena water samples

About 5 L of water samples were collected at random from the Owena River and placed in plastic kegs before being kept in the ultra-low freezer at temperature ranged -40°C to -86°C with model no TSX40086LA, manufactured in USA. After the samples were frozen and then thawed for several days to stress the virus and remove the protein coat, two layers with a line in between were created by centrifugation and the addition of lyses buffer, ammonium acetate, phenol, and chloroform.

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After adding acetic acid so as to stabilize the RNA and hinder its degradation, and ethanol was added to precipitate RNA thereby enhancing its visibility. The mixture was promptly frozen and centrifuged at 13,000 revolutions per minute (rpm) to produce white pellets, transparent precipitate, the ethanol was removed and dried, and then add RNAse-free water. After thawing, all solutions were quickly centrifuged and gently vortexed to begin the amplification process. The following ingredients were combined (apart from the template Deoxyribonucleic acid) in a tube at room temperature to create a reaction master mix. After, it was completely mixed the master mix, the necessary amounts were measured and transferred into PCR tubes or plates. Template DNA (≤ 500 ng/reaction) was put into the individual PCR tubes or wells holding the master mix. Stir the mixture was gently stirred in order to avoid bubbles formation (do not vortex). If necessary, briefly centrifuge. The thermocycler was set up in accordance with the guidelines, the samples were inserted and the program began. Agarose gel and tray casing were used next. Loading of dye, molecular weight ladder into wells then gently place under ultra violet (UV) light so as to observe DNA fragments. The concentration of the virus detected ranged from 0.71 to 2.40 $\times 10^6$ copies per 100 ml during a ten-week period.

Microbial risk assessment

Dose-response data are fitted into mathematical models in Quantitative microbial risk assessment (QMRA), which establish a relationship between the mean ingested dose and the probability of infection. The two most used models are the exponential and the β-Poisson (DuPont *et al*., 1971; Haramoto *et al*., 2005). An exponential model is based on the following assumptions, according to (DuPont *et al*., 1971; Haramoto *et al*., 2005), at least one pathogen must survive within the host; and the probability of infection per ingested or inhaled organism is constant. Microorganisms are distributed in water randomly and thus follow the Poisson distribution for infection to occur. Every bacterium in the exponential model has an equal fixed probability (r) of surviving and making it to a host location, where infection may ensue. Similar assumptions underpin both the exponential and *β*-Poisson models; the only difference is that the likelihood of infection for each ingested or breathed pathogen varies with population. The two parameters (a and b) of the beta distribution are included in this model because the likelihood of surviving and reaching a host site (referred to as "r" in the exponential model) is beta distributed (WHO, 2001). Human enteric viruses (HEntVs) have been linked to waterborne infection epidemics around the world (Tornevi *et al*., 2014). A significant risk of waterborne diseases for users or consumers might arise from viral contamination of source waters. Therefore, the presence of HEntVs in source water poses a risk to the public's health. The term "hazard" refers to a pathogen's propensity to have negative effects on ordinarily healthy persons (United State Environmental Protection Agency, 2012). According to (USEPA, 2012) this potential poses a risk that varies depending on the host's age/life stage, immune system, natural microbiota, nutrition, social and behavioral characteristics, and more. Dose-response models were adopted to calculate microbial health risks from exposures to pathogens in water from the borehole and well. According to DuPont *et al*. (1971), the microbial health risk(MHR) of *E. coli* was assessed through *Beta*-Poisson model, where the value of α = 0.200 and β = 1000. Also, the MHR of the HAV was determined using the *Beta*-Poisson model, where the α = 1.55E-1 and β = 2.11E6 (Haramoto *et al*. (2005). Mathematically,

The *beta*-Poisson model equation was utilized to determine the probability of infection.

$$Pi=1-\left(1+\frac{N}{\beta}\right)^{\alpha}$$

Where: *Pi* = Probability of infection; α (Alpha), β (Beta), and N = Viral load (copies per milliliter).

Results

The enumeration of *E. coli* in water samples collected from River Owena for the period of ten weeks range from 18 to 26 counts colonies per 100 ml. The concentrations of *E. coli* showed a drastic decrease from week 5 to 8. Also, there was uniformity in the concentrations week 3 to 4 and week 8 to 10. The detection of HAV in water sample collected from River Owena for the period of ten weeks range from 0.71 to 2.40 $\times 10^6$ copies per 100 ml. The concentrations of HAV showed a progressive decrease from week 1 to 3. Also, there was progressive increase in the concentrations from week 3 to 6 and further decrease HAV from week 8 to 10. The concentration of HAV appeared to be higher than those of fecal indicator bacteria in the water samples.

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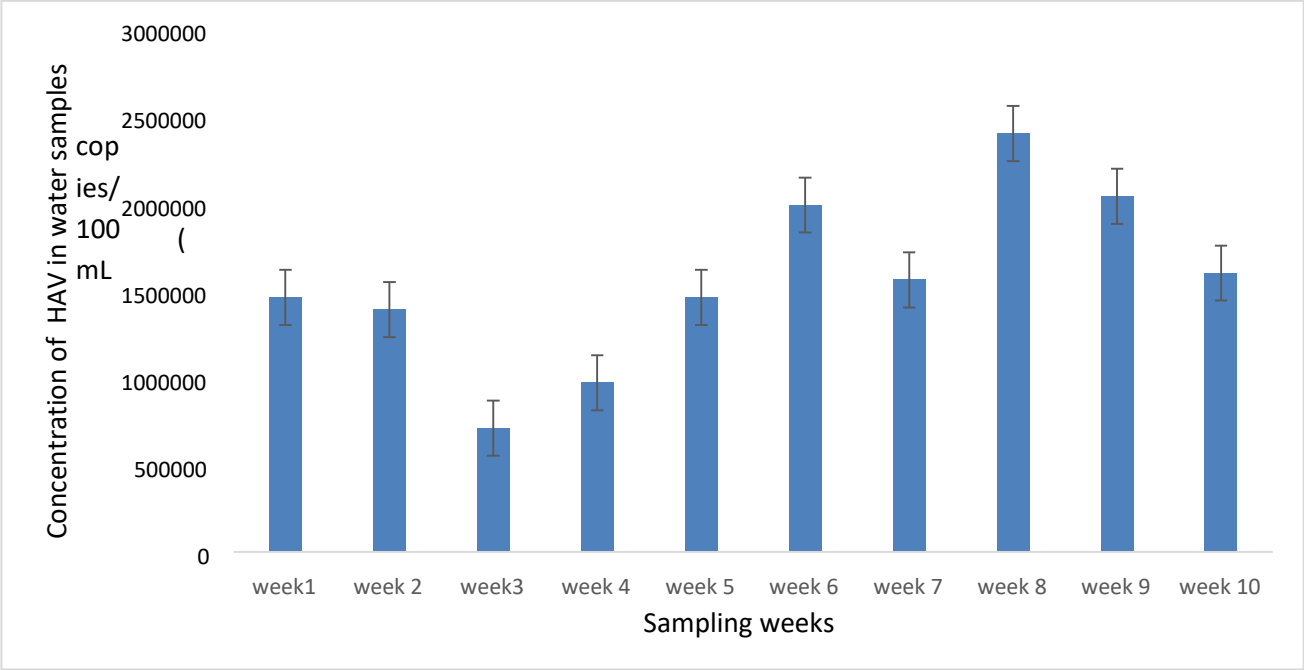

Figure 2: The average levels of the HAV during a ten-week period were measured in a water sample from the River Owena

Table 1: Values showing probability of viral load and *Escherichia coli* concentrations in water samples from River Owena during a period of 10 weeks

Sampling (Week)	Hepatitis A Virus (HAV) Load through *beta*-Poisson model equation		*Escherichia coli* concentrations through *beta*-Poisson model equation	
	Viral load	Pi = (1- (1+ N/B) E-0.2	*E. coli* count	Pi = (1- (1+ N/B) ^1.55E-1
1.	1.46 E6	0.77	2.30 E4	1.7E3
2.	1.39 E6	0.76	2.60 E4	1.9E3
3.	0.71 E6	0.73	2.00 E4	1.5E3
4.	1.46 E6	0.77	2.00 E4	1.5E3
5.	1.99 E6	0.78	2.30 E4	1.7E3
6.	1.56 E6	0.77	2.20 E4	1.6E3
7.	2.40 E6.	0.79	2.00 E4	1.5E3
8.	2.40 E6	0.79	1.80 E4	1.3E3
9.	2.04 E6	0.78	1.80 E4	1.3E3
10.	1.60 E6	0.77	1.80 E4	1.3E3

Keys: α (Alpha) for HAV = 0.200; β (Beta) =1000; E6= $x10^6$; α (Alpha) for *E. coli* = 1.55E-1; β (Beta) =2.11E6; E3= $x10^3$; E4= $x10^4$
^= Raise to power

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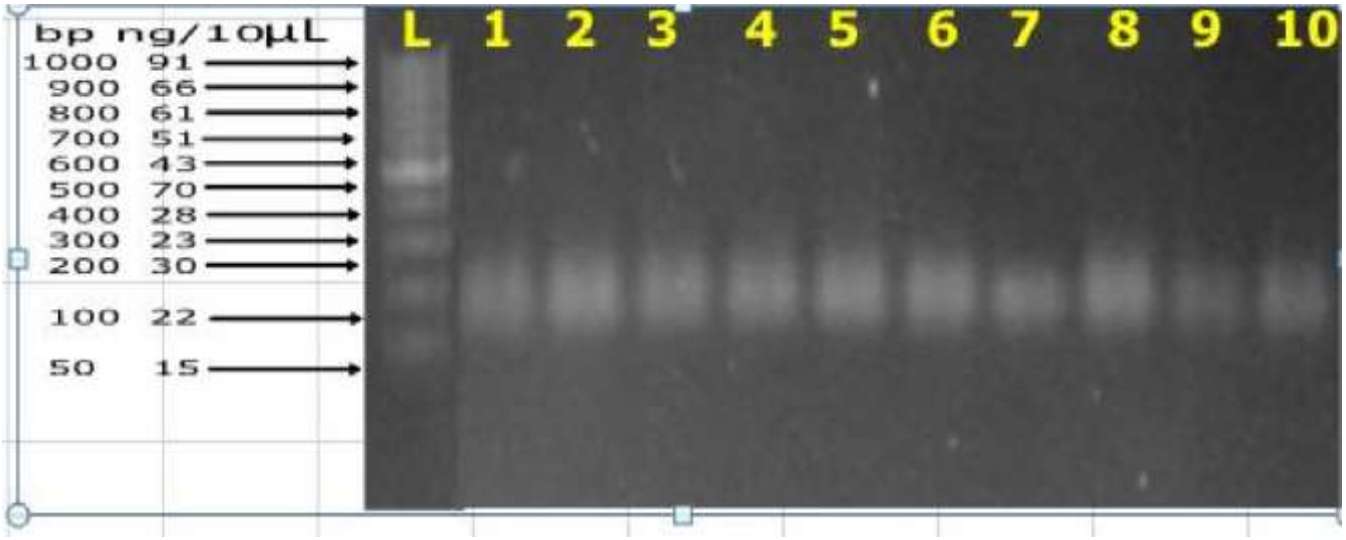

Plate 1: Gel electrophoresis image of HAV on an Agarose gel with amplicon size of 1kbp

Keys: kbp = Kilo-base-pair; µL= microliter; ng = nanogram; L = ladder; 1 to 10 = water samples (from week 1 to 10)

Discussion

My recent research showed that the River Owena, Osun State, Nigeria contained both fecal indicator bacteria and enteric virus. There was more HAV and *E. coli* in the river's upstream sections. The highest colony count of *E. coli* and HAV were observed at week two and eight respectively. The colony count and viral concentration increased from week three and achieved a peak at fifth and sixth week respectively; this could have evolved as a result of heavy rainfall at this period which had led to increased inflow of effluent from different sources. Colony counts gradually reduced at sixth week to the tenth, this could have low amount or absence of rainfall during this period. This was similar to the study carried out (Yeo *et al*., 2020), rainfall is associated with the exponential increase in concentrations of indicator pathogen while the effect on turbidity could evolved due to heavy rainfall. The impact of precipitation on elevated levels of indicator microorganisms was considerable throughout the ten weeks, and distinct correlations were also seen between successive days of rainy weather and lower quality.

It was observed that at varying volume of the collected water sample from the cite, the concentration of *E. coli* ranged from 1.8×10^4 to 2.6×10^4 cfu/100 ml and the concentration of HAV ranged from 0.71×10^6 to 2.40×10^6 copies/100 mL. HAV is shed in an extremely high numbers in the faeces of infected individuals and patients suffering from gastroenteritis may excrete about 10^5 to 10^{11} virus particles per gram of stool which then allow tainted effluent to be released into environmental media (Wu *et al*., 2020; Yeo *et al*., 2020).

Conclusion

This research showed that the River Owena contained both HAV and *E. coli.* From the results, it was observed that the concentration of HAV was more than the fecal indicator bacteria (i.e., *E. coli*) in the river's upstream sections. The various amount of these microorganisms were determined through real-time PCR and culture based method. The implication of this findings has evidenced that people dependent on River Owena for domestic use which includes cooking, bathing, washing, drinking or for agricultural purposes such as fishing and farming are most likely to experience public health risks. Proper treatment is imperative for the river to be appropriate for potable, domestic and industrial purposes.

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