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Detection of Rotavirus in Sewage Water Within Maiduguri Metropolis, Borno State Nigeria

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Abstract

A modified adsorption- elution method for the concentration of enteric viruses in sewage and water samples was used to concentrate water samples and detect the presence of Rotavirus virus using the Enzyme linked immune-sorbent assay (ELISA). The viruses in water were concentrated by negatively charged membrane filtration, eluted with 0.05M Glycine at pH 11.5, and re-concentrated by centrifugation at 12,000rpm for one hour. The presence of Rotavirus was determined by an ELISA. A total of 170 sewage and water samples (underground and surface waters) were collected from various sources in high density and low density areas of the metropolis in Maiduguri. Of the 60 domestic sewage samples collected from high-density areas, 8.3% were positive for Rotavirus ELISA antigen, and none of the samples collected from low-density areas were positive for Rotavirus. Rotavirus was not detected in underground and surface water samples. This study has demonstrated the potential usefulness of the virus concentration method, and ELISA for the determination of Rotavirus antigen in environmental water samples. The implications of these findings for environmental health are discussed from public health viewpoints.

Keywords: Detection; Rotavirus; Sewage water.

1. Introduction

Sewage water harbors over 100 different virus species which cause a wide variety of illnesses in man including hepatitis, gastroenteritis, meningitis and fever. Human enteric viruses enter the water environment through the discharge of sewage contaminated water. Viruses are shed in extremely high numbers in the feces of infected individuals. Many viruses cause in apparent or silent infections that go unrecognized until secondary person to person spread finally leads to overt diseases. Human enteric viruses enter the water environment through the discharge of sewage contaminated water. Viruses are shed in extremely high numbers in the feces of infected individuals. Many viruses cause in apparent or silent infections that go unrecognized until secondary person to person spread finally leads to overt diseases. Rotavirus infections can occur throughout life: asymptomatic infections in adults may maintain the transmission of infection in the community [1].

The roles of sewage, underground and surface waters in the occurrence and spread of Rotavirus infection in this environment have not been studied previously .Enteric viruses are able to persist under environmental conditions, and the release of infectious viruses into the water environment may cause public health problems, particularly with regard to the use of contaminated water for drinking, recreation, park irrigation, and road washing [2]. Outbreaks of viral acute gastroenteritis by consumption of contaminated water have been frequently reported all over the world, and viruses are known as the etiological agents of infectious gastroenteritis [3]. The presence of Rotavirus in water and sewage indicates contamination of the environment by the virus. Viral diarrhea is highly contagious and the faeces of an infected person can contain more than 10 trillion infectious particles per gram; fewer than 100 of these are required to transmit infection to another person. Worldwide more than 450,000 children under five years of age still die from Rotavirus infection each year [4] most of whom live in developing countries [5] and almost two million more become severely ill [6]. In this study the environmental surveillance and epidemiology of Rotavirus within Maiduguri Metropolis was carried out to obtain information that could be useful in the effective management of public and domestic water supplies as well as implementation of appropriate preventive and control measures. Sanitary measures adequate for eliminating bacteria and parasites seem to be ineffective in control of Rotavirus, as the incidence of Rotavirus infection in countries with high and low health standards is similar [7]. The main objective of this study is to detect the presence of Rotavirus in sewage, underground and surface waters in the study area and to determine its distribution in different localities within Maiduguri Metropolis.

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2. Materials and Method

The research was conducted within Maiduguri Metropolitan council of Borno State, north eastern Nigeria.

A total of 170 samples were collected from selected high and low density areas of the Metropolis From the high density area, 60 samples were collected from sewage (Plate 3.1), 10 samples from underground (bore-holes), and 22 samples from surface water and from low density area, 50 Samples were collected form sewage, 10 samples bore-hole, and 18 samples from surface water. Samples were collected in sterile one liter bottle. The samples were filtered during collection to remove large debris (Plate 3.2). The back of the sample containers were decontaminated with 10% hypochlorite. Samples were transported to the laboratory on ice pack and stored at -20^{o}C before analysis. The temperature and pH of all the samples (sewage and water) were measured and recorded accordingly. The samples were later concentrated by adsorption - elution method as previously described by Yoshida (2013) and analyzed by Enzyme Linked Immunosorbent Assay (ELISA) for the presence of Rotavirus in the water elutes.

2.1. Sample Processing: Concentration of Water Samples

The concentration of water samples was carried out essentially as described in the manual version: 2013.10.08 by Yoshida (2013). Briefly, approximately 500 mL of water sample was centrifuged in 50 mL tubes at 3000rpm for 30 minutes at 4^{o}C. The supernatant was transferred into glass beaker and 10 ml of 2.5M $MgCL_2$ was added and mixed well using magnetic stirrer. The pH was adjusted to 3.5 using 1N HCL (3-5 mL) on magnetic stirrer. The mixture was filtered using 47 mm filter using 50 mL syringe (Adsorption). The filter cartridge was opened and the filter was cut on petri dish. The pieces of filter membrane were placed in a 50 mL centrifuge tube, and 10 mL of the elution buffer 0.05M Glycine pH 11.5 was added and glass beads: (1-3mm) were added. The content of the tube was mixed on votex mixer for 1-3 mins and the supernatant was transferred immediately to 15mL centrifuge tube (Elution). The pH of the elutes was immediately adjusted to 7.0 using 1N NaoH or 1N Hcl. The water elutes obtained were subjected to hi- speed centrifugation (re-concentration) at 12000 rpm for 15 min at 4^{o}C, and was filtered using MILLIPORE: millex filter, SLHV) 33RS, 0.45µm. The elute was finally aliquoted into 2mL cryovial tube and stored at -20^{o}C until tested.

2.2. Testing of Sample Elutes by Enzyme Immunoassay for Rotavirus

2.1.1. Procedure of the Assay

The ELISA for detection of Rotavirus antigen in the sample elutes was carried out as follows. The micro wells were pre-coated with anti-Rotavirus polyclonal antibody. One hundred microliters of each sample (elute), negative control, and positive control were distributed to the respective microwells and100 µL of the conjugate was added to each well the plate was then covered and incubated at 20-30^{o}C for 60 minutes. The content of the wells was decanted and washed with diluted wash buffer (350-400 µL) per well. The fluid from each well after each wash was shaken out and the washing was repeated 5 times and after the last wash the plate is inverted and tapped on an absorbent paper to remove traces of wash buffer. 100 µL of the substrate was added to each micro well and the plate was covered and incubated at 20-30^{o}C for 10mins. The substrate reaction was stopped by the addition of 100 µL of stop solution (Sulphuric acid) to each well. The plate was immediately read on an ELISA plate reader at a wavelength of 450nm.

2.1.2. Interpretation of Results

The presence or absence of Rotavirus antigen was determined by relating the absorbance of the unknown sample to the cut off value. The cut off value was calculated by adding 0.200 absorbance units to the negative control value. The negative control value or mean of the negative control values should be less than 0.150 absorbance units the positive control value must be greater than 0.500 absorbance units.

2.1.3. Statistical Analysis

Data obtained from the study was presented in tabular forms and the percentage of positive samples in relation to site of sample collection were compared accordingly.

3. Results

The modified adsorption-elution method was used to concentrate water samples for the determination of the presence of Rotavirus antigen in water samples collected from various sources and localities within Maiduguri Metropolis. The concentration method entails the adsorption of the virus particle onto a negatively charged filtermembrane at a pH of 3.5 and addition of Mg+; which enhances the adsorption of the virus particle to the filter membrane. The adsorbed viruses were eluted from the membrane using the buffer; 0.05M glycine at pH 11.5,and the water elutes re-concentrated by high speed centrifugation at 12,000rpm for 15 mins. One hundred and seventy water samples collected from various sources of high density and low density areas of Maiduguri metropolis were analyzed for the presence of Rotavirus. Rota virus was detected in five out of one hundred and seventy (5/170) samples by ELISA. Of all the water samples collected and analyzed, 110 were sewage samples, 40 underground water (bore-hole and Dam) and 20 surface water samples. Out of the 60 sewage samples obtained from high density areas, 5 (8.3%) samples were positive for Rotavirus antigen, while none of the samples collected from underground and surface water were positive for Rotavirus. None of the samples collected either from sewage, underground or surface water in the low density areas of the Metropolis was positive for Rotavirus. In this study, Rotavirus was not

detected in any of the underground (bore-hole and Dam water) and surface water samples obtained from both high and low density areas of the Metropolis.

Table-1. ELISA test for the detection of Rotavirus in water samples obtained from high density areas

Water Source	No. of Samples	No. of positive samples (%) Rota virus
Sewage	60	5(8.3.)
Underground		
Bore-hole	10	0
Surface water	12	0
Dam water	10	0
Total	92	5(5.4)

*ELISA: Enzyme linked immune-sorbent assay, *high density area

Table-2. Comparison of water samples collected from high density and low density areas

	Sewage (% positive)	Underground	Surface
High density	60(8.3)	10(0.00)	22(0.00)
Low density	50(0)	10(0)	18(0)
Total	110(5.5)	20(0.00)	40(0.00)

*high density areas: congested areas of the metropolis *low density areas: non congested areas

Table-3. Temperature ranges of samples collected

Temperature range	No. of samples(+) high density
20-23	31(1)
24-27	46(2)
28-31	13(2)
31-34	2(0.00)
	92

Table-4. pH values for water samples collected from both high and low density areas

pH	No. of samples (High density)	No. Samples (low density)
7	16(0.00)	19
8	69(4)	50
9	5(1)	9
	92	78

*high density areas: congested areas of the metropolis, *low density areas: non congested areas

Table-5. Characterization of positive samples for Rotavirus

Sample No. concentrated	Type of water	Temp.	pH	Original volume	Rotavirus detection
5	sewage	23	8.0	500mL/4mL	+
6	sewage	26	8.0	500mL/4mL	+
10	sewage	27	9.0	500mL/4mL	+
15	sewage/man-hole	30	8.0	450mL/4mL	+
18	sewage/man-hole	29	8.0	400mL/3mL	+

*original volume of water samples collected, concentrated: volume of elutes obtained after water concentration

Table-6. Ranges of optical density (OD) values for Rotavirus positive samples (n=5)

OD Range	No.(%)Positive
0.048 - 0.248	0
0.249 - 0.449	1
0.450 - 0.650	2
0.651 - 0.851	2
0.852 - 1.052	0

Table-7. Localities where water samples were collected for the detection of Rotavirus

Localities	Rotavirus detection
Abbaganaram Ward	+
Budum	+
GoniKachallari ward	-
GwangeWard	-
Kaleri	-
Umarari Ward	-
Bolori Ward	-
Polo	-
Bullumkuttu	-
Shuwari	-
Sulaimanti Ward	-
Ngomari Ward	-

4. Discussion

Since the densities of viruses in water are usually low, a virus concentration method is required. Several methods for concentration of enteric viruses from water have been proposed, and the most promising method is the virus adsorption - elution technique [8, 9]. In an experiment, by Kittigul, *et al.* [10], the recovery efficiency of rotavirus in the adsorption- elution method using 0.05 M glycine at pH 11.5, was found to be (53%). The application of a commercial ELISA kit has reported for the determination of Rotavirus antigen in environmental water samples with high sensitivity and specificity [11]. In this study, 500mLs of each 170 water samples were collected from different sources: sewage, underground (Bore-hole and water Dam) and surface waters in different areas of the metropolis. The water samples were concentrated by adsorption – elution method. Viruses in the water samples were adsorbed onto a negatively charged membrane with diameter 47mm; the adsorbed viruses were eluted with 10mLs of Glycine at pH 11.5. The pH was quickly adjusted to 7.0 to avoid virus inactivation. The water elutes were re-concentrated by high speed centrifuging at 12,000rpm for 15 minutes. The supernatant was filtered through 0.45µm Millipore filter, and the final volume was stored at -70^{o}C before usage. A sandwich Enzyme immunoassay was used to detect the presence of Rotavirus in the water elutes. We found that 5 out of 60 sewage samples (8.3%) that were collected from high density areas were positive for Rotavirus while none of the sewage samples collected from low density areas was positive for Rotavirus. This is compared to the study by Kittigul, *et al.* [12] who reported 8% (4/48) of Rotavirus antigen in domestic sewage using ELISA in congested areas of Bangkok, Thailland. Using this technique, rotavirus antigen has been detected in domestic sewage by Kittigul, *et al.* [12] in 6out of 24 (25%) samples; human Rotavirus was also reported in sewage (32.3%, 31/96) in a study by He *et al* 2010 in china.

It is emphasized that the use of modified adsorption-elution technique in optimum condition and speed Vac reconcentration is an effective method for rotavirus concentration from water samples. Rotavirus was not detected in any of the underground (bore-hole and Dam water) and surface water in this study. This is similar to the study reported by kittigul in 2000 in which Rotavirus was not detected in surface water.

Temperature is the most significant factor controlling virus survival. The lower the temperature the longer viruses persist. The temperature of the 5 samples positive for Rotavirus were dispersed evenly within the range of 23-30. This is contrary to the study by Kittigul, *et al.* [12] in Thailland; in which the four positive samples for Rotavirus had a temperature range between 30-31^{o}C, and compared to the study by Kittigul, *et al.* [13] in which the temperature range was between 27-31^{o}C

Enteroviruses can survive at a pH of 11.0-11.5 and 1.0-2.0 for short period of time. In this study, all the five positive samples for Rotavirus were alkaline and had a pH between 8 and 9. This is compared to the study by Kittigul, *et al.* [12] in which out of the four positive samples, three were alkaline and only one sample was acidic.

5. Conclusion

The results of this study on the detection of Rotavirus in sewage, underground and surface water within Maiduguri metropolis and its environments shows that out of 170 water samples analyzed, 5(3%) sewage samples from two areas were positive for presence of Rotavirus, and none from either underground or surface water. the presence of rotavirus in sewage indicates the circulation of the virus in the environment and the persistence of the virus among the communities. This study also demonstrates the potential usefulness of the virus concentration method, and ELISA for the determination of rotavirus antigen in environmental water samples.

Recommendation

From this study, it is recommended that further studies need to be carried out on the distribution of Rota virus in the study area there is need for environmental surveillance and routine diagnoses of rotavirus in patients who present with diarrhea. Molecular characterization of rotavirus serotype circulating in Nigeria. Introduction of rotavirus vaccine in routine immunization schedule is necessary. Community awareness for proper environmental and personal hygiene.

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