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VIRAL POLLUTION EVALUATION OF THE GUANABARA BAY

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SUMMARY

An evaluation of viral pollution of 10 different beaches in the Guanabara Bay was carried out using a system of cartridge filters, in the period from April to September 1972. This system provided a simple method to concentrate out virus in sea-water up to 1,500 times. A sample of 1,200 liters of sea-water was processed from each beach, and reduced to a final volume of 800 ml. From all the sample studied, virus strains have been isolated and titrated, using monkey kidney cells. However, it was possible to identify virus only from 4 samples (ECHO 1 in two samples, ECHO 14, and ECHO 15). Slow growing virus strains were found, with no neutralizable isolates, and doubtful cytopathic effects on monkey cells have been observed. No lethal virus for baby-mice was isolated, which suggests absence of Coxsackie A virus in the samples. The Authors describe the virus concentration method, discuss the importance of virus isolation from beaches used for recreational purposes and marine food gain, recommending larger studies on the factors responsible for the viral dissemination in the Guanabara Bay.

INTRODUCTION

Studies concerning the concentration of virus particles from large volumes of water are done currently through methods allowing the processing of hundreds of liters of water in continuous flow, in a short lapse of time, to just few mililiters as described by WALLIS et al. [11, 12], HILL et al. [3], METCALF et al. [8] and HOMMA et al. [4] Such studies, however, were carried out mainly on an experimental basis adding exogeneously virus to the processed water.

The objective of the present study was to apply these systems of virus concentration in field experiments. Therefore, some beaches in the Bay of Guanabara were sellected for the evaluation of viral pollution.

The Bay of Guanabara is located at 22°30′/23°S/43°15′W, has many recreational areas and in its surrounding area, lives a population of about 2 million [1, 2]. Poorly urbanized areas in addition to incomplete sewage treatment, cause contamination of the sea-water within the Bay.

In our field studies we used a method similar to that reported by METCALF et al. [8], whom, in preliminary results of virus concentration from sea-water, reported good performance of cartridge-filters in the clarification at very high flow rate, without

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significant virus loss at this stage. Good virus adsorbance was then obtained by a fiberglass cartridge filter.

MATERIAL AND METHODS

1. Sample collection places

Among the several beaches in the Bay of Guanabara were selected those which have more visitors and, most probably, a higher level of sewage contamination. Figure 1 shows their distribution according to its geographic position in the Bay. A single sample was collected from each of these beaches.

2. Sea-water

The volume of the sample to be processed was set at 1,200 liters and the flow rate controlled to 400 liters per hour.

The collection point was set at 5 to 10 meters from the beach, 0.5 to 1 meter from the soil and about 30 to 50 cm under water level.

The field studies were done in Autumn and Winter (April through September 1972) when the water temperature ranged from 22° to 24°C and salinity values from 29 to 32‰.

3. Virus adsorption in the concentration system

The method of virus concentration was already published in detail [8, 11]. Briefly the system consists of four cartridge filters and a motor-pump, as follows:

a) *Clarifiers*: length — 25 cm; diameter — 6 cm; inner — 3 cm. Three cartridge filters of polyester are mounted serially, according to their decreasing porosity from input (30 um, 15 um, and 1 um) towards the output.

b) *Virus adsorbent filter*: a fiberglass cartridge filter from the same size with a porosity of 3 um was used to adsorb virus. This filter was mounted following the clarifying filters.

c) *Motor-pump*: a 3/4 H.P. gasoline motor-pump with operational capacity of 1,200 liters per hour (Montgomery, model EA) was used as the preasure source for the system. It was connected by 3/4 inch plastic tubing to the filters and a constant flow rate of 400 liters per hour was obtained by controlling the monitoring valve (Fig. 2).

4. Virus elution from fiberglass cartridge filter

After processing the desired volume of sea-water the fiberglass filter was unhooked and transported to the laboratory in a refrigerated box. At the laboratory, the virus adsorbed to this cartridge was recovered using, as eluent, 800 ml of glycine/NaOH buffer ajusted to pH 11.5, added with 10% of bovine calf serum [8, 11].

To enhance virus recovery, the same eluent was passed through the virus adsorbent filter three times after being readjusted to pH 11.5.

The eluent which now supposedly contained the virus samples was then adjusted to pH 7.0 by adding 1N-HCl drop by drop and then inoculated in cells and baby-mice for virus isolation and quantification.

5. Primary monkey cells

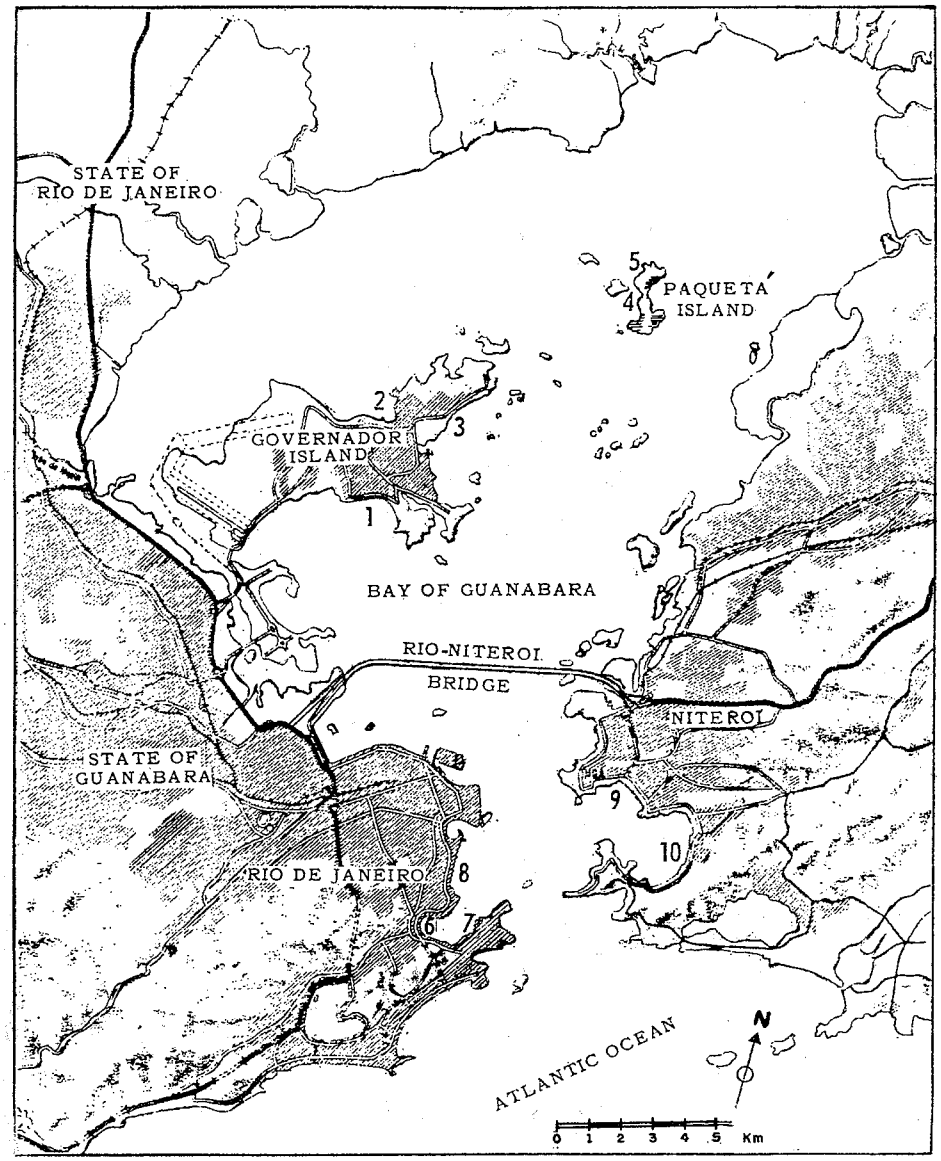
Rhesus monkey (*) kidneys were trypsinized and the cells grown in Eagle medium, supplemented with 5% bovine calf serum and 0.5% lactoalbumin as described by MELNICK & WENNER [7].

6. Virus isolation and quantification

The concentrated samples were serially diluted from 10^{-1} to 10^{-4} and 0.1 ml of each dilution was inoculated in 5 tubes of cells. Microscopic examination was carried out during 20 days and the tubes which did not present cytopathic effect (CPE) were frozen at −20°C and reinoculated in new tissue culture tubes before being considered negative.

The first dilution of each samples was simultaneously inoculated intracerebrally and subcutaneouslly in 2 different groups of new-

(*) Obtained from the Instituto Oswaldo Cruz (FIOCRUZ) vivarium

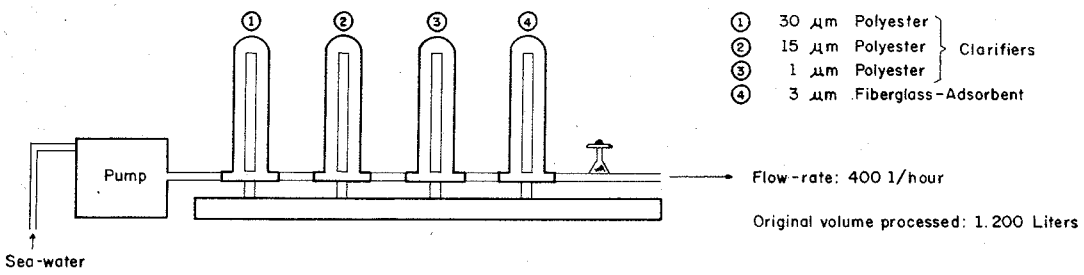


1, 2, 3 Governador Island
4, 5 Paquetá Island
6, 7, 8 Rio de Janeiro (State of Guanabara)
9, 10 Niteroi (State of Rio de Janeiro)

Fig. 1 — Geographic distribution of places where samples were collected

Figure 2

Virus concentration from sea-water



born mice which were then daily examined for signs of illness up to 15 days.

The virus concentration in the samples showing CPE in the tissue culture tubes calculated according to the method of REED & MUENCH [10].

7. Virus identification

Specific enterovirus antisera have been kindly supplied us by Dr. J. L. MELNICK, from the Baylor College of Medicine, Department of Virology and Epidemiology, Houston, Texas, U.S.A. through the Pan American Health Organization.

These sera (equine origin) were used as a "pool" as described by LIM & BENYESH-MELNICK [5]. After neutralization, the mixture virus + antiserum was placed in presence of cells on microplates. After 72 hours of incubation at 37°C, microscopic examination was performed and absence of cytopathic effect in comparison with virus controls was considered as specific neutralization.

TABLE I

Virus quantification and identification from water samples collected at Guanabara Bay (April-September 1972)

Origin of the water samples		TCD50/0.1 ml	Infectious particles per liter	Virus identification
1. Praia da Bica	(a)	$10^{2,43136}$	1.800	ECHO 15
2. Praia do Dendê	(a)	$10^{0,55630}$	24	—
3. Praia da Freguesia	(a)	$10^{0,55630}$	24	—
4. Praia da Moreninha	(b)	$10^{1,98227}$	640	ECHO 1
5. Praia do Pintor	(b)	$10^{1,98227}$	640	—
6. Praia de Botafogo		$10^{0,98227}$	24	—
7. Praia da Urca		$10^{0,45484}$	19	—
8. Praia do Flamengo		$10^{0,78675}$	41	ECHO 14
9. Praia de Icaraí	(c)	$10^{2,14598}$	933	ECHO 1
10. Praia de Jurujuba	(c)	$10^{2,14598}$	933	—

(a) Governador Island

(b) Paqueta Island

(c) Niteroi City

RESULTS

The results of virus isolation from the samples and the titrations are presented in Table I. In the third column of this Table we report the virus amount obtained in the concentrated sample in relation to the original volume collected.

It was particularly remarkable, at times, the presence of a transitory cytopathic effect on the inoculated monkey kidney cells, which did not destroy the entire cell sheet; in many cases, the cells apparently recovered, exhibiting a normal appearance one day later. These unknown agents, if any was present, might include some of not human origin.

The final virus identification was achieved only in a few samples. As far as polioviruses are concerned, no strain was identified in our samples. No virus strain pathogenic for baby-mice have been isolated either. ECHO virus type 1 (from places 4 and 9 — Fig. 1), type 14 (place 8) and type 15 (place 1) constitute the viral strains identified.

DISCUSSION

The possibility of human virus survival in sea-water has been already demonstrated (MOSLEY [9] and METCALF et al. [8]). However, most of the studies are related to virus isolation from oysters or in experimental procedures.

Once standardized a method to process large volumes of water aiming at the isolation of virus [4, 11, 12], the field application of such methodology became highly desirable. Indeed we were able to isolate and quantitate virus in all selected areas in the Guanabara Bay.

Some samples present a very high number of viral infectious particles per liter. On the other hand, the identified viruses were shown to be of the ECHO group. At the same time when this experiment was being carried out (April through September 1972), our laboratory established a pilot project in poliomyelitis oral vaccine evaluation in young children, and ECHO virus was then shown to be also highly prevalent in rectal swabs taken before the vaccination (SCHATZMAYR, HOMMA & MESQUITA, unpublished data), which might be related with the ECHO virus identifications from our sea-water samples.

It seems very difficult to discuss the virus quantification results of our samples. Although it was been tried to obtain uniform conditions by the samples collection, we are perfectly aware of the many different factors which may influence the results like ebb and high tide and physico-chemical properties of each water sample. From the practical point of view, it was not possible to carry out the collections in a very short period of time like one week for instance and under comparable weather conditions.

These factors certainly should be taken in account in the evaluation of our data.

Some experimental virus inactivation carried out in water, showed that ECHO viruses as some other enteric viruses, behaves differently to the chlorine inactivation, as described by LIU et al. [6]. Although we have so far insufficient data for comparisons in the areas studied, the apparent higher resistance of the ECHO group might have contributed for the final results obtained. Comparable results have been also achieved by us at the city of Santos, São Paulo State, where we attained the isolation of several kinds of enteric virus in samples collected close to sewage discharge in the ocean (Poliovirus, Coxsackie and ECHO virus) but only ECHO and a few Coxsackie virus strains were isolated in the samples collected on the sea and at beaches, far away from the discharge point (HOMMA, SCHATZMAYR & FRIAS, unpublished data).

It seems very important to establish the role of sea-water as primary source of virus dissemination among beach visitors, by direct contact in polluted areas.

On the other hand Guanabara Bay furnishes marine foods to large pollutions dwelling at its border and the viral transmission by food has been already described in other areas (MOSLEY [9]).

The cartridge filter system here applied to field conditions is economically and operationally feasible to process large volumes of sea-water.

RESUMO

Avaliação da poluição de natureza viral da Baía da Guanabara

Avaliação da poluição viral através de um sistema de filtros tipo cartucho foi realizada em 10 diferentes praias da Baía da Guanabara, no período de abril a setembro de 1972. Esse sistema permite concentrações de cerca de 1.500 x do volume total de água do mar trabalhado. De cada praia processou-se uma única amostra de 1.200 litros de água, reduzindo-se o volume a 800 ml. Isolaram-se amostras virais de todas as coletas realizadas, através de inoculação em células de rim de macaco *Rhesus*, sistema igualmente usado para as titulações do vírus. No entanto, foi possível a identificação final de apenas 4 amostras virais (ECHO 1 em duas coletas, ECHO 14 e ECHO 15).

Agentes citopatogênicos de crescimento lento, não neutralizáveis pelo esquema de identificação utilizado, bem como agentes de efeito incompleto e reversível sobre as células, foram observados em várias das amostras. Em nenhuma das amostras observou-se a presença de agentes letais para o camundongo recém-nascido.

Os Autores descrevem o método utilizado para concentração de vírus e discutem a importância do isolamento de vírus de praias usadas com finalidade recreacional e de fornecimento de alimentos de origem marinha, recomendando estudos mais amplos sobre os fatores envolvidos na disseminação de vírus no interior da Baía da Guanabara.

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