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Detection of Enteric Viruses in Freshwater and Ground Water from Four Departments in Tucuman, Argentina

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Abstract: Freshwater and ground water are continuously affected by human faecal contamination. Numerous studies have documented the presence of enteric viruses in water from different sources. Due to the high incidence of hepatitis A and other viral diseases attributed to enterovirus, the sanitation authorities requested an assessment of the virological quality of the water in four departaments of Tucumán, Argentina. The aim of this study was to recover and characterize the viruses present in freshwater and ground water in the aforementioned districts. Ten water samples of 20 l each were taken from the areas with the highest number of cases of hepatitis registered. Each sample was concentrated by adsorption-elution with glass wool. Isolation of the viruses was carried out using Vero, Hep2 and Rd cell lines and the immune electron -microscopy technique (IEM) was used. four samples were positive for cytopathic effect viruses in cell cultures, confirming: Echoviruses 18, Poliovirus 1 and 2 non-typified Enteroviruses. Immune electron microscopy in 2 samples revealed aggregations of viral particles morphologically compatible with Hepatitis A. Recovery of cytopathic viruses demonstrates the presence of infective viruses or virus particles, which create a potential source of infection.

Key words: Enteric viruses, contamination, freshwater, ground water

INTRODUCTION

Enterovirus, Hepatitis A and E, Adenovirus, Rotavirus and Norwalk are among the enteric viruses transmitted by water sources and the cause of a diverse range of pathologies[1-4]. These viruses cause gastroenteritis, hepatitis, meningitis, respiratory infections and encephalitis[5]. Their way of transmission is faecal-oral and although generally transmitted from person to person they are also passed on by contaminated food products or water[6]. The risk of transmission by water is potentially high, thus creating epidemic outbreaks[5,7,8].

Due to the high incidence of hepatitis A and other viral diseases attributed to enteric virus, the sanitation authorities requested an assessment of the virological quality of the water in four Departaments of Tucumán, Argentina.

The aim of this study was to recover and characterize the viruses present in freshwater and ground water in the aforementioned districts.

The amount of viruses present in water is too low for samples and a previous concentration step is needed in order to reduce the sample volume.

In our study a glass wool filter was chosen for viral concentration, followed by an elution technique, which does not require any modification of the water quality[9]. The efficiency of this technique is known[10,11]. In order to establish any association between the disease and consumption of contaminated water 10 samples of 20 l each of freshwater and ground water were taken from departaments of Famaillá, Lules, Yerba Buena and Leales in Tucumán, areas where the first cases of hepatitis A were detected.

The results obtained indicated the presence of human viral pathogens in various water types used for drinking purposes.

MATERIALS AND METHODS

Cells: Vero (Green African monkey kidney), Hep 2 (human epitelial) and Rd (human rhabdomyosarcoma) cells were used in all experiments. They were propagated in Eagle's minimal medium (EMM), supplemented with 5 % foetal calf serum.

Water samples: Samples consisted of 20 l of freshwater and ground water from the Departaments: Famaillá (3 samples), Lules (3 samples), Yerba Buena(1 sample) and Leales (3 samples).

Filtration system: Stainless steel holders (Sartorius SM

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16249, d=42 mm) contained 50 g of glass wool compacted until a specific weight of 0.5 g cm^{-3}. Each filter was subsequently washed with HCl (1N) and NaOH (1N) and then rinsed with sterile distilled water until neutral pH. The glass wool used in our experiments did not provide technical information of the manufacturer unlike the material proposed by the authors of the method[12].

Concentration procedure: Water was passed through the glass wool filter under pressure at a flow rate of 30 l/h. Elution was performed with 50 mM glycine-0.5 % beef extract, gentamicin (100 ug mL^{-1}), pH 9.5 (250 mL). Organic flocculation was used as a second concentration step.The precipitate was recovered by centrifugation at 3,500 x g for 35 min and resuspended in 5 mL of a sterile Phosphate Buffer Saline solution, pH 7.5, with penicillin-streptomycin (100 ug mL^{-1}) and amphotericin B (2 ug mL^{-1}).

Viral recovery: Qualitative assays (in duplicate) of the virus concentrate were achieved by infection of Vero Hep2 and Rd cell monolayers grown in 25 cm^2 plastic flasks, with 0.5 mL of the concentrate. The flasks were incubated at 37°C for 72 h and transfers to complete monolayers were made from all cultures.Virus detection in each concentrate was performed in EMM supplemented with 2% foetal calf serum.

The qualitative assay was used to confirm virus recovery and presence of cytotoxicity.

A Vero, Hep2 and Rd cell suspension was prepared at a concentration of 2x 10^5 cells mL^{-1} with EMM with 2% foetal calf serum. This suspension was then poured into six wells of a microplate, each well were inoculated with the undiluted concentrate (50 ul per well). All microplates were incubated at 37°C under a CO_2 atmosphere and examined every day for a period of 5 to 8 days. Presence of cytophatic effects (CPE) on cell monolayers was used as virus recovery criterion for assaying.

After 14 days of incubation plates were given a final reading of wells with CPE.

Neutralization tests: Typing of virus isolate by the neutralization test in six wells microplate was attempted, using standard methods[13]. For the virus dilution neutralization 10 Log serial dilutions, containing from 100, 50% tissue culture infectious dose, were prepared and each dilution was tested in a standard microneutralizationtest with each of the Lim and Benyesh-Melnick (LBM) antiserum pools, A to H[14,15]. Isolates were considered typed if the pattern of neutralization made sense according to the LBM scheme[13].

Electron microscopy (EM) and Immune electron microscopy (IEM): conventional negative staining electron microcopy was used for tentative detetion of virus in concentrates CPE negatives in cell cultures.

Immune Electron immune microscopy was used to identify human viruses in concentrates, using a pool of sera from ill people with a high titre of IgM HAV[16].

RESULTS

Four samples were positive for viral Cytopathic Effect in cell cultures, showing Echoviruses 18, Poliovirus 1 and 2 non-typified Enteroviruses, typified with the Melnick pool[14].

The sample areas and sources and the subsequent results are given in Table 1.

IEM revealed aggregates from viral particles in two of the 3 Famaillá samples. They had a diameter of 20-22 nm and were morphologically compatible with Hepatitis A. Immune electron microscopy appears to be a sensitive and simple techinque for the detection of enteric viruses[16] (Fig.1 and 2).

These results would imply faecal contamination of the drinking water.

DISCUSSION

The aim of this study was to recover and typing the viruses present in freshwater and ground water in four districts of Tucumán.

Enterovirus and Hepatitis A are waterborne enteric viruses and their detection is of crucial importance in Public Health. Their number is highly variable and depends on multiple factors.

Freshwater and ground water are continuously affected by human faecal contamination. Knowledge of the viral genera and/or species present in water from different sources contributes essential elements for the existing risk assessment from a sanitation point of view. Different factors are involved in viral

Table 1: The sample areas and sources and the subsequent results

Sample Area	Sample Source	Results
Famaillá	ground water	Positive IEM
	ground water	Positive IEM
	freshwater	Positive
Lules	ground water	Positive
	ground water	Positive
	freshwater	Positive
Yerba Buena	freshwater	Negative
Leales	ground water	Negative
	ground water	Negative
	freshwater	Negative

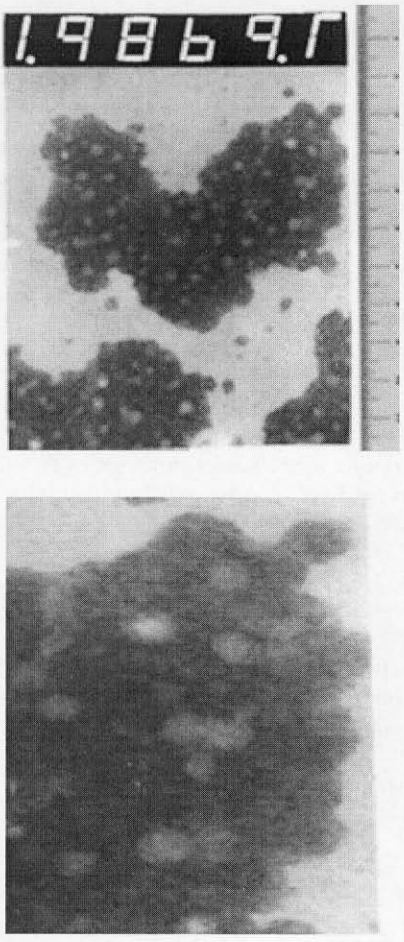

Fig. 1A: Immuno Electron microphotography,Sample: Famailla (ground water) 96,300 X
B: Immuno Electron microphotography,Sample: Famailla (ground water) 286,00 X

survival, such as temperature, pH, biochemical and biological activities and solid suspensions, being the most important ones temperature and virus adsorption to solid suspensions.

Numerous studies have documented the presence of enteric viruses in water from different sources.Our study showed four samples positives for cytopathic effect viruses in cell cultures, confirming: Echoviruses 18, Poliovirus 1 and 2 non-typified Enteroviruses. IEM in 2 samples revealed aggregations of viral particles morphologically compatible with Hepatitis A.

The results of this study have demonstrated that the water was contaminated and the source of contamination were the viruses detected.

Prevention should include vaccines as well as

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for environm
ental cleaning
up with
virus

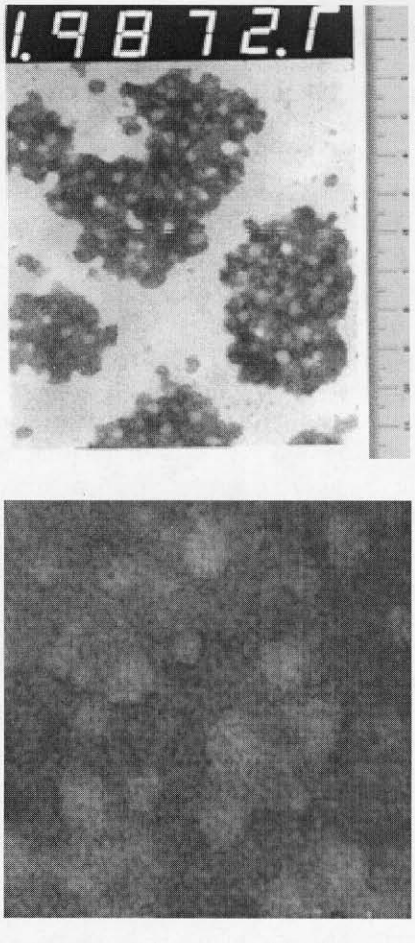

Fig. 2A: Immuno Electron microphotography,Sample: Famailla (ground water) 96,300 X
B: Immuno Electron microphotography,Sample: Famailla (ground water) 286,00 X

monitoring, especially in the case of epidemic outbreaks.

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