SURVEILLANCE OF ENTERIC VIRUS IN SOURCE WATERS FOR DRINKING WATER SUPPLY IN METROPOLITAN REGIONS OF SÃO PAULO STATE, BRAZIL.

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Aims

The demand for surface water intended to drinking water production is critical in the eastern region of the Sao Paulo State where about 70.0% of the population is concentrated and the rivers and reservoirs are contaminated by sewage discharges inadequately treated. The waterborne pathogens are of great concern in these source waters, mainly those more resistant to the water treatment process and with low infectious dose, such as protozoan and enteric virus. The Brazilian Directive for Drinking Water Quality doesn't require the monitoring of pathogenic microorganisms in source or drinking water neither establishes a level of risk infection, however recommends the analysis of *Giardia*, *Cryptosporidium* and *Enterovirus* in treated water with a maximum contaminant level goal of zero. The present study aims to evaluate the occurrence of *Enterovirus* (EV), *Adenovirus* (AdV) and *Hepatitis A virus* (HAV) in surface waters from urban catchments and to estimate the risk of Echovirus (*Human enterovirus B)* in drinking water, to give support to decision makers.

Methods

Sampling was performed during 12 months, bimonthly, at 10 locations in the intake area of Water Treatment Plants (WTP) at urban areas in the eastern region of Sao Paulo State. Forty litre volumes of water were collected in polyethylene bottles and transported to the lab on ice. Water samples were concentrated in 24 hours using the electronegative filter adsorption-elution method and reconcentrated by ultracentrifugation. Viral nucleic acids were extracted using guanidinium thiocyanate as lysis agent and adsorbed onto silica particles. Amplification of viral nucleic acids was performed by nested PCR (AdV*)* and nested RT-PCR (EV and HAV) using oligonucleotide primers described previously. Two additional sampling campaigns were performed in order to determine the concentrations of infectious viruses by tissue culture for the sites with a high percentage of PCR positive results. The methodology employed was a monolayer plaque assay using rhabdomyosarcoma (RD) cells. Thermotolerant coliforms were determined by membrane filter technique using mFC agar.

Quantitative microbiology risk assessment (QMRA) for Human echovirus infection was calculated considering that it is the most common group of Enterovirus genus isolated in surface water and sewage samples in São Paulo. The following assumptions were used in the model: a 4.0 log virus reduction by the conventional water treatment, a lognormal distribution for consumption of drinking water (Adults ≥21 years old - 1.21 (SD 0.29) L/day; Children <5 years old - 0.44 (SD 0.92) L/d and the Beta Poisson dose-response model (α=1.06; N50=921.4). The Monte Carlo analysis was performed using Crystal Ball® software, considering 10,000 simulations.

Results

During 2006 to 2007 a total of 60 source water samples taken from 10 different locations were analysed for enteroviruses, adenoviruses and hepatitis A viruses by the nested (RT) PCR assay. Enteric viruses were detected in 51 (85%) of these samples with a prevalence of positive samples for AdV (41/60) and EV (37/60). HAV was detected only in two samples from different locations but at the same watershed (Piracicaba River). The majority of the samples presented contamination for both AdV and EV (26/60). Figure 1 shows the distribution of the enteric viruses at the different location sites. All the collection sites but point 3 presented high percentages of positivity (≥80%). The evaluation of the seasonal behaviour of these viruses during the period of study showed a decay of positive samples in July and August, at winter time, when the temperature and rain precipitation are low.

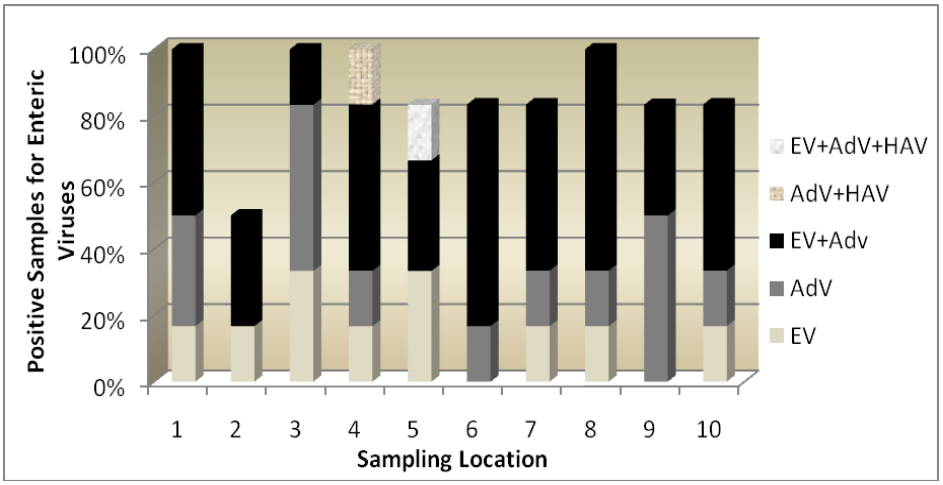

Figure 1 – Percentage of positive samples for enteric viruses per sampling location taken during 2006 to 2007. (1) Aparecida WTP: Paraiba River; (2) Atibaia WTP and (3) Campinas WTP: Atibaia River; (4) Piracicaba WTP and (5) Americana WTP: Piracicaba River; (6) Campinas WTP: Capivari River; (7) Cajamar WTP: Cristais River; (8) Carapicuiba – Baixo Cotia WTP: Baixo Cotia River; (9) São Paulo – Alto Boa Vista WTP: Guarapiranga Reservoir; (10) Cubatão WTP: Billings Reservoir/Canal de Fuga.

The geometric mean of thermotolerant coliform (TtC) concentrations varied from 19 CFU/100 mL (location 9) to $2.0x10^4$ CFU/100 mL (location 4), with most of the sites with geometric mean above 1000 CFU/100 mL (except locations 5, 9 and 10). Therefore, it was not observed a correlation between TtC concentrations and the presence of enteric viruses in the sites studied.

The viruses were quantified in two campaigns during 2008 at the four locations that presented 100% of positive samples. Concentrations of *Enterovirus* ranged from 9.58 PFU/L at Paraiba River (Point 1) to 0.23 PFU/L at Atibaia River (Point 3) and all samples presented counts ≥ 1.0 PFU/L in the second campaign. The mean concentration of viruses at the four locations was 2.59 (SD 3.37) PFU/L, and this value was multiplied by the frequency of Echovirus (about 40%) to calculate the QMRA. The CI95% of annual estimated infection risk is $7.6x10^{-6}$ to $4.1x10^{-4}$ and the median is $5.5x10^{-5}$ for adults and for children, the CI95% ranged from $1.9x10^{-7}$ to $1.2x10^{-4}$ and the median is $4.9x10^{-6}$. The sensitivity analysis showed that the driver of risk for adults was the concentration of Echovirus (98.2%) followed by the ingestion rate (1.8%) while for children it was the ingestion rate (63.5%) and the pathogen concentration (36.3%).

Conclusions

Enterovirus and *Adenovirus* were detected in a high percentage of source waters samples which supplies metropolitan regions of Sao Paulo, indicating the impact of point and diffuse fecal pollution in these highly urbanized areas. These results provide valuable information for WTP managers that need to guarantee the virus removal in the treatment process and for health authorities during outbreak investigations. The risk analysis shows a maximum probability of infection by ingestion of Echovirus close to the value of pathogen tolerable risk established by USEPA (10^{-4}), but much lower than the diarrheic disease rate at Sao Paulo State population (1.2%). Therefore it is important to extend the monitoring for other pathogenic enteric viruses, such as norovirus, as well as to perform a molecular characterization of the viral isolates to understand better the implication of them in human health.