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Waterborne viral diseases resulting from the consumption of contaminated drinking water, inadequate supply of water for personal hygiene and poor sanitation take heavy toll worldwide and developing countries are the major sufferers. Though presence of human enteric viruses has been demonstrated in water bodies as well as drinking water supplies throughout the world and several outbreaks of enteric viral diseases attributed to virologically contaminated water have been recorded, routine examination of water samples for the presence of enteric viruses is not carried out in both developed and developing countries. The main reason for not carrying out such an examination remains unavailability of a rapid, cost-effective and efficient standard procedure. The basic steps of the virological analysis of water are sampling, concentration, and specific virus detection. Concentration of water samples is a critical step, since the viruses may be present in such low numbers that it is necessary to use very large volumes and concentrate it up to a few milliliters.

Recognizing the necessity of an assay for virological evaluation of water samples, this study undertook the task of developing an efficient, sensitive, quick and relatively inexpensive protocol for concentration of viruses from large amount of water samples followed by simultaneous detection of Hepatitis A Virus (HAV), Hepatitis E Virus (HEV), entero and rotaviruses. The efficacy of water supply system of Pune, India and the prevalence of enteric viruses in Mutha River flowing through Pune city was also assessed. Evaluation of point-of-use water purification devices for their efficiency to remove the virus from seeded water samples was another objective.

A membrane filtration based two-step virus concentration protocol, a Real time PCR assay for quantification of HEV (the model virus for the study) and a multiplex PCR for the simultaneous detection of HAV, HEV, Entero and Rotavirus were developed.

Drinking water samples (n=662, 40 liters each) were collected during January 2007-December 2007 from three water treatment plants (Parvati, Warje and lashkar) and from points of common public use located
downstream to the respective water treatment plants. Water samples (n=5, 40 liters each) were collected from Kha dakwasla dam. The samples were concentrated up to ~3ml by the two step concentration protocol. Water samples (n=64, 500 ml each) were collected twice in a month during March – December 2007 from 4 points along Mutha River. The concentrated drinking water samples and un-concentrated river water samples were subjected to multiplex nested PCR for simultaneous detection of HAV, HEV, enteroviruses and rotavirus. Only enterovirus RNA was detected in 2/662 (0.3%) drinking water samples and the samples from the city’s water reservoir tested negative for all four viruses. Among 64 Mutha river samples, 49 (76.56%) were positive for Hepatitis A Virus, 36 (56.25%) were positive for Rotavirus, 33 (51.56%) were positive for Enterovirus and 16 (25%) were positive for Hepatitis E Virus RNA.

Eight domestic water purification systems widely used in India were evaluated using Hepatitis E Virus (HEV) as a model virus. For HEV concentration and detection, membrane filtration and real time PCR were used respectively. Viral log reduction value (LRV) was calculated for each unit. The parameter for virological evaluation of water purifiers, established by United States Environmental Protection Agency (USEPA) is minimum 4-log reduction of seeded virus, while India does not have any such standards. Viral log reduction value was 0.21 for unit 6 (polyester + carbon), 1.45 for unit 4 (filter + UV), 1.52 for unit 3 (filter + chlorine), 1.70 for a carbon + exhaust indication contact disinfection unit, 2.20 for an iodine resin unit, 2.51 for a dual filter unit and 6.53 for a hollow fibre membrane unit and a gravity-fed filter unit. Thus, only the technologies employed by the latter two were efficient in complete removal of HEV.

The study suggests good performance of Pune city’s water supply system and documents high prevalence of enteric viruses in river water, posing threat to the community. The majority of the water purifiers under use are inadequate. Virological standards in evaluating such devices need to be established urgently, in order to help manufacturers to improve the performance of such products and most importantly, to help consumers. The rapid, sensitive and relatively inexpensive protocol developed for virological evaluation of water seems extremely useful and should be adapted for
evaluating viral contamination of water for human consumption. This will lead to development of adequate control measures thereby reducing disease burden due to enteric viruses.