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Virological evaluation of domestic water purification devices commonly used in India emphasizes inadequate quality and need for virological standards

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Summary

OBJECTIVES To evaluate the performance of domestic water purification units with respect to contaminating enteric viruses.

METHODS 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.

RESULTS 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.

CONCLUSIONS 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.

keywords water purifier, virological evaluation, hepatitis E virus, India

Introduction

Enteric infections resulting from the consumption of contaminated drinking water, inadequate supply of water for personal hygiene and poor sanitation take a heavy toll worldwide and developing countries are the major sufferers. Diarrhoeal diseases are one of the leading causes of morbidity and mortality in less developed countries, accounting for an estimated 4 billion cases and 2.5 million deaths each year (Prüss *et al.* 2002; Kosek *et al.* 2003) especially among children aged <5 years (Parashar *et al.* 2003). Consumption of microbiologically contaminated water leads to diseases such as amoebiasis, cryptosporidiosis, giardiasis, leptospirosis, legionellosis, campylobacteriosis, cholera, typhoid, paratyphoid, gastroenteritis and viral infections leading to hepatitis A, hepatitis E, diarrhoea as well as several enteroviral diseases. Hepatitis E virus (HEV) infection by the faecal/oral route is an important cause of epidemic and sporadic acute viral hepatitis among Indian adults (Arankalle *et al.* 1993, 1994). During epidemics, the virus is associated with high mortality among pregnant women (Khuroo *et al.* 1981). HEV is responsible for a substantial proportion of sporadic fulminant cases of hepatitis among Indian men and non-pregnant women (Arankalle *et al.* 1995). Hepatitis A is highly endemic in developing countries and a large proportion of the population acquires immunity through asymptomatic infection early in life (Gust 1992). However, with changing epidemiology of hepatitis A in these countries, epidemics even in adults (Arankalle *et al.* 2006) are being recorded.

In India the average incidence of viral hepatitis is 12 cases per 100 000 people, while in urban communities incidence of viral hepatitis might be around 100 per 100 000 people (Mudur 2003). Just 30% of wastewater from India's cities is treated before disposal. The rest flows into rivers, lakes and groundwater. Figures from India's Central Bureau of Health Intelligence show that the incidence of diarrhoea, enteric fever, viral hepatitis and cholera has stayed at the same level over the past decade (Water Resources Division 2002). The evaluation of drinking water supply in Indian cities shows evidence of microbial contamination (Jothikumar *et al.* 2000; Brick *et al.* 2004).

Practices of water purification for improving the microbial quality of water at the user level is an effective approach ensuring availability of safe drinking water to households without access to a reliable supply of safe drinking water (Clasen & Bastable 2003; Sobsey *et al.* 2003). A World Health Organization-sponsored literature review concluded that simple, acceptable, low-cost interventions at the household and community level are capable of dramatically improving the microbial quality of household stored water and reducing risks of diarrhoeal disease and death (Sobsey 2002). The efficacy of household water treatment has been documented in several reports (Synder *et al.* 1995; Grabow *et al.* 1999; Luby *et al.* 2001; Clasen *et al.* 2004; Crump *et al.* 2004; Matsui *et al.* 2004; Hörman *et al.* 2005; Doocy & Burnham 2006).

Various types of water purification units are currently used in India for treatment of water at the household level. Activated carbon filter, ceramic candle filter, sediment filter, iodine resin gravity filter, polyester filter, ultra violet irradiation, reverse osmosis and hollow fibre membrane filters are different techniques employed either singly or in combination. The parameters for performance evaluation of water purifiers, established by United States Environmental Protection Agency (USEPA) Guide Standard and Protocol for Testing Microbial Purifiers (USEPA 1987) are 6-log reduction of bacteria, 4-log reduction of viruses and 3-log reduction of protozoan cysts (USEPA 1987, 2001). However in India the only standard (IS: 7402) available for microbial performance evaluation of water purifiers requires the filtrate to be bacteria free when the unit is challenged with 1×10^5 CFU/ml of *Escherichia coli* and compliance with the standard is not obligatory. No standards are currently available for the virological evaluation of water purification units in India.

This paper describes the development of a sensitive method for virological evaluation of domestic water purifiers using HEV as a model virus and evaluates various domestic water purification systems.

Materials and methods

Preparation of hepatitis E virus stock

A stool sample collected from a sporadic case of Hepatitis E was used to prepare the virus stock. One gram of stool was added to 10 ml of phosphate buffered saline (PBS) pH 7.4 and sonicated (SONIFIER, Branson, USA). After centrifugation at 16 000 ***g*** for 15 min at 4 °C, the supernatant was removed and stored at −70 °C in aliquots until further use.

Water purification devices

Eight water purifiers (Table 2) that are commonly used in Indian households were selected and purchased from the local market. Technology used for water purification was an important criterion for selection of water purifiers. Unit 1 is a faucet-mounted filter; it uses iodinated resin to chemically disinfect water. The unit has a design life of 7500 l, after which the entire product is to be replaced. Unit 2 is an on-line water filter-cum-purification system with dual filter to remove odour and impurities followed by UV deactivation of microorganisms. Unit 3 involves a candle filter, followed by activated carbon filter and chlorine dispenser. Unit 4 is a three-stage water purification system, the first stage removes impurities such as dust, sand using a multi-layered filter candle; the second stage involves silver-activated carbon granules, the third, an ultraviolet lamp of 8 W. The unit operates on supply voltage from 200 to 240 V and a maximum water flow rate of 1.5 l/min. Unit 5 is an on-tap water purifier, it comprises of a sediment filter that removes suspended impurities from water, followed by elimination of organic impurities and chlorine traces via activated carbon treatment. The final purification process comprises of exhaust indication contact disinfection (EICD) consisting of active iodinated resin. The replaceable filter cartridge has a design life of 9000 l and best performance is achieved at a flow rate of 2 l/min. Unit 6 is also an on-tap water purifier; it employs a combination of multi-stage polyester filter and silver-coated activated carbon filter. It has a design life of 10 000 l and works at a flow rate of 2 l/min. Unit 7 employs hollow fibre membrane filtration technology: the water passes through tubes of Poly Acrylo nitrile membrane and microbes are removed on the basis of size exclusion. Unit 8 is a gravity-fed water treatment system; water is initially filtered through a microfibre mesh made from polyester non-woven fibre, then it passes through a moulded carbon block pre-filter and a chlorine dispenser cartridge. The level of free available chlorine is about 6 ppm. The water then passes through a polisher containing silver-impregnated granular-activated carbon, the polisher removes excess chlorine and chlorination by-products. The cartridges have a design life of 1500 l. All the purification units were installed in the laboratory and their operation was demonstrated by authorized representatives/engineers of the concerned manufacturers.

Quantification of hepatitis E virus stock

Hepatitis E virus stock was 10-fold serially diluted up to 10^{-5} dilution in PBS. Viral RNA was extracted from 140 µl neat virus stock as well as serially diluted stock virus using

QIAmp viral RNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. RNA was eluted in 40 μl AVE buffer (elution buffer) and 5 μl RNA was subjected to in-house real-time RT-PCR assay as described earlier (Arankalle *et al*. 2009). Sensitivity of the assay in detecting transcribed HEV RNA was 100 copies/ml. The copy number of HEV viral particles present in 1 ml of sample was calculated both for virus stock and 10-fold serial dilution series (copy number/ml multiplied by dilution factor) and arithmetic mean of all observations was considered as number of viral particles present in 1 ml of virus stock. All samples were screened in triplicate.

Testing of purification units with hepatitis E virus-spiked distilled water

Five litres of distilled water was spiked with 10 μl of HEV virus stock containing approximately 10^6 viral particles. This water was subjected to the purification protocol of the unit being tested according to the manufacturer's instructions. The purified water (approximately 5 l) was concentrated to 2–3 ml by two-step concentration protocol. In the first step the sample was concentrated to about 300 ml using polyacrylonitrile (PAN) membrane-based tangential flow filtration technology with a spirally wound membrane of 60 000 Da exclusion limit (Membrane filters, Pune, India). Subsequently, 300 ml was concentrated to about 2 ml in an amicon 8200 stirred cell unit (Millipore, USA) using PAN membrane disc of 63.5 mm diameter, providing 28.7 cm^2 surface area for filtration. Each purification unit was tested twice and the volume of the concentrate was recorded for each experiment. Number of RNA copies in purified and concentrated sample was estimated by real time PCR as described above.

Amplification of hepatitis E virus full genome by RT-PCR

Almost the complete HEV genome was amplified and sequenced for the purified/concentrated sample for the units yielding HEV RNA positivity. For cDNA synthesis 10 μl of RNA was added to a reaction mix (mix A) containing 1 μl of 10 μM Reverse primer and 0.5 μl RNasin (Promega, Madison, USA; 40 unit/μl) and incubated at 65 °C for 5 min. Another reaction mix (mix B) containing 4 μl 5x First Strand buffer, 1 μl of 25 mM dNTP mix (Promega), 2 μl of 0.1 M dithiothreitol (DTT), 1 μl RNasin was added and incubated at 45 °C for 2 min. Following addition of 1 μl of Super Script™ II Reverse Transcriptase (Invitrogen; 200 Unit/μl) the reaction mix was incubated at 45 °C for 1 h.

For PCR amplification, 2 μl of cDNA was added to 98 μl of a reaction mixture containing 68.5 μl of triple distilled water, 10 μl of 10X Pfx Amp buffer, 5 μl of 10X PCR enhancer solution, 2 μl of 50 mM $MgSO_4$, 1 μl of 25 mM dNTP mix, 0.5 μl RNasin, 1 μl Platinum® Pfx polymerase (Invitrogen; 2.5 Unit/μl), 5 μl of 10 μM Reverse primer and 5 μl of 10 μM Forward primer. Thermal cycling was performed on GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, CA) as follows: 5 min at 94 °C for initial denaturation; 35 cycles of 1 min at 94 °C, 1 min at 50 °C (different annealing temperatures were used for different primer pairs) and 3 min at 68 °C; and a final extension step of 7 min at 68 °C. Nested PCR was performed by adding 10 μl first PCR product to 90 μl of PCR reaction mixture (composition was same as PCR mix for first PCR except triple distilled water was reduced to 58.5 μl) and thermal cycling was performed as described above.

The PCR products of expected size from water samples treated using six of the eight units evaluated and not resulting in complete virus removal were column purified (Qiagen). Both strands were sequenced using Big Dye Terminator cycle sequencing Ready Reaction Kit (version 3.1; Applied Biosystems) and automatic sequencer (ABI 3130 xl; Applied Biosystems).

Results

Quantification of hepatitis E virus virus stock

Hepatitis E virus RNA copies/ml of neat or 10-fold serial dilution series of the virus stock varied between 3.02×10^8 and 3.74×10^8 copies/ml. The arithmetic mean of all observations was 3.36×10^8 copies/ml (Table 1).

Virus log reduction by water purification units

Log reduction values were calculated based on HEV RNA copies present in the seed virus and the purified concentrate

Table 1 Estimation of No of RNA copies in the seed virus

Sample	Quantification result (no. of virus copies/ml seed virus)		Estimated copy no./ml seed virus	Arithmetic mean copies/ml seed virus
	Experiment1 (triplicates)	Experiment2 (triplicates)		
Neat virus stock	3.65×10^8	2.92×10^8	3.29×10^8	3.36×10^8
−1 dilution	3.79×10^7	3.54×10^7	3.65×10^8	
−2 dilution	2.87×10^6	3.25×10^6	3.02×10^8	
−3 dilution	2.95×10^5	3.33×10^5	3.14×10^8	
−4 dilution	4.5×10^4	3.01×10^4	3.74×10^8	

Table 2 Performance of different units as judged by recovery of the seed virus in treated water

Purification unit	(Price in INR/USD)	Design life	Flow rate	Virus particles added	Virus particles recovered			Log reduction value
					Experiment 1 (triplicates)	Experiment 2 (triplicates)	Average	
Unit 1 (iodine resin)	295/5.78	7500 l	2 l/min	3.36×10^6	2.29×10^4	1.95×10^4	2.12×10^4	2.20
Unit 2 (dual filter)	6750/132.3	NA*	3 l/min	3.36×10^6	1.13×10^4	1.02×10^4	1.07×10^4	2.51
Unit 3 (filter + chlorine)	1750/34.3	NA*	2 l/min	3.36×10^6	1.09×10^5	0.95×10^5	1.02×10^5	1.52
Unit 4 (filter + UV)	2990/58.6	NA*	1.5 l/min	3.36×10^6	1.16×10^5	1.22×10^5	1.19×10^5	1.45
Unit 5 (carbon + EICD)	690/13.5	9000 l	2 l/min	3.36×10^6	7.56×10^4	6.09×10^4	6.82×10^4	1.70
Unit 6 (polyester + carbon)	205/4	10 000 l	2 l/min	3.36×10^6	1.96×10^6	2.19×10^6	2.07×10^6	0.21
Unit 7 (hollow fibre membrane)	5900/115.6	NA*	3 l/min	3.36×10^6	Not detected	Not detected		6.53
Unit 8 (gravity-fed filter)	1800/35.2	1500 l	NA**	3.36×10^6	Not detected	Not detected		6.53

*Depends on water quality.
**Gravity-based storage device.
NA, not available.

for each unit (Table 2). Log reduction values for different water purifiers ranged between 0.21 and 6.53 logs (Figure 1). Maximum log reduction (complete removal of the seeded virus) was observed for units 7 (hollow fibre membrane) and 8 (gravity-fed filter), low (0.21) log reduction was observed for unit 6 (polyester + carbon), units 1 (iodine resin) and 2 (dual filter) showed approximately 2 logs reduction, while units 3 (filter + chlorine), 4 (filter + uv) and 5 (carbon + EICD) were removing approximately 1 log viral RNA.

Amplification of hepatitis E virus full genome

Nearly complete genome (7.2 kb) of HEV was amplified in five overlapping fragments from each of the concentrated samples showing HEV RNA positivity (units 1–6). Amplification of almost the complete genome confirms the presence of intact HEV in water samples treated using these six units. All amplified products were partially sequenced to confirm the presence of HEV genome sequence (data not shown).

Discussion

Use of household water purification systems is a common practice in India, especially in the urban and semi-urban areas. Many Non-Government Organizations have come forward to provide such units in the schools from rural areas as well. This indicates the concern of the population about the quality of drinking water available and efforts to remove the disease-causing microbes at the household level. The manufacturers routinely follow testing of such units for the removal of bacteria, as it is easily available and inexpensive. Though enteric viral diseases are of immense public health importance, because of the difficulties in concentrating large volumes of water as well as

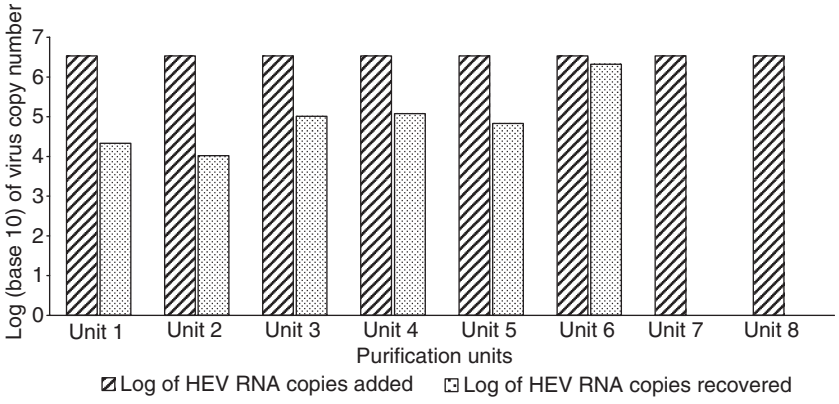

Figure 1 Hepatitis E virus RNA copies in the seed virus (virus particles added) and the treated concentrates from the eight units examined.

non-availability of rapid and highly sensitive techniques for the quantification of these viruses, evaluation of domestic water purification units for the removal of viruses is usually not followed in India. Moreover, no guidelines are provided for the certification of these units as virus-free.

The first important issue was to standardize a reproducible method for rapid concentration of the viruses present in approximately 5 l of water. This was accomplished by a two-step membrane-filtration method. As we were planning to seed the known quantity of virus, the water volume was fixed to 5 l. The entire protocol takes only 2 h and the concentrate is kept on ice thereby minimizing the possibility of loss of virus with time and at room temperature.

Development of a highly sensitive and specific real time PCR assay enabled the quantification of HEV RNA, an integral part of the evaluation protocol. With both methods in place, we were able to clearly document that only two of the eight units were able to remove HEV completely as evidenced by real time PCR. In the absence of a cell-culture system or a small laboratory animal (except monkeys) it was not possible to assess the infectivity of the virus-recovered from the various units. However, as we were able to amplify almost the entire genome from all these virus positive concentrates, we believe that the PCR positivity did not indicate partially degraded virus but the intact virus. There are evidences to demonstrate that signals generated after RT-PCR amplification of viral genome correlates well with the presence of infectious virus in the sample. Viral samples inactivated by heat or UV treatment produce significantly lower signal strength that paralleled infectivity of the sample in cultured cells (Bhattacharya *et al.* 2004).

Unit 1 (iodine resin) and unit 2 (dual filter) were able to remove two logs of seeded HEV particles while unit 3 (filter + chlorine), unit 4 (filter + UV) and unit 5 (carbon + EICD) were showing one log reduction. Performance of unit 6 (polyester + carbon) was poorest showing no log reduction at all. Thus, only units 7 (hollow fibre membrane) and 8 (gravity-fed filter) were able to fulfill the log reduction value requirements laid by USEPA (4 log reduction). These results indicate superiority of the techniques employed for designing unit 7 and unit 8 as against those employed for other units. It may be noted that ultra violet irradiation, activated carbon filtration and other filtration techniques were not able to either remove or destroy seeded HEV particles beyond two logs.

These purification units were evaluated under controlled laboratory conditions and distilled water spiked with HEV was used as test water, which does not mimic *in situ* conditions where the feed water quality is poor. The maintenance and the frequency of the replacement of filter medium of purification units may also be inadequate. In practice, these limitations may result in even poorer performance of the units. Employing Male-specific MS2 coliphage as a surrogate marker for enteric viruses, Clasen *et al.* (2006), evaluated Pureit^{TM} water purifier developed by Hindustan Lever Limited and three other units (Clasen & Menon 2007). The removal of the phage was 99.9% with the former unit whereas none of the three later devices achieved 4.0 Log Reduction Value. The Pureit^{TM} water purifier (unit 8) and one of the three units tested subsequently (unit 1) were also evaluated in the present study employing HEV as the test virus. Although the methods employed were very different, the results obtained were similar; complete virus removal by unit 8 and the Viral log reduction value of 2 for unit 1. These results suggest that the present method developed to evaluate purification systems for virus removal using HEV as an enteric virus is in accordance with the accepted protocol, employing a surrogate for enteric viruses. The possibility of use of actual enteric virus in place of a surrogate organism in evaluation programmes may be considered following extensive comparison of both methods.

As a rule, viruses persist longer than enteric bacteria in water environments (Bosch 1998). It is therefore unsafe to rely on bacteriological standards to assess the virological quality of water. Importantly, waterborne outbreaks related to potable water that met bacteriological standards have been reported (Hejkal *et al.* 1982; Bosch *et al.* 1991). During an outbreak of infectious hepatitis among a military community, HAV, rotaviruses and enteroviruses were detected in water samples that were consistently free of indicator bacteria (Bosch *et al.* 1991). These same samples showed free and total chlorine levels that were adequate to ensure proper elimination of bacterial contaminants, but were unable to remove pathogenic viruses. These reports clearly document a definite need for a separate, well-defined virological standard for drinking water as well as for the evaluation of water treatment plants and domestic water purifiers. The minimum standards established by USEPA were not designed for developing countries where the microbiological quality of public water supply may not be as good as in developed countries. India and other developing countries should formulate their own standards and ensure strict adherence by all those concerned. This will help both manufacturers and consumers to be quality conscious with respect to drinking water, a basic need for every population and the major source of a variety of infectious diseases taking heavy toll every year in all the under-developed and developing countries. Similar study needs to be extended to the water treatment plants/systems used in villages, small cities and the Metros to truly understand the quality of water made

available to the people. It would be worthwhile judging the performance of the domestic units in field, i.e. houses, with respect to water quality, adherence to the recommended maintenance of the units as well as time period of usage. We would like to point out here that we have evaluated one unit of each type. The batch-to-batch or unit-to-unit variation was not evaluated. This is a limitation of this study and needs to be extended to several units from one batch as well as different batches.

In conclusion, our study suggests that even with the limitation of the study pointed out above, the results indicate that six of eight units tested (one unit/type) do not confirm to USEPA standards and emphasizes the need for a definite national policy for the evaluation of such devices by the regulatory authorities as well as at factory level. Such an exercise will ensure availability of quality-assured domestic water purification units to the community, thereby reducing the burden of water-borne infections. It is desirable to set up our own national virological standards as well as evaluation of the protocol developed by us in several laboratories followed by strict adherence to the method accepted and approved by the regulatory authorities.

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