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UV disinfection and flocculation-chlorination sachets to reduce hepatitis E virus in drinking water

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ABSTRACT

Hepatitis E Virus (HEV) is a major cause of waterborne outbreaks in areas with poor sanitation. As safe water supplies are the keystone for preventing HEV outbreaks, data on the efficacy of disinfection treatments are urgently needed. Here, we evaluated the ability of UV radiation and flocculation-chlorination sachets (FCSs) to reduce HEV in water matrices. The HEV-p6-kernow strain was replicated in the HepG2/C3A cell line, and we quantified genome number using qRT-PCR and infectivity using an immunofluorescence assay (IFA). UV irradiation tests using low-pressure radiation showed inactivation kinetics for HEV of 99.99% with a UV fluence of 232 J/m^2 (IC 95%, 195,02–269,18). Moreover, the FCSs preparations significantly reduced viral concentrations in both water matrices, although the inactivation results were under the baseline of reduction (4.5 LRV) proposed by WHO guidelines.

1. Introduction

Hepatitis E Virus (HEV) is an emerging virus causing water- and food-borne disease of global significance. The World Health Organization (WHO) estimates there are 3 million acute cases of HEV and 56,600 HEV-related deaths per year (WHO, 2014). Although the majority of HEV infections are subclinical, when HEV does cause clinical symptoms, they can have severe consequences, including fulminant hepatic failure and death, most often in pregnant women (Kmush et al., 2015). In addition, extra-hepatic manifestations of HEV have been observed, including neurological injury (Kamar et al., 2011).

According to the International Committee on Taxonomy of Viruses (ICTV), HEV has four classical genotypes (1, 2, 3 and 4) belonging to the *Orthohepevirus* genus, and it includes a diverse array of viral variants that can infect different hosts (primarily mammalian and avian). Genotypes 1 and 2 are strictly human, whereas strains corresponding to genotypes 3 and 4 are zoonotic, with pigs being the primary host (Kamar et al., 2014).

The epidemiology of hepatitis E differs between low- and high-income countries. In areas with poor/limited sanitation and hygiene practices, including large parts of Asia, Africa and South America, HEV has caused medium- to large-sized waterborne outbreaks. Over the last decade, outbreaks have occurred in areas of humanitarian emergencies, such as camps for refugees or internally displaced populations (Boccia et al., 2006; Guerrero-Latorre et al., 2011; Howard et al., 2010). The most recent example was an HEV outbreak that spread across South Sudan between 2012 and 2014, resulting in over 10,000 cases and cross-border infections into neighbouring countries, including 367 cases in South-Sudanese refugee camps in Ethiopia (UNHCR, 2014).

Moreover, the increased prevalence of HEV among populations in high-income countries has been well documented, with sporadic patterns of cases due to zoonotic transmission following consumption of raw meat, close contact with infected animals or hepatic transplantation (Kamar et al., 2012).

The fecal-oral route is the predominant mode of transmission for HEV, and as there are currently no efficient curative therapies for Hepatitis E infection, measures aimed at proper treatment of drinking water, safe disposal of human excreta and improvements to personal hygiene are the keystones for prevention and control of this disease (WHO, 2014).

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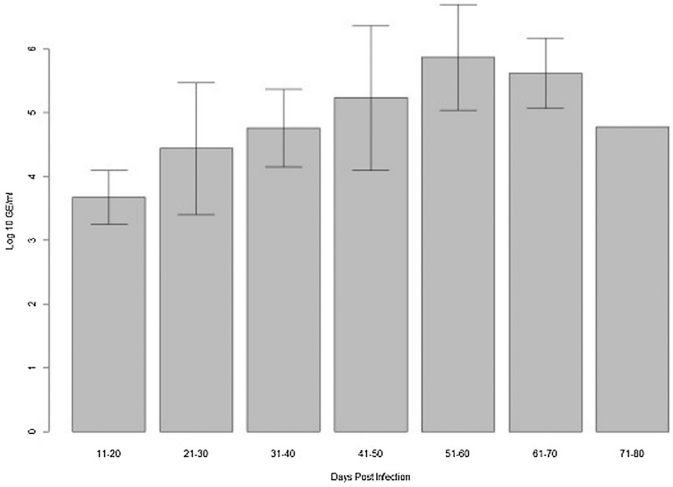

Fig. 1. Infections of Hep2G/C3A cell line with HEV p6-kernow strain. Bars represent average values of HEV concentration of supernatants at different times post-infection.

With respect to water treatment, chlorination was the first option used in many HEV outbreak scenarios until a study suggested that chlorinated water does not reduce the risk of infection (Guthmann et al., 2006). During that period, there were no studies addressing HEV disinfection, due to methodological limitations and a lack of cell culture assays to evaluate virus viability after treatment. The above study caused some organizations that were assisting HEV outbreaks to use other water treatment methods instead, such ultraviolet (UV) radiation. However, there is no available experimental information concerning the efficiency of UV disinfection for HEV.

Recently, cell culture systems have been developed that allow for the replication of low titres of HEV using a recombinant strain of a HEV, Kernow-p6-HEV, which is derived from the human hepatoma cell line HepG2/C3A (Shukla et al., 2012). These advances in laboratory techniques now make it possible to study HEV disinfection. One study has already shown that HEV is susceptible to chlorination, with a 99% reduction in virus levels following current treatment guidelines (Girones et al., 2014), supporting the use of chlorine as an effective strategy for controlling HEV waterborne transmission.

UV disinfection is now accepted as a major disinfection process for drinking water. Irradiation of water by low-pressure monochromatic UV radiation (253.7 nm), also known as UVC radiation, is a common water treatment procedure, which damages the genomes of microbial pathogens by producing photodimers between adjacent pyrimidine nucleotides, blocking RNA and DNA replication. Additional factors are likely to contribute on virus inactivation by UV such as damage to virus capsid proteins by hydroxyl radicals produced during photocatalysis (Mayer et al., 2015). Unlike ozone or chlorine, UVC radiation does not create any known toxic disinfection by-products, although it also leaves no residual protective effects in the treated water. Most regulatory bodies now specify a fluence or UV dose of 400 J/m^2 to assure at least 4 logarithms of inactivation (4-log) for any pathogenic microorganism (DVGW, 2006; EPA, 2003; ÖNORM, 2001). Although the majority of viruses do not show further inactivation at fluences above 500 J/m2, there are some DNA viruses (e.g., adenovirus) that are resistant to standard UV doses due to their capacity to repair their genomes following damage (Calgua et al., 2014; Hijnen et al., 2006).

Moreover, in humanitarian emergency settings, when the risk of waterborne outbreaks is increased and efficient common drinking water treatments are not available, point-of-use (POU) water treatments can be used as a rapid response to reduce waterborne disease transmission. Flocculation-chlorination sachets (FCSs) are premade formulations that combine flocculating agents to reduce turbidity and a chlorine-based disinfectant. FCSs are commercialized as single-use packages that can purify 10 or 20 L of untreated water to remove 6, 5, 4 logarithms of bacteria, viruses and protozoa respectively, and they are both well-accepted and properly used in many remote communities (Colindres et al., 2007; Powers et al., 1994; Souter et al., 2003). However, no specific studies have been performed to validate the inactivation of HEV by these treatments.

This manuscript describes the first experimental data on the efficiency of HEV disinfection in water using UV and FCS treatments. We also discuss the best options for HEV outbreak management based on the obtained experimental data.

2. Materials and methods

2.1. Viral suspensions

Hepatitis E Virus suspensions were produced by culturing the HEV-p6-kernow strain, genotype 3 (originally provided by Sue Emerson, National Institutes of Health, Bethesda, USA) in HepG2/C3A cells (ATCC reference: CRL-10741). This virus contains a 171-nucleotide insertion that encodes 58 amino acids of the human S17 ribosomal protein in the hypervariable region, which confers a significant growth advantage (Shukla et al., 2012).

HepG2/C3A cells were grown in flasks pre-coated with collagen (rat tail collagen type 1, Millipore, Billerica, MA) and cultured in Dulbecco modified Eagle medium (DMEM) (Gibco, Frederick, MD), L-glutamine, penicillin-streptomycin, gentamicin, and 10% fetal bovine serum (Ultra-Low immunoglobulin G, Invitrogen).

The viruses present in the media after a minimum of 10 days post-infection were passed through a 0.45-μm filter to avoid aggregation, concentrated by ultracentrifugation for 1 h at 100,000*g*, and then resuspended in phosphate buffer (pH 7, 1:2 v/v 0.2 M Na2HPO4 and 0.2 M NaH2PO4). The final virus suspensions were quantified and stored at −20 °C until use.

MS2 bacteriophage was used as a control process and reference for the disinfection experiments. MS2 stocks were produced in Salmonella WG49 following the standard ISO 10705-1 (ISO, 1995).

2.2. Quantification of viral suspensions

To evaluate the efficacy of the water treatments, both HEV and MS2 were analysed in duplicate for all experiments. HEV genomes

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