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Solar water disinfection (SODIS): Impact on hepatitis A virus and on a human Norovirus surrogate under natural solar conditions

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Summary. This study evaluates the effectiveness of solar water disinfection (SODIS) in the reduction and inactivation of hepatitis A virus (HAV) and of the human Norovirus surrogate, murine Norovirus (MNV-1), under natural solar conditions. Experiments were performed in 330 ml polyethylene terephthalate (PET) bottles containing HAV or MNV-1 contaminated waters (10^3 PFU/ml) that were exposed to natural sunlight for 2 to 8 h. Parallel experiments under controlled temperature and/or in darkness conditions were also included. Samples were concentrated by electropositive charged filters and analysed by RT-real time PCR (RT-qPCR) and infectivity assays. Temperature reached in bottles throughout the exposure period ranged from 22 to 40ºC. After 8 h of solar exposure (cumulative UV dose of ~828 kJ/m^2 and UV irradiance of ~20 kJ/l), the results showed significant ($P < 0.05$) reductions from 4.0 (±0.56) $\times 10^4$ to 3.15 (±0.69) $\times 10^3$ RNA copies/100 ml (92.1%, 1.1 log) for HAV and from 5.91 (±0.59) $\times 10^4$ to 9.24 (±3.91) $\times 10^3$ RNA copies/100 ml (84.4%, 0.81 log) for MNV-1. SODIS conditions induced a loss of infectivity between 33.4% and 83.4% after 4 to 8 h in HAV trials, and between 33.4% and 66.7% after 6 h to 8 h in MNV-1 trials. The results obtained indicated a greater importance of sunlight radiation over the temperature as the main factor for viral reduction. [**Int Microbiol** 2015; 18(1):41-49]

Keywords: Solar water disinfection (SODIS) · water disinfection · hepatitis A virus (HAV) · murine Norovirus (MNV-1)

Introduction

Water scarcity and the lack of access to sanitation in developing countries continue to be global health challenges. Despite progress towards the Millennium Development Goals, more than 768 million people (~11% of the global population) remain without access to safe drinking water sources [42]. Consumption of untreated or improperly treated water is one of the most common routes for enteric disease outbreaks and is a priority issue to solve in order to reduce morbidity and mortality in the developing world [7,18].

Household water treatment and storage (HWTS) have demonstrated to be among the most effective ways to reduce the incidence of waterborne diseases in regions without access to adequately treated drinking water. It constitutes a low cost, easy to use and sustainable water treatment, complying with basic criteria for acceptance in these developing zones

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[9,18,32]. Solar disinfection (SODIS) is a HWTS method that has been gaining popularity over the last 30 years [1,32,41]. The SODIS technique consists of exposing small-volumes (up to 3 l) of contaminated water with low turbidity (<30 NTU) in transparent containers (usually polyethylene-terephthalate [PET] bottles) to direct sunlight for at least 6 h (or 2 consecutive days if there is more than 50% of cloud cover) during the maximum intensity of radiation [32]. Biocidal effects of SODIS are attributed to optical (UVA) and solar mild-heating mechanisms [8,26]. The disinfection efficacy of SODIS depends principally on the solar irradiance, water temperature, turbidity, dissolved oxygen and resistance of the type of microorganism [31].

Solar UV radiation consists of UV-C (λ = 100–280 nm), UV-B (λ = 280–320 nm) and UV-A (λ = 320–400). However, only UV-A, and a small part of UV-B reach the earth surface [20,40]. UV-B may directly damage nucleic acids through formation of pyrimidine dimers. UV-A photons, the main component responsible for the disinfecting action of SODIS, are not sufficiently energetic to modify directly nucleic acids like UV-B and UV-C. However, it causes indirect damage to structural components and DNA of cells through photosensitizers and may generate reactive oxygen species (ROS) in water including singlet oxygen ($^1O_2$), superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH), which can form single strand breaks, nucleic base modifications as well as induce oxidations in proteins and membrane lipids [15,26,36].

Previous studies reported reductions in the incidence of diarrhoea using the SODIS method [10]. Recently, new research proved that SODIS can significantly reduce rates of childhood dysentery and infantile diarrhoea by 45% [13]. Beyond its health benefits through reduction of waterborne diseases, additional studies have also demonstrated that SODIS has positive health impact for Kenyan children under 5 years as they showed significant increase of weight and height [13].

SODIS efficacy has been already demonstrated for a wide range of microorganisms including bacteria, fungi and protozoan parasites [8,19,28,37]. Studies evaluating SODIS against human viruses under real field conditions are scarce. Some research has been done using enteric viruses and viral indicators such as bacteriophages under simulated sunlight and laboratory conditions or real sunlight in natural conditions [2,22, 23,29,45]. From a public health perspective, only a few human enteric viruses have been shown epidemiologically to be waterborne transmitted and widely detected in the environment. Hepatitis A virus (HAV) (fam. *Picornaviridae*) and Norovirus (NoV) genogroups I and II (fam. *Caliciviridae*) are among the leading aetiological viral pathogens transmitted by water and food [6,34,43,44]. HAV is the main cause of acute hepatitis worldwide and the WHO regard it as reference pathogen for drinking water risk analysis [17]. NoV is the most important foodborne infectious agent of gastroenteritis outbreaks worldwide [3,34]. Human NoV are non-culturable under laboratory conditions, however, murine norovirus (MNV) has been demonstrated to be an useful human NoV surrogate due to its similarity in genetic and environmental stability properties [4]. In addition, viral pathogens, especially non-enveloped viruses such as hepatitis A virus and noroviruses, besides having a high environmental stability and a low minimal infective dose, are resistant to commonly disinfection processes and persistent in water supply systems [6,43].

The main objective of this study was to evaluate and compare the effectiveness of SODIS method for the disinfection of HAV and MNV-1, two high resistant pathogens, in distilled water under natural solar conditions.

Materials and methods

Cell culture and viral stocks. HAV HM-175/18f was obtained from the ATCC as a cell culture-adapted cytopathic clone of strain HM-175. MNV-1, a culturable calicivirus genetically similar to human NoV [4,47] was kindly provided by Dr Herbert W. Virgin IV (University of Washington, USA). A mutant non-virulent infective strain of Mengovirus (vMC_0), kindly provided by Dr. Albert Bosch (University of Barcelona) was employed as RNA extraction control as it was previously described [11]. Stocks of each viral strain were generated by inoculation onto confluent monolayers of appropriate cell lines (FRhK-4, RAW 267.4 and HeLa for HAV HM-175, MNV-1 and vMC_0, respectively). The stocks were purified previously to their use performing 5 series of freezing/thawing to complete release of the viral particles from the cells and centrifuged at 2000 ×*g* for about 20 minutes at 37°C to remove cellular debris and titrated by plaque assay [12]. (Plaque forming units: PFU.) Final concentration of each viral stock was 1×10^5 PFU/ml for mengovirus vMC_0, and 1×10^6 PFU/ml for HAV and MNV-1.

Solar experiments. All assays were carried out at Plataforma Solar de Almería, Tabernas dessert (Spain) (37.09º N, 2.36º W). SODIS experiments were performed contaminating volumes of 330-ml distilled water contained in PET bottles at initial HAV or MNV-1 concentration of 10^3 PFU/ml and then exposing the bottles (from here SODIS-bottles) on concrete surface directly to the action of natural solar radiation during 2, 4, 6 and 8 h. The distilled water used had a conductivity of <10μS/cm, Cl^- = 0.7–0.8 mg/l, NO_3^- = 0.5 mg/l and dissolved organic carbon <0.5 mg/l. Water temperature was measured during the experiments every two hours with a thermometer (model HI 98509-1, Hanna Instruments, Eibar, Spain).

In order to discriminate the effect of solar radiation and temperature, parallel assays under controlled temperature and in darkness conditions were also included. Bottles under controlled temperature (from here Bath-bottles) were maintained at 25 ± 1°C in a cooled water bath. The cooled water bath consisted of a plastic container filled with water up to half of the bottles, which were placed in a horizontal position as the rest of the bottles not cooled. The water was renewed every so often to maintain the temperature.

Bottles in darkness (from here Dark-bottles) were wrapped in opaque aluminium foil and exposed to solar radiation. Control-bottles were wrapped in opaque aluminium foil and maintained at 25 ± 1°C in a cooled water bath. Each treatment was performed in triplicate and in perfectly clear sunny days of July 2012 at Plataforma Solar de Almería (South of Spain). A solar energy unit, Q_{UV}, is a term commonly used to compare results under different conditions [16]. UV radiation was measured in continuum with a global UV-A radiometer (295–385 nm, Model CUV3, Kipp & Zonen, Netherlands) on a horizontal platform, with a typical sensibility of 264 µV/W m^2. The radiometer provides data in terms of incident W/m^2, which is defined as the solar radiant energy rate incident on a surface per unit area. UV dose (kJ/m^2) is dependent on UV intensity and time, and is given by Equation 1:

$$dose = I \times \Delta t$$

where I is the average irradiation intensity, W/m^2, and Δt is the experimental time, in seconds. Moreover, the inactivation kinetics can be plotted as function of cumulative energy per unit of volume (Q_{UV}, kJ/l) received by the bottles, and calculated by Equation 2:

$$Q_{UV,n} = Q_{UV,n-1} + \frac{\Delta t_n \overline{UV}_{G,n} A_r}{V_t} \qquad \Delta t_n = t_n - t_{n-1}$$

where $Q_{uv,n}$, $Q_{uv,\,n-1}$, are the UV energy accumulated per unit volume (kJ/l) at times n and $n-1$, respectively, $UV_{G,n}$ is the average incident irradiation on the irradiated area, Δt_n is the experimental time of sample, A_r is the illuminated area of the solar bottle (m^2), and V_t is the total volume (l) of treated water.

Virus concentration and RNA extraction. Virus recovery from water samples was carried out following the principles outlined in the recently developed standard method for virus detection in foodstuffs, included bottled water (ISO/TS 15216-1:2013) with minor modifications. The concentration of viral particles from each sample was performed by filtration using electro-positive charged filters (Virocap filters, Scientific Methods, USA). After the adsorption to filters, the viral particles were eluted by an alkaline solution of pH = 9.5 (Beef extract 1.5%, 0.25 mol/l glycine, Tween 80 0.1%) in a final volume of 5 ml. Additional 2 ml of the alkaline solution were added to the empty bottle, shaked during 10 min and added to the previous eluate. Then, pH was adjusted to 7.5 with 0.1 mol/l HCl and viral particles were concentrated by PEG 8000 (8%) with a vigorous stirring for 2 h. After centrifugation at 10,000 ×*g* for 1 h the pellet was resuspended in 1 ml of PBS.

The viral RNA from each sample was extracted using a commercial kit (NucleoSpin RNA virus, Macherey-Nagel, Germany). This method is based on the guanidine thiocyanate disruption and the adsorption of RNA to silica columns. Known amounts of mengovirus clone (vMC_0) (10 µl of mengovirus stock) were previously spiked to each sample as an independent nucleic acid extraction efficiency control [11]. To determine the extraction efficiency, cycle threshold (C_t) value for the Mengovirus-positive amplification control and the C_t value of each sample for the Mengovirus were compared and classified as valid (> 5%) or invalid (<5%). Following the ISO technical specifications, samples with a < 5% extraction efficiency were re-extracted again.

Reverse transcriptase-real time PCR (RT-qPCR). RT-qPCR method was carried out according to the CEN/ISO standard method. RT-qPCR was performed on an Mx3005p QPCR System (Stratagene, USA) thermocycler, using TaqMan probes and Platinum Quantitative RT-PCR Thermoscript One-step System kit (Invitrogen, Saint Aubin, France) (25 µl final volume) with 5 µl of extracted RNA. Primer set and probe used were: 0.9 µmol/l of reverse primer HAV240 (5′-GGAGAGCCCTGGAAGAAAG-3′), 0.5 µmol l^{-1} of forward primer HAV68 (5′-TCACCGCCGTTTGCCTAG-3′) and 0.45 µmol l^{-1} of probe HAV150 (6-FAM-CCTGAACCTGCAGGAATTAA-MGB) for HAV [11]. For MNV-1, 0.2 µmol/l of reverse primer Rv-ORF1/ORF2 (5′-GCGCTGCGCCATCACTC-3′), 0.2 µmol/l of forward primer Fw-ORF1/ORF2 (5′-CACGCCACCGATCTGTTCTG-3′) and 0.2 µmol/l of probe MGB-ORF1/ORF2 (6-FAM-CGCTTTGGAACAATG-MGB) [5].

Amplification conditions for HAV were: reverse transcription at 55ºC for 1 h, denaturation at 95ºC for 5 min, followed by 45 cycles of amplification with a denaturation at 95ºC for 15 s, annealing at 60ºC for 1 min, and extension at 65ºC for 1 min. Amplification conditions for MNV-1 were as previously described [5] with minor modifications. Briefly, after a RT step at 45ºC for 1 h, PCR amplification was carried out with a initial denaturation at 95ºC for 5 min, and 50 cycles of amplification with a denaturation at 95ºC for 15 s and annealing-extension step at 60ºC for 1 min. Primers/probe set and amplification conditions for Mengovirus are those specified in the standard method ISO/TS 15216-1:2013.

The presence of RT-PCR inhibitors and the determination of the RT-qPCR efficiency were tested by means of the external controls (EC) included for each reaction. Briefly, 2.5 µl of EC, containing 10^3 genome copies of appropriated virus (HAV or MNV-1), were mixed with 2.5 µl of each sample extracted RNA and the C_t values of these reactions were compared with the C_t value obtained for the EC in RNA-free sterile water. Then the efficiency was classified as valid (> 25%) or invalid (< 25%). Following the ISO technical specifications, samples with < 25% RT-qPCR efficiency were tested again. Negative controls containing no nucleic acid as well as positive controls were also introduced in each run. A sample displaying a $C_t \leq 41$, with no evidence of amplification in the negative controls, was considered as positive. Quantification was estimated by standard curves constructed with serial dilutions of HAV or MNV-1 RNA, plotting the number of genome copies against the C_t. This quantification was not corrected with the extraction or RT-qPCR efficiencies, following the recommendations of the ISO standard method.

Infectivity assays. The infectivity of HAV and MNV-1 remaining in water samples at the end of each experimental period was evaluated in confluent FRhK-4 cells for HAV and RAW 267.4 for MNV-1 in 48-well cell culture plates. Each sample was tested in duplicate using 100 µl of viral concentrate per well. Once inoculated, plates were incubated 1 h at 37°C with slow agitation to promote virus attachment and internalization. After this period, the cells were washed with PBS (pH 7.4) to avoid the toxicity of beef extract components [25], and then maintenance medium was added to each well. Maintenance medium consisted on DMEM supplemented with 2% foetal bovine serum, 1X non-essential amino acids, 2 mmol/l L-glutamine and 100 UI-100 UI µg/ml penicillin-streptomicin (Lonza-BioWhittaker, Belgium). The plates were then incubated at 37°C and 5% of CO_2 and microscopically examined daily for cytopathic effect (CPE) during 21 and 5 days for HAV and MNV-1, respectively. Negative samples were subjected to a blind passage to avoid false negative results. Appropriate negative and positive controls were included. Negative controls consist on FRhK-4 or RAW 267.4 cells inoculated with sterile water filtered and subjected to the same conditions than the samples. Positive controls consist on the appropriate cell line inoculated with HAV or MNV-1 stock solutions.

Statistical analysis. One-way ANOVA analysis was performed to compare the differences in the percentage of viral elimination obtained between viruses and exposure conditions. Moreover, *post-hoc* tests were employed to determine the statistical significance of the viral reduction between each exposure conditions using the Tukey's and Dunnett's tests. Significance level was established at $P < 0.05$. All statistical analyses were performed using the SPSS v20.0.0 software statistical package (IBM Corp., Madrid, Spain).

Results

The maximum local noon UV irradiances recorded for HAV and MNV-1 experiments were 35.4 W/m^2 and 38.2 W/m^2, respectively (Fig. 1). The accumulated UV dose and Q_{UV} at the end of the exposure period were 820.692 kJ/m^2 and 19.9 kJ/l for HAV, and 834.876 kJ/m^2 and 20.24 kJ/l for MNV-1 (Fig. 2). The average water temperature profiles reached in bottles along the exposure time for HAV and MNV-1 experiments are shown in Figure 1. Maximum water temperatures recorded within the PET bottles in HAV trials were 38.6°C in SODIS-bottles, 37.4°C in Dark-bottles, 29.1ºC in Bath-bottles and 27.3ºC in Control-bottles. In MNV-1 trials, the maximum water temperatures reached were: 40.7°C in SODIS-bottles, 39.7°C in Dark-bottles, 30.9°C in Bath-bottles and 27.8°C in Control-bottles.

All samples yield valid extraction and RT-qPCR efficiency values. Extraction values ranged from 20 to 100% for HAV and from 26 to 100% for MNV-1. RT-qPCR efficiencies ranged from 52 to 100% for HAV and from 43 to 100% for MNV-1. The average viral quantification at initial time (0 h of solar exposure) was 4.0 (± 0.56) ×10^4 and 5.91(± 0.59) × 10^4 RNA copies/100 ml (RNAc/100 ml) for HAV and MNV-1, respectively. After 8h of solar exposure, average quantification values in SODIS bottles were 3.15 (± 0.69) × 10^3 and 9.24(±3.91) × 10^3 RNAc 100 ml^{-1} for HAV and MNV-1, respectively. These values represent an average decrease of 92.1% (1.1 log) for HAV (Table 1) and 84.4% (0.81 log) for MNV-1 (Table 2). The average decreases for Bath-, Dark- and Control-bottles were 85.1% (0.83 log), 36.7% (0.20 log) and 17.4% (0.08 log) for HAV; and 61.6% (0.42 log), 37.4% (0.20 log) and 10.3% (0.05 log) for MNV-1 (Tables 1 and 2; Fig. 2).

Statistical analyses did not show significant differences between HAV and MNV-1 removal rates ($P > 0.05$). However, statistical differences were observed with Dunnet's and Tukey tests between treatments, both in HAV and MNV-1 trials (Table 1 and 2). Significant differences with regard to the control were

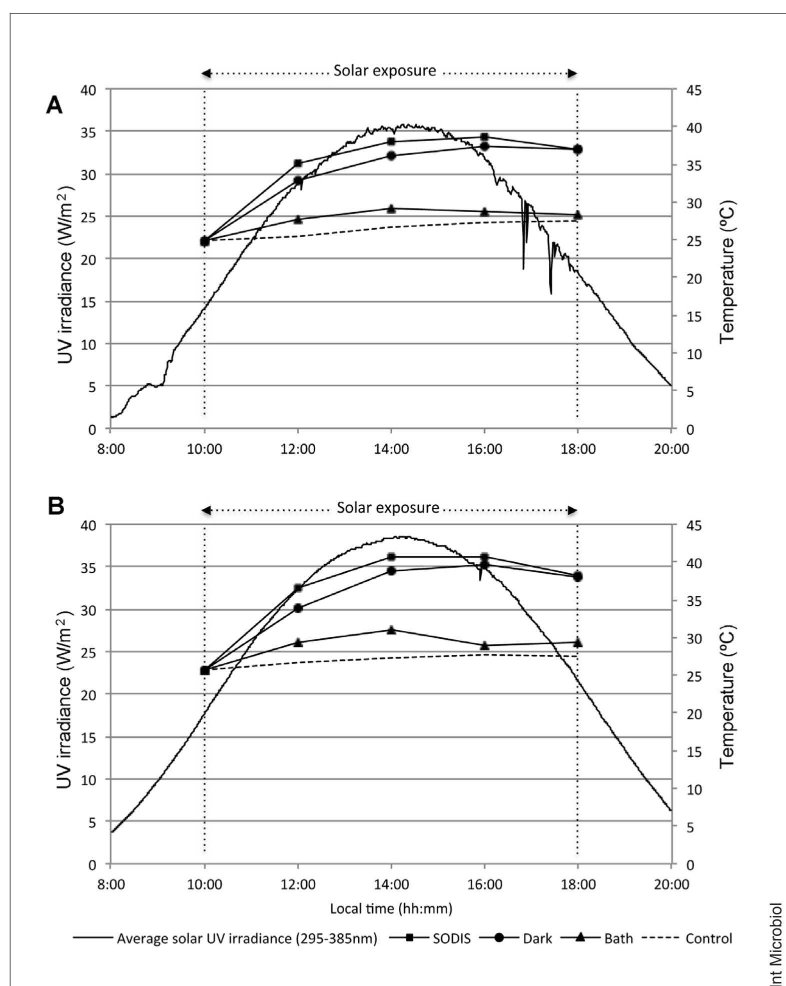

Fig. 1. Average solar UV irradiance (295–385 nm) along the solar exposure period and profiles of the mean water temperatures recorded in bottles for each exposure condition performed with HAV (**A**) and MNV-1 (**B**).

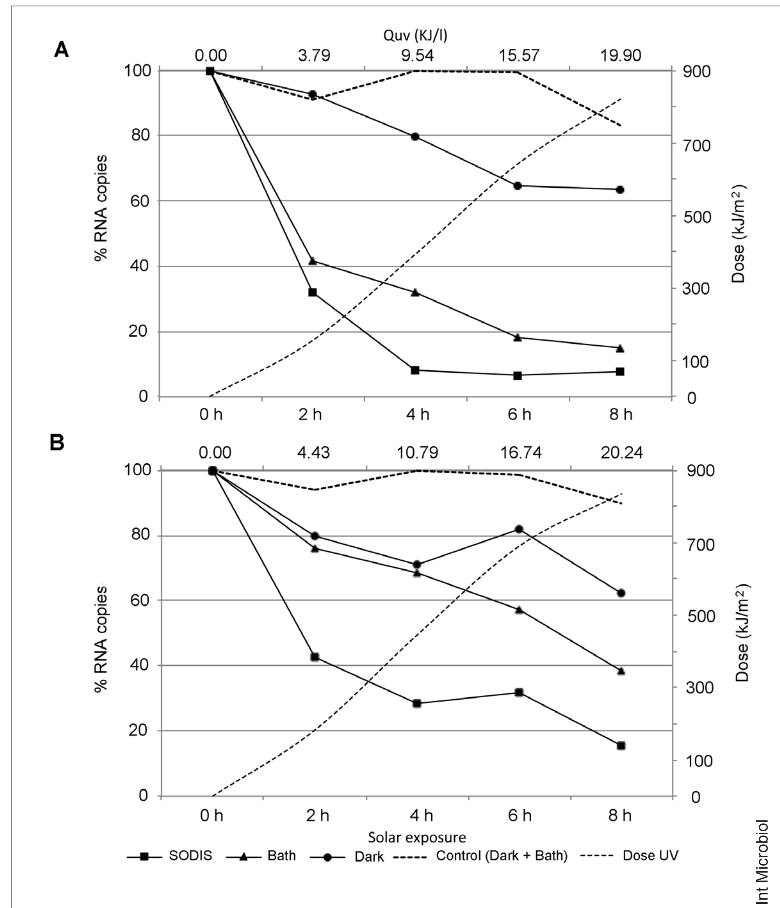

Fig. 2. HAV (**A**) and MNV-1 (**B**) RNA removal expressed as % RNA copies along the exposure time (2, 4, 6 and 8 h). Solar UV dose (W/m²) and cumulative UV radiation (Q_{UV}, kJ/l) are also shown in secondary axis.

observed for SODIS ($P < 0.01$) and constant temperature conditions (Bath-bottles) ($P < 0.05$), but no for darkness conditions (Dark-bottles) in HAV trials. For MNV-1, all conditions showed significant differences, $P < 0.001$ for SODIS, $P < 0.01$ for constant temperature conditions and $P < 0.05$ for darkness conditions.

With regard to infectivity assays, water samples from control-bottles and Dark-bottles maintained their infectivity capacity along the study period for both viruses. Water samples from Bath-bottles showed a one third decrease (33.4%) in their infectivity capacity after 6 and 8 h of exposure in HAV

Table 1. Quantification data of HAV expressed as viral RNA copies/100 ml for each solar exposure condition along the study period (t)

HAV	RNA copies/100 ml¶			
t	SODIS	Bath	Dark	Control
0 h	4.00 (± 0.56) × $10^{4\ A,a}$	4.00 (± 0.56) × $10^{4\ A,a}$	4.00 (± 0.56) × $10^{4\ A,a}$	4.00 (± 0.56) × $10^{4\ A,a}$
2 h	1.27 (± 0.41) × $10^{4\ B,a}$	1.66 (± 1.74) × $10^{4\ AB,a}$	3.70 (± 3.77) × $10^{4\ A,a}$	3.64 (± 0.21) × $10^{4\ A,a}$
4 h	3.27 (± 0.75) × $10^{3\ C,b}$	1.28 (± 3.10) × $10^{4\ AB,ab}$	3.18 (± 1.92) × $10^{4\ A,ab}$	4.00 (± 1.21) × $10^{4\ A,a}$
6 h	2.65 (± 0.31) × $10^{3\ C,a}$	7.30 (± 1.78) × $10^{3\ BC,a}$	2.58 (± 6.23) × $10^{4\ A,a}$	3.97 (± 1.40) × $10^{4\ A,a}$
8 h	3.15 (± 0.69) × $10^{3\ C,b}$	5.97 (± 0.11) × $10^{3\ C,b}$	2.53 (± 3.79) × $10^{4\ A,a}$	3.30 (± 0.43) × $10^{4\ A,a}$
%r*	92.1 (1.1 Log)	85.1 (0.83 Log)	36.7 (0.20 Log)	17.4 (0.08 Log)

¶All data correspond to the geometric mean of three replicates. Standard deviation is shown in parentheses.
*Percentage (and log units) of total viral removal. Statistical differences among results of the different sampling times within the same treatment are indicated by capital letters. Statistical differences among results of the different treatments within the same sampling time are indicated by small letters. Results with the same letter did not show significant differences ($P > 0.05$).

Table 2. Quantification data of MNV-1 expressed as viral RNA copies/100 ml for each solar exposure condition along the study period (t)

MNV-1	RNA copies/100 ml¶			
t	SODIS	Bath	Dark	Control
0 h	$5.91 (\pm 0.59) \times 10^{4\,A,a}$	$5.91 (\pm 0.59) \times 10^{4\,A,a}$	$5.91 (\pm 0.59) \times 10^{4\,A,a}$	$5.91 (\pm 0.59) \times 10^{4\,A,a}$
2 h	$2.52 (\pm 3.20) \times 10^{4\,AB,a}$	$4.52 (\pm 0.72) \times 10^{4\,AB,a}$	$4.73 (\pm 0.60) \times 10^{4\,AB,a}$	$5.58 (\pm 0.71) \times 10^{4\,A,a}$
4 h	$1.68 (\pm 3.01) \times 10^{4\,AB,b}$	$4.06 (\pm 0.40) \times 10^{4\,B,ab}$	$4.21 (\pm 0.43) \times 10^{4\,B,ab}$	$5.91 (\pm 0.21) \times 10^{4\,A,a}$
6 h	$1.88 (\pm 2.07) \times 10^{4\,AB,c}$	$3.36 (\pm 0.50) \times 10^{4\,BC,bc}$	$4.85 (\pm 0.51) \times 10^{4\,B,ab}$	$5.85 (\pm 0.51) \times 10^{4\,A,a}$
8 h	$9.24 (\pm 3.91) \times 10^{3\,B,d}$	$2.27 (\pm 3.92) \times 10^{4\,C,c}$	$3.70 (\pm 0.33) \times 10^{4\,B,b}$	$5.30 (\pm 0.41) \times 10^{4\,A,a}$
% r*	84.4 (0.81 Log)	61.6 (0.42 Log)	37.4 (0.20 Log)	10.3 (0.05 Log)

¶All data are the geometric mean of three replicates. Standard deviation is shown in paretheses.
*Percentage (and log units) of total viral removal. Statistical differences among results of the different periods within the same treatment are indicated by capital letters. Statistical differences among results of the different treatments within the same sampling time are indicated by small letters. Results with the same letter did not show significant differences ($P > 0.05$).

trials and after 8 h in MNV-1 trials. Bottles exposed to SODIS conditions showed a decrease in the infectivity capacity of their water samples after 4 h (infectivity loss of 33.4%), 6 h (66.7%) and 8 h (83.4%) in HAV trials, and after 6 h (33.4%) and 8 h (66.7%) in MNV-1 trials (Table 3). In addition, a delay in the appearance of the CPE from 2–3 days to 6 days in water samples from Bath-bottles (after 8 h of exposure) and SODIS-bottles (after 4, 6 and 8 h of exposure) was observed for MNV-1 (data not shown).

Discussion

The aim of this study was to obtain a preliminary picture of the water disinfection for HAV and MNV-1 by SODIS. To our knowledge, this is the first study that evaluates and compares by RT-qPCR the efficacy of this method to reduce and inactivate HAV and MNV-1 under natural solar conditions. In an attempt to establish the baseline of the impact of these conditions on viral RNA and infectivity, the study was carried out using distilled water as an approach with as fewer variables as possible (i.e., organic matter that could interfere in the process). On the basis of the results obtained, future studies with natural waters (ground, river or tap water), which are likely to be used in developing countries, could be designed in order to optimize SODIS method for viral elimination.

The procedures employed involved the inclusion of reliable controls of RNA extraction and amplification steps. Results showed that extraction and RT-qPCR efficiencies did not showed important variations between HAV and MNV-1, making data consistent and suitable for quantification. SODIS is recommended to be practiced in regions with > 500 W × m^{-2} of global sunlight irradiance during 35 h [14]. Here, global sunlight irradiance values between 800 and 1000 W × m^{-2} were recorded during 3–4 h (data not shown). A strong synergistic effect has been observed between optical and thermal inactivation processes at water temperatures above 45ºC [33,45]. However, the maximum temperatures reached in SO-

Table 3. Infectivity assays carried out with HAV and MNV-1

	HAV					MNV-1				
	Solar exposed for					Solar exposed for				
Treatment	0 h	2 h	4 h	6 h	8 h	0 h	2 h	4 h	6 h	8 h
Control	100	100	100	100	100	100	100	100	100	100
Dark		100	100	100	100		100	100	100	100
Bath		100	100	66.6	66.6		100	100	83.3	66.6
SODIS		100	66.6	33.3	16.6		100	100	66.6	33.3

Results are expressed as bottles that showed infectivity/total bottles × 100.

DIS bottles were between 38 and 40ºC and at least 4 h were necessary to reach the temperatures between 35–40ºC from the beginning of the exposure. This is an important fact since, as it was also previously suggested [8], it may be a cause of the disparity between simulated and natural sunlight results.

Results showed a significant reduction in RNA levels after 8 h under SODIS conditions (1.1 log units for HAV and 0.81 log units for MNV-1) although final RNA counts remained relatively high (~10^3 RNAc/100 ml). In addition, infectivity assays reflected a decrease in the infectivity capacity of water samples from bottles in SODIS conditions 4 h after the beginning of the exposure (Table 3). Harding and Schwab [21] have reported 0.4 log and 1.4 log reduction in infectious MNV by plaque assay after a 2.5 and 6 hour of SODIS, respectively. They have also reported better reductions in MS2 than MNV, suggesting that MNV would be highly resistant to damage by SODIS. Here, RNA removal rate and infectivity assays seem to indicate a higher resistance of MNV-1 than HAV, but without statistical differences.

The SODIS principle relies on the action of the solar UV radiation and the water temperature. The comparison between exposure conditions suggests a greater importance of sunlight radiation over the temperature as a principal factor of viral reduction. Previous studies have reported that high temperatures have a major effect on viral capsids proteins but limited effect on the viral genome [5,24,35]. In addition, a synergistic effect between heating and UV inactivation over 45ºC which leads to improved disinfection has been reported [33,45]. Although these temperatures never were reached in this study, better results were achieved with the combination of radiation and heat. Wegelin et al. [45] have reported similar findings with other viruses at comparable temperatures (<40°C).

Viral inactivation by heat relies basically in the loss of ability to bind with its host cell, by structural changes in the viral capsid proteins that disrupt the specific structures needed to recognize and bind the host cells [35,46]. Nevertheless, in this study, water samples from bottles maintained in darkness retained its infectivity capacity. Although high temperatures clearly denature capsids, the natural mode of indirect transmission of enteric viruses like HAV and NoV confers high stability in harsh environments outside the host's body, including food and water at physiological temperatures as recorded here.

On the other hand, UV radiation seems to be crucial in this study for viral reduction. The mechanisms involved in viral inactivation may be either by direct UV damage on viral components, by indirect damage by reactive intermediates, such as ROS, or both [39]. The main components of non-enveloped viruses (proteins and nucleic acids) do not absorb light at wavelengths > 320 nm, so direct damage mechanisms are conducted by UV-B light portion. The ROS mechanism, on the other hand, is initiated by UV-A light [27]. ROS mechanism was the major destroying viral capsid, as •OH have a high reactivity and the oxidative action alters membrane permeability reacting and oxidising capsid proteins, therefore diffusion of viral components to the medium occur, ending in viral inactivation.

It was reported that, although certain modifications in viral proteins can occur, the exposure to UV irradiation and ROS damages by $^1O_2$ seems to be a more strong-genome damage component, since it transforms RNA itself by dimers or RNA−RNA and RNA–protein cross-links [46]. This suggests that RT-qPCR amplification loss by UV genome damages could be an appropriate proxy for SODIS evaluations in certain conditions, like low or middle temperatures, when damages to viral capsids are minimal. However, note that other genome damages outside the amplification regions can be underestimated. In this sense, long range RT-qPCR could be a useful solution [48].

From a viral perspective, subtle differences in viral genome and capsid composition affect disinfection kinetics and mechanisms between closely related viruses [38]. Differences in nucleic acid type (single- or double-stranded DNA or RNA), genome length and structure (longer genomes offer more targets for attack) and composition (% of adjacent pyrimidines or content of guanines, the most easily oxidized bases) could account for the variability in direct and indirect sunlight damages and inactivation rates [27,30,38]. HAV HM-175 and MNV-1 have similar genome lengths (7478 and 7382 bases, respectively) and with a similar % of pyrimidine bases (51 and 48%, respectively) but with different base composition (HAV 32.9% U; 16.1% C; 29.3% A; 21.8% G and MNV-1 22.2% U; 28.9 C; 21.1% A; 27.8 G). How these differences in genomic composition could affect to the disinfection rates remains unclear and future research is needed in this sense.

With regard to the inactivation kinetic, for HAV and in lesser extent for MNV-1, a clear stabilization of the inactivating effect after 4 h is observed. This effect could not be related in a direct way to solar exposure since values of solar exposure at 6 h are still quite high (Fig. 1). Factors such as viral aggregation could affect the inactivation kinetics through subpopulations of non-easily-inactivated viruses. In this sense future experiments are needed with longer exposure times.

In summary, our results indicate that, under appropriate conditions, SODIS may be an effective and acceptable intervention against certain waterborne viruses including HAV

and NoV. Viral disinfection rates are relatively lower than bacterial disinfection rates reported in other studies, and the requireder exposure periods are longer. Although the drinking water requirements for a total protection against viral illness are not completely fulfilled, our results point out that SODIS could contribute to reduce the risk of viral infection, supporting its use as an emergency intervention for vulnerable communities. Viral inactivation of non-enveloped viruses is scarce and this study provides new comparative data on HAV and MNV-1 RNA damage and infectivity after sunlight exposure. Further research is required to determine and validate the efficacy and limits of SODIS to eliminate HAV and MNV-1 under different conditions, including in natural waters.

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