

HUMANITARIAN INNOVATION FUND Final Report

Reference Number	HIF/L/2012/4-019
Organisation Name	Intermón Oxfam

Project Title	Water Disinfection Protocols for Hepatitis E Virus (WADHE)
Location	Spain and South Sudan
Start Date	01 March 2013
Duration	18 Months
Total Funding Requested	£124,805

Partner(s)	University of Barcelona, Intermon Oxfam
Total Funding	Total HIF contribution: 124,805 £. Other contributions: 9,630 £.

Innovation Stage	Development of Standard Protocols for Hepatitis E Virus Water Disinfection
Type of Innovation	Service innovation as it would improve Water Treatment Services in a particular context based on scientific evidence
One sentence description of the innovation	Creation of evidence-based Standard Operational Procedures for Hepatitis E Virus Water Disinfection in Humanitarian Context
Project Impact Summary	The development of experimental data on HEV stability in water brings to humanitarian actors specific protocols for interventions on water or food borne HEV epidemics as well as a better understanding of risk factors and routes of HEV infection.

Reporting Period	1st March 2013 until 31st December 2014
Total Spent	134,435 £

ACTIVITIES CARRIED OUT

WADHE project have carried out several activities divided into different *Workpackages*, the details from each one are described below:

- **Workpackage 1: Create an *in vitro* model of Stability for Hepatitis E Virus.**

In order to evaluate the stability of Hepatitis E Virus to different water treatments, WADHE team have created a biological system to replicate Hepatitis E Virus and measure its presence and infectivity. In particular we produced suspensions of infective viruses concentrated by direct infection as well as by transfection and implemented infectivity assays to quantify viruses in cellular cultures of human hepatoma cells by Immunofluorescence.

- **Workpackage 2: Kinetics of disinfection for UV treatment, Chlorine treatment as mass water disinfection technologies and rapid flocculation + chlorination sachets will be described.**

To evaluate the most effective treatment to disinfect HEV from water matrices we have evaluate HEV stability towards different treatments: Chlorine treatment was evaluated with different water matrices (low and high organic material concentration) and different chlorine starting dosages.

HEV stability to UV treatment was tested by assays carried out in collaboration with Professor Regina Summer from the Medical University Vienna (Water Hygiene Institute). And rapid flocculation and chlorination sachets were evaluated in two different matrices (low and high organic material concentration) by two different commercial products for Household water usage in Emergencies.

- **Workpackage 3: Developing Standard Operational Protocols for Drinking Water Treatment in HEV outbreak settings.**

In July 2014 an internal discussion meeting among Oxfam International WaSH coordinators was taken place in Oxford to discuss in detail experimental results obtained and how to develop an operational protocol for its dissemination. After this meeting a first draft was shared among operational partners and feedbacks were included. Final protocol has become a Oxfam Technical Brief, that will be available in Oxfam online public database.

- **Workpackage 4: Characterization of Hepatitis E Virus transmission patterns in an outbreak context.**

An HEV outbreak was identified between the refugee populations in South Sudan at the beginning of 2013. In April 2013 a field study was organized by WADHE team in order to establish potential sources of faecal contamination and evidence of transmission. Epidemiological information supplied by MSF-Holland was

analysed to identify potential sources of HEV infection and water and food samples were collected in the most affected camps (Jamam and Batil camps). Samples were shipped to the reference laboratory for analysis of human viruses (Human Adenovirus as index of faecal contamination and Hepatitis E Virus). Results were shared among NGOs and submitted as a scientific manuscript for publication.

ACHIEVEMENTS

1. The field study in refugee camps has been completed. Information on the presence of faecal contamination, measuring HAdV and HEV in selected samples of drinking and source water and food has been obtained showing the presence of HAdV in water and food from household samples. These results will help to understand human viral transmission routes in these contexts and to adapt disinfection protocols. Hepatitis E Virus was not detected in the samples collected probably related to the reduction in the HEV incidence observed in this period and also the practically absence of superficial water and rain (dry season) and high temperatures. However, specific human viruses used as indicators of human faecal contamination, Human adenoviruses (HAdV), have shown that risk of transmission is higher at household level. Those findings are consistent with the epidemiological study carried out by *Epicenter*¹ in the same outbreak scenario, showing lack of obvious point-sources of infection and higher risk by close contact with infected individuals.
2. Adaptation of the HEV Kernow-C1 strain to growth in human hepatoma cells (Hep2G/C3A) has provided the bases for an *in vitro* system of Hepatitis E Virus stability evaluation. Moreover, an efficient system to evaluate infectivity by immunofluorescence has been reproduced to perform stability experiments.
3. Water disinfection treatments have been tested for HEV stability showing:
 - A. Chlorine treatment: HEV inactivation data shows a biphasic kinetic of inactivation. In order to achieve 1-log HEV reduction (90% inactivation) a chlorine concentration between 0.15-0.12 mg x min/l is needed. Current protocols establish 0,5 mg/l after 30 minutes of contact time will be sufficient to inactivate the virus.
 - B. UV treatment: Experiments performed to evaluate the effect on HEV from UV radiation have produced data on inactivation kinetics

¹ Epicentre, 2013. Risk factors for Hepatitis E in Yusuf Batil refugee camp, October-November 2012.

that shown 99 % disinfection with low fluences (100 J/m²). To reach those levels, standard UV radiation fluences for water treatment (400-600 J/m²) will be sufficient.

- C. Rapid Flocculation-Chlorination: data on HEV inactivation kinetics have shown different efficiencies between commercial preparations: PUR® inactivation of 91.39% in low turbid water and 79.58% in high turbid water. WaterMaker® inactivation 93.48% in low turbid water and 91.51% in high turbid water. All assays were done in parallel with a surrogate virus (MS2 bacteriophage) as a control of process, showing reductions of 99,8% (SD=0,23).

4. A technical protocol has been developed for better understanding and management of water sources in HEV outbreak settings.
-

METHODOLOGY

Different methodologies have been used to each activity:

- Workpackage 1: Create an *in vitro* model of Stability for Hepatitis E Virus.

The assays *in vitro* have been performed following published methods (Shukla et al 2010) and have been validated with internal controls, assuring the correct performance.

- Workpackage 2: Kinetics of disinfection for UV treatment, Chlorine treatment as mass water disinfection technologies and rapid flocculation + chlorination sachets will be described.

The experimental methodology for chlorine experiment is described in the scientific manuscript *Chlorine inactivation of hepatitis E virus and human adenovirus 2 in water*. Girones R. et al. J Water Health. 2014 Sep;12(3):436-42. doi: 10.2166/wh.2014.027.

The assays to evaluate UV disinfection were carried out in the Medical University of Vienna. UV apparatus was equipped with three low-pressure (LP) mercury vapor lamps (36 W each, ozone-free, EK 36, Katadyn, Switzerland) emitting quasi-monochromatic radiation at 253.7 nm oriented normal to the surface of the test suspension. Viral suspension was placed in a 90 mm diameter Petri dish under constant stirring during UV exposure. The viral suspensions were irradiated by duplicate with increasing fluences (0, 100, 200, 300, 400, 600, 800, 1,000 and 1,400 J/m²). For each UV fluence, samples were quantify by quantitative RT-PCR (qRT-PCR) assays and immunofluorescence assays were used for quantifying infectious HEV in cell cultures.

Finally, in order to evaluate the efficiency of the Flocculant-Chlorination commercial sachets for viral removal, we have used MS2 bacteriophage as a surrogate of RNA virus and Hepatitis E Virus. Two water matrices have been

used following EPA guidelines (Environmental Protection Agency, USA) to evaluate different scenarios: Model Surface Water (EPA#1) and Stressed Surface Water (EPA#2).

- Workpackage 3: Developing Standard Operational Protocols for Drinking Water Treatment in HEV outbreak settings.

In order to develop a practical guide for humanitarian workers we have start by writing a primary draft between WADHE team members based on experimental evidence and literature review. Afterwards, the manuscript was presented to Oxfam WASH cluster in Oxford for major comments and finally shared among Oxfam Emergency Manager Network that have completed inputs before final edition.

- Workpackage 4: Characterization of Hepatitis E Virus transmission patterns in an outbreak context.

Regarding the field study the sampling points for the environmental study were mainly sources of drinking water: 5 boreholes and 2 pipelines before treatment, and 9 surface water points from the *hafirs* (local ponds). Moreover, water and food samples (9 water and 13 food samples) were collected from 5 households in each camp with at least two reported onsets of jaundice in the last month. The methods used for the environmental study are based on viral concentration by glass wool filtration at field level with a further elution and molecular analysis. All processes were validated with internal controls at process and detection level, assuring the correct performance of the sampling protocols.

MAJOR OBSTACLES

Regarding WP1, the Hepatitis E Virus strain Kernow-p6, used in the experiments replicates more slowly *in vitro* (20-25 days) than expected, expanding the time to obtain high titres and limiting disinfection experiments. To overcome this problem new protocol of infection was used based on transfection of infectious cDNA by electroporation.

Regarding the activities carried in WP4, the study was carried out during the late phase of the South Sudan HEV outbreak (April 2013), therefore HEV was no detected in any sample in this particular outbreak setting. However, the results obtained on human viral contamination (HAdV) highlight the risk of faecal-oral transmission at household level and disinfection at this level has to be considered a priority.

BENEFICIARIES/HUMANITARIAN INTERVENTIONS IMPACTED

Humanitarian Organizations facing HEV outbreaks will have more information based in experimental data to adapt their interventions and improve their impact to prevent HEV transmission by water matrices.

PARTNERSHIPS AND COLLABORATION

Collaboration between Oxfam Intermon and University of Barcelona has been very fluid throughout the project by frequent meetings to discuss accomplished steps. MSF (Holland and Belgium) collaborated with the program when HEV outbreak in South Sudan was identified as a potential research scenario. Following the study, MSF collaboration was extended by training WASH teams on monitor viral contamination in Brussels, September 2013 (Appendix E).

DISSEMINATION

Major scientific results produced during the project have been published or/and submitted in specialized journals: *Chlorine inactivation of hepatitis E virus and human adenovirus 2 in water*. Girones R. et al. Published in Journal of Water and Health 2014 Sep;12(3):436-42. doi: 10.2166/wh.2014.027 (Appendix A). *Investigation during a Hepatitis E Outbreak in Maban refugee camps, South Soudan*. Guerrero-Latorre L. et al. Submitted 2014 (Appendix B).

Moreover, the whole project was presented as an oral presentation in the Humanitarian Innovation Conference 2014 (Appendix C), chosen as a pertinent meeting to present final results of the study and discuss among humanitarian stakeholders about the final parameters for a Standard Protocol of Water Disinfection of Hepatitis E Virus in Emergencies.

And finally, the complete protocol will be available online in Oxfam database (Appendix D).

TRANSFERABILITY

WADHE experience has create evidence-based data on water treatment towards Hepatitis E Virus, an important water-borne pathogen affecting areas with low sanitation. Taken this generated information, organizations and enterprises in charge of water & sanitation can apply improved management of disinfection in their interventions based on our original research.

