April 2013

Environmental Investigation in Maban, South Soudan

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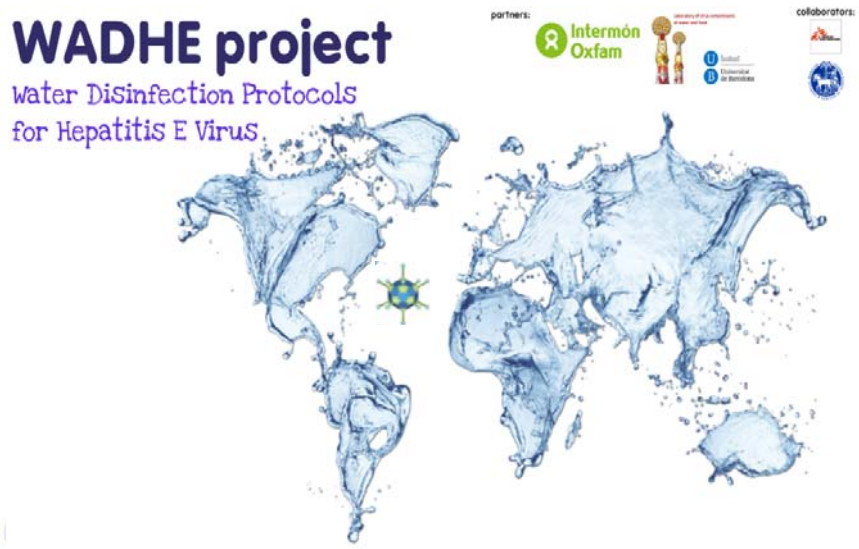
Preliminary Results



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Introduction

HEV is recognized as an important pathogen in tropical and subtropical regions and is one of the two leading causes of acute hepatitis in adults in North Africa, Asia and the Middle East. No much information on stability in water and food neither environmental circulation of this pathogen is being described due to the complexity of analytical methods.

WADHE project (Water Disinfection protocols for Hepatitis E) has as a main objective to develop protocols for Hepatitis E Virus (HEV) disinfection in outbreak settings. Complementarily, a field study is programmed to evaluate potential routes of HEV transmission in a real outbreak scenario.

Our group of research has developed a range of protocols for detect and track viral human contamination applicable to field studies in unstable settings as refugee's camps (Pina et al 1998; Hundesa et al., Albinana-Gimenez et al, Guerrero-Latorre et al, 2011). Moreover methods to identify Hepatitis E Virus and to quantify viral indicators by Microbial Source Tracking tools in water and food are ready to use to characterize the sources of transmission.

Just after the project initiation (March 2013), it was notified by Intermon Oxfam that significant HEV epidemics were active in several refugee camps in South Sudan (Maban Contry). A quick field study was organized with MSF to obtain information on the potential transmission routes for the virus, and potential management problems. Laura Guerrero from the UB team travelled to South Sudan to perform the environmental evaluation.

Since the epidemic starts in July 2012, Maban Country has been the most affected area with >8000 cases of HEV. At the moment of the field visit (April w14), Batil camp presents and incidence of 61 cases in epi week 14, which suggest that transmission, could be still active within the area although the trend is clearly decreasing. In other hand, at Jamam camp has been slightly increasing numbers of cases in the last 3 weeks (w14: 76 cases).

Methods

Data collection and sampling

Epidemiological information from Maban refugee camps was collected from MSF-Holland and MSF-Belgium medical teams. Hepatitis E case definition was defined as a patient with a recent onset of scleral icterus or 'yellow eyes' plus the presence of one or more typical symptoms of hepatitis E (malaise, anorexia, epigastric discomfort, or nausea) and exclusion of malaria by rapid test or microscopy examination.

Sampling programs were defined in two selected settlements: Batil and Jamam refugee camps. The analysis took place in April 2013 at the pick of the dry season and when the epidemiological curve was decreasing in all the affected camps.

Sampling points were mainly sources of drinking water such as, wells, boreholes and pipelines before treatment, and surface water from the *hafirs* (local ponds). Moreover, water and food samples were collected from households in each camp with at least two reported onsets of jaundice in the last month. Samples were treated to concentrate viruses and kept at -20ºC until shipping to the reference laboratory.

Samples from Batil Refugee Camp

Three surface water samples were collected from the water point drainages, not being used for consumption but we reported domestic animals drinking and children playing. Another 4 groundwater samples were taken from hand pumps inside the camp plus one groundwater sample of an open well by the river bed few kilometers out of the camp. Four households with at least two Acute Jaundice Syndrome reported in the last month were visited and water storage at home and food prepared in the same day were collected for analysis. Moreover, stool from a domestic donkey was sampled (Appendix A).

Samples from Jamam Refugee Camp

Four *hafirs* (local ponds) in Jamam camp were sampled as they had water remaining from the raining season. Two groundwater points outside the camp were sampled before treatment as they were motor-pumped sources of the pipeline water supply being treated by chlorination before distribution. Four households with at least two Acute Jaundice Syndrome reported in the

last month were visited and water storage at home and food prepared in the same day were collected for analysis. Moreover, stool from a domestic pig was sampled (Appendix B).

Virus concentration

The method used to concentrate virus particles from all types of water samples was chosen on the basis of previous studies and used glass wool columns and gravity flow (Albinana-Gimenez et al. 2009, Gillian et al 1988).

Food samples were processed to concentrate virus attached following method described in ISO/TS 15216-1:2013. Faecal material was treated as previously published (Guerrero-Latorre et al. 2011).

Enzymatic amplification of viral genomes

Viral RNA and DNA were extracted using QIAamp Viral RNA Extraction kit (Qiagen). Human faecal contamination was quantified using HAdV as a viral indicator of human faecal contamination (Pina et al. 1998; Albinana-Gimenez et al. 2009).

Water and faecal samples were tested for evidence of HEV by RT-PCR using primers targeting HEV ORF2/ORF3 overlapping region (Inoue et al 2006) capable of detecting a wider spectrum of HEV types.

Control process

The MS2 bacteriophage (RNA virus) was used as a control process and two field water samples were collected and spiked with MS2 in Sudan and were treated, transported and analysed together with other viral parameters at the UB. MS2 is determined by quantitative PCR in the initial seed and from processed samples. Although the protocol used for virus concentration has not been optimized for MS2 (more sensitive to pH than other viruses analysed), still MS2 may be used as control process for virus in water. Two samples of 10-L pre-acidified water were seeded with a final concentration of 3E+08 GC/L.

Results

Epidemiological data

Clinical assessments from MSF-Holland indicate that first onsets of Acute Jaundice syndrome appeared in Jamam camp in week 22 (June) of 2012. A real cluster of patients began in August 2012 in both camps, increasing incidence, especially in Batil, until the highest pick reported in January 2013 with 500 cases reported per week in Batil MSF-clinic. Since then, the number of cases has decreased consistently, coinciding with the dry season. The punctual sampling has taken place when the outbreak was reduced significantly in number of cases (Figure 1).

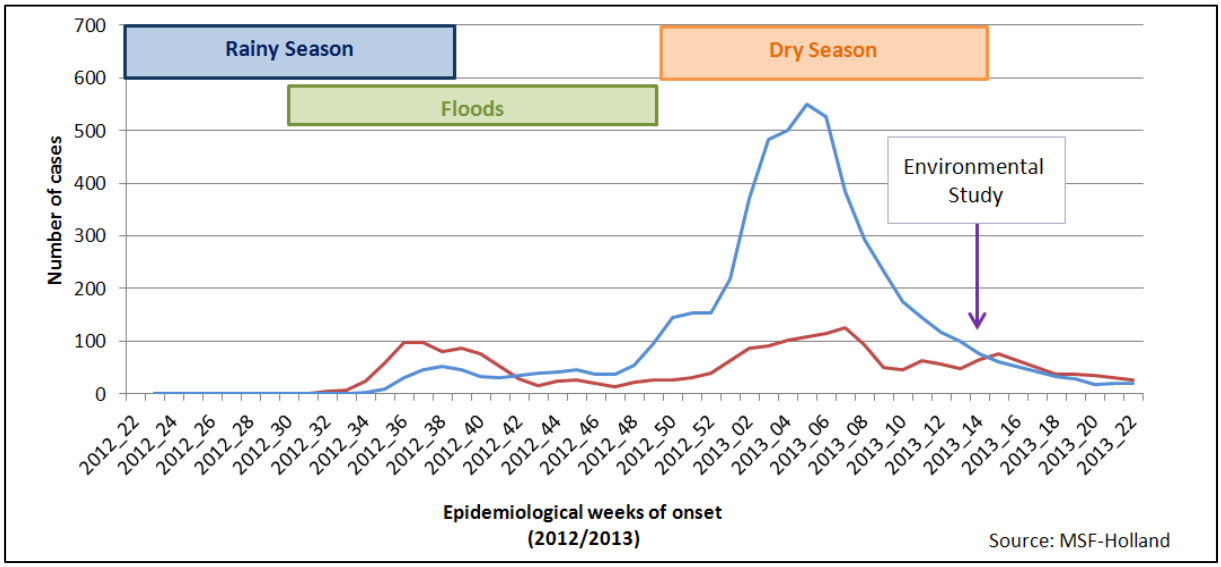

Figure 1 | Incidence of acute jaundice syndrome in Batil (Red line) and Jamam (Blue line) camps

Detection of Human Contamination in Water and Food Samples

Human adenoviruses were no detected in any of the analyzed groundwater samples neither surface water samples. Although contamination at the points of source of drinking water was no detectable, indicators of human viral contamination were present in 3/4 water samples from households of Batil camp and in two of four analyzed

household water samples from Jamam Camp. Food samples from the same households were also contaminated by HAdV in one sample tested in Batil camp (Table 1).

Table 1 | Microbiological analyses from water sources & food samples

Type of Sample	Camp	Sampling location	Quantity (L o gr)	Human Adenovirus (GC/L or GC/gr)
Surface Water	Batil	SW1. Tapstand runover	10	ND
		SW2. Handpump runover	1	ND
		SW3. Tapstand runover	5	ND
	Jamam	SW4. Hafir	10	ND
		SW5. Hafir	8	ND
		SW6. Hafir	5	ND
		SW7. Shallow well	10	ND
		SW8. Hafir	5	ND
Ground Water	Batil	GW1. Open Well	10	ND
		GW2. Borehole	10	ND
		GW3. Borehole	20	ND
		GW4. Borehole	20	ND
		GW5. Borehole	10	ND
	Jamam	GW6. Shallow well	10	ND
		GW7. Borehole	10	ND
Household Water	Batil	HW1. Water storage	10	ND
		HW2. Water storage	5	130,75
		HW3. Water storage	2	2,18
		HW4. Water storage	10	2,71
	Jamam	HW5. Water storage	10	71,87
		HW6. Water storage	7	ND
		HW7. Water storage	10	ND
		HW8. Water storage	8	16,55
Household Food	Batil	HF1. Uncooked Kisra*	34,45	ND
		HF2. Uncooked Kisra	24,3	ND
		HF3. Uncooked Kisra	30,8	ND
		HF4. Uncooked Kisra	30,5	0,7
	Jamam	HF5. Cooked Kisra	26,25	ND
		HF6. Cooked Kisra	30,6	ND
		HF7. Cooked Kisra	28,5	ND
		HF8. Cooked Kisra	30,8	ND

* Kisra is a thin pancake-like made from whole sorghum flour

HEV analysis

Hepatitis E Virus was not detected in any water, food or stool sample neither from Batil camp nor from Jamam camp. Controls were correct. Theoretical limits of detection were between 1.76 and 24 viral particles per liter of water sample (mean = 7.24 GC/L) and between 1.7 and 0.9 viral particles per 10 grams of food sample (mean = 1.02 GC/ 10gr).

The control process, MS2 coliphage was positive in the two spiked samples. Calculated recoveries in the two pre-acidified water samples: surface water: 0.5%, household water: 1%. These results are similar to results described in previous studies analysing lake water with recoveries of 4% (Francy et al. 2013).

Discussion / Conclusions

1. The environmental reservoir of Hepatitis E Virus was not found probably related to the reduction in the HEV incidence observed in this period and the absence of superficial water and rain (dry season) as well as high temperatures, environmental conditions which have already indicated low stability for HEV (Emerson et al 2005). In fact, active transmission and higher levels of HEV are reported during the rains (Bile et al 1994, Ippagunta et al 2007)
2. Human adenoviruses used as indicators of human faecal contamination have been detected in household water samples and food. The presence of HAdV, have confirmed that risk of transmission is high at household level. Those findings are consistent with the epidemiological study carried out by *Epicentre* (Epicentre 2013) in the same outbreak scenario, showing lack of obvious point-sources of infection and higher risk by close contact with infected individuals. Moreover, a case-control study carried out in last HEV outbreak in Uganda has found major risk factors of infection in variable related to hygiene practices at household level (Howard et al. 2010). Overall, findings suggest that, in outbreaks scenarios, transmission may be multifactorial and include household-level.
3. The information obtained contributes to the identification of water and food as sources of viral contamination at household level in the analysed period, with independence of the potential role of flooding or heavy rain may have on the microbiological quality of water sources.
4. The project will continue at the laboratory level by further research on HEV stability by different methods in order to establish disinfection protocols for HEV outbreak based in scientific evidence.

Any further questions or comments, please don't hesitate to contact us:

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Appendix A: Sampling in Batil camp

Sampling has been completed in Batil camp (11th until 16th April). Following samples have been collected:

- Camp level:
  - 3 samples of Surface Water inside the camp
  - 4 groundwater samples inside the camp and 1 groundwater from the riverside (river has dried)
- Household level (5 households with at least 2 HEV cases in the last month)
  - 5 samples from water storage at the households
  - 10 food items taken from the households
  - 1 stool sample from domestic donkey

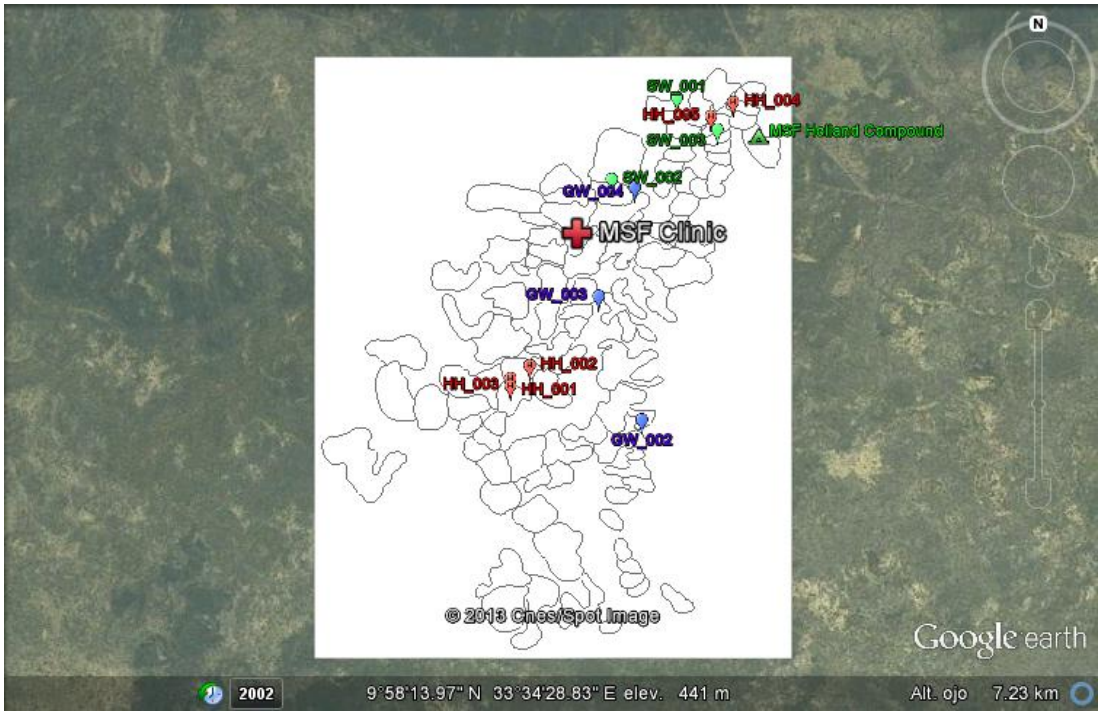

Figure 1. Batil camp sampling points (GW: groundwater, SW: surfacewater, HH: household)

Considerations:

- Main surfacewater have dried already (including the CDC positive pond), instead of those we have collect drain water from handpumps or tapstands.
- Groundwater sources sampled where no treated
- Household storage water sampled had no free residual chlorine (they don't like chlorine taste, so they go to non-chlorinated sources)
- Water samples have been concentrated (10 to 20 liters) by glass-wool non preacidified protocol (I just got the Acid today, so I can do next sampling with preacidification step)
- Glasswools, food items and stools are kept at -20ºC until shipping

Photos:

Dried surface water source Drainage from Water point

Woman cooking Kisra (Sorghum flour) Household water storage recipients

Sampling and concentrating by glass-wool filters

Appendix B: Sampling in Jamam camp

Sampling has been completed in Jamam camp (17th until 21st April). Following samples have been collected:

- Camp level:
  - 5 samples of Surface Water inside the camp (reported use from population as a drinking water when there is not water in Oxfam distribution system)
  - 2 groundwater samples inside the camp (main source for drinking water in Oxfam distribution system)

- Household level (4 households with at least 2 HEV cases in the last month)
  - 4 samples from water storage at the households
  - 4 food items taken from the households
  - 1 stool sample from domestic pigs

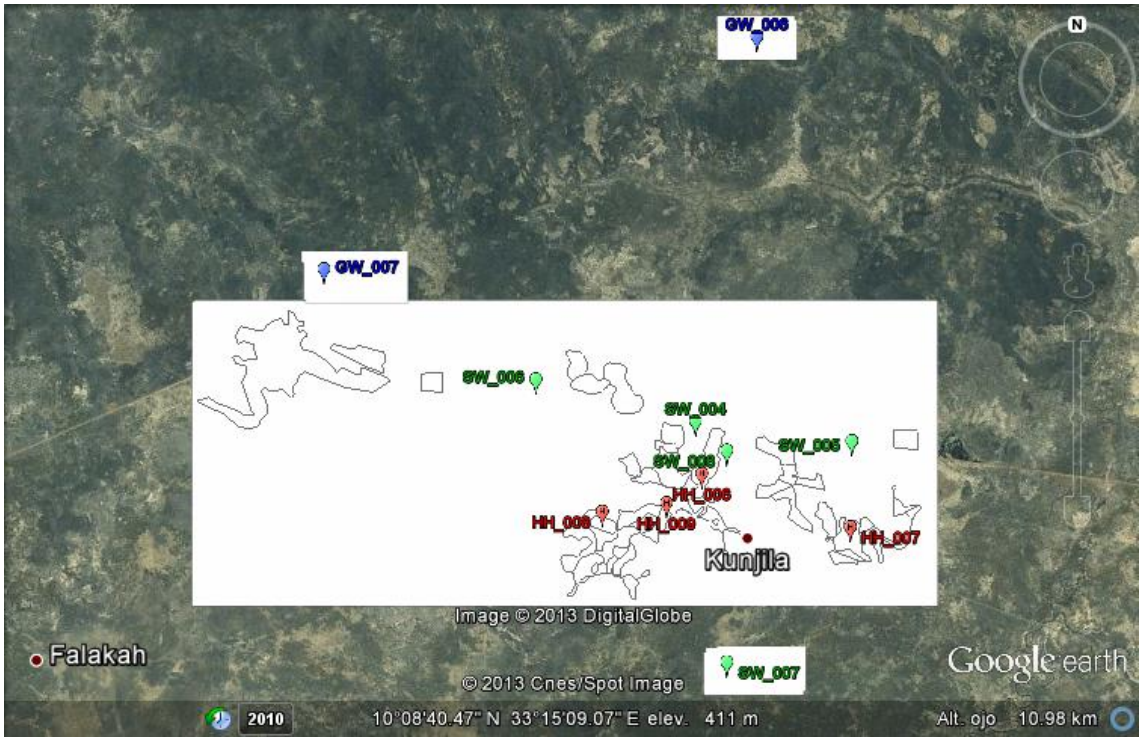

Figure 1. Jamam camp sampling points (GW: groundwater, SW: surfacewater, HH: household)

Considerations:

- Still surfacewater available within the camp and being used when breakdowns of Oxfam distribution system. The organization has experience some cuts on the pumping activities due to the motorpump problems, during those periods, watermaker sachets have been distributed in the surfacewater points to treat water.
- Groundwater sources sampled where taken before chlorination
- Household storage water sampled had no free residual chlorine
- Water samples have been concentrated (10 to 20 liters) by glass-wool with pre-acidified step protocol.
- Glasswools, food items and stools are kept at -20ºC until shipping