MAJOR ARTICLE

A Large Outbreak of Hepatitis E among a Displaced Population in Darfur, Sudan, 2004: The Role of Water Treatment Methods

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(See the article by Boccia et al. on pages 1679–84)

Background. The conflict in Darfur, Sudan, was responsible for the displacement of 1.8 million civilians. We investigated a large outbreak of hepatitis E virus (HEV) infection in Mornay camp (78,800 inhabitants) in western Darfur.

Methods. To describe the outbreak, we used clinical and demographic information from cases recorded at the camp between 26 July and 31 December 2004. We conducted a case-cohort study and a retrospective cohort study to identify risk factors for clinical and asymptomatic hepatitis E, respectively. We collected stool and serum samples from animals and performed a bacteriological analysis of water samples. Human samples were tested for immunoglobulin G and immunoglobulin M antibody to HEV (for serum samples) and for amplification of the HEV genome (for serum and stool samples).

Results. In 6 months, 2621 hepatitis E cases were recorded (attack rate, 3.3%), with a case-fatality rate of 1.7% (45 deaths, 19 of which involved were pregnant women). Risk factors for clinical HEV infection included age of 15–45 years (odds ratio, 2.13; 95% confidence interval, 1.02–4.46) and drinking chlorinated surface water (odds ratio, 2.49; 95% confidence interval, 1.22–5.08). Both factors were also suggestive of increased risk for asymptomatic HEV infection, although this was not found to be statistically significant. HEV RNA was positively identified in serum samples obtained from 2 donkeys. No bacteria were identified from any sample of chlorinated water tested.

Conclusions. Current recommendations to ensure a safe water supply may have been insufficient to inactivate HEV and control this epidemic. This research highlights the need to evaluate current water treatment methods and to identify alternative solutions adapted to complex emergencies.

Since February 2003, the conflict in Darfur, western Sudan, has resulted in 1.8 million persons being internally displaced [1]. In December 2003, Médecins Sans Frontières started health and nutritional programs in Zalingei, Niertiti, Mornay, and El Genina camps for internally displaced persons. Surveys performed in these sites showed high mortality rates [2].

During the last week of June 2004, there were 6 cases of acute jaundice reported from western Darfur to the Early Warning Alert and Response system developed by the World Health Organization (WHO), the Sudanese Ministry of Health, and other organizations. The following week, 18 new cases were reported, with 4 from Mornay Camp. Two were in pregnant women in coma; the women died within 48 h. By the end of July 2004, the diagnosis of hepatitis E [3] was confirmed by PCR performed on serum specimens at the Naval Medical Research Unit 3 (Cairo) [4]. By 7 August 2004, of 1006 cases reported in western, northern, and southern Darfur, 609 (61%) were from western Darfur. Of them, 204 were from Mornay camp.

We conducted an investigation to describe the magnitude of this hepatitis E outbreak and to identify pos-

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sible sources of infection and risk factors for asymptomatic and symptomatic infection with hepatitis E virus (HEV). Clinical findings and the description of the outbreak among pregnant women are reported elsewhere [5]. The investigation was approved by the State Ministry of Health in El Genina and by the WHO in Khartoum and El Genina. Records were made anonymous.

METHODS

Site and population. During June–September 2003, the population of Mornay increased from 6000 (in the village) to 78,800 (in the village and the camp). Most internally displaced persons were living in wood and mud huts covered by plastic sheeting. They were dependent on external aid. They had received plastic sheeting, blankets, soap, and jerry cans. Access to water (<15 L/person/day) and latrines (1 per 40 persons) was insufficient. The camp was divided into 13 geographic districts, grouped into 4 main areas. Water was taken from 2 main sources: pipelines providing unchlorinated groundwater pumped deeply from the *wadi* (a sort of river that partially dries in the dry season) (2 pipelines drilling, respectively, 50 m and 16 m deep), and pipelines established by Médecins Sans Frontières in March 2004 providing surface *wadi* water that was later chlorinated (4 m deep). Other minor sources were water taken directly from the *wadi* and water taken from protected wells (7 hand pumps).

Description of the outbreak. For the purpose of epidemiological surveillance, a patient with hepatitis E with jaundice was defined as a person who developed acute onset of jaundice since 1 July 2004 (defined as a yellow coloration of the sclera). Case patients with a positive result of a malaria rapid diagnostic test (Paracheck-Pf; Orchid Bio-Medical Systems) were excluded. We collected clinical and demographic data from each case patient recorded at the hospital outpatient and inpatient departments. Hospital deaths were recorded.

Sources of infection and risk factors of hepatitis E. We conducted 2 studies to identify the source of and risk factors for HEV infection: a case-cohort study of HEV infection with jaundice and a retrospective cohort study of asymptomatic HEV infection. In the case-cohort study of HEV infection with jaundice, we selected a sample of outbreak cases and a random sample of the Mornay population (denominator) [6, 7]. Assuming that sampling of cases and population was independent of exposure, the OR of exposure between cases and the population sample is the unbiased estimate of the risk ratio (RR) for disease [6, 7]. To identify risk factors for asymptomatic HEV infection, we used the random sample of the Mornay population that was selected for the case-cohort study to perform a retrospective cohort study.

A case of HEV infection with jaundice was defined as a case that occurred in a person >2 years of age, who had lived in Mornay since 15 June 2004, who presented with yellow coloration of the sclera between 14 August and 5 September, and who had ⩾1 of the following symptoms: weakness, abdominal pain, fever or vomiting, and laboratory-confirmed acute HEV infection.

To recruit HEV-infected case patients with jaundice, between 25 August and 5 September, home visitors carried out a house-to-house census visit in all sections of Mornay camp and sent all potential cases of jaundice with onset since August 14 to the study clinic. Cases in persons who had died were eligible for inclusion and were verified by hospital records when available.

Members of the population sample were selected at random from each of the 13 districts of the camp, with probability of selection proportional to size, using a 3-stage random cluster sampling technique [8]. Persons in this group with past immunity were excluded. Persons who had died and hospitalized patients were also eligible for inclusion. Informed consent was requested from case-patients and from members of the population sample (obtained from a relative ⩾18 years old for subjects <18 years old).

We calculated a sample size of 80 cases and 160 members of the population sample, which included a 20% uncertainty margin and assumed the following: ratio population sample/cases, 2; RR worth detecting, 2.5; percentage exposed in the population sample, 50%; power, 80%; and α error, 0.05.

We collected personal and environmental data through a structured questionnaire. Personal information included age, sex, internally displaced person or resident, area of residence, family size, education level, and clinical signs and symptoms. Environmental information included source of drinking water, type and number of containers for storing water, presence and use of soap, and latrine use. One investigator assisted by a translator conducted the interview, performed in Arabic or in the local dialect. If a child was unable to answer or if the case had occurred in a person who had died, the closest relative aged ⩾18 years was interviewed.

Information for each participant was recorded in individual records, entered into EpiData, version 3.0 (EpiData Association), and checked for inconsistencies. Statistical analysis was performed using Stata, version 8.2. We estimated the RR by computing the OR and 95% CIs. To control for confounding, we included all variables significant in the univariate analysis ($P < .3$) in a logistic regression model, along with age and sex, using backwards selection (likelihood ratio test; $P < .05$). The attributable risk percentage was calculated as OR − 1/OR [9].

For the purpose of the second study, a case of asymptomatic HEV infection was defined as acute HEV infection confirmed by laboratory results (see below) but without symptoms of infection.

Laboratory investigations. Blood and stool samples were

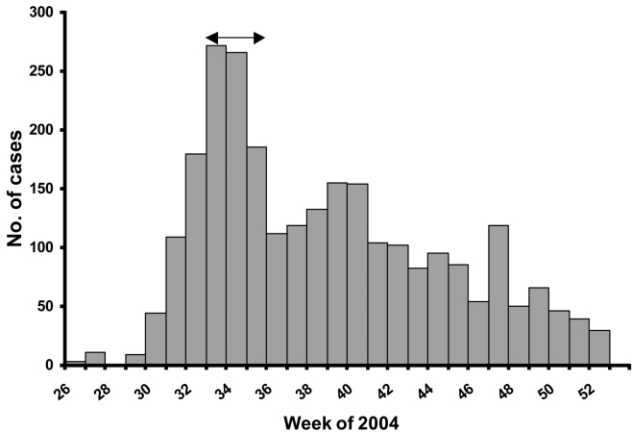

Figure 1. Weekly distribution of 2621 hepatitis E cases recorded at Médecins Sans Frontières hospital, Mornay Camp, western Darfur, Sudan, 26 July–31 December 2004. The arrow indicates the 3-week period (14 August–5 September) of onset of acute jaundice for cases included in the case-cohort study.

collected at the study clinic. We collected 5-mL blood samples from case patients and members of the population sample. Serum was stored at 4°C–8°C after manual centrifugation. Human stool specimens were taken with rectal swabs. Rectal swabs and serum were taken from a nonrandom sample of sick donkeys. Transport was done using a cold chain (4°C–8°C). We did not test for infection due to hepatitis A, B, C, or D viruses. HEV analyses were performed at the National Reference Centre for enterically transmitted hepatitis (Val de Grâce Hospital, Paris, France). All human serum samples were tested with IgG and IgM anti-HEV ELISA kits (Genelabs Diagnostics). All human and animal samples were tested for HEV RNA according to a published method [10, 11]. Acute HEV infection was defined as an optical density (OD) ratio of ⩾3 for HEV IgG, an OD ratio of >2 for HEV IgM, and/or the presence of HEV RNA in stool or serum specimens. Past immunity to HEV was defined by anti-HEV IgG immunoreactivity (OD ratio, >0 and <3), absence of IgM immunoreactivity, and a negative result of PCR for HEV [12].

Environmental investigation. Samples from various water sources were taken in sterile tubes, transported at 4°C–8°C, and analyzed within 48 h for the presence of fecal coliforms at the Central Public Health Laboratory, Ministry of Health (Khartoum, Sudan).

RESULTS

Description of the Outbreak

We identified 2621 cases of acute jaundice with onset between 26 June and 31 December 2004. Among these, 1407 (53.7%) occurred in female patients. We observed a first large peak during weeks 33–34 (mid-August) and a smaller peak during weeks 39–40. Cases decreased steadily thereafter (figure 1). The overall reported attack rate was 3.3% (2621 of 78,800 persons) and varied in the 4 administrative areas: 2.4% (566 of 23,710 persons) in Al salaam, 3.0% (651 of 21,825 persons) in Al wadi, 3.7% (865 of 23,588 persons) in Al almtidade, and 5.2% (508 of 9708 persons) in Al djebel ($P = .67$). The attack rate was 3.1% among males and 3.0% among females (χ^2; $P = .42$). A total of 48.7% of the population was aged ⩾15 years, and 51.3% was aged <15 years [2]; the attack rates were 4.3% (1639 of 38,375 persons) and 2.4% (982 of 40,425 persons) in each respective group ($P < .001$).

During the same period, 253 case patients were admitted to the hospital, of whom 72 (39.1% of those for whom clinical records were available) had hepatic encephalopathy. Of the 45 recorded case patients who died (overall case-fatality rate, 1.7%), 19 were pregnant women (specific case-fatality rate, 31.1% [19 of 61 pregnant women]) [5].

Case-Cohort Study Results

Microbiological analysis. From 25 August to 5 September 2004, 82 case patients were recruited, and 162 persons were randomly selected in the population sample (figure 2). Blood specimen results were interpretable for 75 case patients and for 143 individuals in the population sample. Two case patients (2.7%) and 37 individuals (25.9%) in the population sample had past immunity to HEV (before the outbreak). Acute HEV infection was diagnosed in 73 case patients and in 49 subjects from the population sample (figure 2).

Risk factors for HEV infection with jaundice. Overall, 73 cases and 106 persons in the population sample were included in the case-cohort study (figure 2). Two of the case patients had died of hepatitis. Eleven women were pregnant (4 were case patients). The risk of hepatitis E with jaundice was higher

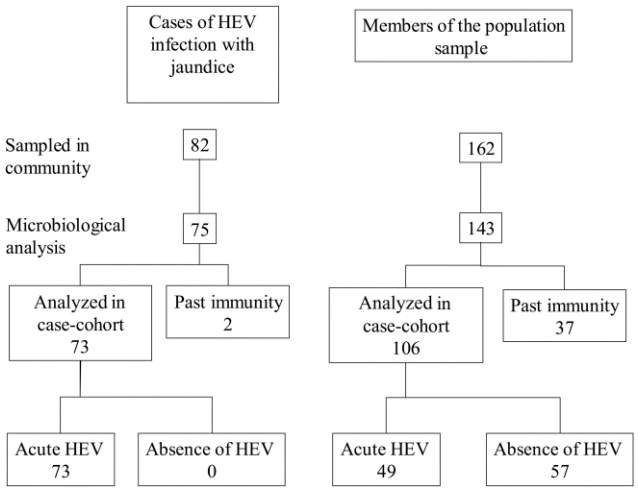

Figure 2. Cases of hepatitis E virus (HEV) infection with jaundice and members of the population sample according to laboratory results, Mornay Camp, western Darfur, Sudan, August–September 2004.

in the group of subjects aged 15–45 years old and among people who drank chlorinated surface water (table 1). The multivariate analysis supports the role of being in the age group of 15–45 years (OR, 2.13; 95% CI, 1.02–4.46) and consumption of chlorinated surface water (OR, 2.49; 95% CI, 1.22–5.08) in the risk for HEV infection with jaundice. Drinking chlorinated surface water potentially accounted for 59.8% (attributable risk percentage) of HEV infection with jaundice. When 7 case patients and 19 population sample members for whom no laboratory results were available were included (case definition of acute jaundice), results did not change (data not shown).

Risk factors of asymptomatic HEV infection. We used 104 members of the population sample as a retrospective cohort (2 cohort members were excluded because of missing values). Among them, 49 (46.2%) had acute asymptomatic HEV infection (figure 2). The risk of asymptomatic HEV infection was higher in those aged 15–45 years (RR, 1.47) and in those aged 0–14 years (RR, 2.08) than in those aged >45 years (table 2). People drinking chlorinated surface water were more likely to acquire asymptomatic HEV infection (RR, 1.26) than were those drinking unchlorinated water group. However, none of these associations was statistically significant.

Table 1. Risk factors for hepatitis E virus (HEV) with jaundice, Mornay population, western Darfur, Sudan, September 2004.

Exposure	Case patients (n = 73)[a]	Members of population sample (n = 106)	RR estimate (95% CI)
Age group, years			
>45	5	17	Reference
15–45	52	52	3.40 (1.17–9.90)
0–14	16	37	1.47 (0.46–4.58)
Sex			
Female	43	74	Reference
Male	30	32	1.61 (0.86–3.01)
Size of the family			
≤6 persons	36	66	Reference
>6 persons	37	40	1.70 (0.93–3.10)
Presence of animals in the house			
No animals	27	55	Reference
At least one animal	46	51	1.84 (1.00–3.38)
Used to collect water from river			
Never	45	78	Reference
At least sometimes	27	28	1.67 (0.88–3.18)
No. of water reservoirs in house			
1	6	20	Reference
2	25	37	2.25 (0.79–6.40)
>2	42	49	2.86 (1.05–7.78)
Source of drinking water			
Borehole, unchlorinated	17	42	Reference
Surface water, chlorinated	51	57	2.21 (1.12–4.36)
Other	2	7	0.71 (0.13–3.75)
Use of latrines			
At least sometimes	62	83	Reference
Never	11	23	0.64 (0.29–1.41)
Wash hands before eating			
At least sometimes	61	82	Reference
Never	12	24	0.67 (0.31–1.45)
Wash hands after defecating			
At least sometimes	65	85	Reference
Never	8	21	0.50 (0.21–1.20)

NOTE. RR, risk ratio.

[a] Values <73 reflect missing data.

Table 2. Risk factors for asymptomatic hepatitis E virus (HEV) infection in a random sample of Mornay population, Darfur, Sudan, September 2004.

Exposure	No. of individuals		Risk of asymptomatic HEV infection, %	RR (95% CI)
	All (n = 104)	Asymptomatic HEV infection (n = 49)		
Age group, years				
>45	17	5	29.4	Reference
15–45	51	22	43.1	1.47 (0.48–4.47)
0–14	36	22	61.1	2.08 (0.67–6.43)
Sex				
Female	73	35	47.9	Reference
Male	31	14	45.2	0.94 (0.45–1.99)
Size of the family				
≤6 persons	65	29	44.6	Reference
>6 persons	39	20	51.3	1.15 (0.57–2.30)
Presence of animals in the house				
No	54	23	42.6	Reference
Yes	50	26	52.0	1.22 (0.62–2.41)
Ever collected water from river				
Never	76	35	46.1	Reference
Yes	28	14	50.0	1.09 (0.51–2.31)
No. of water reservoirs in house				
1	19	6	31.6	Reference
2	37	16	43.2	1.37 (0.46–4.07)
>2	48	27	56.3	1.78 (0.63–5.00)
Source of drinking water				
Borehole, unchlorinated	42	17	40.5	Reference
Surface water, chlorinated	55	28	50.9	1.26 (0.61–2.59)
Other	7	4	57.1	1.41 (0.37–5.45)
Use latrines				
At least sometimes	81	37	45.7	Reference
Never	23	12	52.2	1.14 (0.51–2.54)
Wash hands before eating				
At least sometimes	80	35	43.8	Reference
Never	24	14	58.3	1.33 (0.62–2.88)
Wash hands after defecating				
At least sometimes	83	38	45.8	Reference
Never	21	11	52.4	1.14 (0.50–2.61)

NOTE. RR, risk ratio.

Environmental investigations. Coliforms were found in 5 (41.7%) of 12 water samples. In the 2 *wadi* samples, concentrations were high (56/100 mL and uncountable coliforms/100 mL), whereas, in the 3 other positive samples (2 water lines and 1 house container), concentrations were <10 coliforms/100 mL. The free residual chlorine concentration measured at the tap from the chlorinated system was between 0.3 and 0.6 mg/L.

Two of the 5 serum samples from donkeys exhibited a low reactivity for HEV RNA, suggesting that they had been in contact with HEV. Stool samples obtained from 19 donkeys all tested negative for HEV by PCR.

DISCUSSION

This outbreak of HEV infection is, to our knowledge, the largest documented in displaced populations [13, 14]. The attack rate of acute jaundice is probably higher than the 3.3% rate recorded, because we recorded only cases with acute jaundice who sought treatment at a health facility. Active case finding identified a number of community cases with acute jaundice 6 times higher than that recorded at the hospital OPD. Thus, we cannot exclude the possibility that 15,000 cases ($78{,}000 \times 0.033 \times 6$) of acute jaundice occurred in Mornay. Considering that 49 (34%) of 143 subjects in our population sample had

asymptomatic infection (figure 2), up to 26,520 additional persons could be infected [15–17]. Such a high ratio of asymptomatic to clinical infection has already been described: for example, 4:1 in Pakistan [15] or 2.8:1 in Nepal [16]. That other diagnoses could account for an important part of acute jaundice is not supported by surveillance data in Darfur.

Our results suggest that risk of acute HEV infection with jaundice is higher among persons aged 15–45 years and that risk of asymptomatic HEV infection is highest in those aged <15 years, which was also seen in previous studies [18, 19].

Our most striking finding is the increased risk of asymptomatic and clinical HEV infection with drinking water from chlorinated sources. The methods chosen for the studies and their limitations need to be discussed. The choice of a case-cohort design rather than a traditional case-control study was guided by the high incidence expected. In a case-cohort study design, in which the comparison group is a sample of the population at risk, the OR of exposure is an unbiased estimate of the RR. This is not true in a traditional case-control design (ill versus non-ill), in which the OR overestimates the RR when the disease is frequent (>5%–10%). The assumption of a case-cohort study is that sampling (of cases and denominator) is unbiased with regard to the exposure experience of the source population. We assumed that exposure to water sources measured in our sample of cases and population reflects exposure in the source population at the beginning of the outbreak. We cannot exclude that some people switched sources of water before and during the 5 weeks of the study period. We had, however, no indication of such a pattern during interviews; people continued to use the closest source of water. In addition, drinking patterns may have changed after the end of the study. We cannot, therefore, extend our findings to the entire duration of the outbreak. It is also likely that all sources of water were a vehicle for the epidemic and that surface water only increased the risk. The absence of a truly unexposed population probably lowered the estimation of the RR. Our sample size was small, and 10% had no laboratory results available. However, results did not change when subjects lacking laboratory results were included in the analysis. At the time of the study, the shape of the epidemic curve was consistent with a common source that is likely to be water. Although person-to-person transmission is likely to have occurred, particularly within families, because of limited resources we did not study secondary attack rates in families, particularly during the second phase of the outbreak. Despite a small study size and results of borderline statistical significance, we believe that this study should add to the discussion of the potential role of chlorinated surface water in HEV transmission.

Water originally pumped from the river (the *wadi,* which was ~4 m deep) was contaminated. Human and animal excreta were either deposited directly in the water or washed in by rain. This was confirmed by the presence of fecal coliforms in the environmental analysis. The water was then stored and chlorinated in a bladder to attain a free residual chlorine concentration of 0.3–0.6 mg/L after 30 min (pH, <8) (Pooltester chlorine-pH, using Lovibond tablet reagents; Tintometer) [20] before being distributed through a 20-tap system. Free chlorine concentrations were checked daily at the exit of the bladder and at each tap. In all tap water samples, coliform concentrations were <10 coliforms/100 mL, indicating that levels of free chlorine residual (0.3–0.6 mg/L) were sufficient to reduce coliform load and that water could be consumed without additional treatment. However, the level of chlorination may not have been enough to inactivate HEV. Although no laboratory-based studies have directly examined the effect of chlorine on HEV inactivation, the WHO recommends a free chlorine residual of at least 0.5 mg/L for 30 min (pH, <8.0), with mean turbidity not exceeding 1 nephrometric turbidity unit [21]. Laboratory studies of calicivirus showed significant differences in viral inactivation depending on temperature, pH, water type, and state of the virus (aggregated or dispersed). This suggests that viral inactivation may be more difficult than just ensuring adequate free chlorine residual [22].

There are several other candidate explanations for the increased risk of hepatitis E when drinking chlorinated water. Collection and storage of water in containers contaminated with organic matter, or other unsafe hygiene practices, may have led to recontamination, although these unsafe practices were likely evenly distributed across water sources. A break in the integrity of the chlorinated water distribution system could have led to recontamination of treated water. This is inconsistent with the durable presence of free chlorine residual in tap water and routine verification of the integrity of the distribution system.

Because of limited resources, we did not attempt to isolate HEV in water. This requires sophisticated laboratory technology (i.e., virus filtering systems) and is rarely performed in stable settings [21]. Among animals, only donkeys were tested for HEV markers. The positive results of PCR for HEV in donkeys suggests that additional research is needed to better understand the role of domestic animals in transmission or as reservoirs of HEV.

The outbreak exploded and remained out of control after control measures were instituted at the end of July (construction of new latrines, more frequent distributions of soap). Without drilling equipment, the chlorinated river water remained an important source, perpetuating transmission.

This outbreak highlights the importance of further laboratory studies on inactivation of HEV. In the interim, we should consider that although a free chlorine residual of 0.3–0.6 mg/L is sufficient to eliminate fecal coliforms [20], it may be insufficient to inactivate HEV [21]. Standard treatment protocol

could be changed and require ⩾0.5 mg/L free chlorine residual with an increased contact time (60 min) and additional turbidity reduction measures. However, increasing the level of chlorine may result in such an undesirable smell and taste that residents revert to collecting water directly from the river. An alternate strategy in high-risk populations would be to consider adapted sources and treatment, such as deep drilling or ultraviolet light irradiation.

Hepatitis E is endemic in Sudan [17, 23], and ensuring adequate water treatment and integrity of distribution systems is a priority. The population of displaced persons in Mornay will probably remain in the near future and new population influxes may occur. Adapting the water treatment strategy is a priority to prevent future outbreaks.

Outbreak investigation and research remain difficult in complex emergencies. However, the results of our case-cohort study should help better investigate future outbreaks and assess whether water chlorination is enough to reduce HEV transmission.

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Potential conflicts of interest. All authors: no conflicts.

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