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AN OUTBREAK OF HEPATITIS E IN NORTHERN NAMIBIA, 1983

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Abstract. In 1983 in Namibia's Kavango region, epidemic jaundice affected hundreds of people living in settlements lacking potable water and waste disposal facilities. Many were Angolan refugees. The disease, which after investigation was designated non-A non-B hepatitis, was most common in males (72%), in persons aged 15–39 years, and was usually mild except in pregnant women, who incurred 6 (86%) of the 7 fatal infections. Fifteen years later, archived outbreak-associated samples were analyzed. Hepatitis E virus (HEV) was detected by reverse transcription-polymerase chain reaction in feces from 9 of 16 patients tested. Total Ig and IgM to HEV were quantitated in serum from 24 residents of an affected settlement at the outbreak's end: 42% had IgM diagnostic of recent infection and 25% had elevated total Ig without IgM, consistent with past HEV infection. The Namibia outbreak was typical hepatitis E clinically and epidemiologically. This first report of hepatitis E confirmed by virus detection from southern Africa extends the known range of HEV and highlights its risk for refugees.

INTRODUCTION

An outbreak of illness associated with jaundice occurred during the latter half of 1983 in the Kavango region of northern Namibia, centered on the town of Rundu (Figure 1). Laboratory evidence that the outbreak was neither hepatitis-A nor B infection prompted a request for an epidemiologic investigation that was conducted during a 3-day period in mid-October 1983. The investigating team was impressed by the unexpectedly high case-fatality rate among pregnant women. No etiology for the outbreak was determined, but fecal specimens from patients and serum specimens from persons residing in the affected area were collected in November 1983 and archived at −20°C.

In 1983, a new virus later designated hepatitis E virus (HEV) was identified as the cause of enterically-transmitted non-A, non-B hepatitis.[1] In the past decade, hepatitis E has been shown to be the cause of sporadic and epidemic hepatitis across central and south Asia,[2] and in northern and sub-Saharan Africa.[3–11] One of the largest outbreaks of hepatitis E, in Delhi, India (1955) was identified retrospectively by identification of antibodies to HEV in archived serum specimens.[12] Polymerase chain reaction (PCR) tests to detect HEV RNA[8,13,14] and improved serology for HEV[15–18] have greatly increased the ability of reference laboratories to identify hepatitis E disease.

These methods were applied to the archived fecal and serum specimens from the Namibia hepatitis outbreak. Herein, we characterize the clinical and epidemiological features of this outbreak and identify it as epidemic hepatitis E.

MATERIALS AND METHODS

Description of the area and environmental conditions. The Kavango region of Namibia is situated in the northeastern part of the country at latitude 18° S (Figure 1). The average daily temperature in the hottest month (October) is 37°C and the average annual rainfall is 500–600 mm.[19] Its northern border is formed by the Kavango River which separates this region from Angola. In 1983 the Kavango region was relatively densely populated (up to 4 persons/km^2 compared to the national average of 1.2 persons/km^2), but the population was highly concentrated along the Kavango River.[19] Rundu, the largest town, had one major hospital with laboratory services. In 1981 Rundu proper had a population of about 5,000 who were served in their homes by a treated, piped water supply and sewerage system.[19] In the early 1980s, a number of formal and informal settlements occupied by numerous Angolan war refugees had spread out from Rundu along the river that formed the main domestic water supply for some of the settlements.

Outbreak investigation and description of environmental conditions. A field team carried out investigations in Rundu from 12–14 October, 1983. At that time, the area was subject to military activities with all the consequences thereof, including restricted access, impaired freedom of movement, and hospital staff shortages. No comprehensive records of the outbreak had been kept. The team interviewed medical staff at the Rundu Hospital where all jaundiced patients in the affected area had been treated, mostly as outpatients. They visited two formal and five informal settlements and assessed environmental as well as living conditions. Cases of jaundice had occurred in all but one of these sites. Approximately one month later, in November 1983, the team obtained serum specimens from 24 healthy residents of a heavily affected settlement.

The two formal settlements, which directly adjoined Rundu, also had treated water piped to and waterborne sewerage piped from all dwellings. Treated river water or borehole water which in some cases had not been treated, was available at standpipes in three informal settlements. In the fourth, water was obtained directly from shallow wells, and in the fifth, water had to be collected directly from the river. All the water sources were largely inadequate, so that most people had to fetch additional water from the river. Sanitary facilities varied from nonexistent to inadequate numbers of poorly constructed pit latrines. These were commonly sited on the riverbank or close to boreholes, standpipes, meat stalls, and informal open-air beer breweries. Beer was poured into communally used mugs for on-the-spot consumption by purchasers. Although milk for sale in and around Rundu was pasteurized, recent laboratory tests had shown that pasteurization was ineffective in some samples. Likewise, recent tests of treated borehole water samples for bacteriologic quality had revealed some of these to be sub-

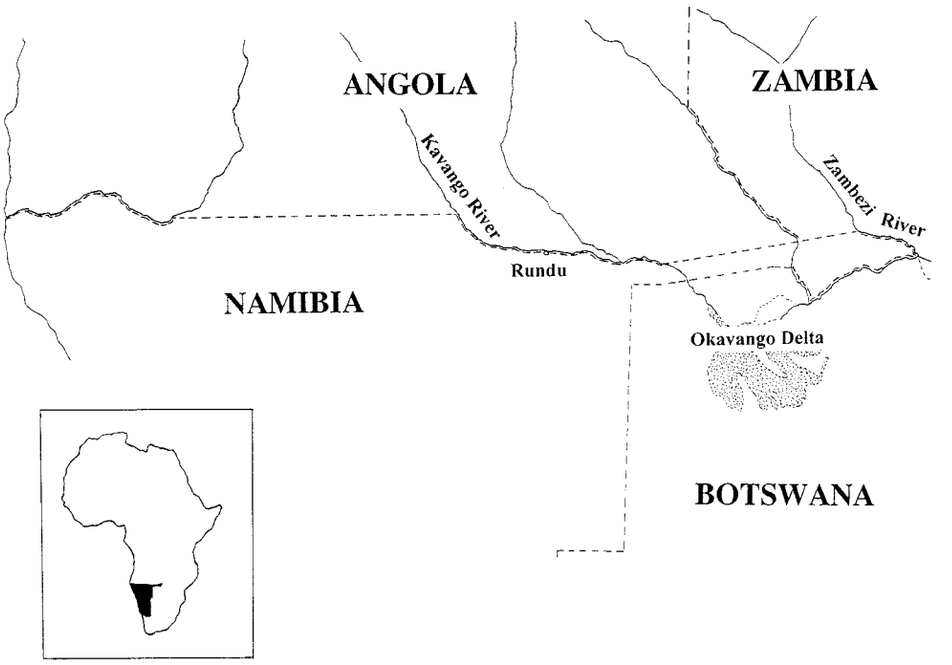

FIGURE 1. Map of the Kavango region in northern Namibia, site of a hepatitis outbreak in 1983. Inset: location of Namibia in Africa.

standard. The river level was very low due to the then prevailing drought. There had been no rain for 6 months and this had caused many people to migrate from inland areas, placing further pressure on existing water supplies and sanitary services.

Patients. Persons who had presented to the Rundu Hospital complaining of jaundice and could be located during the period 12–14 October were selected for further study. Moreover, patients with jaundice arriving at the hospital during the site visit were interviewed and examined. Fecal specimens were collected from 66 patients, regardless of the interval since onset of symptoms. Records of jaundiced patients, who had attended the Rundu Hospital from June 1, 1983, were made available to the investigating team for scrutiny. Laboratory records were utilized to enumerate cases and construct an epidemic curve. Regrettably, as no accurate census was available for the area, no incidence rates could be estimated. In addition, no accurate incidence data were collected for the period after the site visit, although the hospital clinicians' impressions of the course of the epidemic were followed up by telephone.

Clinical laboratory evaluations. Blood specimens from 12 acutely ill, jaundiced patients were assessed hematologically. Results of liver function tests were obtained from the hospital records of 37 patients. Automated blood counts using Coulter instruments and measurement of liver enzymes using the SMA 12/60 autoanalyzer were performed at the South African Institute for Medical Research (SAIMR) branch laboratory in Rundu, Namibia. Differential white cell counts and microscopic examination of blood films for the presence of malaria parasites, trypanosomes, and borrelias were done at the SAIMR, Johannesburg, South Africa.

Serology. Specimens tested serologically comprised 36 acute-phase and 12 convalescent-phase sera, collected shortly before or during the site investigation, and 24 sera of healthy persons collected a month later. There were no paired specimens. Serology for etiologic agents associated with jaundice was performed at SAIMR, the National Institute for Virology, Johannesburg, and the Onderstepoort Veterinary Research Institute, Pretoria, South Africa. Tests included the following: radioimmunoassays for total Ig and IgM to hepatitis A virus (HAV) and for hepatitis B virus (HBV) surface antigen (Abbott Diagnostics, Abbott Park, Illinois); hemagglutination inhibition tests for antibodies to yellow fever virus, chikungunya virus, Middelburg virus, Wesselsbron virus, spondweni virus, Germiston virus, and Rift Valley fever virus; complement fixation tests for antibodies to *Rickettsia conorii* and leptospires; tests for Weil-Felix agglutinins *Proteus* OX-K, OX-2, and OX-19; agglutination for antibodies to *Salmonella typhi* and *Yersinia enterocolitica*; and indirect immunofluorescence tests for antibodies to Crimean-Congo hemorrhagic fever virus, filoviruses, Lassa fever virus, and hantaviruses.

Serology for HEV was performed at the Walter Reed Army Institute of Research, Washington, DC. Total Ig to HEV was measured in U/ml by immunoassay as previously described,[17] but with a slightly different recombinant HEV capsid protein antigen.[20] Total Ig to HEV $\geq$ 20 U/ml was interpreted as evidence of previous infection with HEV. IgM to HEV was measured in a similar fashion, but with an anti-IgM conjugate (Kirkegaard and Perry Laboratories, Gaithersburg, MD) diluted 1:4,000 and a reference antiserum pool defined to contain 860 U/ml of virus-specific IgM. IgM to HEV $\geq$40 U/ml was interpreted as evidence of recent infection with HEV.

Reverse transcription-polymerase chain reaction for HEV. Hepatitis E Virus RNA was detected in 10% fecal suspensions using affinity-capture reverse transcription-poly-

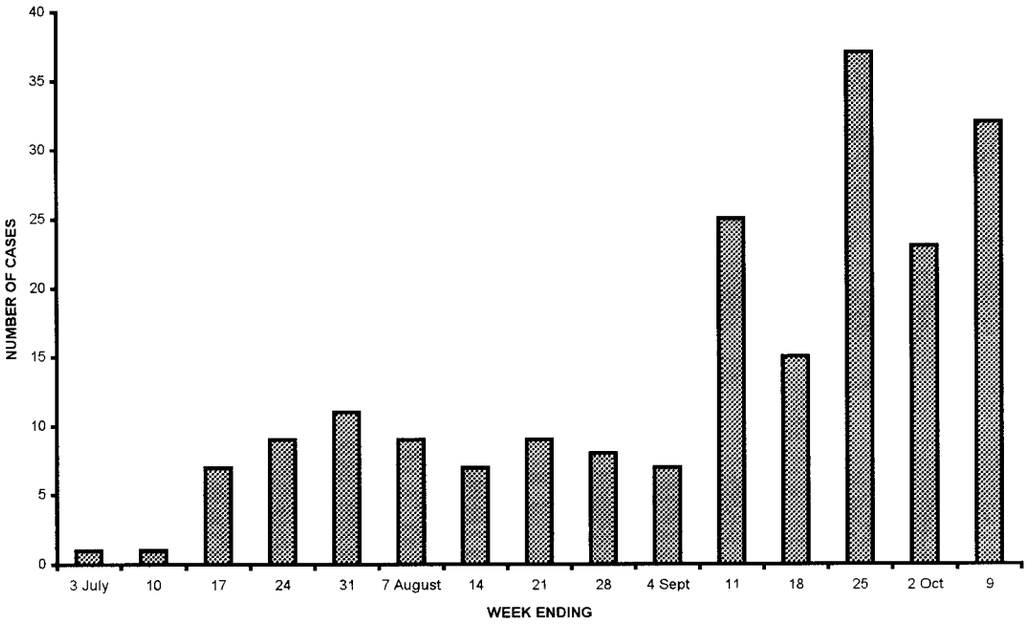

FIGURE 2. Incidence of 201 jaundice cases by week of submission to the Rundu Hospital Laboratory of blood samples for liver function and viral hepatitis investigations.

merase chain reaction (RT-PCR) as previously described.[21] In summary, microcentrifuge tubes coated with anti-mouse IgG were used to capture, in fecal suspensions, virus particles aggregated by antiserum raised in mice against a structural HEV protein.[22] After washing, reverse transcription followed by PCR were performed. Primers used for detection of the HEV genome were derived from the ORF-2 and ORF-3 of the HEV Burma sequence.[10] The amplified products were analyzed using 1% agarose gel electrophoresis. A specimen was considered positive for HEV if independent amplifications of ORF-3 and ORF-2 were positive, and assay controls (n = 5) gave expected results. The positive control was HEV recovered from a patient in Abbottabad, Pakistan in 1988.

RESULTS

Description of the epidemic and its clinical features. Prior to the outbreak, one jaundiced patient per week was normally seen at Rundu Hospital. A review of patient records showed that the epidemic had started in mid-July. Because illness-onset dates were unavailable for many patients, an epidemic curve for 201 patients was constructed using the dates of submission of acute liver function and viral hepatitis specimens to the hospital laboratory, resulting in a mean presentation of 8 cases/wk during the first 8 weeks of the outbreak (Figure 2). In early September, there was a steep increase to a mean of 26 cases/wk for the next 5 weeks after which the epidemic tailed off sharply according to subsequent telephoned reports from the hospital doctors. With the exception of a few nurses, almost all cases occurred in the economically-disadvantaged sector of the population. Analysis of 64 available patient records showed that ages ranged from 15–54 years with only one patient in the 5–9-year age group (Figure 3). The great majority (72%) of these patients were males.

Many patients who had been ill for up to 3 weeks before coming to hospital were apyrexial by then. Common complaints were pleuritic pains, cough, and myalgia that often preceded the onset of jaundice. Less common symptoms were joint pains, sore throat, and sore eyes; nausea and vomiting were uncommon. Hemorrhagic manifestations were rare. Some patients complained only of dark urine. Most cases were managed as outpatients with vitamin B-complex supplementation and bed rest. Of the seven deaths, all but one were pregnant women.

Clinical laboratory data and histopathology. Hematologic examinations of 12 patients showed 10 had low to normal white blood counts, 7 had a relative lymphocytosis, 4 had thrombocytopenia, 7 had atypical lymphocytes, 10 had eosinophilia, and all had target cells. Hepatic enzyme determinations on up to 37 patients suggested most had hepatocellular injury (Table 1). Elevated aspartate aminotransferase levels were present in 36/37 patients and 34/37 patients had raised alkaline phosphatase levels. All 15 patients in whom gamma-glutamyltransferase (GGT) was tested had higher than normal levels; alanine aminotransferase levels were determined in 14 of these and were invariably raised.

Spleen and liver tissues for histologic examination were obtained from 5 fatal cases. Lack of fixation rendered one liver section completely unsuitable for examination. The remaining 4 liver specimens showed massive or sub-massive necrosis with features consistent with those found in hepatitis A and E, but autolytic changes made interpretation uncertain. Splenic tissues appeared to be normal in all five.

Serology. The results of tests for hepatitis A and B markers on 12 of 37 jaundiced patients and on 40 control sera from healthy persons are shown in Table 2. These test results

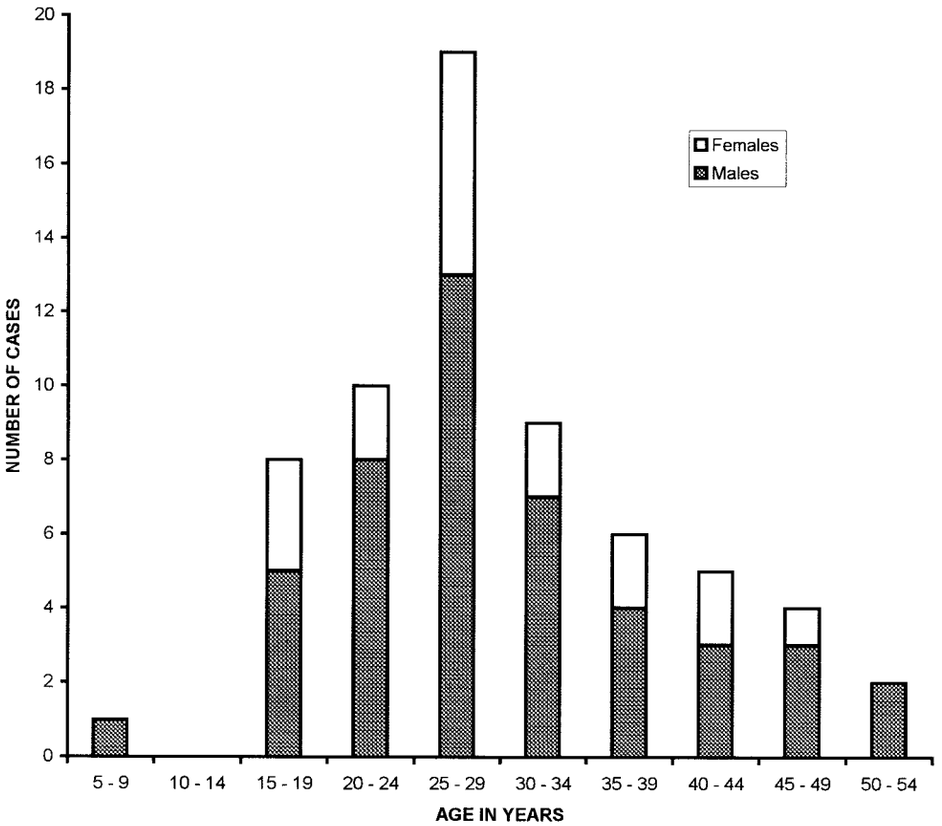

FIGURE 3. Age and gender distribution of 64 cases of jaundice presenting at Rundu Hospital.

failed to explain the etiology of the outbreak. Serology done on 48 patients for selected African arbovirus infections was largely negative or, in a few cases, showed very low titers. Also negative were tests for African viral hemorrhagic fevers, African tickbite fever, and leptospirosis. Weil-Felix tests yielded low positive OX-K titers (1:8 to 1:32) in 64% of 12 patients tested. Other infections excluded by appropriate tests on samples of jaundiced patients included malaria, African trypanosomiasis, relapsing fever, Q fever, typhoid fever, and yersiniosis.

Evaluation of archived specimens for markers of hepatitis E. As no etiology for the outbreak could be identified in 1983, a number of fecal specimens from jaundiced patients and serum specimens from healthy residents were collected one month after the apparent end of the outbreak and stored frozen for future studies in the event of new hepatitis agents being recognized. In 1998, 16 fecal samples were tested for HEV RNA; of those, 9 were positive, yielding amplified fragments of the correct size with both ORF-2 and ORF-3 primer sets. To independently confirm that HEV was the etiology of the hepatitis epidemic, serum specimens from 24 healthy residents of an affected settlement were tested for IgM and total Ig to HEV (Table 3). Ten persons (42%) had elevated levels of total Ig and IgM to HEV consistent with recent infection, 6 (25%) had elevated levels of total Ig without IgM to HEV most consistent with past infection, and 8 (33%) had non-diagnostic low antibody levels.

TABLE 1

Hepatic enzyme values in acute-phase sera of 37 patients with jaundice

Enzyme (normal range, U/L)	No. of samples tested (n = 37)	Mean (U/L)	Range (U/L)
Alanine aminotransferase (2–20)	14	640	33–1,732
Aspartate aminotransferase (2–24)	37	1,062	19–2,940
Gamma glutamyltransferase (5–25)	15	212	46–863
Alkaline phosphatase (75–207)	37	488	137–948

DISCUSSION

A three-month–long outbreak of jaundice, centered in crowded informal refugee settlements along the Kavango River which lacked potable water and sanitation, was investigated in 1983. The epidemic curve suggested that there was an initial wave of illness, followed 8 weeks later by a much larger secondary wave. Numerous lines of evidence sug-

TABLE 2

Hepatitis A and B markers in jaundiced and healthy persons*

Donor	Number positive/number tested		
	Total Ig to HAV	IgM to HAV	HBsAg*
Jaundiced patients	7/7	0/12	2/12
Healthy persons	40/40	Not done	3/10

* HAV = hepatitis A virus; HBsAg = hepatitis B virus surface antigen.

TABLE 3
Serologic classification of hepatitis E virus (HEV) exposure among 24 healthy residents of an affected settlement near the end of the outbreak

No. (%)	Total Ig to HEV (U/ml) median (range)	IgM to HEV (U/ml) median (range)	Serologic diagnosis
10 (42%)	268 (21–719)	166 (50–687)	Recent infection with HEV*
6 (25%)	34 (21–246)	8 (5–33)	Past infection with HEV†
8 (33%)	9 (6–19)	11 (4–14)	No evidence of past infection with HEV‡

* Ig ≥20 U/ml, IgM ≥40 U/ml.
† Ig ≥20 U/ml, IgM <40 U/ml.
‡ Ig <20 U/ml, IgM <40 U/ml.

gested the illness might have been waterborne. Environmental and social conditions in the affected communities were ideal for transmission of an enteric pathogen. The occurrence of afebrile jaundice, with a characteristic hepatic enzyme profile and absence of clinical severity, indicated that the disease was acute viral hepatitis; histopathologic examination of liver tissue collected at autopsy in 4 cases supported this diagnosis. The presence of eosinophilia in 83% of patients on whom hematologic studies were done was unsurprising in view of the high prevalence of intestinal helminth infections in this area.[23] The finding of atypical lymphocytes in 7/12 patients and target cells in 12/12 patients is consistent with a viral infection and hepatic damage respectively. The unusual feature of the illness was its nearly exclusive occurrence in adolescents and young adults and, in fatal form, in pregnant women. Nothing like this had been observed previously in southern Africa.

Serologic tests excluded hepatitis-A and -B viruses as etiologic agents of the outbreak. All evidenced only past infection with HAV. The prevalence of HBV surface antigen was similar in patients and healthy persons. Extensive additional serology incriminated no other infectious disease. The only finding was a high rate (64%) of reactions in the Weil-Felix test to the *Proteus* OX-K antigen, considered insignificant in view of the universally low titers obtained. Similar titers have been found in healthy persons and occasionally in patients with relapsing fever or hepatic damage due to a variety of causes. Relapsing fever was excluded in this outbreak based on negative microscopy of blood films. High titers of OX-K antibodies are typical in scrub typhus, which does not occur in Africa. The current 1983 investigation having reached a dead end, the outbreak was attributed to non-A, non-B hepatitis, and specimens were archived for future reference.

In the past decade, HEV has been increasingly recognized as a major cause of acute viral hepatitis in developing countries. Hepatitis E virus is unrelated to either HAV or HBV, is transmitted by the fecal-oral route, and has caused spectacular water-borne outbreaks of jaundice, primarily in places with crowding and inadequate environmental sanitation.[24] The pathognomonic features of hepatitis E, its propensity to affect adolescents and young adults, and its striking case-fatality rate in pregnant women, match the remarkable aspects of the Kavango outbreak. Therefore, the original field investigators recently submitted some of the archived specimens for HEV testing.

Hepatitis E virus was detected by RT-PCR in the majority of fecal specimens tested (9/16). For each positive specimen, 2 different regions of the virus genome were independently amplified, increasing confidence that the results were accurate. Coupled with the clinical and epidemiological data described above, these molecular virologic data conclusively identify HEV as the etiology of the outbreak. Serology in a convenience sample of healthy residents from a heavily affected settlement supports this conclusion. Of 24 samples tested, 16 (67%) had antibody to HEV. In 10 persons (42%), IgM to HEV was detected, strongly suggesting they were recently infected. In another 6 (25%), Ig to HEV without elevated IgM was detected, suggesting that these persons had been infected more remotely. If the convenience sample was representative, HEV was widely transmitted, as would be expected in a waterborne outbreak.

The presence of both too much and too little surface water may be associated with HEV epidemics.[25] Flooding causes contamination of drinking water with sewage;[26] on the other hand, dry conditions may lead to any HEV present in surface water remaining relatively undiluted. This may have been a factor in the Rundu outbreak; the river level was very low at the time of the epidemic, occurring as it did towards the end of an even drier than usual winter season (normally only 10–20% of the annual total rainfall occurs between March and October).[19]

The age profile of the patients who were predominantly young adults (Figure 3) is typical of HEV infection, and stands in contrast to the pattern of childhood acquisition of hepatitis A virus infection in developing populations. A possible reason for this is waning of the immunity acquired by exposure to HEV in early childhood, allowing later reinfection.[27] The alternative hypothesis that since HEV does not infect children, adults are susceptible, seems unlikely, given the usual pattern of other feco-orally transmitted infections in developing countries. The high rate of HAV seropositivity in our sample of the Rundu population has been noted in other studies in this region.[28] Hepatitis E virus also differs from hepatitis A in the high mortality rate (up to 20%) of infected pregnant women, especially when acquired in the third trimester.[29,30] This has been described in many places, including other parts of Africa.[6,25] In an Ethiopian sample, pregnancy itself appeared to be a risk factor, independent of socioeconomic status, for acquiring HEV infection, which then had an exceptionally high mortality rate (42%).[6] The reason for the high mortality of HEV in pregnancy is unknown.[29] Possibly analogous increased severity of hepatitis A and B has been ascribed to pregnancy-related depression of cell-mediated immunity.[31] Coursaget and others[11] speculate that the superimposition of acute HEV on chronic liver disease caused by HBV may lead to acute or fulminant hepatitis in some patients, including pregnant women. In Chad, these authors found that of 35 patients with sporadic acute or fulminant hepatitis E, 21 (60%) and 5 (14%) had sero-

logical evidence of, respectively, chronic and acute hepatitis B. Hepatitis B virus surface antigen was present in 30% of controls.[11] Hepatitis B virus infection is hyperendemic in Kavango; a 1983 study demonstrated that 98% of the population showed some serological evidence of exposure, with a 13.6% carriage rate of HBV surface antigen.[28] Our small sample (Table 2) showed no significant difference ($P = 0.81$, Fisher's exact test) between patients with overt hepatitis and normal controls with regard to HBV surface antigen carriage, but a much larger study would be required to investigate any association. The reason for the male preponderance of cases in our survey is not known; however, the sample (patients presenting at hospital) may not have been fully representative of the exposed population, and was almost certainly biased towards the more severe end of the clinical spectrum. Military and logistic restrictions precluded our acquiring accurate clinical, socioeconomic, or census data amongst the refugee community who appeared to be most at risk for hepatitis, presumably because of poor living conditions. Displaced populations in other places (for example, Albanians in Greece,[32] Indochinese in Australia[33]) have been shown to have higher rates of HEV antibodies than the native populations, and the risk of HEV to groups affected by natural disasters and wars is well recognised.[5,34]

Previously, sporadic or epidemic hepatitis E had been recognized in northern and central African countries. Between 1980 and 1986, HEV outbreaks occurred in Algeria, Chad, Sudan, and Somalia.[30] More recent reports of hepatitis E have emerged from many other African countries.[3–11] In southern Africa, surveys in South Africans and in Mozambican refugees living in Swaziland have shown serologic evidence of HEV infection,[35–37] but no actual disease has been documented by detection of HEV in clinical specimens. This report of hepatitis E in southern Africa is the first to unambiguously document cases by detection of HEV in patient specimens. Moreover, this report is the third example[37,38] of epidemic hepatitis E among refugees in Africa, highlighting the risk HEV poses to refugees and aid workers ministering to them.

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REFERENCES

1. Balayan MS, Andjaparidze AG, Savinskaya SS, Ketiladze ES, Braginsky DM, Savinov AP, Poleschuk VF, 1983. Evidence for a virus in non-A, non-B hepatitis transmitted via the fecal-oral route. *Intervirology 20:* 23–31.
2. Mast EE, Purdy MA, Krawczynski K, 1996. Hepatitis E. *Baillieres Clin Gastroenterol 10:* 227–242.
3. Hyams KC, Purdy MA, Kaur M, McCarthy MC, Hussain MA, el-Tigani A, Krawczynski K, Bradley DW, Carl M, 1992. Acute sporadic hepatitis E in Sudanese children: analysis based on a new western blot assay. *J Infect Dis 165:* 1001–1005.
4. Mushahwar IK, Dawson GJ, Bile KM, Magnius LO, 1993. Serological studies of an enterically transmitted non-A, non-B hepatitis in Somalia. *J Med Virol 40:* 218–221.
5. Burans JP, Sharp T, Wallace M, Longer C, Thornton S, Batchelor R, Clemens V, Hyams KC, 1994. Threat of hepatitis E virus infection in Somalia during Operation Restore Hope. *Clin Infect Dis 18:* 100–102.
6. Tsega E, Krawczynski K, Hansson BG, Nordenfelt E, 1993. Hepatitis E virus infection in pregnancy in Ethiopia. *Ethiop Med J 31:* 173–181.
7. Gomatos PJ, Monier MK, Arthur RR, Rodier GR, el-Zimaity D, Hassan NF, Quinti I, el-Sahly AD, Sultan Y, Hyams KC, 1996. Sporadic acute hepatitis caused by hepatitis E virus in Egyptian adults. *Clin Infect Dis 23:* 195–196.
8. Tsarev SA, Binn LN, Gomatos PJ, Arthur RR, Monier MK, van Cuyck-Gandre H, Longer CF, Innis BL, 1999. Phylogenetic analysis of hepatitis E virus isolates from Egypt. *J Med Virol 57:* 68–74.
9. Benjelloun S, Bahbouhi B, Bouchrit N, Cherkaoui L, Hda N, Mahjour J, Benslimane A, 1997. Seroepidemiological study of an acute hepatitis E outbreak in Morocco. *Res Virol 148:* 279–287.
10. van Cuyck-Gandre H, Zhang HY, Tsarev SA, Clements NJ, Cohen SJ, Caudill JD, Buisson Y, Coursaget P, Warren RL, Longer CF, 1997. Characterization of hepatitis E virus (HEV) from Algeria and Chad by partial genome sequence. *J Med Virol 53:* 340–347.
11. Coursaget P, Buisson Y, N'Gawara MN, van Cuyck-Gandre H, Roue R, 1998. Role of hepatitis E virus in sporadic cases of acute and fulminant hepatitis in an endemic area (Chad). *Am J Trop Med Hyg 58:* 330–334.
12. Wong DC, Purcell RH, Sreenivasan MA, Prasad SR, Pavri KM, 1980. Epidemic and endemic hepatitis in India: evidence for a non-A, non-B hepatitis virus aetiology. *Lancet 2:* 876–879.
13. Clayson ET, Myint KS, Snitbhan R, Vaughn DW, Innis BL, Chan L, Cheung P, Shrestha MP, 1995. Viremia, fecal shedding, and IgM and IgG responses in patients with hepatitis E. *J Infect Dis 172:* 927–933.
14. van Cuyck-Gandre H, Caudill JD, Zhang HY, Longer CF, Molinie C, Roue R, Deloince R, Coursaget P, Mamouth NN, Buisson Y, 1996. Short report: polymerase chain reaction detection of hepatitis E virus in north African fecal samples. *Am J Trop Med Hyg 54:* 134–135.
15. Bryan JP, Tsarev SA, Iqbal M, Ticehurst J, Emerson S, Ahmed A, Duncan J, Rafiqui AR, Malik IA, Purcell RH, Letger LJ, 1994. Epidemic hepatitis E in Pakistan: patterns of serologic response and evidence that antibody to hepatitis E virus protects against disease. *J Infect Dis 170:* 517–521.
16. Tsarev SA, Tsareva TS, Emerson SU, Kapikian AZ, Ticehurst J, London W, Purcell RH, 1993. ELISA for antibody to hepatitis E virus (HEV) based on complete open-reading frame-2 protein expressed in insect cells: identification of HEV infection in primates. *J Infect Dis 168:* 369–378.
17. Gambel JM, Drabick JJ, Seriwatana J, Innis BL, 1998. Seroprevalence of hepatitis E virus among United Nations Mission

in Haiti peacekeepers, 1995. *Am J Trop Med Hyg 58:* 731–736.
18. Ghabrah TM, Tsarev S, Yarbough PO, Emerson SU, Strickland GT, Purcell RH, 1998. Comparison of tests for antibody to hepatitis E virus. *J Med Virol 55:* 134–137.
19. van der Merwe JH, 1983, ed. *National Atlas of South West Africa (Namibia).* Windhoek, Namibia: Directorate Development Co-ordination, South West Africa, 10,18,45.
20. Robinson RA, Burgess WH, Emerson SU, Leibowitz RS, Sosnovtseva SA, Tsarev S, Purcell RH, 1998. Structural characterization of recombinant hepatitis E virus ORF2 proteins in baculovirus-infected insect cells. *Protein Expr Purif 12:* 75–84.
21. He J, Binn LN, Caudill JD, Asher LV, Longer CF, Innis BL, 1999. Antiserum generated by DNA vaccine binds to hepatitis E virus (HEV) as determined by PCR and immune electron microscopy (IEM): application for HEV detection by affinity-capture RT-PCR. *Virus Res* 62: 59–65.
22. He J, Hoffman SL, Hayes CG, 1997. DNA inoculation with a plasmid vector carrying the hepatitis E virus structural protein gene induces immune responses in mice. *Vaccine 15:* 357–362.
23. Kyrönseppä HJP, Goldsmid JM, 1978. Studies on the intestinal parasites in African patients in Owamboland, South West Africa. *Trans R Soc Trop Med Hyg 72:* 16–21.
24. Purcell RH, 1994. Hepatitis viruses: changing patterns of human disease. *Proc Natl Acad Sci USA 91:* 2401–2406.
25. Corvin AL, Tien NTK, Bounlu K, Winarno J, Putri MP, Laras K, Larasati RP, Sukri N, Endy T, Sulaiman HA, Hyams KC, 1999. The unique riverine ecology of hepatitis E virus transmission in South-East Asia. *Trans R Soc Trop Med Hyg 93:* 255–260.
26. Bile K, Hohamud O, Allebeck P, Nilsson L, Norder H, Mushahwar IK, Magnius LO, 1994. Contrasting roles of rivers and wells as sources of drinking water on attack and fatality rates in a hepatitis E epidemic in Somalia. *Am J Trop Med Hyg 51:* 466–474.
27. Goldsmith R, Yarbough PO, Reyes GR, Fry KE, Gabor KA, Kamel M, Zakaria S, Amer S, Gafar Y, 1992. Enzyme-linked immunosorbent assay for diagnosis of acute sporadic hepatitis E in Egyptian children. *Lancet 339:* 328–331.
28. Joubert JJ, Prozesky OW, Lourens JG, van Straten AM, Theron JW, Swanevelder C, Meenehan GM, van der Merwe CA, 1985. Prevalence of hepatitis virus and some arbovirus infections in Kavango, northern SWA/Namibia. *S Afr Med J 67:* 500–502.
29. Skidmore SJ, 1997. Tropical aspects of viral hepatitis. Hepatitis E. *Trans R Soc Trop Med Hyg 91:* 125–126.
30. Bradley DW, 1990. Enterically-transmitted non-A, non-B hepatitis. *Br Med Bull 46:* 442–461.
31. Weinberg ED, 1984. Pregnancy-associated depression of cell-mediated immunity. *Rev Infect Dis 6:* 814–831.
32. Dalekos GN, Zervou E, Elisaf M, Germanos N, Galanakis E, Bourantas K, Siamopoulos KC, Tsianos EV, 1998. Antibodies to hepatitis E virus among several populations in Greece: increased prevalence in an hemodialysis unit. *Transfusion 38:* 589–595.
33. Moaven L, Van Asten M, Crofts N, Locarnini SA, 1995. Seroepidemiology of hepatitis E in selected Australian populations. *J Med Virol 45:* 326–330.
34. Skidmore SJ, 1999. Factors in spread of hepatitis E. *Lancet 354:* 1049–1050.
35. Tucker TJ, Kirsch RE, Louw SJ, Isaacs S, Kannemeyer J, Robson SC, 1996. Hepatitis E in South Africa: evidence for sporadic spread and increased seroprevalence in rural areas. *J Med Virol 50:* 117–119.
36. Grabow WOK, Favorov MO, Khudyakova NS, Taylor MB, Fields HA, 1994. Hepatitis E seroprevalence in selected individuals in South Africa. *J Med Virol 44:* 384–388.
37. van Rensburg EJ, Lemmer HR, Joubert JJ, 1995. Prevalence of viral infections in Mozambican refugees in Swaziland. *East Afr Med J 72:* 588–590.
38. Khudyakov YE, Khudyakova NS, Fields HA, Jue D, Starling C, Favorov MO, Krawczynski K, Polish L, Mast E, Margolis H, 1993. Epitope mapping in proteins of hepatitis E virus. *Virology 194:* 89–96.