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Genetic Heterogeneity of Hepatitis E Virus in Darfur, Sudan, and Neighboring Chad

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The within-outbreak diversity of hepatitis E virus (HEV) was studied during the outbreak of hepatitis E that occurred in Sudan in 2004. Specimens were collected from internally displaced persons living in a Sudanese refugee camp and two camps implanted in Chad. A comparison of the sequences in the ORF2 region of 23 Sudanese isolates and five HEV samples from the two Chadian camps displayed a high similarity (>99.7%) to strains belonging to Genotype 1. But four isolates collected in one of the Chadian camps were close to Genotype 2. Circulation of divergent strains argues for possible multiple sources of infection. ***J. Med. Virol. 77:519–521, 2005.*** © 2005 Wiley-Liss, Inc.

KEY WORDS: HEV genotype; genetic diversity; refugee camp

INTRODUCTION

Hepatitis E virus (HEV) specimens from outbreaks and sporadic cases have been characterized genetically and have been divided into at least four genotypes. HEV isolates obtained within outbreaks have generally shown a high degree of genetic similarity [Aggarwal et al., 1999; Grandadam et al., 2004]. Beginning in May 2004, a large outbreak of hepatitis E occurred among victims of the civil disturbance in the Darfur region of Sudan. Cases were recorded over a large geographical area both in camps for internally displaced persons within Sudan as well as in refugee camps across the border in Chad where over 10,000 refugees lived. This outbreak provided an opportunity to characterize further the within-outbreak diversity of HEV.

METHODS

Specimens

The genetic diversity was investigated in a camp for internally displaced persons in Sudan (Mornay Camp, West Darfur) and in two refugee camps in Chad (Iriba Camp and Goz Amer Camp). Sera were drawn from patients with acute hepatitis in the following locations: Mornay camp from August to September 2004 (84 specimens); Iriba Camp, in July 2004 (9 specimens); and Goz Amer Camp, in September 2004 (125 specimens).

Diagnosis

After collection, specimens were stored at +4 to 8°C and shipped to the National Reference Centre for Hepatitis E Virus, Val de Grâce Teaching Military Hospital, Paris, France, for testing. The diagnosis of acute hepatitis E was based on serology and amplification of HEV RNA by RT-PCR. IgG and IgM anti-HEV was detected with commercial assays (HEV Elisa and HEV IgM Elisa, Genelabs Diagnostics, Singapore Science Park). RT PCR was performed using the GeneAmp 9600 Thermocycler with primers targeting HEV ORF2 nucleotides 6,653–7,100 as described previously [Tam et al., 1991; Grandadam et al., 2004].

Sequence Analysis

PCR products of the appropriate size were sequenced on both strands with an automated DNA sequencer (CEQ 8000, Beckman Coulter, Inc., Fullerton, CA) using the DTCS sequencing kit. DNA sequences were aligned using Clustal X (Version 1.82). The sequences were compared with other HEV genomic sequences listed in Figure 1. Distance matrices were determined with the DNADIST program of the PHYLIP 3.5 package using Kimura

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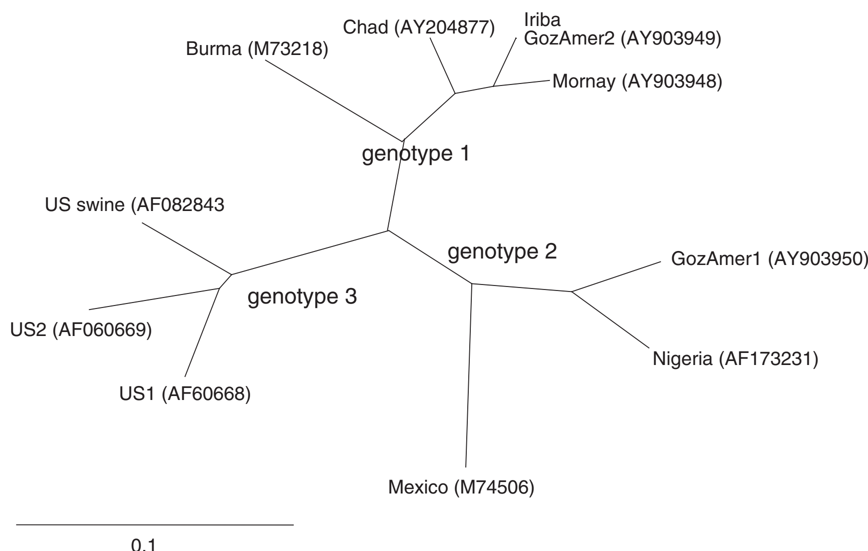

Fig. 1. Unrooted phylogenetic tree depicting the diversity of Sudanese (Mornay) and Chad HEV isolates (Goz Amer 1, Goz Amer2, Iriba) with referenced HEV. The scale bar represents nucleotide substitutions per position. References in brackets represent accession number in Genbank database.

two-parameter algorithm. The robustness of the tree was evaluated with the bootstrap method (1,000 replicates). The final tree was created with TREEVIEW program.

RESULTS

All sera were strongly reactive for IgG and IgM anti-HEV, consistent with recent hepatitis E infection among the patients tested. HEV RNA was amplified successfully from 23/84, 2/9, to 15/125 specimens collected in Mornay, Iriba, and Goz Amer camps, respectively.

The resulting 363-nucleotide sequences of all 23 isolates from Mornay Camp, two isolates from Iriba Camp, and 11 of 15 isolates from Goz Amer Camp shared 99.7–100% similarity (Table I). The comparison of these sequences with other HEV sequences from the same region showed that these isolates are related to Genotype 1, sharing 88% homology with the prototype Burmese strain (Genbank Accession Number M72318) and 93% identity with a Chad strain (Genbank Accession Number AY204877) collected in 1983 from a patient with hepatitis E (Fig. 1) [van Cuyck et al., 2003]. The remaining four isolates from Goz Amer camp shared 100% similarity. Interestingly, these isolates were distinct phylogenetically from Genotype 1 and close to Genotype 2 sharing 93% nucleotide identity with the Nigerian strain (Genbank Accession Number AF173231) and 85% identity for the prototype Mexican strain (Genbank Accession Number M74506).

Based on their high similarity and their geographical origin, three consensus sequences of isolates collected during this outbreak were created using the Sequencer program 5.0 and deposited in Genbank as AY903948 (Mornay, Darfur), AY903949 (Goz Amer2, Chad), and AY903950 (Goz Amer1, Chad).

DISCUSSION

In almost all outbreaks of hepatitis E studied previously, outbreak-related HEV isolates have shown a high degree of genetic similarity (>98%). The sole exception was an HEV isolate collected during an

TABLE I. Percentage Nucleotide Identity Between HEV Isolates Over a 363-Base ORF2 Fragment

	Goz Amer1	Nigeria	Mexico	US1	USswine	US2	Iriba	Goz Amer2	Mornay	Chad
Goz Amer1										
Nigeria	92.6									
Mexico	84.6	82.7								
US1	77.7	78.1	76.3							
USswine	77.0	78.2	75.1	91.0						
US2	78.1	79.3	77.5	90.7	90.0					
Iriba	78.4	77.4	75.8	76.8	79.0	75.1				
Goz Amer2	78.4	77.4	75.8	76.8	79.0	75.1	100.0			
Mornay	77.3	76.2	75.6	77.7	80.3	75.9	95.5	95.5		
Chad	79.2	77.7	77.0	79.3	81.2	79.1	92.8	28.0	94.1	
Burma	78.1	78.2	77.0	76.9	78.3	78.6	87.6	87.6	86.5	87.0

outbreak in India, which was 95% similar to the other outbreak-related viruses in the ORF2 region and which may have represented a sporadic isolate unrelated to that outbreak [Aggarwal et al., 1999]. Recently, the quasi-species organization of HEV was demonstrated within one epidemic in Algeria with a sequence diversity ranging from 0.11% to 3.4% [Grandadam et al., 2004]. By contrast, in the Sudan/Chad outbreak described in this article, widely divergent isolates from at least two genotypes of virus were circulating simultaneously.

Genotype 1, identified in the three camps sampled, is the most common genotype found in Africa. Genotype 2 was first described in specimens from an outbreak in Mexico in 1986 but has not been found in America since then. Genotype 2 has also been found in Africa, in sporadic cases in Nigeria, and in an outbreak in Namibia [Maila et al., 2004]. Both Genotype 1 and Genotype 2 are considered to be human HEV, in that they have only been found in humans and have failed to transmit to animals in the laboratory setting, and the situation of these displaced people continues to be marred by unpredictability. Therefore, the displacement of refugees living on the Chad border is difficult to assess.

The important modes of transmission in this large, multinational outbreak remain unknown. Most such outbreaks to date have been attributed to contaminated water, but no single water source could account for such geographically widely dispersed cases. The occurrence of this outbreak during the May–August rainy season also supports the important role of waterborne transmission. Circulation of such diverse strains of HEV during an outbreak suggests multiple sources of contamination and highlights the importance of improving hygiene in the camps and, in particular, of assuring the safety of drinking water. Vaccination may eventually provide an additional means of outbreak control. One vaccine was highly successful in Phase I trials and has recently completed a large Phase II/III trial in Nepal [Emerson and Purcell, 2001; Purcell et al., 2003].

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