A new hepatitis E virus genotype 2 strain identified from an outbreak in Nigeria, 2017

Bo Wang¹, Olusola Anuoluwapo Akanbi¹, Dominik Harms¹, Olufisayo Adesina¹, Folakemi Abiodun Osundare¹,², Dhamari Naidoo³, Isabel Deveaux⁴, Opeayo Ogundiran⁴, Uzoma Ugochukwu⁴, Nwando Mba⁴, Chikwe Ihekweazu⁴ and C.-Thomas Bock¹,⁵*

Abstract

Background: In 2017 the Nigerian Ministry of Health notified the World Health Organization (WHO) of an outbreak of hepatitis E located in the north-east region of the country with 146 cases with 2 deaths. The analysis of the hepatitis E virus (HEV) genotypes responsible for the outbreak revealed the predominance of HEV genotypes 1 (HEV-1) and 2 (HEV-2). Molecular data of HEV-2 genomes are limited; therefore we characterized a HEV-2 strain of the outbreak in more detail.

Finding: The full-length genome sequence of an HEV-2 strain (NG/17–0500) from the outbreak was amplified using newly designed consensus primers. Comparison with other HEV complete genome sequences, including the only HEV-2 strain (Mex-14) with available complete genome sequences and the availability of data of partial HEV-2 sequences from Sub-Saharan Africa, suggests that NG/17–0500 belongs to HEV subtype 2b (HEV-2b).

Conclusions: We identified a novel HEV-2b strain from Sub-Saharan Africa, which is the second complete HEV-2 sequence to date, whose natural history and epidemiology merit further investigation.

Keywords: Hepatitis E virus, HEV genotype 2, HEV subtype 2b, Outbreak, Nigeria, Complete genome

Main text

Hepatitis E virus (HEV) is the prototype of the family Hepeviridae and a common causative agent of acute viral hepatitis. HEV is a small, (non)enveloped spherical particle of about 34 nm in diameter harboring a single stranded, positive sense RNA genome of approximately 7.5 kb [1]. Eight HEV genotypes are recognized within the species Orthohepevirus A based on the pairwise distances of entire viral genomes (HEV-1 to HEV-8). The different HEV genotypes have various reservoirs, distinct distribution, and transmission patterns. Four major HEV genotypes (HEV-1 to HEV-4) are well recognized as human pathogens while HEV-5 and HEV-6 have been detected only in wild boars so far. HEV-7 from dromedary camels has been reported to infect humans and cause chronic hepatitis E. HEV-8 is identified in Bactrian camels with an unknown zoonotic potential [2–4]. HEV-1 and HEV-2 are transmitted through the waterborne/fecal-oral route and responsible for large HEV outbreaks and epidemics in endemic areas like the Indian subcontinent and Africa, whereas HEV-3 and HEV-4 are linked to zoonotic transmission causing sporadic infections mainly in industrialized countries [5]. HEV-2 was firstly identified during a hepatitis E outbreak in Mexico in 1986 while the complete genome sequence (HPENSSP, GenBank accession No. M74506) was subsequently characterized [6]. Recently, additional HEV-2 full-length genome sequences were obtained from an individual patient of same Hepatitis E outbreak in Mexico (Mex-14, KX578717) showing 99.5%
nucleotide identity to M74506 [7]. However, the sequences for M74506 and KX578717 are from the same isolate. Since M74506 has been referred to as Mexico [8], Mexico-14 [9], and Telixtac-14 [10], the stool sample was re-analysed at the Paul Ehrlich Institute (PEI), Germany, with the isolate designation Mex-14, and the sequences were submitted to GenBank receiving the accession number KX578717. Additionally, several studies have reported that HEV-2 is distributed in Africa. However, only partial ORF2 gene sequences were amplified [11–14]. According to the report of Lu et al. and the International Committee on Taxonomy of Viruses (ICTV) *Hepeviridae* Study Group, Mex-14 is the proposed HEV subtype 2a (HEV-2a) reference sequence.

### Table 1

| Primer | Sequence (5′-3′) | Location | Use | Reference |
|--------|------------------|----------|-----|-----------|
| HEV-07_f | GGTGGTTTCTGGGGTGAC | 5261–5278 | HEV-1 to HEV-4 quantification | [18] |
| HEV-07_r | CACCTGTCAGAGGCGAGTCA | 5261–5278 | HEV-1 to HEV-4 complete genome sequencing | This study |
| HEV-08_f | FAM-TGATTCTCAGCCCTTTCGC-MGB | 5284–5301 | HEV-1 to HEV-4 genotyping in ORF1 | [18] |
| HEV-08_r | AGGGGTGTTTGGATGGA | 5330–5313 |
| HEV-09_f | GARGCYATGTGGAGAARG | 4084–4102 |
| HEV-09_r | GCCATRTCCAGCATGCTTGTCC | 4622–4601 |
| HEV-30_f | CCGACAGAATTTTGCAGG | 6296–6317 |
| HEV-30_r | TCRCCRGARTGYTTCTTCC | 6350–6372 |
| HEV-32_f | GARGCYATGGTBGAGAARG | 4277–4297 |
| HEV-32_r | GCCATRTCCAGCATGCTTGTCC | 4583–4565 |
| HEV-33_f | GCCATRTCCAGCATGCTTGTCC | 4622–4601 |
| HEV-33_r | AGGGGTGTTTGGATGGA | 5330–5313 |
| HEV-34_f | GARGCYATGTGGAGAARG | 4084–4102 |
| HEV-34_r | GCCATRTCCAGCATGCTTGTCC | 4622–4601 |
| HEV-35_f | CCGACAGAATTTTGCAGG | 6296–6317 |
| HEV-35_r | TCRCCRGARTGYTTCTTCC | 6350–6372 |
| HEV-36_f | GARGCYATGTGGAGAARG | 4084–4102 |
| HEV-36_r | GCCATRTCCAGCATGCTTGTCC | 4622–4601 |
| HEV-37_f | CCGACAGAATTTTGCAGG | 6296–6317 |
| HEV-37_r | TCRCCRGARTGYTTCTTCC | 6350–6372 |
| HEV-38_f | GARGCYATGTGGAGAARG | 4084–4102 |
| HEV-38_r | GCCATRTCCAGCATGCTTGTCC | 4622–4601 |

*Forward primer designations end with _f; reverse primer designations end with _r*

*Numbering is according the HEV prototype strain Burma (GenBank accession No. M73218)*
and HEV subtype 2b (HEV-2b) was assigned to the partial sequences AF173231 and AF173232 from Nigeria and AY903950 from Chad [15, 16].

In June 2017, the Nigerian Ministry of Health reported to the World Health Organization (WHO) an HEV outbreak in north-east Nigeria, with 146 laboratory confirmed cases and two outbreak-associated cases of death in pregnant [17]. In order to identify the corresponding viral pathogens of this hepatitis E outbreak, we determined HEV genotypes from outbreak samples. The genotyping results showed mainly HEV-1 and HEV-2 strains being predominant within the outbreak, the genotype distribution of isolates from this outbreak as determined by the Nigerian center of disease control (NCDC) and Robert Koch Institute (RKI) was 40% HEV-1 and 60% HEV-2. However, a number of HEV-positive outbreak samples could not genotyped. Since full-length genome sequences of HEV-2 strains are rare, we here report the full-length genome sequence of the HEV-2 strain (NG/17–0500) from an isolate of the Nigerian outbreak. The virus was detected from an individual from Borno state, Nigeria, and initially tested serologically positive for HEV using Wantai HEV-IgM Rapid Test and Wantai HEV-IgM ELISA (Sanbio, Uden, Netherlands).

Viral RNA from an anonymized serum sample (NG/17–0500) was extracted using High Pure Viral Nucleic Acid Kit (Roche, Mannheim, Germany) following cDNA synthesis using SuperScript™ III First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions. Molecular approaches with sensitive real-time PCR and consensus nested PCR assays were conducted as described recently [18]. To amplify the entire genome sequence of NG/17–0500 and to verify the geno/subtyping results, universal primers were designed based on 38 complete HEV-1 and HEV-2 sequences from the GenBank database. Using genome walking method, gene-specific primers were designed to amplify the gaps. Primers and probe used are listed in Table 1. The complete viral genome of NG/17–0500 was amplified using KAPA HiFi HotStart

**Fig. 1** Phylogenetic relationships of NG/17–0500 within the species Orthohepevirus A. HEV-2 strains are designated with geno/subtype, accession number, country, and collection year. NG/17–0500 of this study is shown in red. Phylogenetic analyses were performed with MEGA software version 7.0.26. Maximum likelihood trees based on General Time Reversible model with Gamma distributed with Invariant sites was inferred. The values at nodes indicate the bootstrap values (using 1000 replications). Values below 70% are hidden for clarity of presentation. Reference sequences for HEV genotypes were as proposed from the ICTV Hepeviridae Study Group. Nucleotide (nt) and amino acid (aa) sequences were aligned using MAFFT software version 7.222.

- **a** Phylogenetic relationships based on complete genome sequences of representative HEV reference strains. HEV-2 strains are highlighted with red branches.
- **b** Phylogenetic relationships based on 641 nt of ORF 2 corresponding to nt positions 6453 to 7093 (numbered according to the HEV prototype strain from Burma GenBank accession No. M73218). HEV-2b strains are highlighted with red branches.
Real-time PCR assay targeting the HEV ORF2 and ORF3 overlapping region (ORF2/3) demonstrated viremic HEV infection with a viral load of $1.2 \times 10^7$ IU/mL. Sequence analysis of partial ORF1 and ORF2 genes indicated that NG/17–0500 preliminary belongs to HEV-2. After de novo assembly of the amplicons, the NG/17–0500 full-length sequence showed 7198 nucleotides, excluding the 3′-poly (A) tail, with a G + C content of 57.5% harboring the typical 3 HEV ORFs. Phylogenetic analysis of the complete genome sequence revealed that NG/17–0500 grouped with HEV-2 Mex-14 strain (Fig. 1a), and this was all true for phylogenetic analysis of individual ORFs (data not shown). These relationships were also observed for sequence identities between NG/17–500 and other HEV complete genome or nucleotide or amino acid sequence identities of individual ORFs (Table 2).

Due to inadequate sanitation and lack of clean drinking water, hepatitis E is a severe public health issue in several regions of Africa [20]. Partial HEV-2 sequences have been reported from Sub-Sahara African countries mainly during HEV outbreaks including Namibia in 1995 [12], Sudan in 2004 [13], and the Central African Republic in 2002 [14]. In addition, a single study reported of the analysis of HEV isolates from ten sporadic cases in Port-Harcourt city, southern Nigeria in 1997. Phylogenetic analysis of partial ORF2 fragments indicated that the Nigerian isolates from 1997 are most closely related to the HEV-2a reference Mex-14 strain and have been proposed as HEV-2b [11, 15, 16]. In this regard, comparison of NG/17–0500 sequence to the previously characterized Nigerian HEV-2b isolates displayed a 91.2% to 92.2% nt identity. Phylogenetic analysis of the

### Table 2 Nucleotide and amino acid sequence identities between NG/17–0500 and reference HEV strains within the family Hepeviridae

| Hepeviridae | Degree of identity (%) |
|-------------|------------------------|
| Complete genome | ORF1 | ORF2 | ORF3 |
| nt | nt aa | nt aa | nt aa |
| HEV-1 | 75.0 | 72.3 | 83.5 | 81.0 | 94.8 | 90.9 | 87.8 |
| HEV-2 | 83.5 | 82.3 | 92.7 | 86.8 | 98.2 | 94.1 | 91.1 |
| HEV-3 | 73.1 | 71.2 | 81.5 | 78.7 | 91.2 | 83.9 | 76.4 |
| HEV-4 | 73.5 | 71.1 | 80.6 | 80.7 | 91.1 | 80.6 | 78.9 |
| HEV-5 | 72.6 | 70.1 | 80.1 | 78.6 | 89.1 | 75.0 | 74.1 |
| HEV-6 | 72.8 | 70.9 | 79.4 | 78.0 | 88.5 | 75.5 | 70.5 |
| HEV-7 | 72.3 | 69.8 | 80.6 | 78.8 | 89.8 | 79.0 | 79.8 |
| HEV-8 | 72.2 | 70.0 | 79.9 | 77.9 | 89.4 | 78.5 | 81.6 |
| Avian HEV | 46.3 | 44.9 | 41.3 | 46.2 | 43.3 | 25.6 | 21.1 |
| Rat HEV | 52.1 | 50.1 | 48.0 | 55.1 | 54.8 | 38.1 | 27.1 |
| Bat HEV | 47.2 | 45.8 | 41.6 | 55.1 | 48.1 | 30.6 | 15.9 |

The sequences were aligned using MAFFT software version 7.222. The evolutionary analyses were conducted using MEGA 7 software version 7.0.26. The GenBank accession numbers are for HEV-1 (M733218), HEV-2 (KX578717), HEV-3 (AF082843), HEV-4 (AJ272108), HEV-5 (AB573435), HEV-6 (AB602441), HEV-7 (KJ496143), HEV-8 (KX387868), Avian HEV (AY153500), Rat HEV (GJU345042), and Bat HEV (JQ001749).

* nt and aa represent nucleotide and amino acid, respectively.
availability of data of partial HEV-2 sequences from Sub-Saharan Africa showed that NG/17–0500 clustered with proposed HEV-2b sequences, indicating NG/17–0500 belongs to HEV-2b (Fig. 1b). Comparison of partial ORF2 sequences of NG/17–0500 to Chad (AY903950) and Central African Republic (DQ151640) HEV-2b isolates shared 90.3% and 88.4% identity, respectively. No evidence of recombination in NG/17–500 was detected by either Identity Plot or Bootstrap analysis (Fig. 2). The complete genome sequence of NG/17–0500 has been deposited in GenBank under the accession number MH809516.

In conclusion, to the best of our knowledge the novel HEV-2b strain NG/17–0500 from Nigerian hepatitis E outbreak 2017 represents the first complete HEV-2 genomic sequence from Sub-Saharan Africa and the second complete HEV-2 sequence worldwide, which contributes to our knowledge of the diversity of HEV-2. Nevertheless, the natural history of NG/17–0500 requires further comprehensive genetic and epidemiological analyses.

Abbreviations
aa: Amino acid; HEV: Hepatitis E virus; HEV-1 to HEV-8: Hepatitis E virus genotype 1 to hepatitis E virus genotype 8; HEV-2a and HEV-2b: Hepatitis E virus subtype 2a and 2b; ICTV: International Committee on Taxonomy of Viruses; NCDC: Nigerian center of disease control; nt: Nucleotide; ORF: Open reading frame; PEI: Paul Ehrlich Institute; RKI: Robert Koch Institute; WHO: World Health Organization

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Availability of data and materials
All data generated or analysed during this study are included in this published article.

Authors’ contributions
CI and CTB conceptualized the study. BW, OA, FAO, DH performed the experiment and data analysis. DN, ID, OX, UU, NM collected specimens. BW, OA, DH drafted the manuscript. CI and CTB revised the manuscript critically. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Not applicable. German public health institute (RKI) is authorized to receive blood residuals from diagnostics for surveillance purposes (Infection Protection Act IfSG §13). All samples analysed were anonymised.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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Author details
1. Division of Viral Gastroenteritis and Hepatitis Pathogens and Enteroviruses, Department of Infectious Diseases, Robert Koch Institute, 13353 Berlin, Germany.
2. Ladoke Akintola University of Technology, Ogbomoso, Oyo State, P.M.B. 4000, Nigeria.
3. Infectious Hazard Management Department, World Health Organization, Geneva, Switzerland.
4. Nigeria Centre for Disease Control, Port, 801, Ebute Ukiwe Street, Jabi, Abuja, Nigeria.
5. Institute of Tropical Medicine, University of Tübingen, 72074 Tübingen, Germany.

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