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Identification of rabbit hepatitis E virus (HEV) and novel HEV clade in Irish blood donors

To the Editor:

We read with interest that Cordes *et al.*[1] undertook a study wherein they performed individual donor nucleic acid testing (ID-NAT) for hepatitis E virus (HEV) in Germany using the Procleix HEV assay (Grifols, Barcelona, Spain). Using a similar approach, between January 4th, 2016 and March 17th, 2017, 172,277 blood donations were screened for HEV by the Irish Blood Transfusion Service. Screening was performed by ID-NAT using the Procleix HEV assay on the automated Procleix Panther System (Grifols). Irish blood donors consented for their samples to be used for the purposes of anonymous research. Forty-three confirmed HEV RNA-positive blood donations were identified, resulting in an overall incidence of 1:4,006. The HEV viral loads ranged from ~0.5-4.3 $\log_{10}$ IU/ml. Twenty-seven donations were window period donations; one was anti-HEV IgM positive; 4 anti-IgG positive; the remaining donations were positive for both IgM and IgG (Fortress Diagnostics, Antrim, Northern Ireland).

Cordes *et al.*,[1] performed molecular typing of the HEV-positive donations with subtype 3c predominating, with a further virus identified as subtype 3f consistent with the prevalence of these subtypes in Germany. Sequence analysis was performed on the HEV RNA-positive Irish donor samples using a combination of methods targeting the methytransferase (Met) and RNA-dependent (directed) RNA polymerase (RdRp) in open reading frame 1 (ORF1).[2,3] Sequences were generated for thirty (70%) of the HEV RNA-positive samples; 13 were HEV subtype 3c, 12 were 3e, 2 were 3f and 3 were unclassified. Of the unclassified viruses, unlike Cordes *et al.*,[1] who reported that they failed to identify rabbit HEV (HEV-3ra) in their cohort, one Irish donor virus (IE-568) was clearly related to HEV-3ra isolates. The Met sequence for IE-568 shares 87.6% nucleotide identity with a HEV-3ra from Japan (LC535077) whilst the RpRp sequence for IE-568 shares 92.58% identity with HEV 3ra sequences obtained from rabbits in China and Mongolia (KX227751 and AB740222, respectively).

Two further unclassified HEV isolates (IE-112 and IE-224) were found to share 88% and 89% nucleotide identity with each other for the Met and RdRp sequences, respectively; however, they were more distantly related to other HEV isolates in the GenBank database. Phylogenetic analysis of concatenated Met and RdRp sequence fragments (525 bp in total) demonstrated that IE-568 lies firmly within the HEV 3ra clade,[4] and IE-112 and IE-224 form a distinct clade clustering outside of HEV-3ra and basal to most HEV-3 sequences (Fig. 1A). In order to improve resolution of this new phylogenetic clade, it was possible to perform next generation sequencing for IE-112 using an HEV-sequence enriched library approach.[7] The full-length sequence shared, at best, only ~77-80% nucleotide identity with HEV-3ra and genotype 3 sequences in GenBank. Phylogenetic analysis of the full-length IE-112 sequence confirmed a clustering outside the HEV-3ra clade and basal to other HEV-3 clades, but distinct from the non-HEV-3 *Orthohepevirus* A genotypes (Fig. 1B); separate analysis of ORF1, ORF2 and ORF3 of IE-112 showed overall similar phylogeny for the novel sequence (not shown). Using several different approaches, there was no evidence of recombination in the IE-112 sequence, including the hypervariable region in ORF1 (Fig. S1-S3). Although IE-112 does not contain the 90/93 bp insert, within the ORF1 X region, characteristic of HEV-3ra viruses, neither does a recently identified virus (rab81) isolated from a wild rabbit in Germany[6] (Fig. S4). Both IE-II2 and IE-224 form a distinct and novel HEV clade distantly related to HEV-3ra.

Re-testing of IE-568, IE-112 and IE-224 using the altona Diagnostics realStar® HEV RT-PCR kit (altona Diagnostics, Hamburg, Germany) yielded viral loads of 1.1, 4.3 and 2.8 $\log_{10}$ IU/ml, respectively. Using the Cobas® HEV/Cobas® 6800 System (Roche Diagnostics, Mannheim, Germany) IE-112 and IE-224 had viral loads of 4.4 and 2.5 $\log_{10}$ IU/ml compared to the World Health Organization International Standard (6329/10), similar to the Altona Diagnostics assay values; IE-568 was not detected since the viral load was below the 95% cut-off of the Cobas® HEV assay (18.6 IU/ml). Nevertheless, we have previously observed reduced sensitivity (~2 $\log_{10}$ IU/ml) for one HEV-3ra isolate (MG211750)[8] with the Cobas® HEV assay, but not the Procleix assay, although it is not known if this is the case for other HEV-3ra viruses.

All 3 donors ate pork with donor IE-112 reported to have consumed “black pudding” (pork/beef blood sausage) as well as cured meats. Whilst donor IE-224 reported eating medium/rare venison, the other donors denied eating game meat including rabbit. One donor (IE-112) owned a dog, the other donors had no pets. No donors reported contact with farm animals; donor IE-568 reported contact with a koala in an Australian zoo ~4

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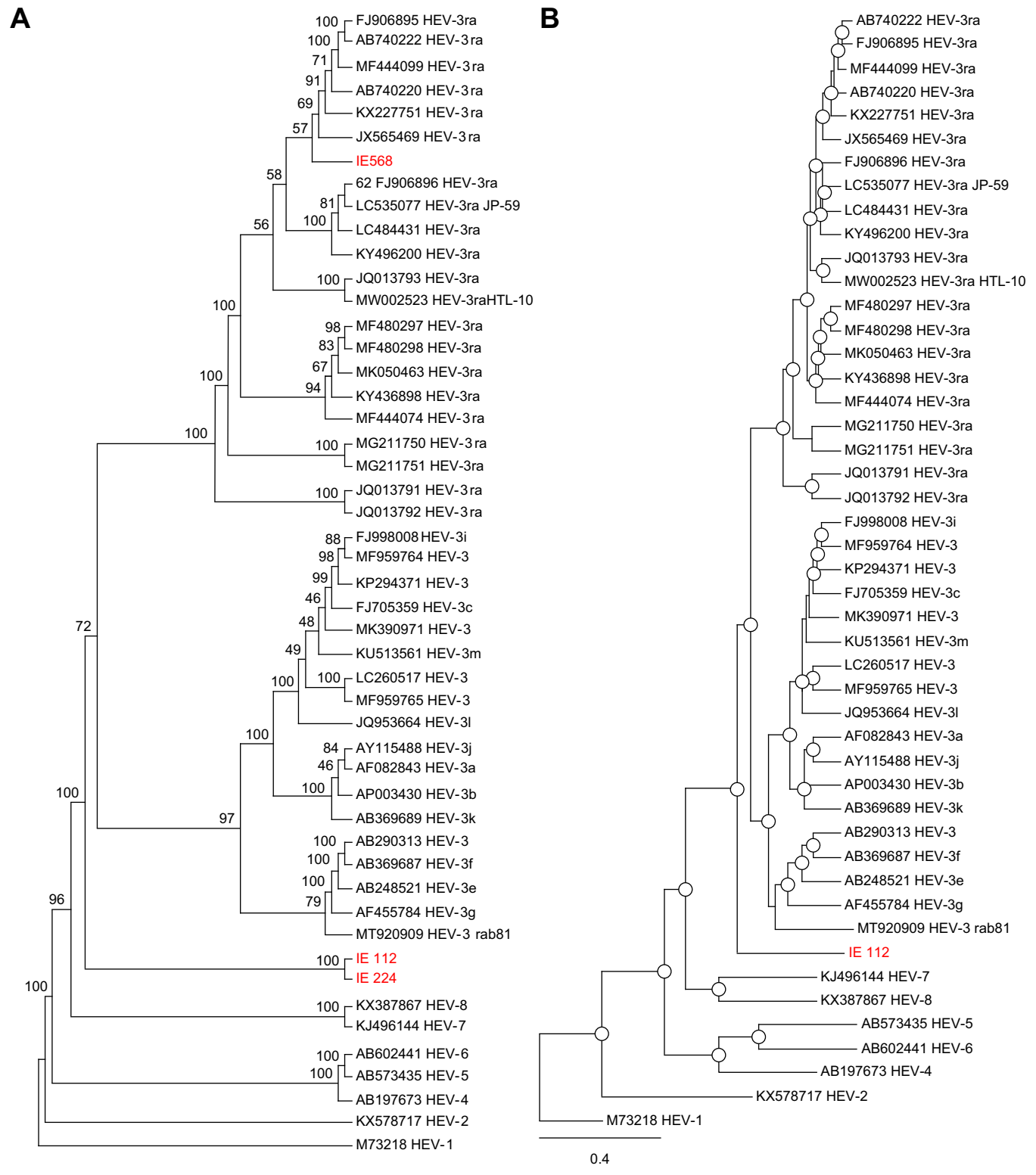

Fig. 1. Phylogenetic analysis of HEV-positive Irish donations IE-568, IE-112 and IE-224. (A) Cladogram of maximum likelihood phylogenetic tree. Phylogenies were generated using Geneious Prime® 2022.0.1 and nucleotide alignments of the Met (242 bp), the RdRp (283 bp) fragments of IE-586 and IE-224, and complete genome data for IE-112 and HEV reference sequences.[5] Numbers at nodes, represent bootstrap values of repetitive analyses for confidence testing. (B) Bayesian phylogenetic analysis of the full-length sequence of IE-112 and reference sequences, here all alignment position containing any ambiguous data or gaps have been removed from the dataset. A GTR model with a γ distribution (G) across sites and a proportion of invariant sites (I) (GTR + G + I) was used as the substitution model; HEV-1 was used as the out-group. Trees were run for 1 million generations and sampled every 500 steps. Circles, at nodes, represent Bayesian posterior probability support of 1 and the scale bar indicates genetic distance. In addition to proposed reference sequences, full-length HEV 3ra isolates (HTL-10 and JP-59), published since the latest HEV reference sequence update,[5] have been included in the analysis. The HEV isolate identified in a wild rabbit in Germany (rab81[6]) has been tentatively proposed as a novel subtype of HEV genotype 3. The red lines indicate the donor isolates described in the study. The sequences of IE-112, IE-224 and IE-568 are listed under GenBank accession numbers OM777188, OM777189 and OM777190, respectively. GTR, general time-reversible.

months prior to donation. Although donors IE-112 and IE-224 donated within 1 week of each other, one lived in Dublin whilst the other lived in a rural area on the West coast. None of the donors travelled abroad in the 9 weeks prior to donation suggesting autochthonous transmission of HEV. No post-donation illnesses were reported. HEV-3ra sequences have mainly been identified in immunosuppressed individuals[9,10] and the detection of IE-568 in a healthy blood donor is noteworthy. With the identification of the novel isolates IE-112 and IE-224, forming a new clade and potentially a tentative new genotype, further investigations are required to understand potential animal origins associated with these Irish isolates and potential routes of transmission which, in the case of HEV-3ra, where it has been identified in humans, remain elusive.

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Conflicts of interest
The authors declare no conflicts of interest.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions
S.A.B - analysis of HEV-positive donor samples, writing and review of manuscript; N.O'F - HEV screening, review of manuscript; L.B. - HEV screening, review of manuscript; B.H. - analysis of HEV-positive samples, review of manuscript; VMC - analysis of HEV-positive donor samples, writing and review of manuscript.

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Supplementary data
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Exacerbation of familial intrahepatic cholestasis in conjunction with COVID-19 vaccination

To the Editor:

The devastating global impact of the COVID-19 pandemic led to the fast development of efficient anti-COVID-19 vaccines. These have in rare instances been associated with side effects including autoimmune hepatitis.[1,2]

Herein, we report the case of a 53-year-old man presenting in our emergency department with jaundice and pruritus. Within 48 hours following BTN162b2 Pfizer-BioNTech mRNA COVID-19 vaccination he felt fatigued, developed nausea, severe ubiquitous pruritus and a temperature (37.5-38°C). Progressive

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