Appendix

This appendix was part of the submitted manuscript and has been peer reviewed. It is posted as supplied by the authors.

Online appendix for “First confirmed case of transfusion-transmitted hepatitis E in Australia”

Molecular characterisation of hepatitis E virus

Hepatitis E virus (HEV) RNA was detected in our patient’s liver biopsy and whole blood from March 2015 using an in-house RT-PCR assay. On retrospective testing using the same assay, HEV RNA was detected in post-transplant plasma from September 2014 but was not detected in pre-transplant serum from July 2014. The HEV RNA detection was confirmed at a national reference centre using a commercial HEV assay (RealStar HEV RT-PCR, Altona Diagnostics, Germany). Nucleotide sequencing of the HEV open reading frame 2 (ORF2) region identified it as HEV genotype 3. Patient liver samples and donor liver samples set aside at the time of transplant were retrieved and tested using the in-house and commercial assays and HEV RNA was not detected.

The Australian Red Cross Blood Service (Blood Service) keeps a repository sample of every donation collected for three years following donation. The archived samples from the units transfused to the patient peri-transplant were retrieved and tested for HEV RNA at the same national reference centre using the commercial assay. HEV RNA was detected in one blood donation and was found by sequence analysis to be HEV genotype 3. This donation was a whole blood donation that was manufactured into red cell, platelet, and fresh frozen plasma (FFP) components. Our patient had received the FFP component of the implicated donation, while the red cell component had been transfused into another recipient who died of an unrelated cause and the platelet component was discarded. The viral load of the HEV positive donation was quantified using a “research use only” RT-PCR with 10-fold serial dilutions of a WHO International Standard for HEV (Paul-Ehrlich-Institut, Germany). The viral load was estimated to be 947 IU/mL.

Sequencing of the ORF2 region of the HEV genome in the blood donation demonstrated 100% homology with the patient HEV where the sequences overlapped (145 base pairs). The donor and patient sequences were compared with other HEV sequences in GenBank® and matched best with European human- and swine-origin strains. The sequences were also compared to HEV sequences from a contemporaneous foodborne outbreak of HEV in NSW and were found to have at most a 90% homology in the ORF2 region overlap (167/186 base pairs). Phylogenetic analysis resolved our sequences and published reference sequences into previously described monophyletic groups despite the small region sequenced (Figure 1). The donor and patient sequences clustered together within the 3chi group of the HEV-3 clade, whereas the foodborne outbreak sequence from NSW was assigned to the 3jab group.

The cause of our patient’s original hepatitis necessitating transplant remains unknown, but is unlikely to be HEV-related as HEV RNA was not detected in blood collected during the acute hepatitis or in patient liver samples set aside at the time of transplant. The detection of HEV RNA in blood collected soon after transplant suggested acquisition peri-transplant, but transmission from the donor liver was excluded. Incidental foodborne acquisition related to a HEV outbreak that occurred in NSW around the time of the transplant is unlikely, as the patient HEV sequence did not show high homology with outbreak strains and clustered within a different monophyletic group on phylogenetic analysis. The Blood Service investigation found HEV RNA in a blood product the patient received peri-transplant at viral loads above the minimum infectious dose associated with transfusion-transmission (TT). The HEV RNA detected in the blood product sample showed 100% homology with the patient’s strain and clinical history suggests the blood product donor acquired HEV infection in France prior to donation. This evidence strongly supports the conclusion that the recipient’s HEV infection was TT.
**Figure 1: Molecular Phylogenetic Analysis**

Molecular phylogenetic analysis was performed as described previously (2) with the percentage of trees (from 500 bootstrap replicates) in which the associated taxa clustered together is shown next to the branches when over 50%.

a) Global view of the unrooted tree using 193 reference sequences cited by (2) covering 117 codons. Donor and recipient sequences (overlapping) are shown with red circle and unrelated NSW foodborne outbreak sequence with blue square.

b) Focused view of donor and recipient sequences (red circles) with the most closely related sequence by BLAST search immediately below and the 12 most closely related reference sequences. Genotype, country of origin, host and year are shown, where available.

**References**