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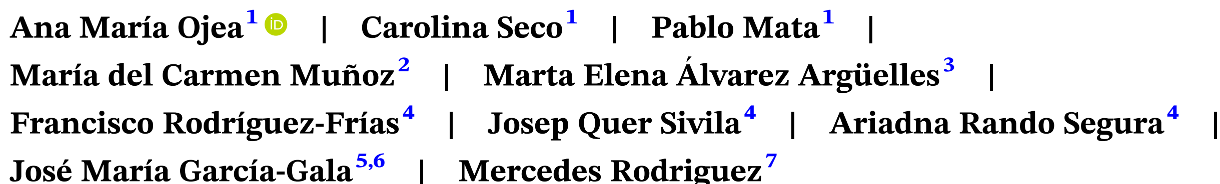
CASE REPORT

TRANSFUSION

Transfusion-transmission of hepatitis E virus through red blood cell transfusion but not through platelet concentrates: A case report from Spain



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Abstract

Background: Few cases of transfusion-transmitted hepatitis E virus (HEV) have been published in Spain. Here, we describe a well-characterized lookback investigation of a transfusion-transmitted HEV case at the Community Centre for Blood and Tissues of Asturias (Spain).

Case Report: A female patient with chronic myeloid leukemia underwent an allogeneic bone marrow transplant in March 2019 and showed alterations in liver function shortly afterwards. This patient received blood components from 30 different donors in the 3 months before the transplant. Frozen plasma samples from these donations were investigated for the presence of HEV-RNA. One frequent donor was identified as asymptomatic HEV RNA-positive at the time of his whole blood donation. The investigation revealed that this donor's plasma unit, originally intended for the fractionation industry, had a viral RNA concentration of 1.9×10^4 copies/mL. HEV RNA was detected initially in the index patient who received the red cell concentrate from this donor 25 days after the transfusion. HEV RNA isolated from both donor and recipient were identified as subtype 3f. The recipient of platelet concentrate (PC), treated with a riboflavin-based pathogen reduction technology (PRT) was not infected, being negative for the presence of HEV IgM, IgG, and HEV RNA before and after the transfusion.

Conclusion: This case study shows that HEV was transmitted through red cell transfusion to a recipient, while the patient who received riboflavin/UV light treated PC did not develop signs of infection. A causal relationship between PRT treatment of the PC and the non-transmission of HEV remains to be established.

Abbreviations: ALT, alanine; AST, amino transferase; BC, buffy coat; CCBTA, Community Centre for Blood and Tissues of Asturias; CML, chronic myeloid leukemia; HEV, Hepatitis E virus; PC, platelet concentrate; PCR, polymerase chain reaction; PRT, pathogen reduction technology; RCC, red cell concentrate; SOPs, standard operating procedures; TT, transfusion-transmitted; UV, ultraviolet.

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KEYWORDS
hepatitis, transfusion-transmitted disease

1 | INTRODUCTION

Hepatitis E virus (HEV) is the main etiological agent of the enterically transmitted viral non-A, non-B hepatitis.[1] It is responsible for 3.3 million symptomatic cases of hepatitis E worldwide.[2] Over the last decade, the incidence of HEV infections has had a 10-fold increase across European countries.[3] Most HEV infections are asymptomatic and resolve spontaneously.[4] The primary route of the viral transmission is fecal/oral, associated with the contamination of the drinking water (mainly by genotype 1 and 2 viruses).[2] Where sanitation and water supply are better, HEV infection is sporadic. In these cases, the infection is primarily caused by the genotype 3 virus that is acquired by consumption of contaminated food, frequently undercooked animal meat.[2] Additionally, HEV can also be transmitted through blood transfusion. Cases of HEV infection by contaminated blood components have been described since 2004, initially in Japan (genotype 3 and 4) and Europe (genotype 3).[5–10] A retrospective study, with 225,000 blood donations from Southeast England (UK) that were collected between 2012 and 2013, detected 79 infected blood donations with genotype 3 HEV.[11] Of the 43 recipients who received HEV-positive blood components, 18 (42%) showed evidence of transfusion-transmitted HEV (TT-HEV). The risk for diagnosed acute hepatitis E after being transfused with a contaminated blood component appears to depend on the anti-HEV status of the donor, the virus dose transfused as well as the recipient's immunologic state.[11] Accordingly, some countries, such as Ireland, UK, Japan, or the Netherlands, have implemented HEV RNA screening for all blood donations.[12] Alternatively, pathogen reduction technology (PRT) aims to improve the safety of transfused blood products through the inactivation of bloodborne pathogens as well as contaminating white blood cells.[13] Currently available PRTs use ultraviolet (UV) light with a photosensitizer to cause irreversible breakage of nucleic acids blocking further replication of cells and pathogens.[14]

Anecdotal cases of TT-HEV have been published from blood centers in Spain.[15,16] Here, we describe a well-characterized lookback investigation of an HEV case transmitted via transfusion, carried out at the Community Centre for Blood and Tissues of Asturias (CCBTA) in Oviedo, Spain. Moreover, we show that the platelet concentrates (PCs) produced by pooling 5 units of buffy coat (BC), including one BC derived from the HEV-contaminated donation, did not transmit the infection to the recipient. This PC was treated with the PRT method based on riboflavin and UV light.

2 | METHODS

2.1 | Collection and screening of blood donations for infectious diseases

In the Spanish region of Asturias, whole blood donations are screened and fractionated at the CCBTA. Donations are routinely screened for human immunodeficiency virus, hepatitis B virus, hepatitis C virus, and *Treponema pallidum* with serological and nucleic acid tests, Elecsys® anti-HCV/HBsAg II/HIV Duo/Syphilis and Cobas® TaqScreen MPX Test (Roche Diagnostics, Switzerland), respectively. One plasma sample of 1,000 μL is automatically aliquoted from each whole blood donation with the Aliquot STARlet (Hamilton Robotics, USA) and stored at −20°C for 30 years.

2.2 | Blood component preparation

Whole blood donations were collected and processed at CCBTA in accordance with the site's standard operating procedures (SOPs), and following European and Spanish guidelines for blood processing. The PCs derived from whole blood were generated by pooling five BC units with the TACSI PL device (Terumo Blood and Cell Technologies, USA). Each BC unit contributed approximately 25–30 mL of donor plasma. The PCs were treated with the Mirasol® PRT System (Terumo Blood and Cell Technologies, USA),[17] following the manufacturer's instructions, and were stored in platelet additive solution (SSP+, MacoPharma, France) up to 7 days.[18] This PRT system is based on the use of riboflavin and UV light; its ability to reduce pathogens has been reported.[19,20]

2.3 | Investigation of TT-HEV case

Frozen plasma samples from the suspected donors were sent to the Department of Microbiology at the University Hospital Vall d'Hebron (Barcelona, Spain), and screened

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for the presence of HEV RNA (Cobas® HEV NAT assay, on Cobas 6800/8800 systems, Roche Diagnostics, Switzerland). The viral RNA concentration was determined using an in-house quantitative polymerase chain reaction (PCR) assay performed at the University Central Hospital of Asturias (Oviedo, Spain). This in-house method used automatic nucleic acid extraction with MagNApure 96 Instrument (Roche Diagnostics, Switzerland) and TaqMan Fast Virus 1-Step Master Mix (Applied Biosystems by Thermo Fischer Scientific, USA). Primers used to amplify a sequence at the ORF1 region were previously described.[21] The limit of detection of this assay was determined to be less than 50 HEV RNA copies/mL.

The anti-HEV status of the blood donor and patients was determined using HEV IgM/HEV IgG ELISA kits (DiaPro Diagnostic Bioprobes, Italy). All anti-HEV ELISA positive samples were subsequently confirmed using the recomLine HEV IgG/IgM kit (Mikrogen Diagnostik, Germany). Levels of aspartate and alanine aminotransferase in the serum of patients were measured with Roche/Hitachi Cobas c701/702 (Roche Diagnostics, Switzerland), according to International Federation of Clinical Chemistry guidelines. The final PC containing one BC unit from the index HEV donor was prepared and treated with the Mirasol PRT system in accordance with the site's SOP.

Bootstrap analysis of donor and recipient's HEV isolates was performed at Hospital Vall d'Hebron. For this purpose, RNA was extracted using the QIAamp® Viral RNA kit (Qiagen, Netherlands), a nested RT-PCR using the Transcriptor One Step RT-PCR kit (Roche, Switzerland) and the FastStart™ High Fidelity PCR System, dNTPack (Roche, Switzerland). Purification of the amplified DNA was carried out with QIAquick Gel Extraction kit (Qiagen, Netherlands). The purified, amplified product was sequenced by Macrogen Europe (Amsterdam, Netherlands). For the characterization of the subtype of isolates from both donor and recipient, a phylogenetic tree was constructed using sequences of both isolates as well as reference sequences of all HEV genotypes and subtypes from GenBank. To conclude the relationship between the two isolates, the sequence of a local isolate (HEV-025) from the same subtype was included and a relationship was established using the Hamming distance method (Table S1).

3 | CASE PRESENTATION

A female patient diagnosed with chronic myeloid leukemia (CML) underwent an allogeneic bone marrow transplant on March 6th, 2019. After the intervention she showed a gradual increase in levels of alanine (ALT) and aspartate aminotransferase (AST), culminating with 2,138 U/L and 1,557 U/L for ALT and AST, respectively on April 19th, 2019 or day +43 post-transplant. The patient had received 30 blood components from 30 distinct donors during the 3 months before the transplant. One of the donors, a 34-year-old male, frequent donor, who resided in a rural area was diagnosed to be HEV RNA-positive. This donor consumed homemade, freshly slaughtered food derived from raw pork meat. He was asymptomatic both before and after the blood donation. The donor's anti-HEV positive status was confirmed 18 months after the index donation.

The examination of the donor's plasma unit (recovered from the fractionation industry) revealed the presence of viral RNA at a concentration of 1.9×10^4 HEV RNA copies/mL. Although the suspected PC unit was not tested for HEV-RNA it has been calculated that the one BC coming from the index donation contributed approximately with 25–30 mL of plasma to the final pooled PC (a total volume of 415 mL), which is similar to the volume of residual plasma contained in the red cell concentrate (RCC) unit according to the standard manufacturing protocol. Therefore, the transfused HEV dose in the final PC was estimated to be between 4.8 and 5.7×10^5 HEV RNA copies, which is similar to the estimated dose in RCC that led to the infection of the index patient. The RCCs (a total volume of 260 mL) was transfused to the female patient with CML. The PC was transfused to a 79 years old male patient who underwent surgery. Both blood components were completely transfused to their respective recipients.

Retrospective examination of RCC recipient's plasma showed low HEV RNAemia from day +1 to day +20 post-transplant (day +25 to day +45 post-index transfusion), but on day +46 post-transplant (day +70 post-transfusion), no more virus could be detected. Weak anti-HEV IgM positivity was detected on day +36 post-transplant (day +60 post-transfusion) and finally a clear anti-HEV IgM positivity with confirmed immunoblot was observed on day +47 post-transplant (day +71 post-transfusion). In a later laboratory analysis (17 months after transplant), the patient was still seropositive for IgG and IgM.

Two samples from the PC recipient, one obtained approximately 18 months before and another 5 months after the transfusion were screened for HEV IgM and IgG (ELISA kits, DiaPro). The results from both samples were negative for HEV IgM and IgG. Moreover, HEV RNA tests were also negative in both samples.

The phylogenetic tree investigation performed with the donor and RCC recipient isolates revealed that both belong to subtype 3f and shows a clear cluster (bootstrap

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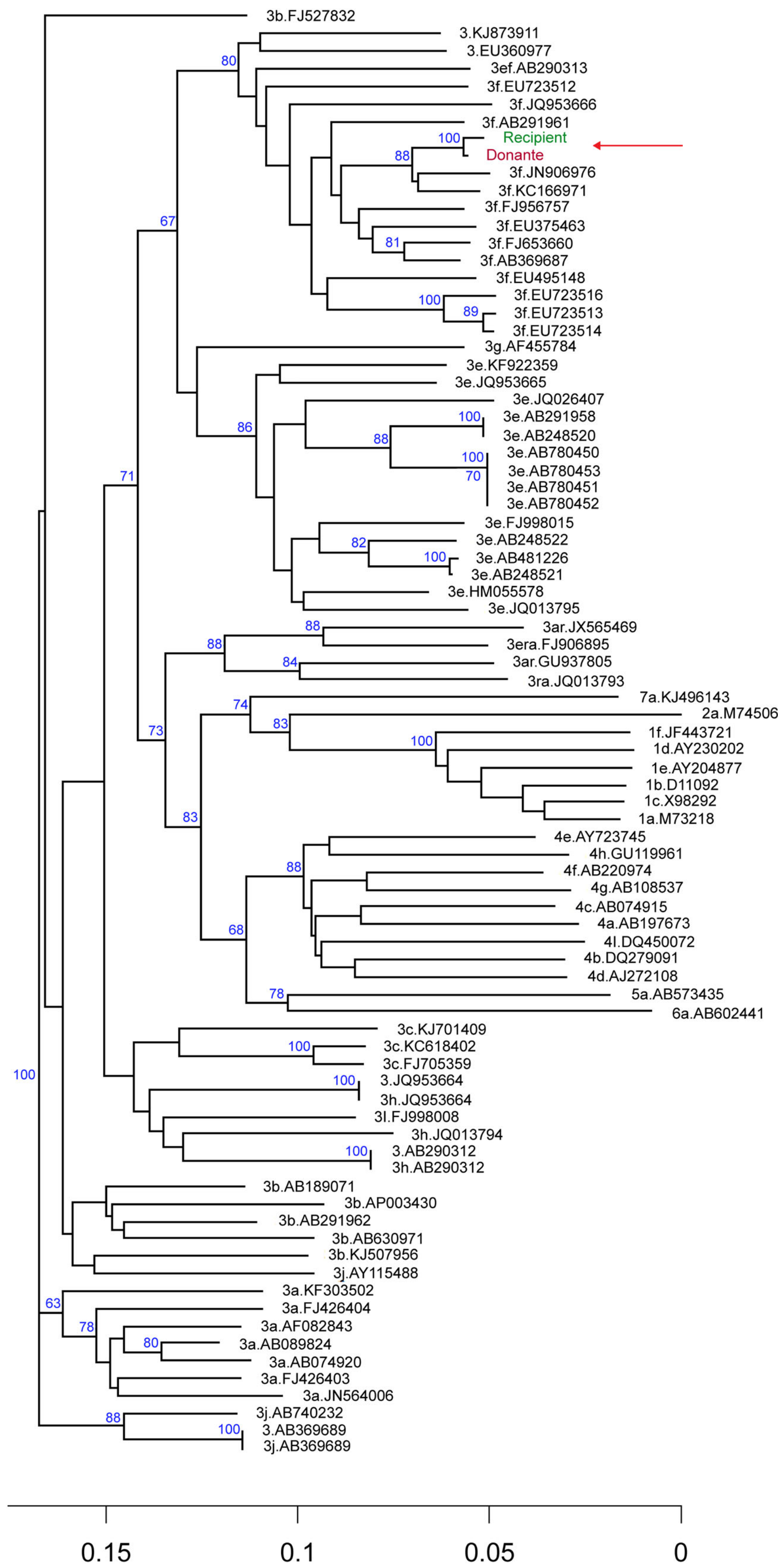

FIGURE 1 Bootstrap phylogenetic tree with subtype 3f isolates, including some from the same geographical region. Neighbor-joining tree on p-distances. The arrow shows the pair donor/recipient isolates. [Color figure can be viewed at wileyonlinelibrary.com]

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100%) between them: only two mutations were observed between these isolates whereas nine mutations were identified to the next closest local sequence, and 14 mutations with a sequence from the Genbank (Figure 1).

4 | DISCUSSION

The TT-HEV potentially represents a serious health problem for transfusion recipients due to the fact that many transfused patients are immunosuppressed or are in poor health; thus, being susceptible to more severe complications from TT-HEV.[22] To our knowledge, few reports have evaluated HEV infection associated with transfused blood components in Spain, where the adoption of HEV screening in blood donations is still under evaluation.[15,16,23] Riveiro-Barciela *et al.*[15] reported in 2017, the case of a 61-year-old male who was diagnosed with acute hepatitis E after transfusion of 8 units of RCCs. A look-back investigation revealed that one of the eight archived samples was HEV RNA-positive, with an estimated viral load of 75,000 IU/mL. The donor was a 41-year-old male who was asymptomatic for hepatitis E before and after the blood donation. He was working in a pork sausage factory and frequently ate raw pork meat. Rivero-Juarez *et al.*[16] investigated prospectively 11,313 healthy blood donors between 2017 and 2018. HEV RNA was detected in four blood donations (0.04% of total). Therefore, the estimated prevalence in this blood donor population is 1:2,828 donations.

Our present case adds further evidence of TT-HEV occurrence in Spain. The implicated whole blood donation was collected at the asymptomatic acute phase of infection and was highly infectious with a titer of 1.9×10^4 HEV RNA copies/mL. Phylogenetic analysis of isolates from donor and recipient determined the same subtype differing by only two mutations, which is in agreement with the estimation of HEV mutation rates from clinical isolates and establishing the causal relationship.[24]

In respect to the non-transmission of HEV to the PC recipient, the estimated dose of transfused HEV in the final PC unit was similar to that in RCC. Hewitt *et al.*,[11] observed that HEV transmission is related to the levels of RNA in donors whose components transmitted HEV. In this current report, the level determined in the plasma of the co-component correlated to levels similar to median levels described by Hewitt *et al.*[10] for donors who transmitted the infection.

This case study suggests that the Mirasol treatment of the PC could have contributed to abrogate the HEV transmission; an effect firstly postulated by Owada *et al.*[25] Yet, a causal relationship between PRT treatment of the PC and the non-transmission of HEV to the second patient cannot be completely demonstrated. Other factors might have played a role, for example the recipient might have been able to produce a powerful immunoresponse. In addition, processing of BC-derived platelet concentrates requires additional steps, such as a second centrifugation, which might influence the final pathogen load in this component.

This report, on the other hand, highlights the importance of routine nucleic acid screening of all blood donations and/or the use of a proactive safety measure like PRT to reduce the risk of transmission of HEV through blood transfusion.

Meanwhile, CCBTA's commitment to transfusion security led to the implementation in January 2020 of HEV-NAT mini-pool screening while continuing to apply Mirasol PRT to 100% of the produced PC.

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CONFLICT OF INTEREST STATEMENT

Authors have no conflict of interest to declare.

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REFERENCES

1. Kumar S, Subhadra S, Singh B, Panda BK. Hepatitis E virus: the current scenario. Int J Infect Dis. 2013;17(4):e228–33.
2. World Health Organization. Hepatitis E. Available from https://www.who.int/news-room/fact-sheets/detail/hepatitis-e
3. European Centre for Disease Prevention and Control. ECDC report: 10-fold increase of hepatitis E cases in the EU/EEA between 2005 and 2015. Available from https://www.ecdc.europa.eu/en/news-events/ecdc-report-10-fold-increase-hepatitis-e-cases-eueea-between-2005-and-2015
4. Horvatits T, Schulze Zur Wiesch J, Lütgehetmann M, Lohse AW, Pischke S. The clinical perspective on hepatitis E. Viruses. 2019;11:617.
5. Mitsui T, Tsukamoto Y, Yamazaki C, Masuko K, Tsuda F, Takahashi M, et al. Prevalence of hepatitis E virus infection among hemodialysis patients in Japan: evidence for infection with genotype 3 HEV by blood transfusion. J Med Virol. 2004;74:563–72.
6. Matsubayashi K, Nagaoka Y, Sakata H, Sato S, Fukai K, Kato T, et al. Transfusion-transmitted hepatitis E caused by apparently indigenous hepatitis E virus strain in Hokkaido, Japan. Transfusion. 2004;44:934–40.
7. Boxall E, Herborn A, Kochethu G, Pratt G, Adams D, Ijaz S, et al. Transfusion-transmitted hepatitis E in a 'nonhyperendemic' country. Transfus Med. 2006;16:79–83.

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8. Colson P, Coze C, Gallian P, Henry M, de Micco P, Tamalet C. Transfusion-associated hepatitis E, France. Emerg Infect Dis. 2007;13:648–9.
9. Huzly D, Umhau M, Bettinger D, Cathomen T, Emmerich F, Hasselblatt P, et al. Transfusion-transmitted hepatitis E in Germany, 2013. Euro Surveill. 2014;19:20812.
10. Sakata H, Matsubayashi K, Iida J, Nakauchi K, Kishimoto S, Sato S, et al. Trends in hepatitis E virus infection: analyses of the long-term screening of blood donors in Hokkaido, Japan, 2005–2019. Transfusion. 2021;61(12):3390–401.
11. Hewitt PE, Ijaz S, Brailsford SR, Brett R, Dicks S, Haywood B, et al. Hepatitis E virus in blood components: a prevalence and transmission study in Southeast England. Lancet. 2014;384: 1766–73.
12. Denner J, Pischke S, Steinmann E, Blümel J, Glebe D. Why all blood donations should be tested for hepatitis E virus (HEV). BMC Infect Dis. 2019;19:541.
13. Alter HJ. Pathogen reduction: a precautionary principle paradigm. Transfus Med Rev. 2008;22:97–102.
14. McCullough J. Progress with pathogen-reduced blood. Blood. 2018;132:119–21.
15. Riveiro-Barciela M, Sauleda S, Quer J, Salvador F, Gregori J, Pirón M, et al. Red blood cell transfusion-transmitted acute hepatitis E in an immunocompetent subject in Europe: a case report. Transfusion. 2017;57:244–7.
16. Rivero-Juarez A, Jarilla-Fernandez M, Frias M, Madrigal-Sanchez E, López-López P, Andújar-Troncoso G, et al. Hepatitis E virus in Spanish donors and the necessity for screening. J Viral Hepat. 2019;26:603–8.
17. Terumo BCT. Mirasol. Available from https://www.terumobct.com/mirasol
18. Garcia-Gala JM, Muñoz-Turrilas C, Buesa-Garcia C, et al. Observational study comparing clinical performances of mirasol and intercept-treated platelet concentrates in routine. Vox Sang. 2015;109:184.
19. Hauser L, Roque-Afonso AM, Beylouné A, Simonet M, Deau Fischer B, Burin des Roziers N, et al. Hepatitis E transmission by transfusion of intercept blood system-treated plasma. Blood. 2014;123:796–7.
20. Singh Y, Sawyer LS, Pinkoski LS, Dupuis KW, Hsu JC, Lin L, et al. Photochemical treatment of plasma with amotosalen and long-wavelength ultraviolet light inactivates pathogens while retaining coagulation function. Transfusion. 2006;46:1168–77.
21. Fogeda M, Avellón A, Cilla CG, Echevarría JM. Imported and autochthonous hepatitis E virus strains in Spain. J Med Virol. 2009;81(10):1743–9.
22. Westhölter D, Hiller J, Denzer U, Polywka S, Ayuk F, Rybczynski M, et al. HEV-positive blood donations represent a relevant infection risk for immunosuppressed recipients. J Hepatol. 2018;69:36–42.
23. Domanović D, Tedder R, Blümel J, Zaaijer H, Gallian P, Niederhauser C, et al. Hepatitis E and blood donation safety in selected European countries: a shift to screening? Euro Surveill. 2017;22:30514.
24. Primadharsini PP, Nagashima S, Okamoto H. Genetic variability and evolution of hepatitis E virus. Viruses. 2019;11:456.
25. Owada T, Kaneko M, Matsumoto C, Sobata R, Igarashi M, Suzuki K, et al. Establishment of culture systems for genotypes 3 and 4 hepatitis E virus (HEV) obtained from human blood and application of HEV inactivation using a pathogen reduction technology system. Transfusion. 2014;54:2820–7.

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