Prevalence of Hepatitis E Virus Infection Among Blood Donors in the Eastern Province of Saudi Arabia

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Purpose: Hepatitis E virus (HEV) causes acute hepatitis in humans and constitutes a major problem for immunocompromised patients, patients with hematological diseases, and pregnant women. It is transmitted mainly through fecal oral route; however, transmission through blood and blood products is reported globally and becoming a health concern. We sought to determine the prevalence of HEV among blood donors in the Eastern Province of Saudi Arabia using molecular as well as serological assays to assess the safety of blood transfusion and the need for HEV screening among blood donors.

Patients and Methods: A total of 806 whole blood samples were collected from blood donors between May and November 2020 and tested for anti-HEV IgG and IgM antibodies by ELISA and for HEV RNA by RT-PCR.

Results: The overall seroprevalence of HEV IgG antibodies was 3.2% with no statistically significant difference between the non-Saudis (3.28%) and Saudis (3.17%) (p value 0.929) or between males (3.14%) and females (4.88%) (p value 0.527). None of the IgG positive individuals had IgM antibodies. HEV RNA was not detected in any of the blood donors.

Conclusion: HEV seroprevalence is low among blood donors in the Eastern Province of Saudi Arabia and may constitute minimal risk for transfusion associated infections.

Keywords: HEV, IgG, IgM, seroprevalence, ELISA, RT-PCR

Introduction

Hepatitis E virus (HEV) is a single-stranded positive sense RNA, non-enveloped, icosahedral virus that belongs to the genus orthohepeivirus of the family Hepeviridae. Like other hepatitis viruses, members of this genus affect the liver and cause acute hepatitis in humans and various mammals. Immunocompromised patients and pregnant women are also a cause of chronic hepatitis.1,2 A key feature of HEV, unlike other hepatitis viruses, is its ability to infect animals as well. Phylogenetically, the genus Orthohepeivirus A is divided into eight genotypes (HEV 1–8) with different host specificity and geographical localization. HEV-1 and HEV-2 are mostly associated with human infection, while HEV-3 and HEV-4 can infect humans and animals, such as swine, deer, goats, Bottlenose dolphins and boars.3–5 HEV-5 and HEV-6 were found in wild boar in Japan, while HEV-7 and HEV-8 have only been isolated from camels in China.6 Geographically, genotype 1 is most commonly reported in the countries of Asia and Africa, while genotype 2 is most common in Mexico, Nigeria, and Chad.3,5 Genotype 3 is limited to Japan, Korea, and Taiwan, while genotype 4–8 is restricted to Asia.3,6

The first epidemic of HEV infection was reported in India as icteric hepatitis in 1955, after which the oral route of infection was documented in a Russian military
Although HEV infection is mostly self-limiting and can cause asymptomatic disease, infection of immunocompromised, thalassemic, HIV patients, and pregnant women can cause mild forms of hepatitis, extra-hepatic manifestations, and death in some cases.\textsuperscript{9}

HEV infection is mostly ecologically dependent and is associated with travel history to endemic regions or low sanitary conditions, involving water-borne and fecal oral routes as the predominant route of transmission.\textsuperscript{9} However, evidence of HEV transmission through blood and plasma has been reported globally.\textsuperscript{7,10–13} According to a report from the World Health Organization (WHO), about 118.2 million blood donations were collected in 118 countries globally in the year 2013, and nearly 21 million blood components were transfused annually in the USA alone.\textsuperscript{14} But HEV is not included in routine testing for pathogens before blood transfusion. A number of surveillance studies have been done to detect the prevalence of HEV in blood donors, indicating that the sero-positivity of HEV among blood donors ranges from 2\% to 49\% in different parts of the world.\textsuperscript{4,9,11–55} Some studies reported active viremia in blood donors, indicating a direct risk to blood or blood components recipients.\textsuperscript{7,12,18,21–24,29,30,32,34–41,45,46,48,52–54,56–58} Table 1 summarizes studies in the literature concerning HEV-seroprevalence among blood donors.

The transfusion-related HEV transmission, which is reported in several studies, strongly suggests the need for HEV screening of donated blood. No study from Saudi Arabia so far has investigated the prevalence of HEV RNA in blood donors to estimate the risk of HEV transmission from blood transfusion. In our study, we sought to determine the prevalence of HEV among blood donors in the Eastern Province of Saudi Arabia using molecular as well as serological assays.

**Materials and Methods**

**Inclusion and Exclusion Criteria**

All blood donors attend the blood bank section of the Laboratory department of king Fahd Hospital of the University (KFHU) in Al-Khobar, Saudi Arabia. Blood samples were centrifuged, then the plasma was separated and stored at \(-80^\circ\text{C}\). Written ethical consent was taken from the participating volunteers, including information on their age, gender, and nationality. All the volunteers were informed about the purpose of study in accordance with the declaration of Helsinki.

Ethical approval for the study was obtained from ethical committee of the Institution Review Board (IRB) at Imam Abdulrahman Bin Faisal University (IAU) (number IRB-2020-01-149).

**Serological Tests**

All plasma samples were tested qualitatively for anti-HEV antibodies of IgG type using an indirect enzyme-linked immuno-sorbent assay, Human Hepatitis E Virus IgG (HEV IgG) ELISA Kit (Abbexa Ltd, Cambridge, UK).\textsuperscript{60} Positive HEV IgG plasma samples were further tested for Anti-HEV IgM antibodies against Orthohepevirus A genotypes, using Human Hepatitis E Virus IgM (HEV IgM) ELISA Kit (Abbexa Ltd, Cambridge, UK) following the manufacturer’s instruction.\textsuperscript{60,61}

The HEV IgG & IgM ELIZA plates are coated with Recombinant HEV ORF-2/ORF-3 antigen and Mouse-anti-human IgM (\(\mu\) chain), respectively. These ELIZA kits can detect IgG/IgM antibodies against Orthohepevirus A genotypes. The sensitivity and specificity of HEV-IgM (abx055720) is 99.6\% and 99.2\%, while HEV-IgG (abx364866) is 99.5\% and 99.3\%, respectively.

**NAT Testing**

RNA was extracted from plasma samples using QIAamp Viral RNA mini kit (Qiagen, Hilden Germany), as per the manufacturer’s instructions. All samples were spiked with internal control from the employed detection kit. All plasma samples were tested for HEV RNA using RealStar HEV RT-PCR Kit 2.0 (Altona Diagnostics GmbH, Hamburg, Germany).\textsuperscript{62} The assay was run on the Applied Biosystem QuantStudio™ 5-Realtime PCR system (Thermo Fisher Scientific, MA, USA). Quantification standards provided with the kit were used with each run. The standards are designed in accordance with the first World Health Organization International Standard for Hepatitis E Virus RNA Nucleic Acid (NAT)-Based Assays (PEI code 6329/10). The analytical sensitivity of the kit is 95\%. It specifically detects all relevant genotypes of HEV and does not cross-react with viruses causing similar symptoms.\textsuperscript{62}
Table 1 Summary of the HEV Seroprevalence Studies in Blood Donors in Multiple Geographical Areas

| Continent | Country               | Year and Study Duration | HEV IgG Seroprevalence | HEV IgM Seroprevalence | HEV Viremia |
|-----------|-----------------------|-------------------------|------------------------|------------------------|-------------|
|           |                       |                         | Sample Size            | % Positive             | Sample Size | % Positive |
|           |                       |                         |                        |                        |             |            |
| Europe    | Central Italy         | Feb-Mar 2013             | 198                    | 3.5                    | 198         | 1.01       | 198        | 0.5        | [15]        |
|           | Central Italy         | Feb-Mar 2014             | 313                    | 49                     | 313         | 0.6        | 313        | 0.6        | [46]        |
|           | Overall Italy         | 2015–2016                | 10,011                 | 8.7                    | 10,011      | 0.4        | 10,011     | 0          | [47]        |
|           | Ireland               | Dec 2013-Jun 2014        | 1076                   | 5.3                    | 57          | 2          | 24,985     | 0.02       | [54]        |
|           | Serbia                | 2010                    | 200                    | 15                     | ND          | ND         | 200        | 0          | [55]        |
|           | Spain                 | Jun-Dec, 2013            | 1082                   | 19.96                  | 216         | 13         | 9998       | 0.03       | [58]        |
|           | Bulgaria              | Jun-Oct, 2020            | 555                    | 25.9                   | ND          | ND         | ND         | ND         | [69]        |
|           | Germany               | 2009–2010               | ND                     | ND                     | ND          | ND         | 1185       | 1.18       | [31]        |
|           | Germany               | Jul-Sept 2011            | 349                    | 6.3                    | 349         | 4.3        | 16,125     | 0.08       | [32]        |
|           | France                | Nov-2012–14              | 183 pools              | 175 pools              | 175 pools   | 2 pools    | 53,234     | 3.94       | [35]        |
|           | France                | Sept, 2003-May, 2004    | 512                    | 52.5                   | ND          | ND         | ND         | ND         | [36]        |
|           | Southern France       | Oct 01–14, 2011          | 3,353                  | 39.1                   | 3353        | 3.31       | 591        | 0.16       | [68]        |
|           | Paris                 | Jan 12-Feb 13, 2015     | 11                     | 45.45                  | 11          | 36.36      | 25,637     | 0.04       | [37]        |
|           | Upper Austria         | Feb 2013-Apr 2014       | 1203                   | 13.55                  | 7           | 6          | 58,915     | 0.01       | [34]        |
|           | UK/ Southeast England | Oct 8, 2012-Sep 30, 2013| 79                     | 29                     | 79          | 29         | 225        | 0.04       | [7]         |
|           | Netherland            | Jan 2013-Dec 2014       | 45                     | 24                     | ND          | ND         | 59,474     | 0.0069     | [39]        |
|           | Netherland            | Nov 2011-Jan 2012       | 5229                   | 26.7                   | 1401        | 3.5        | 45,415     | 0.028      | [40]        |
|           | UK/ Scotland          | 2004–2008               | 1559                   | 4.7                    | 1559        | 0          | 43,560     | 0.0069     | [29]        |
|           | UK/ Scotland          | 2nd set 2012            | 528                    | 5.7                    | 528         | 0          | 0          | 0          | [20]        |
|           | UK/ Scotland          | Aug 2014 to Sept 2015   | 1714                   | 6.1                    | 38          | 21.05      | 94,302     | 0.04       | [30]        |
|           | UK/ Scotland          | Feb 2016-Feb 2017       |                        |                        |             |            |            |            |            |
|           | Africa                | Egypt                   | ND                     | ND                     | 760         | 0.45       | 3*         | 66.66*     | [21]        |
|           | Egypt/ Dakahlia       | Jan 2017-Jan 2018       | 200                    | 25                     | 200         | 5          | 200        | 3          | [22]        |
|           | Sudan                 | Apr-Jul, 2014           | 90                     | 26.7                   | ND          | ND         | ND         | ND         | [26]        |
|           | Ghana                 | Unknown                 | 239                    | 4.6                    | 239         | 5.9        | 239        | 0          | [51]        |

(Continued)
| Continent | Country | Year and Study Duration | HEV IgG Seroprevalence | HEV IgM Seroprevalence | HEV Viremia |
|-----------|---------|-------------------------|------------------------|------------------------|-------------|
| Asia      | Nepal   | Feb-Mar 2014            | 581                    | 9.5                    | 27°         | 1.54        | [23] |
| Asia      | Pakistan| Jan-Jun 2020            | 5230                   | 3.49                   | 107°        | 0.70        | [24] |
| Asia      | India, Northern region | Jun-Jul, 2016 | 633                   | 60.5                   | ND          | 1799        | 0        | [44] |
| Asia      | India, Pune | Jan-Aug, 2017       | 2447                   | 17.70                  | 2447        | 0.20        | 5°        | 40        | [45] |
| Asia      | Philippines | Unknown            | 85                     | 11.8                   | 85          | 2.4         | ND        | ND        | [25] |
| Asia      | Thailand | Oct-Dec 2015            | 26                     | 23.08 Emurium          | 7.69 Emurium| 30,115      | 0.086     | [41] |
| Asia      | China    | Dec 2002-Oct 2008      | 44,816                 | 32.60                  | 44,816      | 0.94        | 420°       | 7.14       | [48] |
| Asia      | China    | Jan-Dec, 2012          | 816                    | 21.1                   | 816         | 0.5         | 816        | 0         | [49] |
| Asia      | East China, Jiangsu Province | Jan-Jun, 2011 | 486                   | 23.3                   | ND          | ND          | ND        | ND        | [79] |
| Asia      | Japan    | 2004–2014              | 36                     | 19.44                  | 36          | 5.5         | 620,140    | 0.007      | [52] |
| Asia      | Cambodia | Jul-Aug 2014            | 301                    | 28.2                   | 301         | 0.3         | 301°        | 0.3        | [53] |
| Asia      | Saudi Arabia | Al-Qassim      | Total: 1078             | 5.7                    | 1078        | 1.3         | ND        | ND        | [65] |
|           |         | Saudis: 85.7 Non-Saudis: 14.3 |         |                        |             |             |           |           | |
|           |         |                           | 900                    | 18.7                   | 900         | 4.3         | ND        | ND        | [42] |
|           |         | Saudis: 15.18 Non-Saudis: 23.32 |               |                          |             |             |           |           | |
|           |         |                           | 530                    | 14.3                   | ND          | ND          | ND        | ND        | [43] |
|           |         |                           | 400                    | 11.5                   | ND          | ND          | ND        | ND        | [20] |
| Australia | Australia | May 16- Dec 02, 2016  | 1                      | 100                    | 1           | 100         | 74,131    | 0.0013     | [80] |
| Australia | Australia | Sept-Oct 2014           | 1                      | 0                      | 1           | 0           | 14,799    | 0.0068     | [38] |
| Australia | New Zealand | Nov 11, 2014-Mar 10, 2015 | 1013                  | 9.7                    | 8.1         | ND          | ND        | 5103        | 0        | [27] |
Additionally, a 10-fold serial dilution of the highest HEV RNA standard concentration was performed and tested in triplicates using the above-mentioned kit and thermocycler (Figure 1). The limit of detection of the kit was 0.1 IU/ul (Figure 1). The dilution of 0.01 IU/ul gave a positive RT-PCR result in one out of the three measurements at a Ct of 40.23.

**Statistical Analysis**
All data were tabulated in Excel spreadsheets to calculate frequencies. The chi-square test was calculated using the OpenEPI webpage (https://www.openepi.com/Menu/OE_Menu.htm). For the age groups, the chi-square for linear trend was used. The post-hoc analysis was used to calculate the chi-square for the difference among nationalities by testing each value of one nationality versus the sum value of all other nationalities. P value was considered significant if less than 0.05.

**Results**
Of the 806 blood donors, 765 (94.91%) were males and 41 (5.08%) were females. 410 (50.8%) of the blood donors were Saudis, while 396 (49.1%) were non-Saudis from India, Yemen, Philippine, Egypt, Syria, Sudan, and Bangladesh (Table 2). Median age of the participants was 32 years (range 18–85 years).

![Figure 1](https://www.dovepress.com/figure-a-10-fold-serial-dilution-of-hev-rna-detected-by-realstar-hev-rt-pcr-kit-the-results-show-a-ct-from-three-measurements-the-dilution-0.01-iul-gave-a-positive-result-in-one-out-of-the-three-readings-with-a-ct-of-40.23.png)

**Figure 1** A 10-fold Serial dilution of HEV RNA detected by RealStar HEV RT-PCR Kit. The results show the average Ct from three measurements. The dilution 0.01 IU/ul gave a positive result in one out of the three readings with a Ct of 40.23.

| Continent | Country | Year and Study Duration | HEV IgG Seroprevalence | HEV IgM Seroprevalence | HEV Viremia |
|-----------|---------|-------------------------|------------------------|------------------------|-------------|
| North America | USA | Jul 20-Aug 29, 2015 | 3 | 33.33% | 3 | 0% | 128,021 | 0.002 | [56] |
| USA | Feb-Jul, 2013 | 4499 | 7.3% | 4499 | 0.58 | 18,829 | 0.39 | [57] |
| Canada | Jul 2013- Dec 2015 | 4102 | 5.9% | 241 | 1.65 | 13,993 | 0 | [33] |
| USA/South Caribbean | Unknown | 600 | 4.2% | 600 | 0.17 | 25 | 0 | [59] |

**Notes:** a Only IgM positive samples were tested for HEV viremia, b only IgG reactive samples were tested for HEV viremia, c random samples were tested for HEV viremia.


Table 2: Prevalence of Anti-HEV IgG Antibodies Among Blood Donors in the Eastern Province of Saudi Arabia

| Gender     | Positive | Negative | Total | P value |
|------------|----------|----------|-------|---------|
|            | N        | %        |       |         |
| Male       | 24       | 3.14     | 741   | 96.86   | 765     | 0.527 |
| Female     | 2        | 4.88     | 39    | 95.12   | 41      |       |
| Age groups |          |          |       |         |
| 18 to <25  | 5        | 3.76     | 128   | 96.24   | 133     | 0.589* |
| 25 to <35  | 10       | 2.90     | 336   | 97.1    | 346     |       |
| 35 to <45  | 8        | 3.96     | 194   | 96.04   | 202     |       |
| 45 to <55  | 3        | 3.16     | 92    | 96.84   | 95      |       |
| ≥55        | 0        | 0        | 30    | 100     | 30      |       |
| Nationality |          |          |       |         |
| Philippine | 2        | 6.25     | 30    | 93.75   | 32      | 0.356 |
| Sudan      | 1        | 5.26     | 18    | 94.74   | 19      | 0.589 |
| Bangladesh | 1        | 5.26     | 18    | 94.74   | 19      | 0.589 |
| India      | 3        | 4.48     | 64    | 95.52   | 67      | 0.532 |
| Egypt      | 2        | 3.51     | 55    | 96.49   | 57      | 0.838 |
| Saudi Arabia | 13   | 3.17     | 397   | 96.83   | 410     | 0.929 |
| Syria      | 1        | 2.27     | 43    | 97.73   | 44      | 0.906 |
| Yemen      | 2        | 2.04     | 96    | 97.96   | 98      | 0.522 |
| Kuwait     | 1        | 100      | 0     | 0       | 1       | 0.064 |
| Others     | 0        | 0        | 59    | 100     | 59      | 0.268 |

Notes: *Chi square for linear trend. **The p value is calculated for each nationality against the sum of all other nationalities.

Anti-HEV IgG antibodies were detected by ELISA in 26 (3.23%) donors, and there was no statistically significant difference between non-Saudis (3.28%) and Saudis (3.17%). Anti-HEV IgG antibodies were detected in 3.14% (24/765) of the males and in 4.88% (2/41) of the females with no statistically significant difference (Table 2). Additionally, there was no statistically significant tendency with different age groups.

The highest IgG antibody prevalence was observed among the blood donors from the Philippines followed by blood donors from Sudan, Bangladesh, India, Egypt, and Saudi Arabia (Table 2). One case was from Kuwait and was positive for HEV IgG. There was no statistically significant difference in the positivity among different nationalities (Table 2).

Anti-HEV IgM antibodies were not detected in any of the 26 samples IgG positive samples.

HEV-RNA was not detected in any of the 806 samples.

Discussion

In the current study, we tested 806 blood donors from the Eastern Province of Saudi Arabia for hepatitis E virus RNA, and HEV IgG and IgM antibodies. The seroprevalence for Anti-HEV IgG was found to be 3.23%. None of the donors was positive for HEV RNA or Anti-HEV IgM. Additionally, there was no significant difference in IgG seroprevalence between Saudis (3.17%) and non-Saudis (3.28%) with any age or gender presence. Despite the fact that we have looked for IgM antibodies in IgG positive donors only, we believe that it is less likely to have missed IgM positive donors who are IgG negative for the following reason: According to the known information about the course of the HEV infection, IgM antibodies appear with the peak of RNA titer.63–65 IgG antibodies appear at the peak of IgM antibodies and continue to be positive for a long time. IgM antibodies can still be detected until week 14 post-infection along with IgG antibodies.63–65 Therefore, there is no window where IgG antibodies are positive and IgM antibodies are negative during an acute infection and hence it is less likely that we have missed a patient with an acute infection because all patients were negative for RNA, and the IgG positive samples are negative for IgM. This means that all IgG positive blood donors in our study had past infections.

An approximately similar HEV IgG antibody prevalence was reported from another recent study from the middle region (Qasim) of Saudi Arabia (5.7%).66 This is lower than the previously reported HEV IgG seroprevalence before 2013, where it ranged between 14% and 18% from the southern and western regions, respectively.16,42,43,67 This could indicate a reduction in the HEV exposure in the past few years or a regional difference in the country. The western region (Mecca and Jeddah) receives the majority of Saudi Arabia’s visitors for religious purposes, who may import a silent infection and increase the exposure of the local population. Two studies have previously reported the detection of anti-HEV IgM antibodies without the confirmation of a current HEV infection with RNA detection.16,43 It is worth noting that HEV seroprevalence in countries of the expatriate who are involved in our study is also moderate, such as Philippines (11.8%), Sudan (26%), and Egypt (25%).21,25,26

It is also important to note that the seroprevalence of Anti HEV-IgG in our study is less than neighboring
Middle Eastern countries like Qatar (20.5%) Iran (14.3% and 11.5%), Egypt (25%) and Abu-Dhabi (10.69%) and African countries like Sudan (26%), Ghana (4.6%) and Asian countries from where most of the non Saudis belong to like Nepal (9.5%) Pakistan (3.5%) Philippines (11.8%) China (23.3%, 21.1%) Cambodia (28.2%) Japan (3.7%) and India (17.70% and 60.5%) and European countries like Scotland (4.7%, 6.1%) France (3.94% and 52.5%) Italy (8.7% and 49%) Netherland (26.7%) Ireland (5.3%) Spain (19.9%) and Bulgaria (25.9%).

However, this tremendous variation in seroprevalence among different countries is expected due to differences in assays, sample size and geography.

Our study was the first to look for HEV RNA in blood donors in the country. The lack of RNA detection in our study cannot be attributed to the reduced sensitivity of the employed RT-PCR kit, especially with the absence of IgM antibodies among the donors. Our analytical sensitivity analysis shows that the kit can detect as low as 0.1 IU/ul of HEV RNA consistently in three different measurements. This could, however, suggest the need for a much larger sample size to detect such a low prevalent virus. Additionally, a short duration of HEV viremia during the course of infection could have been missed by our one-time sampling strategy.

Transfusion-transmitted hepatitis E is gaining growing attention, particularly among blood donors, because of the increased number of reported cases in multiple countries. More importantly, HEV infections in high-risk individuals, such as pregnant women, immunocompromised patients and patients with hematological diseases, have been associated with fulminant hepatitis and chronic hepatitis, which can lead to liver cirrhosis and liver failure with high fatality. Furthermore, vertical transmission in pregnant women has been reported with high risk of transmission and high neonatal fatality.71,72

In response to the emerging pattern of transfusion-transmitted hepatitis E, screening programs for HEV in blood donors are being implemented in many countries including Austria, the Netherland, Ireland, UK, France, Spain, Germany, Luxembourg,73,74 Switzerland75 and Japan76 and being evaluated in others. Hepatitis E screening programs for blood donors have been implemented using different modalities, which include universal screening vs selective screening and individual vs pooled samples screening.77 Cost-effectiveness studies showed variable impact78,79 primarily due to variations in HEV prevalence in different parts of the world.

This study reports a low seroprevalence of HEV in the blood donor population in the eastern province of Saudi Arabia and no detectable HEV viremia, which alone cannot rule out the risk of transfusion associated with hepatitis E in the area, particularly for high-risk individuals. One main limitation of this study is the sample size, which contributes to the lack of detection of HEV viremia and this necessitates the need for large-scale studies and evaluation of the cost-effectiveness of blood donor HEV screening programs in the kingdom in order to quantify the risk and propose a cost-effective preventive tool.

Acknowledgments
We are grateful to Mr. Lauro Bartolome for his technical support. We would also like to thank the technical staff of the blood bank at King Fahd Hospital of the University for their help in sample collection and the blood donors for their participation in the study. This project was funded by the Deanship of Research at Imam Abdulrahman Bin Faisal University (Project number: 2020-183-Med).

Disclosure
The authors report no conflicts of interest in this work.

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