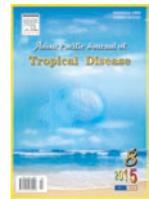




Contents lists available at [ScienceDirect](http://www.sciencedirect.com)

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd



Original article doi: 10.1016/S2222-1808(15)60896-9

©2015 by the Asian Pacific Journal of Tropical Disease. All rights reserved.

Detection of IgM and IgG antibodies against hepatitis E virus in donated blood bags from a national voluntary blood bank in Metro Manila, Philippines

Annalyn Aler Lorenzo¹, Teresita Santiago De Guzman^{1*}, Glenn Lo Sia Su²

¹Department of Medical Microbiology, College of Public Health, University of the Philippines, Manila

²Biology Department, College of Arts and Sciences, University of the Philippines, Manila

ARTICLE INFO

Article history:

Received 2 Apr 2015

Received in revised form 14 Apr 2015

Accepted 28 May 2015

Available online 9 Jul 2015

Keywords:

Blood

Virus

Antibodies

ABSTRACT

Objective: To assess the blood bags obtained from a national voluntary blood bank for the presence of immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies against hepatitis E virus (HEV).

Methods: Plasma from the blood bags provided by a voluntary blood bank was examined for the IgM and IgG antibodies against HEV through the ELISA technique.

Results: Results showed HEV IgG and IgM antibodies with prevalence of 11.8% and 2.4% positives, respectively.

Conclusions: Screening of HEV antibodies of donors is a must to safeguard blood products and reduce the possible transmission of HEV infections.

1. Introduction

Hepatitis E is an emerging disease in many countries worldwide. It is estimated to bring about more than 3 million symptomatic cases of acute hepatitis E and approximately 70 000 deaths annually[1]. In the Philippines, a high prevalence of hepatitis E virus (HEV) antibodies was observed among those household-raised pigs in the rural areas[2]. Documentations of HEV infections in the Asian countries have likewise indicated that pigs are an important source of zoonotic infections[3,4], indicating that hepatitis E infection can be attributed to foodborne transmission[5,6]. The virus is primarily transmitted through oral-fecal route[5], and is commonly associated with sporadic infections and epidemics in areas with poor sanitation and weak public health infrastructures[7]. Other reports presented that the occurrence of HEV may also develop in polluted water environments[8], and in cases of transmission through blood transfusion[9]. As of date, there are limited epidemiological studies regarding the HEV infection in the Philippines. In the Philippines, studies pertaining to HEV were limited to detect the HEV antibodies in swine[2], water environments[8], and among jaundiced patients[10]. No study has looked into the detection of HEV antibodies in donated blood bags from voluntary blood banks. This study aimed to detect and assess the presence of HEV antibodies in the blood

bags obtained from blood banks. Results of this study are vital, as they provide baseline information on the current prevalence of HEV infection of blood donors through the blood bags available in blood banks. Results likewise provide valuable information on the possible sources of infection and new HEV transmission routes that may help in safeguarding the general public health from being exposed to the transmission of HEV infections.

2. Materials and methods

The study was approved by the ethical review board of authors' institution. All the blood bags were obtained in a national voluntary blood bank facility situated in Metro Manila. All the blood donors were previously screened by the facility following the international standards for blood donor eligibility. All blood bags provided were cleared of any transfusion transmissible infections such as HIV, hepatitis B and C, syphilis, and malaria. The OpenEpi version 3.01 was used to calculate for the sample size. A previous point prevalence study method on blood donors was used to estimate the minimal sample size required in this study[11]. The point prevalence of 32.6% at a confidence level of 95%, with a maximum allowable error of 10%, was used to estimate a minimal sample size requirement of 85 blood bags. Anonymized blood samples collected from blood donors were provided by the blood bank facility for the detection of hepatitis E immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies. Aliquots of three containing 100 μ L per plasma sample placed on microcentrifuge tubes were

*Corresponding author: Teresita Santiago De Guzman, Department of Medical Microbiology, College of Public Health, University of the Philippines, Manila.
Tel: +63 25255874
E-mail: tessdeguzman04@yahoo.com

stored at -20 °C. Plasma samples were quickly thawed for a few minutes at 18–24 °C. All plasma samples were ensured to be free of hemolysis and turbidity. Plasma samples were then tested for the presence of antibodies against HEV by the ELISA technique through using the GenWay Biotechnologies® ELISA kit (California, USA). Each plasma sample was tested in duplicate for both the IgM and IgG antibodies against HEV. The tests were performed according to the manufacturer's instructions. Positive and negative controls were both run together in each test plate. The HEV antibodies were determined by reading the optical density at 450 nm using the Zenyth 200 ELISA Reader. The presence or absence of HEV antibodies was determined by the ratio of the optical density of each sample to the calculated cutoff value. Data analysis included descriptive statistics.

3. Results

A total of 85 blood bags obtained from a national voluntary blood bank facility situated in Metro Manila were assessed for the presence of HEV IgG and IgM antibodies. About 10 blood bags (11.8%) were tested positive for the IgG antibodies against HEV, and 2 blood bags (2.4%) were tested positive for IgM antibodies against the HEV.

4. Discussion

This was a cross-sectional study, and its scope was limited to investigate the HEV infection by detecting the HEV IgG and IgM antibodies in the plasma from accepted blood bags from a national voluntary blood bank in Metro Manila. This study used only anonymized donated blood bags provided by the blood bank. The authors had no direct contact with the blood donors nor access to their records. Our findings have shown that, among the blood bags examined, the prevalence of acute HEV infection using the IgM anti-HEV was 2.4% (2/85), whereas the prevalence of chronic or convalescent HEV infection using the IgG anti-HEV was 11.8% (10/85). The prevalence of HEV infection was indicated by the presence of antibodies against the HEV in donated blood bags examined, posing a potential risk of transfusing HEV-contaminated blood to individuals especially if these blood turn positive for the active HEV, since routine screening tests of donated blood do not include test for the presence of HEV infection. As estimated by use of IgM anti-HEV, the rate of acute HEV infection is 2.4%. The occurrence of more HEV IgG antibodies than the HEV IgM antibodies may likely due to that, during a HEV infection, the IgM antibodies usually occur at the Week 4 of the infection, followed by the IgG antibodies at Week 5 of infection[12]. It is probable that the 2 blood bags tested positive for IgM antibodies against HEV were from donors who had the HEV at the early onset of infection. Of the 2 positive blood bags, only 1 blood bag that was anti-HEV IgM-reactive was also found to be anti-HEV IgG positive. This may be due to the collection of blood during the transition period when there is IgM switch to IgG or that the donor was previously infected with HEV thereby having anti-HEV IgG and was re-infected shortly before the time of blood collection as manifested by the presence of anti-HEV IgM. All the other 9 blood bags examined may have had donors who have the HEV infection longer than 4 weeks.

HEV presents a risk of infection especially to those individuals needing blood transfusions, especially if available blood bags are contaminated with HEV. This study has shown a relatively low prevalence of anti-HEV in the blood bags obtained from a national

voluntary blood bank. Despite this result, the risk of HEV-infected blood products can be prevented if HEV screening of blood bags is instituted to evaluate the safety of blood products that will be given to patients who need them.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

We would like to express our gratitude to all who have assisted us in the course of this study especially Mary Ann C. Sison and Rubelia A. Baterna of the Department of Medical Microbiology, College of Public Health.

References

- [1] Rein DB, Stevens GA, Theaker J, Wittenborn JS, Wilersma ST. The global burden of hepatitis E virus genotypes 1 and 2 in 2005. *Hepatology* 2012; **55**(4): 988-97.
- [2] Liu XF, Saito M, Sayama Y, Suzuki E, Malbas FF, Galang HO, et al. Seroprevalence and molecular characteristics of hepatitis E virus in household-raised pig population in the Philippines. *BMC Vet Res* 2015; **11**: 11.
- [3] Conlan JV, Jarman RG, Vongxay K, Chinnawirotpisan P, Melendrez MC, Fenwick S, et al. Hepatitis E virus is prevalent in the pig population of Lao People's Democratic Republic and evidence exists for homogeneity with Chinese Genotype 4 human isolates. *Infect Genet Evol* 2011; **11**(6): 1306-11.
- [4] Hinjoy S, Nelson KE, Gibbons RV, Jarman RG, Chinnawirotpisan P, Fernandez S, et al. A cross-sectional study of hepatitis E virus infection in pigs in different-sized farms in northern Thailand. *Foodborne Pathog Dis* 2013; **10**(8): 698-704.
- [5] Kasorndorkbua C, Guenette DK, Huang FF, Thomas PJ, Meng XJ, Halbur PG. Routes of transmission of swine hepatitis E virus in pigs. *J Clin Microbiol* 2004; **42**: 5047-52.
- [6] Brassard J, Gagné MJ, Généreux M, Côté C. Detection of human food-borne and zoonotic viruses on irrigated, field-grown strawberries. *Appl Environ Microbiol* 2012; **78**: 3763-6.
- [7] Dalton HR, Stableforth W, Thurairajah P, Hazeldine S, Remnarace R, Usama W, et al. Autochthonous hepatitis E in Southwest England: natural history, complications and seasonal variation, and hepatitis E virus IgG seroprevalence in blood donors, the elderly and patients with chronic liver disease. *Eur J Gastroenterol Hepatol* 2008; **20**(8): 784-90.
- [8] Li TC, Yang T, Shiota T, Yoshizaki S, Yoshida H, Saito M, et al. Molecular detection of hepatitis E virus in rivers in the Philippines. *Am J Trop Med Hyg* 2014; **90**(4): 764-6.
- [9] Kaufmann A, Kenfak-Foguena A, André C, Canellini G, Bürgisser P, Moradpour D, et al. Hepatitis E virus seroprevalence among blood donors in southwest Switzerland. *PLoS One* 2011; **6**(6): e21150.
- [10] Barzaga NG, Cabanban A, Graham RR, Florese RH. Hepatitis E virus infection diagnosed by serology: a report of cases at the San Lazaro Hospital, Manila. *Philipp J Microbiol Infect Dis* 1997; **26**: 169-72.
- [11] Guo QS, Yan Q, Xiong JH, Ge SX, Shih JW, Ng MH, et al. Prevalence of hepatitis E virus in Chinese blood donors. *J Clin Microbiol* 2010; **48**(1): 317-8.
- [12] 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.