Seroprevalence of anti-HEV among blood Donors in Lagos, Nigeria– A Pilot Study

SO John-Olabode¹, O Ajie², V Osunkalu³, A Akinbami³, K. Aile³
¹ Department of Haematology and Blood transfusion, College of Medicine, University of Lagos, Lagos State, Nigeria
² Department of Clinical Pathology, College of Medicine, University of Lagos, Lagos State, Nigeria
³ Department of Haematology and Blood transfusion, Lagos State University College of Medicine (LASU/LCOM), Lagos, Nigeria

Abstract

Background: Hepatitis E is a common infection in developing countries like Nigeria because of poor sanitation and weak public health facilities. Presently, in Nigeria the mandatory infectious disease screening test for voluntary blood donors does not include HEV, with the emerging evidence of the transfusion transmissibility of HEV because potentially infected donors may never have shown clinical signs and considering the fact that HEV cannot be fully inactivated in blood-derived products, this virus has recently emerged as a transfusion-transmitted pathogen of concern and a potential threat to transfusion safety. To establish whether HEV may be a risk to transfusion safety and, more in general, a problem for public health, the first step is to assess its real seroprevalence in blood donors and the general population.

Aim: To characterize the seroprevalence of anti-HEV IgM and IgG in blood donors in Lagos State, Southwest, Nigeria.

Materials and Methods: Sera from 151 donors were screened for anti-HEV IgM and IgG by enzyme-linked immunosorbent assay (ELISA). Data were expressed as the mean ± standard deviation and all statistical analysis was performed using SPSS version 23.0 statistical software (SPSS, Inc., Chicago, IL, USA) where P-value of 0.05 was accepted as statistically significant.

Results: In this study 151 blood donors were enrolled, overall seroprevalence for HEV of 6.6% was determined. HEV IgG had a significant prevalence of 5.3% as against HEV IgM 1.3% (P = 0.00). Higher prevalence was found among males compared to females. A higher HEV detection rate was observed in the younger age group with 80% (8/10) of the anti-HEV positive donors aged 20 – 40 years old (P = 0.9). In this study, no significant association was observed (p > 0.05) between seropositivity and the risk factors associated with HEV infection.

Conclusions: This pilot study of HEV seroprevalence in blood donors in Lagos, South-west Nigeria has revealed that though low the potential for HEV contamination in the blood supply to recipients does exist. However as the presence of HEV RNA was not examined in this study, no conclusive data can be generated on active HEV prevalence in blood donors. Further studies will be needed in determining active HEV prevalence in blood donors so as to determine if HEV screening will be necessary and cost effective on a larger scale to reduce the risk of on-going infection to a vulnerable population in Nigeria.

Keywords: Prevalence, Blood transfusion, Hepatitis E Virus, Nigeria.

INTRODUCTION

Hepatitis E Virus (HEV) is a small, spherical, non-enveloped, single stranded RNA virus. Originally recognized as a member of the genus Calicivirus, of the Caliciviridae family but recently reclassified into the Hepeviridae family in the Hepesviridae genus [1, 2]. Hepatitis E Virus (HEV) infection is responsible for over 50% of cases of enterically transmitted acute viral hepatitis in endemic countries [3, 4] and Africa is among the most severely affected regions in the world because of poor sanitation and weak public health facilities.

Traditionally, HEV has been known to cause self-limited acute infection in humans, but new reports show evidence that HEV infection can result in significant morbidity and mortality in certain high risk group with compromised immune systems. These include patients with pre-existing liver disease (where HEV infection can result in death with mortality rate can be as high as 60%, or evolve to a chronic state), immunocompromised subjects [5, 6] and transplant recipients [7]. Another characteristic clinical feature of HEV is its high frequency and severity in pregnant women in low income countries with mortality rates around 10-20 % [8, 9] particularly in the third trimester.

HEV has the potential to be transmitted by the transfusion of contaminated blood since it has an asymptomatic blood borne phase and may survive in blood components during processing and storage.
Indeed, the transmission of hepatitis E through transfusion therapy has been suggested by a number of studies done in both non-endemic countries \(^{10-15}\) and endemic countries \(^{16, 17}\).

Many studies show that any blood product, including red blood cells \(^{18-21}\), platelets \(^{10, 21}\) and fresh-frozen plasma \(^{22-24}\) can transmit HEV. The presence of HEV-RNA has been reported from Europe and North America in both mini- and large-plasma pools \(^{9, 22}\) including those for fractionation \(^{25}\). Interestingly, a recent work provides, for the first time, indirect evidence of HEV transmission through solvent/detergent plasma in two patients with thrombotic thrombocytopenic Purpura \(^{26}\).

The common finding of an age-dependent increase in Seroprevalence suggests that many infections occur in middle age, and thus during the period of blood donation activity. This issue is of great significance to transfusion transmissibility of HEV because potentially infected donors may never have shown clinical signs. Hepatitis E should therefore be considered as a risk to transfusion safety, especially in high-risk recipients (pregnant females, patients with pre-existing chronic liver disease, and immunocompromised patients), where it is thought to be associated with considerable morbidity and mortality \(^{27, 28}\).

Implementing universal screening for HEV might be a difficult decision to make for blood providers in Nigeria due to the financial implication but selective screening of blood components for patients at high risk may be an acceptable strategy that is more cost effective than universal screening.

**MATERIALS AND METHODS**

**Study population and Sample collection**

Anonymized blood samples were collected consecutively from April to May 2016, from 151 blood donors who meet the criteria for blood donation in Lagos University Teaching Hospital (LUTH).

**Ethical consideration**

All donors had previously completed the pre-donation screening questionnaire to verify that they fulfilled the criteria for blood donation and all had provided written consent for the use of blood samples in medical research after anonymization. The study was approved by the Ethical Committee of the Lagos University Teaching Hospital (LUTH) assigned NO: ADM/DCST/HREC/APP715.

**Detection of HEV antibodies**

Serum anti-HEV IgG and IgM was detected by the enzyme-linked immunosorbent assay (ELISA) kits containing HEV genotype 1 antigens manufactured by Beijing Wantai Biological Pharmacy Enterprise Co., Ltd. China. All assay procedures were carried out according to manufacturer’s instructions. In the ELISA assay, samples were considered reactive if Sample / Cut-off (S / Co) >1, indeterminate if S / Co = 0.9-1.1 and non-reactive if S / Co <1. Samples were considered positive if it was reactive with the WanTai assay.

**Data collection**

For each blood donation information was obtained on demographic and behavioural characteristics as well as possible associated risk factors such as source of drinking water, type of toilet, personal hygiene, and interaction with animals was also recorded. Other factors include smoking, consumption of alcohol, blood transfusion. The administered questionnaires were filled by the individual before sample collection.

**Statistical analyses**

The minimum sample size was calculated from the general formula as described by Antonisamy B et al.\(^{28}\) Assuming an anti-HEV prevalence of 4.9\% \(^{28}\), we estimated a minimal sample size requirement of 71.5 individuals \(^{30}\). Data analysis included descriptive statistics of means (with standard deviation) and medians, depending on the distribution of the data. Categorical data were assessed using Pearson X\(^2\) tests to compare the anti-HEV positive proportions between different groups. The student T-test; Chi-square or Fisher’s exact test was used for hypothesis testing as appropriate to describe differences between negative and positive individuals.

Data were expressed as the mean ± standard deviation and all statistical analysis was performed using SPSS version 23.0 statistical software (SPSS, Inc., Chicago, IL, USA) where P-value of 0.05 was accepted as statistically significant.

**RESULTS**

In this study 151 blood donors were enrolled, including 140 (92.7\%) males and 8 (5.3\%) females. Their age ranged from 19 to 55 years (mean 31.01 ± 7.49 years; median 30 years) (Table1). Majority of the blood donors were single but no significant association was recorded between marital status and HEV seropositivity (P = 0.9).

**Table 1: Demographic characteristics of blood donors**

| Demographic variable | Frequency | Percentage (%) |
|----------------------|-----------|---------------|
| **Gender**           |           |               |
| Male                 | 140       | 92.7          |
| Female               | 8         | 5.3           |
| Missing              | 3         | 2             |
| **Age Group**        |           |               |
| <20                  | 1         | 0.7           |
| 20-40                | 132       | 87.4          |
| >40                  | 14        | 9.3           |
| Missing              | 4         | 2.6           |
| **Marital Status**   |           |               |
| Married              | 59        | 39.1          |
| Single               | 86        | 57.0          |
| Missing              | 6         | 3.9           |

**Figure 1: Gender and seroprevalence**

Overall seroprevalence for HEV of 6.6\% was determined. HEV IgG had a significant prevalence of 5.3\% against HEV IgM of 1.3\% (P < 0.05) (Table 2). Although not statistically significant (P > 0.05), males accounted for a higher seropositivity for anti-HEV in contrast to
females as all samples which tested positive for anti-HEV were from men. Figure 1) Also, although not significant a higher HEV seroprevalence was observed in the younger age group with 80% (8/10) of the anti-HEV positive donors aged 20 – 40 years old (P = 0.6) Table 3. The mean age for donors that were IgM positive was 29±12.73 (median 29 years) while for IgG positive donors the mean age was 33±8.56 years (median 32 years).

Table 2: Distribution of of anti-hepatitis E virus in blood donors of Lagos State

| Anti-HEV   | IgM n(%) | IgG n(%) | P-value |
|------------|----------|----------|---------|
| Positive   | 2(1.3)   | 8(5.3)   | 0.03*   |
| Negative   | 149(98.7)| 143(94.7)|         |
| Total      | 151      | 151      |         |

No significant association was observed (p > 0.05) between seropositivity and the variables evaluated (Table 3) including occupation, education, source of water (public or other) and sewer (public service or pit). Most subjects lived under adequate sanitary housing conditions in view of the high rate of presence of a sewage system (94%) and public water (86.8%). However, worthy of note is the significant association of IgM seropositivity and alcohol consumption (P = 0.03) whereas no association was observed between HEV infection and previous blood transfusion in this study population. (P = 0.6).

Table 3: Sociodemographic characteristics of 151 blood donors of the Blood Bank of Lagos University Teaching Hospital, Lagos, Nigeria, distributed according to HEV seroprevalence

| Demographic variable | Anti-HEV positive n(%) | Anti-HEV negative n(%) | Total n(%) | P-value |
|----------------------|------------------------|------------------------|------------|---------|
| Gender               |                        |                        |            |         |
| Male                 | 10(100)                | 130(92.1)              | 140(92.7)  | 0.718 NS|
| Female               | 0                      | 8(5.7)                 | 8(5.3)     |         |
| Missing              | 0                      | 3(2.1)                 | 3(2.0)     |         |
| Age Group            |                        |                        |            |         |
| <20                  | 0                      | 1(0.7)                 | 1(0.7)     |         |
| 20-40                | 8(80)                  | 124(87.9)              | 132(87.4)  | 0.635 NS|
| >40                  | 1(10)                  | 13(9.2)                | 14(9.3)    |         |
| Missing              | 1(10)                  | 3(2.1)                 | 4(2.6)     |         |
| Source of water      |                        |                        |            |         |
| Public               | 10(100)                | 121(85.8)              | 131(86.8)  | 0.863 NS|
| Other (stream, well) | 0                      | 14(9.9)                | 14(9.3)    |         |
| Missing*             | 0                      | 6(4.3)                 | 6(3.9)     |         |
| Previous blood transfusion |                |                        |            |         |
| Positive             | 3(30)                  | 21(14.9)               | 24(15.9)   | 0.825 NS|
| Negative             | 7(70)                  | 120(85.1)              | 127(84.1)  |         |
| Sanitary conditions  |                        |                        |            |         |
| Sewer                | 10(100)                | 132(93.6)              | 142(94)    | 0.911 NS|
| Pit                  | 0                      | 3(2.1)                 | 3(2.0)     |         |
| Missing*             | 0                      | 6(4.3)                 | 6(4.0)     |         |

*chi-square, NS not significant, missing no data inputted

DISCUSSION

Many studies show that any blood product, including red blood cells [18-21] platelets [20, 21] and fresh-frozen plasma [22-24] can transmit HEV. Indeed evidence shows that HEV infection can result in significant morbidity and mortality in recipients of blood transfusion with compromised immune systems. Such high risk recipients include patients with pre-existing liver disease, immunocompromised subjects [5, 6] and transplant recipients. In Nigeria, there have been a few studies on prevalence of HEV in the general population but there is a dearth of data on the potential risk of HEV transmission in contaminated blood from HEV infected asymptomatic blood donors. This study was conducted to provide some preliminary data on actual risk of transfusion transmittable HEV.

HEV seroprevalence of 6.6% obtained among blood donors in this study is similar to that reported in a developed country by Kaufmann et al. who obtained prevalence of 4.9% among blood donors in Switzerland [31]. This low seroprevalence is contrary to the figure of 49.7% obtained among the general population by Junaid et al, Jos, Nigeria [32]. This difference might be due to the variation in the target population as sampling in the Jos study was performed in general population including urban and rural participants. In contrast, in the present study we studied only urban participants; another reason for the disparity noted could be as a result of variation in regional seroprevalence even within the same country as supported by other studies done in Brazil and China [33, 34]. Factors that have been identified that could be responsible for these regional differences include differences in living and sanitary conditions. Hence, the low seroprevalence obtained in this study could actually be a reflection of a population residing in an urban area that has better sanitary conditions.

In addition, comparisons between studies may become challenging due to differences in the HEV antibody detection assays used. The Junaid study used an MP Diagnostics HEV ELISA kit as this assay is not the same as the Wantai HEV ELISA kit used in our study, we cannot rule out disparity in overall HEV prevalence due to differences in the sensitivity of HEV ELISA kits used in the two studies.

In this study, Anti-HEV IgM prevalence of 1.3% is comparable to the prevalence of 0.9% among the general population recorded by Junaid et al, Jos, Nigeria [32] and 0.5% among blood donors in China by Li et al [35] but lower compared to 4.78% obtained in a study among 460 blood donors in Western India by Maitrey et al [36]. This disparity may be attributable to well documented variation in the geographic distribution of HEV or may be due to differences in the study sample size.

In accordance with previous researches [31-35], this study revealed males accounted for a higher rate of anti-HEV seropositivity than females. The low number of females observed in this study could account for this but most importantly, the reason for this could be due to the fact that majority of blood donors in Nigeria are men although previous studies have documented that HEV infections are predominantly reported in men [9], the reason for this gender bias still remains unclear.

In developing countries, the age-specific seroprevalence profiles reveal that HEV infection is largely limited to adult population between ages 15 –35 years [9]; this fact is corroborated in the present study as the mean ages for the donors were 29±12.73 years and 33±8.53 years for IgM and IgG seropositivity respectively. This finding is also consistent with Adesina et al [37] who observed the prevalence of anti-HEV antibodies to be highest in ages 20–40 years.

The increase in HEV Seroprevalence in this age group that is the peak period of blood donation activity is of great significance to transfusion transmissibility of HEV because infected donors appear asymptomatic thus posing a potential threat to transfusion safety when blood collected from such infected donors are contaminated with viral proteins.
In line with the findings of Junaid et al on the association between alcohol consumption and presence of anti-HEV, this study recorded a significant association between alcohol consumption and HEV IgM seropositivity (P=0.03). This observation is also consistent with Dalton et al who associated alcohol consumption with enhancement of positive anti-HEV. Although the reason for this observation cannot be fully explained, alcohol consumption has been identified as a strong risk factor for HEV genotype 3 infections [11]. HEV genotypes 1 & 2 have been known to be common in Africa with HEV genotype 2 particularly common in West Africa [11]. The discovery of this risk factor that has been associated with HEV 3 underscores the need for further genotypic characterisation of HEV in Nigeria.

Contrary to previous studies [12, 39] finding of a strong association between previous history of blood transfusion and positive anti-HEV, the present study did not reveal any such association. In addition, no significant association was observed (p > 0.05) between seropositivity and the variables evaluated including marital status, occupation, education, source of water and sewer (water closet or pit).

In summary, HEV seroprevalence of 6.6% was observed in this study. Although this result shows a low prevalence of HEV infection among blood donors in Lagos, South-west Nigeria, it still demonstrates the existence of the risk of HEV transmission by blood transfusion which merits further study.

CONCLUSION

This pilot study of HEV seroprevalence in blood donors in Lagos, South-west Nigeria has revealed that though low the potential for HEV contamination in the blood supply to recipients does exist. However as the presence of HEV RNA was not examined in this study, no conclusive data can be generated on active HEV prevalence in blood donors. Further studies will be needed in determining active HEV prevalence in blood donors so as to determine if HEV screening will be necessary and cost effective on a larger scale to reduce the risk of on-going infection to a vulnerable population in Nigeria.

Conflict of Interest

The authors have no conflict of interest.

REFERENCES

1. Teshale EH, Hu DJ. Hepatitis E: Epidemiology and prevention. World J Hepatol 2011; 3:285-291.
2. Meng XJ, Anderson DA, Arankalle VA, et al. Hepeviridae: In: King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ, editors. Virus taxonomy: classification and nomenclature of viruses: Ninth Report of the International Committee on Taxonomy San Diego, CA: Elsevier; 2011: 1021-8.
3. Aggarwal R, Naik SR. Epidemiology of hepatitis E: past, present and future. Trop Gastroenterol 1997; 18: 49–56.
4. Yarborough PO. Hepatitis E virus. Advances in HEV biology and HEV vaccine approaches. Intervirolology 1999; 42: 179–84.
5. Kumar N, Bendall R, Legrand-Abraham F, et al. Hepatitis E. Lancet. 2012; 379(9835):2477-2488.
6. Dalton HR, Bendall RP, Keane FE, Tedder RS, Ijaz S. Persistent carriage of hepatitis E virus in patients with HIV infection. N Engl J Med. 2009;361:1025–7.
7. Koennecke C, Pischke S, Heim A, Raggub L, Bremer B, Raupach R et al. Chronic hepatitis E in hematopoietic stem cell transplant patients in a low-endemic country? Transpl Infect Dis 2012; 1:103–106.
8. Hoofnagle JH, Nelson KE, Purcell RH. Hepatitis E. Current concepts. N Engl J Med 2012; 367: 1237-44.
9. Kamar N, Dalton HR, Abravanel F, Itoep J. Hepatitis E virus infection. ClinMicrobiol Rev 2014; 27: 116–38.
10. Matusubayashi K, Kang JH, Sakata H, Takahashi K, Shindo M, Kato M, et al. A case of transfusion-transmitted hepatitis E caused by blood from a donor infected with hepatitis E virus via zoonotic food-borne route. Transfusion. 2008; 48:1368–75.
11. 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 a genotype 3 HEV by blood transfusion. J Med Virol. 2004; 74:563–72.
12. Tamura A, Shimizu YK, Yamaka T, et al. Persistent infection of hepatitis E virus transmitted by blood transfusion in a patient with T-cell lymphoma. Transfusion 2007; 37: 113-120.
13. 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.
14. Colson P, Coze C, Gallian P, Henry M, De Micco P, Tamalet et al. Transfusion-associated hepatitis E, France. Emerg Infect Dis 2007; 13: 648-649.
15. Coilly A, Haim-Boukobza S, Roche B, et al. Post transplantation hepatitis E: transfusion-transmitted hepatitis rising from the ashes. Transplantation 2013; 96: e4-e6.
16. Arankalle VA, Chobe MS. Retrospective analysis of blood transfusion recipients: evidence for post-transfusion hepatitis E. Vox Sang 2000; 79: 72-4.
17. Khuroo MS, Kamili S, Yattoo GN. Hepatitis E virus infection may be transmitted through blood transfusions in an endemic area. J Gastroenterol Hepatol 2004; 19: 778-84.
18. Tamura A, Shimizu YK, Tamaka T, et al. Persistent infection of hepatitis E virus transmitted by blood transfusion in a patient with T-cell lymphoma. Hepatol Res 2007; 37: 113-120.
19. 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.
20. Kumar N, Kumar Sarin S. Hepatitis E - Is it a risk to transfusion safety? Asian J TransfusSci 2013; 7: 1-3.
21. Haim-Boukobza S, Ferey MP, Veltiard AL, et al. Transfusion transmitted hepatitis E in a misleading context of autoimmunity and drug-induced toxicity. J Hepatol 2012; 57: 1374-8.
22. Baylis SA, Gartner T, Nick S, Ovemyr J, Blumel J. Occurrence of hepatitis E virus RNA in plasma donations from Sweden, Germany and the United States. Vox Sang. 2012; 103:89-90. http://dx.doi.org/10.1111/j.1423-0409.2011.01583.x
23. Matusubayashi K, Nagaya K, Sakata H, et al. Transfusion transmitted hepatitis E caused by apparently indigenous hepatitis E virus strain in Hokkaido, Japan. Transfusion 2004; 44: 934-940.
24. Kimura Y, Gotoh A, Katagiri S, et al. Transfusion-transmitted hepatitis E in a patient with myelodysplastic syndromes. Blood Transfus 2014; 12: 103-6.
25. Baylis SA, Koc O, Nick S, Blumel J. Widespread distribution of hepatitis E virus in plasma fractionation pools. Vox Sang. 2012; 102:182-3.
26. Andonov A, Rock G, Lin L, et al. Serological and molecular evidence of a plausible transmission of hepatitis E virus through pooled plasma. Vox Sang 2014; doi: 10.1111/vox.12156.
27. Kumar N, Kumar Sarin S. Hepatitis E - Is it a risk to transfusion safety? Asian J TransfusSci 2013; 7: 1-3.
28. Antonisamy B, Christopher S, Samuel PP. Biostatistics: principles and practice. New Delhi: Tata McGraw-Hill; 2010.
29. Annatina Kaufmann, Alain Kenfak-Fouguen, Cyril Andre’, GiorgiaCanellini, Philippe Bu’ risgier, Darius Moradpour, Catherine E. A. Darling, Matthias CavassiniHepatitis E Virus Serore prevalence among Blood Donors in Southwest Switzerland. PLoS ONE 2011; 6(6): e21150.
30. Thrustfield M, Veterinary Epidemiology, 3rd Edition, Blackwell Science, Oxford, UK 2005; 233-234.
31. Kaufmann A, Kenfak-Fouguen A, Andre’ C, Canellini B, Bu’ risgier, et al. Hepatitis E Virus Serore prevalence among Blood Donors in Southwest Switzerland. PLoS ONE 2011; 6(6): e21150.
32. Surajudeen A. Junaid, Samuel E. Agina, Khadijah A. Abubakar. Epidemiology and Associated Risk Factors of Hepatitis E Virus Infection in Plateau State, Nigeria. Virology: Research and Treatment 2014:5 15–26 doi:10.4137/VRT.S15422
33. Andre’ Luiz BORTOLIERO, Ana Maria BONAMETTI, Helena KaminamiMORIMOTO,Tieni MATSUO, Edna Maria Vissoci REICHE. Serore prevalence for Hepatitis E Virus (HEV) infection among volunteer blood donors of the regional blood bank of Londrina, state of Paraná, Brazil. Rev. Inst. Med. Trop. S. Paulo March-April, 2006; 48(2):87-92.
34. Chen Dong, Xing Dai, Jiuhong Liang, Min Dong, Jiuhong Meng. Serore prevalence of Hepatitis E Virus Varies Considerably Among Chinese Provinces. Hepat Mon.2012;12(6):386-390. DOI: 10.5812/hepatmon.6194
35. Li Ma, Pan Sun, Fangzhao Lu, Hongjie Wang, Xia Rong, Yudong Dai et al. Prevalence of hepatitis E virus in Chinese blood donors Journal of International Medical Research 2015, Vol. 43(2) 257–262.
36. Maitrey D. Gajjar, Nidhi M. Bhatnagar, Rajesh V. Sonani, Shweta Gupta,Tarak Patel. Hepatitis E serore prevalence among blood donors.
donors: A pilot study from Western India Asian J Transfus Sci. 2014 Jan-Jun; 8(1): 29–31.

37. Adesina OA, Japhet MO, Donbraye E, Kumapayi TE, Kudoro A. Anti-hepatitis E virus antibodies in sick and healthy individuals in Ekiti State, Nigeria. *Afr J Microbiol Res*. 2009; 3:533–556.

38. Dalton HR, Bendall RP, Rashid M, et al. Host risk factors and autochthonous hepatitis E infection. *Eur J Gastroenterol Hepatol*. 2011; 23(12):1200–1205.

39. Cosme Alvarado-Esquite, Luis Francisco Sanchez-Anguiano, Jesus Hernandez-Tinoco. Seroepidemiology of Hepatitis E Virus Infection in General Population in Rural Durango, Mexico. *Hepat Mon*. 2014 June; 14(6): e16876.