

A Population-based Seroepidemiological Study on Hepatitis E Virus in Iran

SG Sepanlou¹, H Rezvan², S Amini-Kafiabad², MR Dayhim², S Merat^{1*}

1. Digestive Disease Research Center, Tehran University of Medical Sciences, Shariati Hospital, Tehran, Iran
2. Research Center, Iran Blood Transfusion Organization, Tehran, Iran

ABSTRACT

BACKGROUND

Little is known about HEV seroprevalence and its determinants in Iran. Considering the fact that Iran is among the countries in which HEV infection is endemic, a large-scale population-based study in this regard is justified.

METHODS

This survey was conducted in 2006 in Tehran and Golestan Provinces, Iran. Stored sera of subjects were tested for serological markers of anti-HEV. The baseline data were recorded in structured questionnaires. Weighted seroprevalence and weighted logistic regression coefficients were calculated.

RESULTS

A total of 1423 samples were included. The overall seroprevalence in two provinces was 7.4%. Age with an odds ratio equal to 1.59 (95% CI: 1.26-2.02) and history of traditional phlebotomy with an odds ratio equal to 2.28 (95% CI: 1.13-4.60) were independent predictors of HEV seropositivity.

CONCLUSION

Considering the high rate of HEV seroprevalence in Iran, further studies on the cost-effectiveness of vaccination among vulnerable groups are mandatory.

KEYWORDS

Hepatitis E; Seroepidemiological studies; Prevalence; Iran

INTRODUCTION

During 1955 to 1956, a large epidemic of water-borne hepatitis was reported in New Delhi, India.¹ The epidemic was initially believed to be related to hepatitis A. However, subsequent testing of stored sera from this epidemic and another epidemic from 1978 to 1979 failed to demonstrate a serological marker, neither for

hepatitis A nor for hepatitis B.²

³ The new agent was named non-A non-B hepatitis virus.⁴ Later, the agent was renamed hepatitis E virus (HEV) and in 1990 its genome was cloned.⁵

Acute HEV infection often presents with jaundice, dark urine, anorexia, enlarged and tender liver, elevated ALT, and abdominal pain and tenderness.⁶ It is associated

*Corresponding Author:

Shahin Merat, MD
Digestive Disease Research Center,
Tehran University of Medical Sciences,
Shariati Hospital, Tehran 14117, Iran
Tel: +98 21 82415173
Fax: +98 21 82415400
E-mail: merat@ams.ac.ir
Received: 8 July 2010
Accepted: 1 September 2010

with nausea, vomiting and fever, and is self-limiting in most cases with a low mortality rate and very low rates of progression to chronic liver disease.⁶

However, the severity and mortality rates are higher in pregnant women (up to 20%)^{6, 7}, organ recipients^{8, 9} and in patients who receive blood products.¹⁰ HEV infection is associated with abortion, stillbirths and low birth weight in infected pregnant women.^{6, 7}

The prevalence of HEV is as high as 70% in Egypt.¹¹ The prominent route of HEV transmission is fecal–oral in endemic countries^{6, 12} which leads to medium to large epidemics, and the majority of sporadic cases in these countries.² Parenteral or perinatal transmission are less common.⁶

Person-to-person transmission is distinctly uncommon.^{13, 14} HEV, primarily a zoonotic disease, rarely occurs in industrialized countries.¹⁵ HEV is detected serologically through the titration of anti-IgG antibodies in suspected patients.

Iran is classified among the endemic regions, as in several studies the seroprevalence of HEV has been reported to be above 5%. Suspected outbreaks have been reported in Iran.¹⁶ HEV infection was for the first time reported in pregnant women in Kermanshah in 1991.¹⁷

In 1992, 154 cases were reported from Lordegan.^{16, 17} Studies on blood donors in Iran have demonstrated that the prevalence of HEV infection varies, ranging from 7.8% in Tehran¹⁸ and Tabriz¹⁹, to 11.5% in Khuzestan²⁰.

However, the HEV seroprevalence is more varied in population-based studies.

The reported seroprevalence ranges from 3.8% in Isfahan²¹ 9 to 7.3% in Mazandaran among adults between the ages of 20 to 25 years²² and lastly, from 9.6% and 9.3%, respectively in 2004 and 2008 in Nahavand.^{23, 24}

HEV seroprevalence is believed to be higher among patients with chronic liver or kidney disease. A study in Tabriz reported that HEV

was serologically prevalent in 27.5% of patients with chronic liver disease.²⁵ However another report from the same city demonstrates that seroprevalence of HEV among hemodialysis patients is 7.4%.²⁶ A study in Ahvaz shows that HEV seroprevalence is 13.5% in drug addicts²⁷, not much higher than the general population, and it is amazingly low among Iranian soldiers (1.1%).²⁸

The apparent variation in previous studies on HEV seroprevalence has prompted large-scale population-based studies in this regard. The merit of the present study lies in its relatively large sample size and its distribution across two large provinces in Iran.

MATERIALS AND METHODS

Design and setting:

This secondary analysis is a cross-sectional study that was conducted in 2009 in the Digestive Disease Research Center (DDRC) of Tehran University of Medical Sciences. The source survey of this study was performed on the general population of Iran and sera were collected in 2006.

In the original survey, participants were selected from the general population of three provinces of Iran: Golestan in Northeast Iran, Tehran in North central and Hormozgan in Southern Iran. The survey included Iranian nationals between the ages of 18 and 65 who were permanent inhabitants of their households.

Randomly selected from all participants in the source survey, this study has included 1423 adults who resided in two provinces of Tehran and Golestan. Baseline information on Iran and the three provinces studied is given in Table 1.

Sampling and sample size

The original survey was targeted at liver diseases that had an estimated prevalence of about 0.5%; therefore, 4596 participants were enrolled by clustered random sampling and

Table 1: Census information on Iran in the three provinces studied*.

	Area (km ²)	Population	Percent rural	Percent male	Literacy
Tehran	18,814	13,422,366	8.7%	51.4%	91.3%
Golestan	20,367	1,617,087	50.8%	49.7%	82.1%
Iran	1,648,195	70,495,782	31.5%	50.9%	84.0%

*Source: Iran national population and housing census, 2006, http://www.sci.org.ir/portal/faces/public/sci_en, reproduced from Merat et al.²⁹

100 clusters were selected from each province. Each cluster consisted of 20 to 25 eligible subjects who lived within a block of adjacent households and the first household of the cluster was selected by random sampling. Plasma samples were then obtained and a questionnaire was filled in which common risk factors were recorded. Sampling methods and data collection has been explained in detail elsewhere.²⁹

For HEV, the estimated prevalence was 9%^{30,31}, and based on our calculations, a sample size of 1400 would be sufficient ($\alpha=0.05$). Thus, we selected 25% of the originally collected sera for our study by systematic random sampling.

Baseline data:

Baseline data in this study included sex, age, educational level, marital status, birth rank, and possible risk factors such as history of blood transfusion, non IV-drug addiction, IV-drug addiction, previous surgery, phlebotomy, tattooing, imprisonment and hepatitis among family members.

Laboratory tests:

Serum samples from selected participants were transferred to the Iran Blood Transfusion Organization (IBTO) Research Center, where they were tested for total anti-HEV antibodies (Dia.Pro Diagnostic Bioprobes srl, Milano, Italy).

Statistical analysis:

The age specific seroprevalence of HEV was calculated in each province. Data were then

weighted based on the population size in each province. Weighted prevalence of HEV in the entire population and weighted logistic regression were calculated to investigate the potential effect of determinants on HEV seroprevalence. All analyses were done by SPSS 16.0 (SPSS, Inc., Chicago, IL).

Ethics

The study was approved by the Institutional Review Board of the Digestive Disease Research Center, Tehran University of Medical Sciences. Questionnaires and serological tests were registered unanimously. All participants signed an informed consent.

RESULTS

A total of 1423 serum samples were included in the study. The demographics of the study population in the two provinces are presented in Table 2.

Table 2: Characteristics of study participants.

	Subjects interviewed	Samples randomly selected	Percent male	Percent rural	Meanage±SD (years)
Tehran	2561	789 (55.4%)	39.7%	2.7%	35.3±13.3
Golestan	2035	634 (44.6%)	34.4%	37.1%	4.1±12.8
Total	4596	1423 (100%)	37.3%	18.0%	37.9±13.4

The prevalence of HEV seropositivity was 7.3% in Tehran, 9.2% in Golestan and 7.4% in the entire sample, adjusted for the populations of both provinces and residential location (urban or rural; Table 3).

Table 3: The prevalence of anti-HEV antibodies in the two provinces.

	Tehran*	Golestan*	Total**
Anti-HEV antibody	7.3%	9.2%	7.4%
Male	7.6%	8.3%	7.7%
Female	6.9%	11.0%	7.2%

* Percentages adjusted for location (urban or rural).

**Totals adjusted for population of provinces and location (urban or rural).

The difference between males and females was not significant.

The prevalence of HEV seropositivity increased by age in both provinces, both resi-

dential locations (urban and rural) and both genders (Figures 1 to 3).

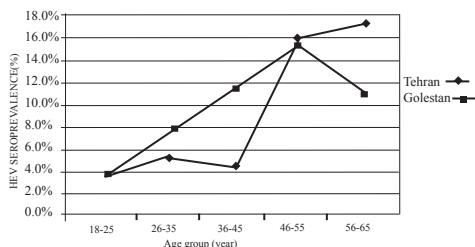


Figure 1: Age specific HEV seroprevalence by province, weighted according to location (urban or rural).

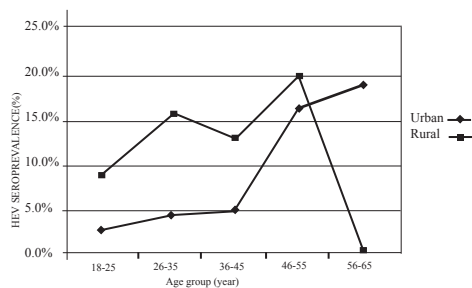


Figure 2: Age specific HEV seroprevalence by residential location weighted according to province population.

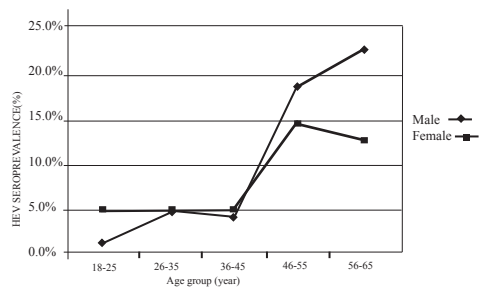


Figure 3: Age specific HEV seroprevalence by gender weighted according to both province population and residential location (urban or rural).

In univariate logistic regression, the determinants of HEV seropositivity consisted of age, subjects' educational levels, marital status, mother's educational level, and history of traditional phlebotomy (Table 4). In multivariate logistic regression however, only age and history of traditional phlebotomy remained in the model (Table 5).

Table 4: Determinants of HEV Seropositivity in univariate analysis.

	Number (%)	Anti-HEV positive Odds ratio (95% CI)	p-value
Province			
Tehran	58 (7.4%)	1	
Golestan	70 (11.0%)	1.52 (0.95-2.44)*	0.08
Gender			
Male	46 (8.7%)	1	
Female	82 (9.2%)	0.93 (0.52-1.69)**	0.81
Location			
Urban	95 (8.1%)	1	
Rural	33 (12.9%)	1.99 (0.79-4.86)#	0.15
Age (for each 10 years)	37.7±13.4	1.66 (1.33-2.08)**	<0.001
Education			
<6 years	66 (14.1%)	1	
6 years or more	44 (6.3%)	0.31 (0.17-0.57)**	<0.001
Marital status			
Unmarried	4 (1.8%)	1	
Married	105 (11.1)	5.48 (1.76-17.13)**	0.003
Father's education			
<6 years	46 (8.3%)	1	
6 years or more	12 (5.2%)	0.56 (0.26-1.18)**	0.13
Mother's education			
<6 years	52 (8.9%)	1	
6 years or more	6 (3.0%)	0.32 (0.12-0.83)**	0.02
Blood transfusion			
No	96 (9.0%)	1	
Yes	14 (14.0%)	1.66 (0.71-3.90)**	0.25
Addiction			
No	100 (9.2%)	1	
Yes	10 (12.7%)	1.84 (0.51-6.64)**	0.35
IV addiction			
No	57 (7.3%)	1	
Yes	1 (20.0%)	3.25 (0.28-37.59)**	0.35
Surgery			
No	53 (8.4%)	1	
Yes	57 (10.7%)	1.43 (0.79-2.59)**	0.23
Traditional phlebotomy			
No	40 (6.0%)	1	
Yes	18 (15.8%)	3.20 (1.64-6.25)**	0.001
Tattooing			
No	54 (7.4%)	1	
Yes	4 (7.0%)	0.97 (0.30-3.12)**	0.96
Imprisonment			
No	57 (7.4%)	1	
Yes	1 (7.1%)	0.99 (0.10-9.62)**	0.99
Family history			
No	48 (6.8%)	1	
Yes	9 (13.2%)	1.91 (0.79-4.61)**	0.15

*OR weighted by location (urban or rural).

**OR weighted by both province population and location (urban or rural).

OR weighted by province population.

Table 5: Determinants of HEV seropositivity by multivariate analysis.

	OR (95% CI)*	p-value
Age (for each 10 years)	1.59 (1.26-2.02)	<0.001
Traditional phlebotomy	2.28 (1.13-4.60)	0.021

*OR weighted by both province population and location (urban or rural).

DISCUSSION

The results of our study on the overall HEV prevalence are concordant with several previous population-based studies and reports among blood donors in Iran.^{18-21, 23,24} The HEV prevalence in Iran is apparently higher than developed countries (0.4%-3.9%), higher than the prevalence in Israel (2.8% in Jews and 1.8% in Arabs)³² or Turkey (3.8%)³³, but lower than Kurdish refugees in Iraq (14.8%)³⁴, blood donors (16.4%), the general population (18.8%) in Saudi Arabia^{35,36}, and finally the general population in Pakistan (17.5%).³⁷

Variations in reports somehow reflect different levels of exposure in different regions. However, alternative evidence may cast doubt on this assumption. It is reported that anti-IgG serological tests have 100% specificity but varied sensitivity.³⁸ The fact that HEV seroprevalence in developed countries and in Egypt is higher than expected raises the possibility of cross-reaction with other antibodies. On the other hand, seroprevalence is lower than expected in endemic regions like India, which indicates the existence of false negative results. Agreement between various tests is moderate³⁹ and several ELISA kits have been previously withdrawn from the market due to high proportions of false negatives.³⁸ The ELISA kit used in this study is documented to be 100% specific, 96% sensitive and 99% accurate. Still, the possibility of inaccurate estimation of HEV seroprevalence in different populations should not be neglected.

HEV seropositivity has been mainly reported in males.^{6, 20} However, most studies in Iran fail to demonstrate any significant difference between the two genders^{18, 21, 22, 25}. The results of one study show that HEV seroprevalence is significantly higher among females.²³ In our study, although more prevalent among males, the seroprevalence is not significantly different between the two genders.

The results of some studies in Iran have

demonstrated no significant difference in HEV seropositivity between urban and rural residents^{21, 25, 27}, which is supported in the current study.

Previous studies have shown that HEV seropositivity peaks among 15-35 year-olds and is not seen in children.^{2, 25, 27} However, other studies in Tehran, Khuzestan, and Nahavand show that HEV seroprevalence is highest among 35-49 year olds.^{18, 20, 23} Also, in two other studies, the HEV seroprevalence is highest among those over 50.^{19, 21} The results of our study confirm the previous studies in Iran and show that age is a significant risk factor for HEV seroprevalence.

Results of previous studies in Iran show that HEV seropositivity is lower in the educated compared to the uneducated, but the difference is not significant.^{21, 23, 25, 27, 28} In two studies, the difference in seroprevalence between educational level of subjects and their parents is significant.^{19, 22} Our results support the first finding that the difference between the educated and the uneducated is not significant.

The results of a study in 2004 in Tabriz failed to show any significant association between history of blood transfusion and HEV seroprevalence which negates the possibility of parenteral transmission for HEV.²⁶ On the other hand, the reported high prevalence of HEV seropositivity among drug addicts, particularly among injecting drug users in Ahvaz, supports the possibility.²⁷ In our study, the significantly high seropositivity in subjects who presented with a history of traditional phlebotomy supports this hypothesis, although the results on other related risk factors such as history of blood transfusion, addiction, imprisonment, surgery and surgery are insignificant, probably due to the high proportion of missing data.

Insignificant results on the role of familial history of hepatitis support the assumption that person-to-person communication is uncommon in HEV hepatitis.^{13, 14}

The main limitation of the current study is that the seroprevalence among vulnerable groups, including pregnant women and immunosuppressed patients, has not been investigated. Despite the low cost-effectiveness of vaccines for the prevention of HEV infection, further studies are needed to investigate the efficiency of vaccination in groups who are vulnerable to HEV infection in Iran.

ACKNOWLEDGEMENT

This study was jointly supported by grants from the Digestive Disease Research Center of Tehran University of Medical Sciences and the Iranian Blood Transfusion Organization Research Center. We are also indebted to Gonbad Cohort Study Center for their assistance in collecting data and blood samples in Golestan Province. We also thank Saideh Raman, Behnaz Amoohosseini, Mina Moghtadaie and Roshanak Shamriz from the Iranian Blood Transfusion Organization Research Center for their technical support.

CONFLICT OF INTEREST

The author declare no conflict of interest related to this work.

REFERENCES

- Vishwanathan R. Infectious hepatitis in Delhi (1955-1956): a critical study: epidemiology. *Indian J Med Res* 1957;45: 1-29.
- Khuroo MS. Study of an epidemic of non-A, non-B hepatitis. Possibility of another human hepatitis virus distinct from post-transfusion non-A, non-B type. *Am J Med* 1980;68:818-824.
- Wong DC, Purcell RH, Sreenivasan MA, Prasad SR, Pavri KM. Epidemic and endemic hepatitis in India: evidence for a non-A, non-B hepatitis virus aetiology. *Lancet* 1980;2: 876-9.
- Balayan MS, Andjaparidze AG, Savinskaya SS, Ketiladze ES, Braginsky DM, Savinov AP, et al. Evidence for a virus in non-A, non-B hepatitis transmitted via the fecal-oral route. *Intervirology* 1983;20:23-31.
- Reyes GR, Purdy MA, Kim JP, Luk KC, Young LM, Fry KE, et al. Isolation of a cDNA from the virus responsible for enterically transmitted non-A, non-B hepatitis. *Science* 1990;247:1335-9.
- Purcell R, Emerson S. Hepatitis E Virus. Philadelphia, PA: Lippincott:Williams and Wilkins;2001.
- Navaneethan U, Al Mohajer M, Shata MT. Hepatitis E and pregnancy: understanding the pathogenesis. *Liver Int* 2008;28:1190-9.
- Haagsma EB, van den Berg AP, Porte RJ, Benne CA, Vennema H, Reimerink JH, et al. Chronic hepatitis E virus infection in liver transplant recipients. *Liver Transpl* 2008;14:547-53.
- Kamar N, Selves J, Mansuy JM, Ouezzani L, Peron JM, Guitard J, et al. Hepatitis E virus and chronic hepatitis in organ-transplant recipients. *N Engl J Med* 2008;358: 811-7.
- Tamura A, Shimizu YK, Tanaka T, Kuroda K, Arakawa Y, Takahashi K, et al. Persistent infection of hepatitis E virus transmitted by blood transfusion in a patient with T-cell lymphoma. *Hepatology* 2007;37:113-20.
- Stoszek SK, Abdel-Hamid M, Saleh DA, El Kafrawy S, Narooz S, Hawash Y, et al. High prevalence of hepatitis E antibodies in pregnant Egyptian women. *Trans R Soc Trop Med Hyg* 2006;100:95-101.
- Meng X. Hepatitis E Virus (Hepevirus). In: Mahy B, Van-Regenmortel M, editors. *Encyclopedia of Virology*, 3rd ed: Oxford:Elsevier; 2008. p.377-83.
- Aggarwal R, Naik SR. Hepatitis E: does person-to-person spread occur? *Indian J Gastroenterol* 1992;11: 109-12.
- Somani SK, Aggarwal R, Naik SR, Srivastava S, Naik S. A serological study of intrafamilial spread from patients with sporadic hepatitis E virus infection. *J Viral Hepat* 2003;10: 446-449.
- Balayan MS, Usmanov RK, Zamyatina NA, Djumalieva DI, Karas FR. Brief report: experimental hepatitis E infection in domestic pigs. *J Med Virol* 1990;32:58-9.
- Ariyegan M, Amini S. Hepatitis E epidemic in Iran. *J Med Council IR Iran* 1998;15: 139-43.
- Hatami H. Epidemic report of hepatitis E in Kermanshah. *Nabz Journal* 1991;9:23-31.
- Aminiafshar S, Alimaghham M, Gachkar L, Yousefi F, Attarchi Z. Anti hepatitis E virus seropositivity in a group of blood donors. *Iranian J Publ Health* 2004;33:53-6.
- Taremi M, Gachkar L, MahmoudArabi S, Kheradpezhough M, Khoshbaten M. Prevalence of antibodies to hepatitis E virus among male blood donors in Tabriz, Islamic Republic of Iran. *East Mediterr Health J* 2007;13:98-102.
- Assarehzadegan MA, Shakerinejad G, Amini A, Rezaee SA. Seroprevalence of hepatitis E virus in blood donors in Khuzestan Province, southwest Iran. *Int J Infect Dis* 2008;12:387-90.
- Ataei B, Nokhodian Z, Javadi AA, Kassaian N, Shoaei P,

- Farajzadegan Z, et al. Hepatitis E virus in Isfahan Province: a population-based study. *Int J Infect Dis* 2009;**13**: 67-71.
22. Saffar MJ, Farhadi R, Ajami A, Khalilian AR, Babamahmodi F, Saffar H. Seroepidemiology of hepatitis E virus infection in 2-25-year-olds in Sari district, Islamic Republic of Iran. *East Mediterr Health J* 2009;**15**:136-42.
 23. Taremi M, Mohammad Alizadeh AH, Ardalan A, Ansari S, Zali MR. Seroprevalence of hepatitis E in Nahavand, Islamic Republic of Iran: a population-based study. *East Mediterr Health J* 2008;**14**:157-62.
 24. Zali M, Taremi M, Arabi S, Ardalan A, Alizadeh A, Ansari S. Seroprevalence of hepatitis E in Nahavand, Iran: A population-based study. *Proceeding of the Digestive Diseases Week* 2004:15-9.
 25. Somi M, Farhang S, Majidi G, Shavakhi A, Pouri A. Seroprevalence of hepatitis E in patients with chronic liver disease from East Azerbaijan, Iran. *Hep Mon* 2007;**7**:125-8.
 26. Taremi M, Khoshbaten M, Gachkar L, EhsaniArdakani M, Zali M. Hepatitis E virus infection in hemodialysis patients: a seroepidemiological survey in Iran. *BMC Infect Dis* 2005;**5**:36.
 27. Alavi S, Ahmadi F, Ghasemirad M. Seroepidemiological study of hepatitis E virus in drug addicts in Ahvaz, Southern Iran: 2005-2006. *Hep Mon* 2008;**8**: 263-6.
 28. Ghorbani G, Alavian S, Esfahani A, Assari S. Seroepidemiology of hepatitis E virus in Iranian soldiers. *Hep Mon* 2007;**7**:121-4.
 29. Merat S, Rezvan H, Nouraie M, Jamali A, Assari S, Abolghasemi H, et al. The prevalence of hepatitis B surface antigen and anti-hepatitis B core antibody in Iran: a population-based study. *Arch Iran Med* 2009;**12**:225-231.
 30. Malekzadeh R, Khatibian M, Rezvan H. [Viral hepatitis in Iran and the world: epidemiology, diagnosis, treatment, and prevention](in Farsi). *J Med Council Iran* 1997;**15**:183-200.
 31. Farzadegan H, Shamszad M, Noori-Arya K. Epidemiology of viral hepatitis among Iranian population--a viral marker study. *Ann Acad Med Singapore* 1980;**9**:144-8.
 32. Karenyi YV, Favorov MO, Khudyakova NS, Weiss P, Bar-Shani S, Handsher R, et al. Serological evidence for hepatitis E virus infection in Israel. *J Med Virol* 1995;**45**: 316-20.
 33. Cesur S, Akin K, Dogaroglu I, Birengel S, Balik I. [Hepatitis A and hepatitis E seroprevalence in adults in the Ankara area]. *Mikrobiyol Bul* 2002;**36**:79-83.
 34. Chironna M, Germinario C, Lopalco PL, Carrozzini F, Barbuti S, Quarto M. Prevalence rates of viral hepatitis infections in refugee Kurds from Iraq and Turkey. *Infection* 2003;**31**:70-4.
 35. Abdelaal M, Zawawi TH, al Sobhi E, Jeje O, Gilpin C, Kinsara A, et al. Epidemiology of hepatitis E virus in male blood donors in Jeddah, Saudi Arabia. *Ir J Med Sci* 1998;**167**: 94-96.
 36. Arif M. Enterically transmitted hepatitis in Saudi Arabia: an epidemiological study. *Ann Trop Med Parasitol* 1996;**90**: 197-201.
 37. Hamid SS, Atiq M, Shehzad F, Yasmeen A, Nissa T, Salam A, et al. Hepatitis E virus superinfection in patients with chronic liver disease. *Hepatology* 2002;**36**:474-8.
 38. Mast EE, Alter MJ, Holland PV, Purcell RH. Evaluation of assays for antibody to hepatitis E virus by a serum panel. Hepatitis E Virus Antibody Serum Panel Evaluation Group. *Hepatology* 1998;**27**:857-61.
 39. Bouwknegt M, Engel B, Herremans MM, Widdowson MA, Worm HC, Koopmans MP, et al. Bayesian estimation of hepatitis E virus seroprevalence for populations with different exposure levels to swine in The Netherlands. *Epidemiol Infect* 2008;**136**:567-76.