

## SEROLOGICAL EVIDENCE OF HEPATITIS E VIRUS INFECTION AMONG VOLUNTEER BLOOD DONORS AT THE ACCRA AREA BLOOD TRANSFUSION CENTRE, ACCRA, GHANA

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### ABSTRACT

Hepatitis E virus (HEV) infection causes an acute, self-limiting hepatitis that is associated with high mortality, especially in pregnant women. We previously reported high sero-prevalence of HEV among pregnant women and persons who worked with pigs. Therefore we evaluated the prevalence of anti-HEV IgM and anti-HEV IgG among blood donors at the Accra Area Blood Centre, Accra, Ghana. Four hundred and seventy-one volunteer blood donors (males, 427; females, 44) voluntarily provided blood samples for unlinked anonymous testing for the presence of antibodies (IgM and IgG) to HEV by enzyme-linked immunoassay. Among the blood donors, the overall sero-prevalence of HEV-IgG and HEV IgM were found to be 25.6% and 45.9% respectively; this difference is statistically significant ( $P < 0.05$ ). None of the blood donors tested positive for both IgG and IgM. The prevalence rate of HEV-IgM (both male and female) in each age group was almost two times higher than the prevalence rate of HEV-IgG. On multivariate analysis, gender and age were not independent determinants ( $P > 0.05$ ) for HEV infection among the blood donors. The results of our studies demonstrate a high prevalence of anti-HEV among Ghanaian blood donors, particularly anti-HEV IgM suggestive of more recent infection.

### INTRODUCTION

Hepatitis E virus (HEV) was recovered originally from stools of patients with enteric non-A, non-B hepatitis. It is a single-stranded non-enveloped RNA virus of approximately 7.5 kb with 3 open reading frames [ORFs] (Bradley *et al.*, 1995), and is considered a major aetiological agent of enterically transmitted viral hepatitis in several developing countries (Paula *et al.*, 2001). HEV is the leading cause of enterically transmitted, non-A hepatitis worldwide, and is responsible for major outbreaks of acute hepatitis in developing countries, especially in tropi-

cal and sub-tropical regions of the world where outbreaks are usually associated with faecally contaminated drinking water (Irshad *et al.*, 1999; Trinta *et al.*, 2001). In developed countries, sporadic cases of HEV appear to be rare, where they tend to be imported. However, not all isolated reports of HEV-associated hepatitis in developed countries include a history of travel to regions endemic for HEV (Worm *et al.*, 2000; Schlauder *et al.*, 1998; Takahashi *et al.*, 2002; Bradley, 1992; Coursaget *et al.*, 1996).

HEV is prevalent in Africa including Algeria, La Cote d'Ivoire, Sudan, Somalia (8), Djibouti (Coursaget *et al.*, 1996), Tunisia (Coursaget *et al.*, 1996), Morocco (Coursaget *et al.*, 1996), and Nigeria (Buisson *et al.*, 2000). The infection usually presents an acute and benign clinical course and does not provoke chronic infection. However, among all the responsible hepatitis viruses, HEV is associated with the largest fulminant hepatitis cases (3.0%) in the general population, and up to 20% in pregnant women (Bradley, 1990).

Growing evidence indicates that the disease is prevalent in communities lacking portable water, waste disposal facilities and in animal handlers especially swine farmers. Another significant route for transmission of the infection is through blood transfusion (Matsubayashi *et al.*, 2008). Bortolifero *et al.*, (2006) recently reported the presence of antibodies to HEV among volunteer blood donors at the regional blood bank in Londrina, Brazil. Similar findings among blood donors were reported in the United States of America and other countries (Mast *et al.*, 1997; Meng *et al.*, 2002; Zanetti *et al.*, 1994).

The extent to which blood transfusion can be a means of transmission of HEV to recipients is not known. The association of HEV with blood transfusion makes it imperative to screen healthy blood donors for anti-HEV antibodies as significant levels of circulating antibodies of IgG isotype were recently detected among volunteer blood donors (Bortolifero *et al.*, 2006; Meng *et al.*, 2002).

Currently in Ghana, donor blood is screened for only human immunodeficiency virus (HIV) 1 and 2, hepatitis B virus (HBV), hepatitis C virus (HCV) and syphilis in some blood banks. We recently reported high sero-prevalence of HEV-IgM and HEV-IgG among pregnant women and persons who work with pigs in Ghana (Adjei *et al.*, 2009a; Adjei *et al.*, 2009b). Furthermore, anecdotal reports of increased non-A and non-B hepatitis (Unpublished report, Department of Medicine and Therapeutics, Korle-Bu Teaching Hospital, [KBTH], Accra,

Ghana) among patients with human viral hepatitis has also been seen at the Department. Outbreaks of hepatitis E have never been reported in Ghana, although the possibility of the occurrence of such outbreaks exists since there are great social differences and sanitary conditions are quite precarious in many areas. Besides, the disease surveillance is deficient in the country as there are no documented cases or data available. The present study was undertaken in order to determine the prevalence of antibodies to HEV among volunteer blood donors at the Accra Area Blood Transfusion Centre (AABTC) of the National Blood Transfusion Services Centre (NBTSC), Korle-Bu, Accra, Ghana. Such information may be invaluable to health planners and policy makers in Ghana.

## MATERIALS AND METHODS

### Study design and study site

The study was designed as a cross-sectional study and was conducted between the months of June and July 2008 among a sample of male and female blood donors at the AABTC at the Korle-Bu Blood Bank, Accra, Ghana. This centre serves the KBTH, other hospitals/clinics in the Accra metropolitan area, both Governmental and-Non-Governmental, and some parts of the Central Eastern Regions of Ghana. The study was approved by the Ethical and Protocol Review Committee of UGMS, and moreover the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the UGMS human research committee.

In Ghana, blood donors are volunteers (both occasional and periodic repeat non-compensated volunteer donors) and are also sought from family members and friends of patients requiring blood transfusion. They are selected based on the following criteria: age between 18 and 65 years; weight >45 kg; haemoglobin >12.5 g/dl; normal blood pressure [BP], pulse, and body temperature; and not belonging to any high-risk group (homosexually or heterosexually promiscuous, intravenous drug users; history of sexually

transmitted diseases; and history of any severe current or chronic illnesses). Donated blood is routinely screened for HIV 1 & 2, HBsAg, anti-HCV and syphilis antibodies. All individuals enrolled in this study were also questioned about a past clinical history of symptomatic hepatitis. None of the voluntary blood donors have travelled outside the country.

### Sample collection and testing

The samples used for the study were the excess sera from blood samples drawn from the volunteer blood donors (n=471) for their routine blood screening (syphilis, ABO and Rhesus, HBsAg, HCV, HIV 1 & 2) testing, with all identifiers removed except for age and sex, were assayed for antibodies to HEV. Fully informed consent was obtained from each study subject and all blood donors who simultaneously or unilaterally tested positive for HBsAg, HCV, HIV and syphilis were excluded from the study. All of the sera were screened in duplicate for specific anti-HEV IgM and IgG antibodies by enzyme linked immunosorbent assay (International Immunodiagnostics, U.S.A), in accordance with the manufacturer's instructions. The results were scored as positive or negative according to the standard procedures recommended by the manufacturer. Positive and negative controls were included in all the ELISA microplates assayed.

### Statistical analysis

The Statistical Analysis System version 9.1 (SAS Institute) was used to complete all data analysis. We divided the study subjects into five categories of age:  $\leq 20$ , 21-25, 26-30, 31-35,  $\geq 36$  years. In the univariate analysis, the frequency for each of the age categories and the mean, median and maximum and minimum age for the overall sample were determined, as well as SD. We repeated the univariate analysis of age after having stratified the data by serum analysis and compared the mean ages for a statistically significant difference using Student's *t*-test. In the bivariate analysis, we evaluated the relationship between age and serum results categories using Fisher's exact test. Logistic

regression analysis was used to model the relationship between age categories and serum results. The logistic model with a maximum-likelihood estimate was fitted to the ordinal response of age categories and 95% confidence for the odd ratios were calculated with age category  $\leq 20$  years as the reference group. A  $\chi^2$  test for trend over increasing age categories was also performed.

### RESULTS

Between June and July, 2008, 471 (males 427, aged  $33.14 \pm 9.50$  years, median age 32 years, modal age 33 years, age range 18 to 60 years; females 44, aged  $29.09 \pm 5.96$  years, median age 26 years, modal age 26 years, age range 19 to 42 years), blood donors in the AABTC participated in the study. The above stated sex (male:female) ratio, median age, and age range among the blood donors at the AABTC were statistically significantly ( $P < 0.05$ ) different.

Table 1 shows HEV-IgG sero-positivity according to age and gender among the 471 healthy blood donors in Accra, Ghana. Among the healthy blood donors, overall sero-prevalence of HEV-IgG over the 2 month period was 25.6% (122 out of 471). There was no statistically significant ( $P > 0.05$ ) difference in the overall sero-prevalence between male and female sero-positive healthy blood donors (Table 1). Furthermore, among the male blood donors, the sero-prevalence of HEV-IgG was lowest in the 51-60 (18.2%; 4 out of 22) age group and highest in the 31-40 (28.3%; 39 out of 138) age group (Table 1). However, among the female blood donors, the prevalence rate of antibodies to HEV-IgG was lowest in the  $\leq 20$  (16.7%; 1 out of 6) age group and highest in the 21-30 (34.5%; 10 out of 29) age group.

Multivariate analysis (Table 2) further showed that gender was not an independent determinant (adjusted odds ratio [OR] 0.82; 95% confidence interval [CI] 0.40-1.65,  $P > 0.05$ ) for HEV-IgG infection among the healthy blood donors.

Table 3 shows HEV-IgM sero-positivity according to age and gender among the 471 healthy blood donors in Accra, Ghana. Among

the healthy blood donors, overall sero-prevalence of HEV-IgM over the 2 month period was 45.9% (216 out of 471). There was no statistically significant ( $P > 0.05$ ) difference in the overall sero-prevalence between male and female sero-positive healthy blood donors (Table 3). Additionally, among the male blood

donors, the sero-prevalence of HEV-IgM was lowest in the  $\leq 20$  (41.0%; 16 out of 39) and 21-30 (41.8%; 61 out of 146) age groups and highest in the 41-50 (53.7%; 44 out of 82) age group (Table 3). However, among the female blood donors, the prevalence rate of antibodies to HEV-IgM was lowest in the 31-40 (37.5%; 3

**Table 1: Comparison of age-specific distribution of HEV sero-reactivity (IgG)**

Age (yrs)	Gender	Positive	Negative	HEV status (n, %)		p-value
				OR	95% CI	
$\leq 20$ (n=45)	Male	9 (23.1)	30 (76.9)	1.50	0.16-14.56	1.000*
	Female	1 (16.7)	5 (83.3)	1.00	Baseline	
21-30 (n=175)	Male	36 (24.7)	110 (75.3)	0.62	0.27-1.46	0.272
	Female	10 (34.5)	19 (65.5)	1.00	Baseline	
31-40 (n=146)	Male	39 (28.3)	99 (71.7)	1.18	0.23-6.12	0.842
	Female	2 (25.0)	6 (75.0)	1.00	Baseline	
41-50 (n=83)	Male	21 (25.6)	61 (74.4)	NE	NE	1.000*
	Female	0 (0)	1 (100)	1.00	Baseline	
51-60 (n=22)	Male	4 (18.2)	18 (81.1)	NE	NE	NE
	Female	0 (N.E)	0 (N.E)	1.00	Baseline	
Total (n=471)	Male	109 (25.5)	318 (74.5)	0.82	0.41-1.62	0.562
	Female	13 (29.5)	31 (70.5)	1.00	Baseline	

OR, odds ratio; CI, confidence interval; n values indicate number of responses obtained in each category; NE, not estimable

**Table 2: Multivariate Predictors of HEV seropositivity for IgG and IgM**

Characteristics	HEV sero-positivity (IgG)		HEV sero-positivity (IgM)	
	Adjusted OR [95% CI]	p-value	Adjusted OR [95% CI]	p-value
<b>Gender</b>				
Female	1.00		1.00	
Male	0.82 [0.40-1.65]	0.568	1.01 [0.53-1.92]	0.972
<b>Age Group</b>				
$\leq 20$	1.00		1.00	
21-30	1.24 [0.57-2.70]	0.589	1.00 [0.52-1.95]	0.993
31-40	1.39 [0.63-3.07]	0.416	1.23 [0.62-2.41]	0.556
41-50	1.22 [0.51-2.89]	0.657	1.54 [0.74-3.22]	0.249
51-60	0.80 [0.22-2.92]	0.736	1.14 [0.41-3.19]	0.805

OR, odds ratio; CI, confidence interval

**Table 3: Comparison of age-specific distribution of HEV sero-reactivity (IgM)**

Age (yrs)	Gender	HEV status (n, %)				p-value
		Positive	Negative	OR	95% CI	
≤ 20 (n=45)	Male	16 (41.0)	23 (59.0)	0.70	0.12-3.90	0.679
	Female	3 (50.0)	3 (50.0)	1.00	Baseline	
21-30 (n=175)	Male	61 (41.8)	85(58.2)	0.88	0.40-1.97	0.762
	Female	13 (44.8)	16 (52.2)	1.00	Baseline	
31-40 (n=146)	Male	66 (47.8)	72 (52.2)	1.53	0.35-6.64	0.722*
	Female	3 (37.5)	5 (62.5)	1.00	Baseline	
41-50 (n=83)	Male	44 (53.7)	38 (46.3)	NE	NE	0.470*
	Female	0 (0)	1 (100)	1.00	Baseline	
51-60 (n=22)	Male	10 (45.5)	12 (54.5)	NE	NE	NE
	Female	0 (N.E)	0 (N.E)	1.00	Baseline	
Total (n=471)	Male	197 (46.1)	230 (53.9)	1.13	0.60-2.11	0.708

OR, odds ratio; CI, confidence interval; n values indicate number of responses obtained in each category; NE, not estimable

out of 8) age group and highest in the ≤ 20 (50%; 3 out of 6) age group. There was no statistically significant difference in HEV-IgG and HEV-IgM sero-positivity among the male and female blood donors between the different age groups, respectively (P for trend > 0.05).

Multivariate analysis (Table 2) further showed that gender was not an independent determinant (adjusted OR 1.01; CI [0.53-1.92], P> 0.05) for HEV-IgM infection among the healthy blood donors.

From Tables 1 and 3, the overall prevalence rate of antibodies to HEV-IgM (45.9%) in all the age groups was statistically significantly (P< 0.05) higher compared to the prevalence rate of antibodies to HEV-IgG (25.9%) in the study. None of the donors tested positive for both IgG and IgM antibodies to HEV.

**DISCUSSION**

The policy concerning blood supply in Ghana is that prospective donors should be screened clinically before selected to donate blood and all donations should be screened or checked anonymously for all transfusion associated pathogens in order to ensure safety to recipients and staff of the blood transfusion services.

This indicates that the safety of blood supply is of special importance and a major concern to the Ministry of Health, NBTSC and the AABTC. However, because of lack of facilities, the AABTC and NBTC are only able to screen for HIV, HCV antibodies, HBsAg and in some cases syphilis antibodies in donor blood. Ghana, an area of endemicity for viral hepatitis B and C, has never had an epidemic of hepatitis E. However, recent reports from the Gastroenterology Unit of the Department of Medicine and Therapeutics, KBTH, indicate cases of acute hepatitis without a defined aetiology. Although the physicians did not estimate HEV or hepatitis A virus (HAV) antibodies in the patients’ serum, based on clinical examinations, they speculated that HEV or HAV may be the causative pathogen. We also recently reported a higher sero-prevalence of HEV IgM and IgG among pregnant women attending ante-natal clinic at the Obstetrics and Gynaecology Out-patient Clinic of the KBTH (Adjei et al., 2009a) and persons who work with pigs (Adjei et al., 2009b).

Blood transfusion and administration of blood products manufactured from large plasma pools (e.g., coagulation factor concentrates) are possi-

ble modes of HEV transmission, because a short period of viremia occurs in patients with acute hepatitis E. Numerous studies from several countries have detected antibodies to HEV in volunteer blood donors (Bortolifero *et al.*, 2006; Mast *et al.*, 1997; Meng *et al.*, 2002; Zanetti *et al.*, 1994) and more recently, cases of transfusion-transmitted hepatitis E caused by blood from a donor infected with HEV were reported in Japan and France (Matsubayashi *et al.*, 2008; Mitsui *et al.*, 2004; Colson *et al.*, 2007). In Ghana, the demand for blood transfusion is high on account of high incidences of anaemia, malnutrition, and surgical/obstetric emergencies that are associated with blood loss (Mollison *et al.*, 1993). To ascertain whether hepatitis E is a frequent disease in Ghana and in order to establish and commence further studies on HEV epidemiology, we estimated the seroprevalence of HEV among blood donors at the NBTSC Centre in Accra, Ghana.

To our knowledge, this is believed to be the first study to determine the prevalence of HEV infection among volunteer blood donors in Ghana, and demonstrates the high prevalence of and the considerable potential for the transmission of HEV infection in recipients of blood and blood components in Ghana. Although there is no report from the Ministry of Health, Accra, Ghana indicating that Ghana is an endemic area for hepatitis E, this study found very high overall prevalence rates (45.9% for IgM, 26.5% for IgG) of HEV antibody among blood donors, suggesting the possibility of sub-clinical infections in the country. The finding of higher HEV antibody prevalence among volunteer blood donors at the AABTC, Accra, Ghana is consistent with literature, and is widely attributable to poor sanitation and contamination of the water supply (Emerson and Purcell, 2003; Aggarwal and Krawczynski 2000). The overall seroprevalence of HEV infection among the blood donors in Ghana (45.9% for IgM or 26.5% for IgG) is higher than the results of similar studies done in Brazil (Bortolifero *et al.*, 2006) (2.3%), France (Boutrouille *et al.*, 2007) (3.2%), Japan (Gotanda *et al.*, 2007) (7.1%), Iran

(Assarehzadegan. *et al.*, 2008) (11.5%), and England (Dalton *et al.*, 2008) (16.0%) but lower than the prevalence rate (52%) in other countries of the Eastern Mediterranean Region (Ritter *et al.*, 1994). The high seroprevalence of HEV in blood donors at the AABTC and the high prevalence in pregnant women (Adjei *et al.*, 2009a) and persons who work with pigs (Adjei *et al.*, 2009b) may suggest that HEV may be widespread in the country and therefore reasonable to speculate that HEV may circulate in the general population and this calls for population-based study to confirm this speculation. In addition, because the virus is transmitted through the faecal-oral route, transmission of HEV is greatly dependent on the sanitary conditions under which individuals live and work. In Ghana, there are great social differences and sanitary conditions are quite precarious in many areas. Majority of the population live and work in densely populated areas where the sanitary conditions are very deplorable and also where animals, such as, sheep, goats, cows, dogs, rats, and cats share their habitat with humans. No data on work activities of the donors were available during the period of study, but it would be interesting to know whether some of them had occupations related to rearing or working with pigs, sheep, cattle and other animals. In fact, serum anti-HEV antibodies have been found in domestic animals such as rats, sheep, dogs, cats and may serve as reservoirs for the transmission of human hepatitis E (Favorov and Margolis, 1999; Purcell and Emerson, 2001; Thomas *et al.*, 1997).

As mentioned earlier, the main route of transmission of HEV infection is the faecal-oral one, which is also the route of transmission of HAV infection, whose prevalence is associated with quality of individuals' life including housing, drinking water and sewer service (Irshad *et al.*, 1999; Bradley, 1992; Coursaget *et al.*, 1996). There is therefore the possibility of associating the outcome of our findings to HAV infection. Although the two viruses are transmitted by the faecal-oral route, the epidemic models of the two infections are different (Aubry *et al.*, 1997), and moreover several studies (Mast *et*

*al.*, 1997, Aubry *et al.*, 1997; Focaccia *et al.*, 1998 have reported no association between these two infections (HEV and HAV infections). Furthermore, the International Immunodiagnosics ELISA kits we used to assess the seroprevalence of HEV-IgG and HEV-IgM were very sensitive (100% sensitivity) and specific (99.8% specificity) to the investigations under study, and that the test kits were very sensitive, specific, accurate and reliable method of confirming a case of HEV. None of the positive volunteers had a recent history of travel in regions of endemicity and thus it is possible to conclude that HEV was acquired locally.

Growing evidence suggest that the seroprevalence of antibodies to HEV increased significantly with age (Boutrouille *et al.*, 2007; Gotanda *et al.*, 2007; Assarehzadegan *et al.*, 2008; Dalton *et al.*, 2008). Our findings reported herein were not in agreement with the results of similar studies conducted elsewhere. The reason(s) for this disparity cannot be discerned from our study. However, it may likely be due to the small sample size and further studies need to be done to define the increased association of high prevalence of anti-HEV antibodies with age in such population.

Another finding of interest reported herein in our study is that the prevalence rate of HEV-IgM antibodies in all the age groups was almost two times higher compared to the prevalence of anti-HEV IgG. The reason(s) for this disparity cannot be discerned from our study. However, the presence of seropositive IgM anti-HEV usually indicates recent HEV infection or a marker of early seroconversion period (Focaccia *et al.*, 1998) and may signify recent introduction of HEV into the country. It also would have been interesting if alanine aminotransferase (ALT) levels of the seropositive blood donors were determined since elevation ALT levels follows viremia and accompanies or precedes Seroconversion (Gotanda *et al.*, 2007; Chauhan *et al.*, 1993; Sakata *et al.*, 2008). There is therefore the need to investigate other Blood Service Centres in the country to

ascertain whether there had been any earlier infections among recipients of blood and its components or among blood donors. There is also the need for further studies to define the clinical and epidemiological importance and pathogenesis of HEV infection and determine the ALT levels in this population.

The results reported herein are unexpected and thus trigger more questions than answers. It is necessary to answer the question of whether the cases of sero-positive IgM and IgG anti-HEV reported herein represents a local outbreak in Accra or it is an indication and/or a sign of a more widespread problem in the country. The results reported herein are unexpected and thus trigger more questions than answers, such as: 1) Do the presence of circulating antibodies to HEV, IgM and IgG in donor blood provide a sufficient background for a risk of transmission of diseases to recipients, particularly immunosuppressed patients? 2) Do the presence of circulating antibodies to HEV IgM and IgG in donor blood provide a sufficient background for screening of donor blood for HEV infection? 3) Do the cases of seropositive IgM and IgG anti-HEV reported in our study warrant the testing of patients with unexplained hepatitis, whatever their age or travel history for HEV?

## CONCLUSION

Although limited by the small number of volunteer blood donors, the results of our study show a high prevalence of anti-HEV antibodies in our blood donors and that the virus may be circulating among this population. Further studies need to be done to define the clinical and epidemiological importance of HEV infection and to identify risk factors involved in the epidemiology and pathogenesis of this infection in the population.

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