Abstract. The seroprevalence to hepatitis E virus (HEV; IgG and total antibodies) in Nigeria lies between 7.0‑66.75% in different populations. This is due to the fact that only a limited number of studies have been performed on the seroprevalence of (HEV). In the present study, demographics, the associated risk and the behavioral characteristics of blood donors were identified based on direct and indirect questions. These were applied to those who gave their consent to participate in the study. Purposive sampling was employed following the principle of gradual selection and multinomial regression analysis was applied. The seroprevalence of HEV in different centers investigated in the present study indicated the high prevalence of HEV infection among blood donors in Southwest Nigeria. The findings of the present study therefore reveal the risks associated with the presence of HEV in blood donor samples, and the potential for this infection to be transmitted to others via blood transfusion.

Introduction

The most common cause of acute viral hepatitis worldwide (1‑3) is known to be hepatitis E. It is regarded as the fifth known form of viral hepatitis. The mechanisms underlying the replication and pathogenesis of hepatitis E virus (HEV) remain poorly understood despite being a predominant cause of hepatitis (4). Historical records suggest it may not be an ‘old’ disease (5), since it was originally categorized as an emerging disease with a relatively unknown origin. The adaptation and the existence of this empirically transmitted disease were later named hepatitis E.

During the war in Afghanistan in 1983, there was an outbreak of an unusual and unexplained hepatitis virus among Soviet soldiers. Small viral particles were detected in the stool of the affected military soldiers through immune electron microscopy. The viral genome produced infected macaques experimentally obtained from small samples of bile that was cloned (6).

Among the liver diseases, HEV in its original form has a minimum of four different types of genotypes, i.e., 1, 2, 3 and 4. Scientifically, it has been shown that genotypes 1 and 2 exist only in humans, while genotypes 3 and 4 are also found in several animals (including pigs, wild boars, and deer); the infected animals do not exhibit any signs of the disease and can thereby infect humans (7).

The virus enters the human body through the intestine from the shedding of stools of infected persons. The mode of transmission is mainly through the consumption of contaminated drinking water and the fecal‑oral route. Usually, the cycle of the virus develops within 2‑6 weeks. Generally, the signs and symptoms of HEV include jaundice, fever, anorexia, nausea, abdominal pain, vomiting and liver enlargement. Occasionally, acute HEV infection can lead to fulminant hepatitis and acute liver failure, and eventually, death. The presence of specific IgM and IgG antibodies in the blood of patients serves as a means for the diagnosis of HEV infection (8).

Hepatitis E is widespread in the vast majority of individuals, particularly in the developing world. The infection can result in a self‑limited, acute illness; however, the infection can become chronic in rare cases. Chronic infection can occur in pregnant women or in individuals with weak immune systems, including the elderly or those who are ill and primarily, in those who have received solid‑organ transplants. In some cases, infection may be fatal, as reported in a previous study (9), conducted on patients with fulminant hepatic failure.

The incidence of cute HEV infection in humans is estimated to be approximately three million cases per year worldwide, resulting in ~70,000 deaths (10). The majority of these cases are from endemic countries. Recently, an increase in the number of cases was reported in low endemic countries (Afghanistan, India), leading to an outbreak. This was a result of the frequent contamination of food, and poor sanitary conditions that affected the water supply. Developing countries, such as India and Southeast Asia accounted for the high HEV seroprevalence, ranging from 27‑80% (10). HEV, according to previous research, has been found to be a small non‑enveloped virus, 27‑34 nm in diameter, with a single‑stranded RNA genome (11). Of note, some studies in developed countries,
such as the United Kingdom and USA have exhibited an unexpectedly high seroprevalence of HEV (21-25%). The possible reasons for this may be subclinical infection, exposure to animals, cross-reactivity with other agents or false-positive test results. Immunodeficient patients and pregnant women have the highest risk mortality (1-4%) (10).

The limited number of studies performed on the seroprevalence of HEV in Nigeria detected a seroprevalence (IgG and total antibodies) between 7.0 and 66.75%, respectively. Among the healthcare workers in Nigeria, the prevalence of hepatitis B virus (HBV)/HEV co-infection reported was a co-infection rate of 27.3%. In addition, an anti-HEV IgM antibody seroprevalence between 0.4-9.0% was reported. In Nigeria, two different outbreaks of HEV have been documented; the first outbreak occurred in Port Harcourt (southern Nigeria) (12), involving 10 individuals and occurred between November, 1997 and June, 1998; and the second outbreak occurred in 2017 in Bornu State (northeast Nigeria). It involved ~1,815 individuals, including pregnant women with a case fatality rate of 0.6%. Only HEV-2 was reported during the first break, while genotypes 1 and 2 were reported in the second outbreak (13). Hence, the populations may be at risk as there is a dearth of information on HEV.

HEV is an emerging infectious threat to blood safety (14,15); a previous study reported a case of transmission-transmitted hepatitis E which was caused by the blood from a donor infected with hepatitis E virus via the zoonotic food-borne route (14). Cases of transmissibility of HEV via blood transfusion in patients are well documented in countries, such as Japan, the United Kingdom, Saudi Arabia and France (14-16).

At present, four genotypes of HEV has been identified with different causatives. The ones associated with waterborne and faecal-oral transmission (genotypes 1 and 2) are human viruses causing epidemic hepatitis. The swine viruses that are common in domestic and wild pigs are genotypes 3 and 4. There is currently insufficient evidence to advocate universal screening for this virus and the issue of the extent of transmission and its clinical relevance are still under debate (10). The aim of the present study was to investigate the prevalence of hepatitis E through blood examinations in the hospitals in order to improve public awareness.

**Subjects and methods**

**Study population.** The data for the study were obtained from three different locations: Ladoke Akintola University of Technology (LAUTECH) Teaching Hospital, Ogbomoso; UniOsun (University of Osun State) Teaching Hospital, Osogbo; and Bowen Teaching Hospital, Ogbomoso (Nigeria). Consenting participants for the study were recruited at the blood donation centers of the hospitals between January, 2020 and March, 2020. Blood donors were identified and well-structured questionnaires were provided to them to obtain demographic characteristics, such as sex, occupation, age, marital status and educational level. Possible associated risk factors, such as a previous history of hepatitis, interaction with animals, sources of drinking water, and their behavioral characteristics were also recorded. The questionnaires also included other factors, such as smoking habits, the consumption of alcohol, previous surgeries, etc. The consenting individuals completed the administered questionnaire prior to sample collection. The present study was approved by the Health Research Ethics Committee of Redeemer’s University, Ede, Nigeria.

A total of 400 samples were required; however, due to the COVID-19 pandemic, only 280 participants were recruited in the study. The breakdown of the expected sample and the actual samples obtained are presented in Table I.

Purposive sampling was employed following the principle of gradual selection (17). Information was theoretically selected and categorized with relevant criteria such as age, sex, ethnicity, locality, HIV status, history of substance of abuse, a history of blood transfusion, source of drinking water (tap, well, bottled and sachet water), alcohol consumption, contact with animals and sexual status. This was to allow maximum variation.

All the sera were analyzed in duplicate for HEV antigen (Ag), using an ELISA kit (MID-0023; Melsin Medical Co., Ltd.). The kit exhibits a high sensitivity and specificity. The assay employs a double-antibody sandwich technique to analyze the presence of HEV Ag in human serum. Provided with the ELISA kit were the microelisa strip plate, negative control, positive control, sample diluent, HRP-conjugate reagent, 20X wash solution, chromogen solution A, chromogen solution B, stop solution and closure plate membrane. Other materials required for the analysis included a standard microplate reader (Melsin Medical Co., Ltd.; 450 nm), precision pipettes, disposable pipette tips and an incubator set at 37°C.

**Inclusion criteria.** The following factors were considered for the prospective blood donors to be included in the study prior to collecting the samples: i) willing blood donors; ii) irrespective of sex; iii) were evidently healthy/fit [with a negative HIV, HBV and hepatitis C virus (HCV) infection status]; and iv) donors with a moderate or high level of PCV and who were not taking any hard drugs.

**Exclusion criteria.** The following were the factors that disqualified some subjects from being considered for participation in the study: i) Refusal to participate; ii) individuals with a low PCV; iii) individuals with a HIV, HBV and HCV positive status; and iv) individuals with a history of drug immunosuppressive therapy or critical illness, etc.

**Test principle (double-antibody sandwich ELISA).** The microtiter plates provided with the ELISA kit were pre-coated with HEV antibody. The samples, positive control, negative control and horseradish peroxidase (HRP-conjugate) are then added to the wells. Following incubation (as per the instructions provided with the ELISA kit) and washing to remove the uncombined enzyme, chromogen solutions A and B were added. As a result of this, the colour of the solution then changed to blue. Once the stop solution (this terminates the enzyme substrate reaction) was added and took effect, the colour of the solution finally changed to yellow. The colour change was measured spectrophotometrically at the wavelength of 450 nm. The presence or absence of HEV Ag in the samples was then determined by comparing the optical density (OD) of the samples to the cut off.
Sample collection. A total of 280 blood samples (5 ml) were collected from multiple individuals at LAUTECH, UNIOSun and Bowen Teaching Hospitals into EDTA anti-coagulated bottles. The plasma of blood was subsequently harvested into a sterile plain container, and allowed to clot and retract. In order to avoid the hemolysis of the red blood cells, the plasma was separated from the clot as early as possible by centrifugation at 100.62 x g for 10 min. The serum samples were then transferred safely into 2 ml cryovial and stored at -20°C until used in the analysis.

Statistical analysis. The frequencies were standardized, particularly when the number of cases was large by translating them into relative frequencies, such as proportions or percentages. Percentages were used since they are also closely represent raw data. The Chi-squared was used to determine whether the observed results were in line with the expected value. SPSS software (version 27; SPSS, Inc.) was used for statistical analysis. A value of P<0.05 was considered to indicate a statistically significant difference.

Multinomial regression analysis was also performed as follows:
Let Y be positive hepatitis, and X1 represents the sources of drinking water; X2 represents contact with animals; X3 represents sex; and X4 represents alcohol consumption. The following equation was used:

$$\hat{P} = \frac{\exp(a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_4X_4)}{1 + \exp(a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_4X_4)}$$

where $\hat{P}$ is the expected probability that the outcome is present; X1 through X4 are distinct independent variables and $a_0$ through $a_4$ are the regression coefficients.

The aforementioned equation can be further expressed as:

$$\ln\left(\frac{\hat{P}}{1-\hat{P}}\right) = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_4X_4$$

Results and Discussion

The prevalence of HEV Ag was determined from the proportion of the positive individuals in the overall population studied, and expressed as percentages. They were structured and analyzed in terms of frequency and the findings of the study are presented in the tables.

Interpretation of the results. The validity of the study was determined by observing whether the average of the positive control well was 1.00; and the average of the negative control well was 0.15. The cut-off value was determined by the addition of the absorbance value of the negative control wells with 0.15.

The presence or absence of HEV Ag was determined by comparing the absorbance of the sample to the cut-off value, which is the average absorbance of the negative control plus 0.15. The cut-off value however, varied across the microtiter plates used. The measure of colour intensity was proportional to the amount of antigen captured in the wells, and to the amount of antigen in the sample respectively. The samples negative for HEV Ag were observed as colorless in the wells.

The study confirmed the presence of HEV infection among blood donors with a seroprevalence of 15.4% in UniOsun, 20% at LAUTECH and 13.1% at Bowen Teaching Hospital (no. of infected participants out of the total number of actual samples; Table I). The prevalence was higher but comparable to the seroprevalence of 6.6% observed in a recent pilot study conducted among blood donors at the blood bank of Lagos University Teaching Hospital in Lagos State, Nigeria (18). The reason for this contrast in results may be due to the different sanitary conditions amongst the participants in the different locations. For instance, the blood donors in the Lagos pilot study probably had access to better sources of drinking water as compared to those in the present study. Another comparison may be due to similar conditions between the study sites, such as similar socio-economic status and the same geographical region as the study area is in the southwest of Nigeria. Other studies from Nigeria demonstrated a higher anti-HEV seroprevalence than observed in the present study. Other researchers have reported an anti-HEV seroprevalence of 44% among health workers and 93% among non-health workers in Ibadan, Oyo State, southwest Nigeria (19). In another study (20), a seroprevalence of 47.9% was recorded in apparently healthy individuals in Plateau State, north-central Nigeria.

The smaller sample size may stand as the major difference between these previous studies and the present study. Another major difference may be due to variations in the target population. In aforementioned study in Plateau State, north-central Nigeria study above (in Jos, the capital of Plateau State) (20) the target population included urban and rural participants, whereas the present study was restricted to only blood donors visiting the selected teaching hospitals. The resulting variations in the regional seroprevalence may serve as another reason for the disparity even within the same country, as supported by other studies performed in Brazil and China (21,22). Factors that have been identified in previous studies that may be responsible for these regional differences include, but are not limited to, the differences in living and sanitary conditions of the study populations. Hence, the high seroprevalence observed in the present study may be a reflection of a population residing in areas with poor and compromised sanitary conditions. In addition, there were differences in the HEV antibody detection assays. One of the previous studies (18) used an MP Diagnostics HEV-ELISA kit, while, the Melsin Diagnostics HEV-ELISA kit was used in the present study.

In addition, the seroprevalence recorded in the present study was higher when compared to 4.78% obtained in another study among 460 blood donors in Western India (23). This difference may be due to the various sanitary conditions of the participants in the different locations or the population sample size.

Demographic characteristics of the respondents. A total of 280 patients with HEV were identified from the laboratory analysis. These patients were subjected to further analysis. The distribution of the variables examined (age, sex, marital status, educational level and occupational status) in these patients are presented in the tables.

The age-specific seroprevalence profiles among the developing countries reveal that HEV infection is usually limited to the adult population between the ages of 15 and 35 years (24). The present study revealed a distribution of the seroprevalence of HEV across ages (Table II) i.e., 20-25, 26-30, 31-35, 36-40, 41-45, 46-50, with the highest prevalence occurring between...
the ages of 26-30 years. This finding is somewhat consistent with the previous observation that the prevalence of anti-HEV antibodies is highest in ages 20-40 years (25).

In accordance with previous studies (20,24,26), the present study demonstrated that males (Table III) accounted for a higher rate of anti-HEV seropositivity than females in the entire three centers examined. This is in disagreement with other studies (20,27,28) however, where a higher prevalence was recorded among females as compared with males. The low number of females observed in the present study as compared to the males may be the reason for this disparity between the studies. However, the most important reason for this may be due to the fact that the majority of blood donors in Nigeria are males, while other researchers have also documented that HEV infections are predominantly reported in males (29).

As regards marital status (Table IV), a higher prevalence was recorded among the subjects who were married as compared to those who were single across the three centers studied. This finding is in agreement with the reports of other studies (18,30), where a higher prevalence was recorded among married subjects compared with those who were single. This result may be attributed to the increased sexual activity among married participants as compared to those who were single, which may lead to sexual transmission.

In contrast to previous studies (31-33), where participants with a low level of education had an increased risk of HEV seropositivity, the present study demonstrated a higher prevalence (LAUTECH, 67.7%; Bowen, 72.6%; and UniOsun, 70.3%) among the blood donors that were graduates of the tertiary institution; however, no statistically significant differences with HEV positivity were found (Table V).
Occupation-wise, there were 49.5% civil servants in LAUTECH, 57.1% in Bowen and 48.4% in UniOsun. This was followed by unemployment with the highest rate from UniOsun with 31.9%, Bowen with 22.6% and LAUTECH with 18.1% of respondents (participants), as demonstrated in Table VI. There was no significant association (P>0.05) between seropositivity and the participants' occupation.

Logistic multiple regression model analysis. It has been stated that the major route of HEV transmission is via the faucal-oral route (34). In the present study, four different variables/observations were considered. The results of the association between the centers studied and the variables under consideration are presented in Table VII. As demonstrated, the type of drinking water exhibited a highly significant association compared with the other variables (LAUTECH, P=0.008; Bowen, P=0.007; and UniOsun, P=0.004). A significant association between the source of water with HEV positivity was observed, from the positive participants had tap/well water as their source of drinking water (P<0.05). The result was therefore consistent with the findings of other research, which reported contaminated water as the main source of HEV infection (34). This may be attributed to the poor source of the water supply around the community and poor sanitary conditions. Different types of water sources include tap water, well water, bottled water and sachet water, as these are available in the areas studied.
In line with the findings of others (20), as regards the association between alcohol consumption and the presence of anti-HEV antibodies, a statistically significant association was found between alcohol consumption and HEV seropositivity (P<0.05). This observation is in agreement with the findings of previous research associating alcohol consumption with an enhancement of positivity for HEV (31). Alcohol consumption has been identified as a strong risk factor for HEV genotype 3 infections. The reason for this observation cannot be fully explained, although it contributes to the clinical expression of the infection and the severity of hepatitis. Furthermore, a previous study that reported an HEV-3 outbreak on a cruise ship, identified alcohol consumption as a significant risk factor, indicating that excess alcohol consumption may affect hepatic function and predisposes one to symptomatic hepatitis E infection (35). The consumption of alcohol in Nigeria is usually an outdoor activity that is often times accompanied by the consumption of street foods that may have been contaminated.

In the present study variable X3 (multiple sex partners) did not reveal statistically significant differences in all three study centers. This indicates that the rate of transmission through the channel was very low. This finding is in accordance with that of a previous study (36), which observed that the rate of transmission of hepatitis E through multiple sexes partners was very low or does not exist (Table VII).

As regards contact with animals, the variable did not record any statistically significances for all the three areas (P>0.05). This does not support the analysis conducted with HEV that zoonotic transmission may be a possible route of transmission.

In conclusion, the present study demonstrates that among the blood donors in South West Nigeria, HEV seroprevalence in the different centers studied indicated a high prevalence of HEV infection. Thus, this indicates the existence of the risk of HEV transmission via blood transfusions, which merits further investigations.

The present study revealed the risks behind the presence and the potential for HEV contamination via blood transfusions. The seroprevalence was high for all three categories and was statistically significant (Table VII) considering the sample size of the study. Alcohol consumption and the type of drinking water (Table VII) were found to be possible risk factors of HEV infection, as they exhibited strong statistical significance with HEV seropositivity. No statistical significance was found between HEV positivity and age, sex, marital status and the educational status among the study participants.

Acknowledgements

The authors acknowledge the laboratory staff of LAUTECH, BOWEN and UniOsun teaching hospitals for their help and support during the field work, and in administering the questionnaires.

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

IB led the laboratory and experimental team. FA led the team that administered the questionnaire. SAA was the chief analyst and designer of the questionnaire. The research work was collaborative in nature. IB and SAA confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

All participants involved in the study provided their written informed consent before completing the questionnaire and prior to sample collection. The present study was approved by the Health Research Ethics Committee of Redeemer's University, Ede, Nigeria.

Patient consent for publication

Not applicable.
Competing interests

The authors declare that they have no competing interests.

References