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# HEPATITIS E VIRUS IMMUNOGLOBULIN M (IgM) AND ASSOCIATED RISK FACTORS IN SOUTHWEST, NIGERIA

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## ABSTRACT

Hepatitis E is one of the most frequent causes of acute hepatitis worldwide, with an estimated 20 million infections and 70,000 deaths attributed to hepatitis E virus (HEV) genotypes 1 and 2 every year. This study was designed to determine the prevalence of HEV immunoglobulin M (IgM) in southwest (SW) Nigeria. Venous blood samples totalling 359 were collected from pregnant women on routine check, apparently healthy prospective blood donors and sick individuals presenting with fever and abdominal disturbance from health facilities in Ekiti, Lagos, Osun and Oyo states, of Nigeria. A structured questionnaire form was administered to gather socio-demographic data, health and travel history from each consenting participant. The screening for HEV IgM was done using HEV IgM ELISA kit. Statistical analyses, including descriptive analysis, correlations and binary logistic regression were carried out using SPSS version 21. In all, 131 samples (36.5%) tested positive for HEV IgM. Osun state had the highest occurrence of HEV IgM (n = 49; 13.6%,) while Ekiti had the least (n= 22; 6.1%). Apparently healthy participants with detectable HEV IgM were 52 (14.5%) while pregnant women were 51 (14.2%). Risk factors implicated in this study were flooding and injection drug use. The overall HEV prevalence reported in this study was higher than previous reports in Nigeria . Higher HEV prevalence in this study could be due to a larger coverage area in the country as well as detection of ongoing infection. The detection of HEV IgM in pregnant women and apparently healthy prospective blood donors indicated ongoing infection with risk of spread to susceptibles since HEV is neither routinely screened for among pregnant women nor among prospective blood donors.

**Keywords**: Hepatitis E, IgM, Pregnancy, Apparently healthy, ELISA Depth, Geothermal Energy.

## INTRODUCTION

Hepatitis E is the most frequent enterically transmitted, self-limiting, acute, viral hepatitis and currently considered an important public health problem worldwide (Pérez-Gracia *et al*., 2015; Khuroo and Khuroo, 2016). The disease is ecologically dependent, causes large-scale water-borne epidemics of viral hepatitis and is the most common cause of acute sporadic hepatitis and fulminant hepatic failure in resource-poor countries (Khuroo, 1991; Khuroo and Khuroo, 2015). The disease has unique and yet unexplained epidemiological characteristics, including repeated waves of large-scale epidemics, occurrence of disease in adult population and high incidence and severity of disease in pregnant women (Khuroo, 2011). The infection is prevalent in a wide range of animal species and human zoonotic hepatitis E is encountered in many developing and industrialized countries (Pavio *et al.,* 2010; Thiry *et al.,* 2015). Khuroo and Khuroo (2016) reported that one-third of the world population has been exposed to hepatitis E virus (HEV) as a result of varying unhygienic practices and, in some cases, relationship with the animal reservoirs. It has been estimated that two billion people, representing one third of the world's population, live in endemic areas for HEV and, therefore, are at a greater risk of infection than others in non-endemic areas (Holla *et al*., 2013).

In communities with poor sanitation conditions, especially in developing countries, transmission is primarily via the faecal-oral route through contaminated food or water, whereas in developed countries, sporadic and autochthonous cases of hepatitis E also occur in many parts of Europe, Asia, and North America (Dalton *et al.,* 2008; Meng, 2011). Transmission can be water- or food-borne or zoonotic. Other routes of transmission said to account for a smaller number of clinical cases include the ingestion of undercooked meat

or meat products derived from infected animals, transfusion of infected blood to uninfected patients and vertical transmission. The ingestion of raw or uncooked shellfish may be the source of sporadic cases in some endemic regions (WHO, 2010).

Hepatitis E is usually self-limiting but may develop into fulminant hepatitis with a case-fatality rate (CFR) between 1 and 2% in the general population (Skidmore, 2002), which can rise to over 40% in pregnant women, especially during the third trimester of pregnancy (Tsega *et al*., 1993). Maternal mortality due to HEV genotype 1 (HEV1) infection in pregnant women in developing countries has been found to be as high as 30%, with most deaths occurring in the third trimester even though the reason for this is yet unclear (Lhomme *et al*., 2016). Apart from maternal death, stillbirths are common, as well as neonatal morbidity of those that survive after getting infected vertically (Khuroo *et al*., 1995; Khatun *et al.,* 2012; Labrique *et al.,* 2012). While clinical symptoms include fatigue, nausea, and jaundice which begin shortly after the increase of serum alanine aminotransferase (ALT) levels, clinical outcomes of hepatitis E has been known to vary from high endemic areas to low endemic regions. A majority of HEV infection cases either have no clinical consequences or minor illness with no liver damage (Aggarwal, 2013). HEV RNA disappears from the serum with recovery in about three weeks after the onset of symptoms, whereas the virus usually remains detectable longer in stools (Hoofnagle, 2012). Acute hepatitis E is usually self-limiting resolving in 6-7 weeks while the chronic condition ensues in both immunocompromised and immunocompetent patients most of them being asymptomatic and with a rapid progression to liver fibrosis especially among transplant recipients (González *et al*., 2011; de Niet *et al*., 2012; Kamar *et al*., 2012).

There is no known specific treatment capable of altering the course of acute hepatitis E because it is usually self-limiting. A recombinant subunit vaccine, Hecolin®, was registered and licensed in China in December 2011 for use in people aged >16 years. The major way to avoid contracting hepatitis E is to avoid drinking unsanitary water and to maintain a good personal sanitation. Also, travelers to HEV-endemic regions such as Asia, Africa, the Middle East, and Central America should avoid water of unknown purity, food from street vendors, raw or undercooked seafood, meat or pork products, and raw vegetables. Travelers to Europe, on the other hand, should also avoid uncooked and undercooked pork/boar sausage or other wild animal meats that have not been properly cooked. This study was designed to determine the prevalence of HEV IgM antibodies and associated risk factors among different categories of study participants in Ekiti, Lagos, Osun and Oyo states of Nigeria.

## MATERIALS AND METHODS

### Study Locations

The study locations included, at least one health facility of either primary or secondary level in each of the four states involved in this study, namely: Ekiti, Lagos, Osun and Oyo states in southwest Nigeria. These southwest states are homes to the Yoruba race although people of other tribes also live with them. The states have many tertiary institutions and are densely populated, though with a high literacy level. The region belongs to the tropical rainforest zone, rich in plant populations with diverse kinds of forest animals.

### Enrolment of Study Participants

Participants for the study included randomly selected apparently healthy prospective blood donors, pregnant women on routine medical check and sick individuals (presenting with symptoms including fatigue, fever, abdominal disturbance, nausea, and vomiting) all with no known ongoing treatment for hepatitis. Ethical approval (SERC-2017-001 OAUS) and permission to conduct the research were obtained from the health institutions as well as written and/or verbal consents of the participants as applicable.

### Sample Collection

Sample collection was done between October 2016 and February 2018. Three (3) milliliters of venous blood was collected from each consenting subject into well-labelled sterile plain bottles with the help of the medical personnel in the various health facilities. The blood samples were separated into serum and packed cells after spinning at 3,000

revolutions per minute (RPM) for 10 minutes. The serum samples were then collected and stored in appropriately labelled cryovials, transported to the laboratory on ice pack for storage at -20 °C until analysed. Sample size was determined for cross-sectional surveys/studies using Noordhuizen *et al.,* (2001) formular:

$$n = \frac{z^2pq}{d^2}$$

where
n = desired sample size
z = the standard normal deviate at 95% confidence interval (=1.96).
p = the proportion in the target population estimated to have had hepatitis (1.3).
q = 1-p,
d = level of precision (set at 0.05).
A total of 359 venous blood samples were used.

**Data Collection**
A structured questionnaire was administered to each subject to gather information, among others, on socio-demography, knowledge about hepatitis, supposed risk factors, symptoms, travel and medical histories.

**Laboratory Procedure**
*HEV IgM Serology*
The serum samples were analysed to screen for hepatitis E virus immunoglobulin (Ig) M.
The serum samples screened were allowed to attain room temperature. The wells of the ELISA plate were labelled to correspond with blank, cut-off, positive and negative controls according to the kit manufacturer's instructions. The ELISA kit (HEV-IgM kit, Dia.Pro Diagnostic Bioprobes, Italy) was also allowed to attain room temperature before use. According to manufacturers instructions, each sample was separately diluted 1:101 by adding 10 µl of the sample to 1000 µl of the sample diluents (provided in the ELISA kit). For the assay, 100 µl of each diluted sample was used following manufacturer's instructions. Summarily, the procedure included addition of the diluted samples to neutralizing reagent in the prelabelled microwells of the micro-titre plate followed by first incubation, then first washing after which enzyme conjugate was added and then, second incubation, followed by second washing, then addition of chromogen/substrate and a final incubation in the dark after which the reaction was stopped with a stop solution. On addition of the stop solution, the positive samples which were previously blue, turned yellow while the negative ones remained colourless.

**Data Analysis**
Data obtained from the administered questionnaires and the laboratory analysis were analysed using SPSS version 21. Descriptive, correlation and some regression analyses of the data were carried out to be able to draw some inferences and likely conclusions from the study.

**RESULTS**
The HEV IgM screening showed that 131 (36.5%) of the 359 study participants had detectable HEV IgM antibodies while 228 (63.5%) had no detectable HEV IgM. The state-by-state analysis of the HEV IgM distribution showed that Osun had the highest occurrence rate of 13.6% (n= 49/96) out of all the samples screened while Ekiti had the least with 6.1% (n= 22/92) occurrence rate (Figure 1). There is a statistically significant relationship between states of sample collection and HEV IgM positivity with a p value of 0.001 at 95% Confidence Interval (CI) using binary logistic regression.

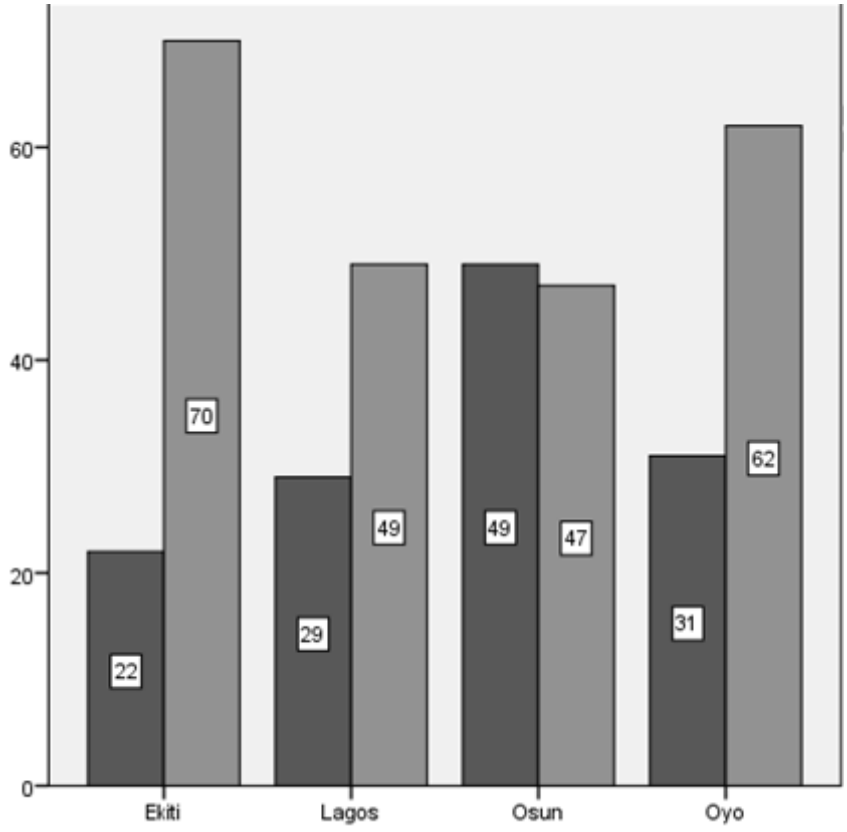


Figure 1: Occurrence of HEV IgM in Ekiti, Lagos, Osun and Oyo States

A general comparison of the HEV IgM positivity among the study participants showed that 14.5% (n=52/165) and 7.8% (n=28 out of 79) cases were observed in apparently healthy prospective blood donors and sick individuals, respectively. Further details are shown in figure 2.

Out of the 165 apparently healthy individuals screened from the four states, 13.3% (n=22), 9.7% (n=16) and 0.6% (n=1) were respectively from Osun, Ekiti and Lagos states. Details of the distribution of the HEV IgM among other categories in the states are shown in table 1.

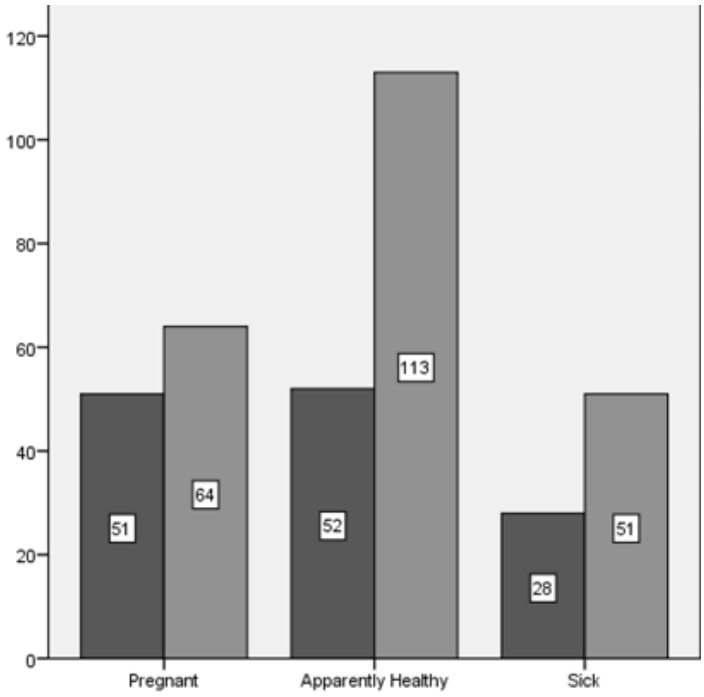


Figure 2: Distribution of Anti-HEV IgM Antibodies Among the Pregnant Women, Apparently Healthy and Sick Individuals in Ekiti, Lagos, Osun and Oyo States, Nigeria

Table 1: Distribution of HEV IgM Positive Individuals Among the Various Categories of Participants Studied in the Four States

| Categories of Participants | | | States of Sample Collection | | | | Total |
|---|---|---|---|---|---|---|---|
| | | | Ekiti | Lagos | Osun | Oyo | |
| Pregnant | HEV IgM | Positive | 5 | 27 | 9 | 10 | 51 |
| | Result | Negative | 5 | 45 | 8 | 6 | 64 |
| | Total | | 10 | 72 | 17 | 16 | 115 |
| Apparently | HEV IgM | Positive | 16 | 1 | 22 | 13 | 52 |
| Healthy | Result | Negative | 57 | 1 | 20 | 35 | 113 |
| | Total | | 73 | 2 | 42 | 48 | 165 |
| Sick | HEV IgM | Positive | 1 | 1 | 18 | 8 | 28 |
| | Result | Negative | 8 | 3 | 19 | 21 | 51 |
| | Total | | 9 | 4 | 37 | 29 | 79 |
| Total | HEV IgM | Positive | 22 | 29 | 49 | 31 | 131 |
| | Result | Negative | 70 | 49 | 47 | 62 | 228 |
| | **Total** | | **92** | **78** | **96** | **93** | **359** |

The incidence of HEV IgM according to gender was observed to be 28.1% (n=101/252) females and 8.4% (n=30/107) males. A general consideration of the distribution of HEV IgM based on gender in this study showed that 40.1% (n=101/252) of the females and 28% (n=30/107) of the males were HEV IgM positive. Gender as a factor was observed to have a significant Pearson Chi-Square p value of 0.03. Osun state recorded the highest HEV IgM occurrence in both genders with 15% (n= 16/107) of the males and 13.1% (n=33/252) of the females screened. There were generally fewer HEV IgM positive males than females observed across the four studied states as shown in figure 3.

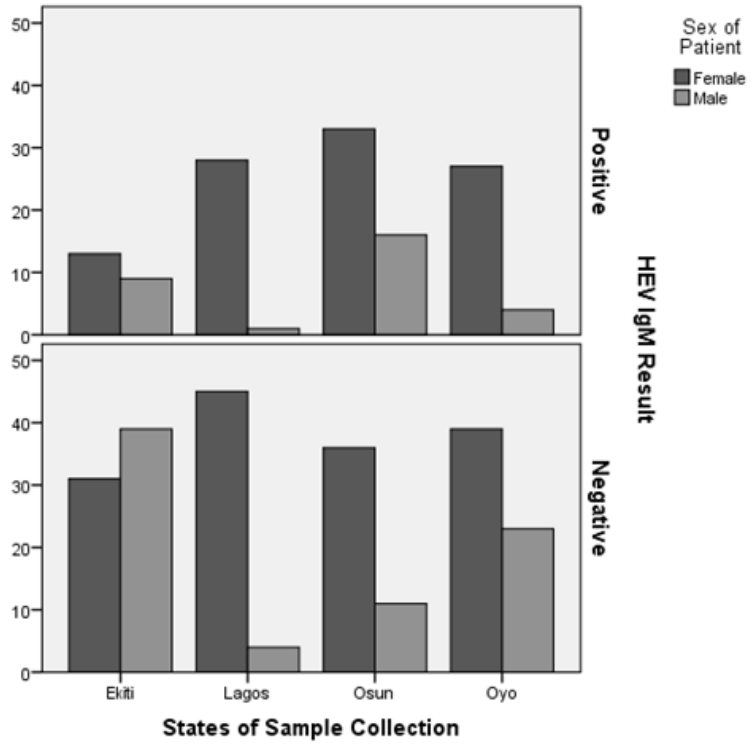


Figure 3: General Distribution of HEV IgM with Gender in Ekiti, Lagos, Osun, and Oyo States

HEV IgM Positive

HEV IgM Negative

This study showed that the most affected age group in the occurrence of HEV IgM are the 41 – 50 years (n=19/35) making 54.3% with 12 (63.2%) out of the 19 from Osun state while it is followed by 31 – 40 years (n=44/106) making 41.5% with 17 (38.6%) out of the 44 coming from Osun state thus having the highest in both age groups. The occurrence of HEV IgM among the age groups in the states are as shown in table 2. Injecting drug use and flooding were observed to have significant association with HEV positivity. Further details are as shown in table 3.

Table 2: Distribution of HEV IgM According to Age Range of Study Participants

| Age Range | | | States of Sample Collection | | | | Total |
|---|---|---|---|---|---|---|---|
| | | | Ekiti | Lagos | Osun | Oyo | |
| 0-10 | HEV IgM | Positive | 0 | 0 | 0 | 0 | 0 |
| | Result | Negative | 0 | 1 | 2 | 0 | 3 |
| | Total | | 0 | 1 | 2 | 0 | 3 |
| 11-20 | HEV IgM | Positive | 1 | 1 | 2 | 5 | 9 |
| | Result | Negative | 3 | 4 | 3 | 18 | 28 |
| | Total | | 4 | 5 | 5 | 23 | 37 |
| 21-30 | HEV IgM | Positive | 13 | 12 | 14 | 11 | 50 |
| | Result | Negative | 49 | 20 | 20 | 9 | 98 |
| | Total | | 62 | 32 | 34 | 20 | 148 |
| 31-40 | HEV IgM | Positive | 5 | 16 | 17 | 6 | 44 |
| | Result | Negative | 11 | 23 | 12 | 16 | 62 |
| | Total | | 16 | 39 | 29 | 22 | 106 |
| 41-50 | HEV IgM | Positive | 3 | 0 | 12 | 4 | 19 |
| | Result | Negative | 4 | 1 | 3 | 8 | 16 |
| | Total | | 7 | 1 | 15 | 12 | 35 |
| 51-60 | HEV IgM | Positive | 0 | 0 | 2 | 1 | 3 |
| | Result | Negative | 2 | 0 | 2 | 8 | 12 |
| | Total | | 2 | 0 | 4 | 9 | 15 |
| >60 | HEV IgM | Positive | 0 | 0 | 2 | 4 | 6 |
| | Result | Negative | 1 | 0 | 5 | 3 | 9 |
| | Total | | 1 | 0 | 7 | 7 | 15 |
| Total | HEV IgM | Positive | 22 | 29 | 49 | 31 | 131 |
| | Result | Negative | 70 | 49 | 47 | 62 | 228 |
| | **Total** | | **92** | **78** | **96** | **93** | **359** |

Table 3: Some of the Risk Factors Associated with HEV IgM Positivity in Southwest, Nigeria

| RISK FACTORS | | HEV IGM | | P VALUE |
|---|---|---|---|---|
| | | Positive (%) | Negative (%) | |
| Had blood transfusion* | No | 122 (37.2) | 206 (62.8) | 0.423 |
| | Yes | 5 (31.3) | 11 (68.7) | |
| Organ transplant* | No | 126 (37.4) | 211 (62.6) | 0.200 |
| | Yes | 1 (14.3) | 6 (85.7) | |
| Medical history of hepatitis* | No | 123 (36.6) | 213 (63.4) | 0.233 |
| | Yes | 4 (57.1) | 3 (42.9) | |
| Family diagnosed of hepatitis* | No | 116 (36.5) | 202 (63.5) | 0.175 |
| | Yes | 12 (48) | 13 (52) | |
| Injecting drug use* | No | 122 (36) | 217 (64) | **0.012**** |
| | Yes | 6 (85.7) | 1 (14.3) | |
| Eat game meat* | No | 101 (36.2) | 178 (63.8) | 0.266 |
| | Yes | 28 (41.2) | 40 (58.8) | |
| Had flooding* | No | 91 (38.4) | 146 (61.6) | **0.023**** |
| | Yes | 14 (23.7) | 45 (76.3) | |
| Drinking water source* | Tap | 77 (33) | 156 (67) | 0.335 |
| | Stream | 9 (47.4) | 10 (52.6) | |
| | Bore hole | 6 (40) | 9 (60) | |
| | Well | 22 (42.3) | 30 (57.7) | |
| | Sachet | 8 (53.3) | 7 (46.7) | |
| | Others | 6 (50) | 6 (50) | |
| Eating out* | Always | 36 (40.4) | 53 (59.6) | 0.123 |
| | Sometimes | 65 (32.8) | 133 (67.2) | |
| | **NEVER** | **27** | **31** | |

** indicate statisticaly significant values
*Responses to questions were less than expected 359

## DISCUSSION

We report a high HEV prevalence of 36.5% in south west Nigeria. To the best of our knowledge, this study is the first reported HEV single study covering four (4) states in Nigeria, hence the possible reason for the high prevalence, since HEV occurrence was found to be associated with location in the study. Some of the previously reported hepatitis E prevalence in Nigeria included 0.4% in pregnant women among Anambra and Oyo community dwellers in two different geographical regions of Nigeria, although there was 0% prevalence among other participants in the same study (Ifeorah *et al*., 2017). In addition, 0.9% was reported among different populations including apparently healthy

individuals in Plateau state (Junaid *et al*., 2014) and 9% was reported by Fowotade *et al*. (2018) among restaurant food handlers in Ibadan, Oyo State.

In a pilot study on the seroprevalence of anti-HEV among blood donors in Lagos, Nigeria, John-Olabode *et al*. (2017) reported an overall HEV seroprevalence of 6.6%, HEV IgG prevalence of 5.3% and HEV IgM of 1.3% among 151 blood donors. Also, Oladipo *et al*. (2017) reported HEV antibody prevalence of 2.7% (5/186) among apparently healthy individuals in Ogbomoso, Oyo state. In a much earlier study, Adesina *et al*. (2009) reported HEV IgM prevalence of 13.4% (N = 186) among sick and healthy individuals in Ekiti state. This study is thus positing a possible increase in hepatitis E prevalence in SW Nigeria in recent times, however, a single study may not be sufficient to conclude on this, hence the need for further studies spanning some years. From this study, state based HEV occurence rate showed the highest prevalence of 13.6% in Osun state compared to a recent study which reported a prevalence of 3.8% (Osundare *et al.,* 2020). On the other hand, Ekiti State showed the lowest occurrence of 6.13% compared to 13.4% (25/186) reported by Adesina *et al*. (2009).

Although, paucity of data exist on the occurrence and the distribution of HEV antibodies in Oyo state, it can be observed that there is an increase in the prevalence in relation to some of the limited reports available and the observed occurence of 8.64% in this study. Oluremi *et al*. (2020) reported an occurrence of 0.2% (2/904) in a recent report. Increase in HEV IgM prevalence as observed in the states with previous HEV published data shows the need for putting a robust HEV surveillance system in place, to prevent hepatitis E outbreak in Nigeria. The increase could have been probably due to limited hepatitis E awareness, diagnosis and prevention compared to hepatitis B and C.

Fewer (7.8%) sick individuals were observed to have hepatitis E compared to apparently healthy (14.5%) and pregnant women (14.2%) in this study. This is different from what Adesina *et al*. (2009) reported in Ekiti state where 20 of the 25 HEV IgM positive individuals were sick of fever and abdominal disturbance while the remaining 5 were apparently healthy individuals on routine medical checkup. The higher HEV prevalence reported among the apparently healthy and pregnant women than the sick individuals is an issue for consideration in the epidemiology of hepatitis E in Nigeria. It has been said that hepatitis E is often asymptomatic and self limiting but the reason for this has not been fully deciphered. Regular molecular studies of HEV is important so as to understand the likelihood of and how to handle the emergence of resistance HEV strains due to natural pressure on the virus (being an RNA virus). This will hopefully make clinical diagnosis less difficult and enhance vaccine development/ effectiveness.

A higher fatality of 42.1% was reported in Ethiopia (Tsega *et al*., 1993) while it was 12.5% during an outbreak in Kenya (Ahmed *et al*., 2013) and10 % or more in south Asia (Labrique *et al.,* 2012; Gurley *et al.,* 2012).

Flooding and injecting drug use showed significant association with HEV positivity and hence could be referred to as likely risk factors for the spread of HEV in this study. Injecting drug use is an uncommon route of HEV transmission in developing countries like Nigeria as this group of people have not been studied for HEV transmission. The significant relationship between injecting drug use and HEV IgM obtained in this study is suggesting that it is also a potential route of transmission of hepatitis E in developing countries because it was only reported among injecting drug users of ages 18 – 40 years in California, USA (Mahajan *et al.,* 2013).

Different types of occupation were considered to see their effects on the transmission of HEV. Not much has been known about the role of occupation in HEV transmission but a significant association between occupational exposure to swine and HEV IgG seroprevalence was reported in some developed countries (De Schryver *et al*., 2015). Due to the zoonotic form of hepatitis E, most occupations that involve animals that have been implicated in HEV transmission are potential risk factors if necessary precautionary measures are not taken. However, those that are self employed, students, traders and civil servants

were found to have HEV IgM prevalence ranging from 6.4% to 8.1% in this study. Furthermore, Junaid *et al.* (2014) reported rural dwelling, attending to animals, blood transfusion and waste disposal as associated risk factors in the seroprevalence of HEV IgM in Plateau state, Nigeria.

In conclusion, our study being the first single study covering four states in south western Nigeria shows that HEV occurrence was associated with location, hence a high HEV IgM prevalence in the study population. It also implicated injecting drug use as a possible major means of HEV transmission in Nigeria, which was formerly adduced to the developed countries, suggesting the need to encourage more epidemiological HEV studies.

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