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Seroprevalence and Related Risk Factors of Hepatitis E Virus Infection among Pregnant Women Attendees at Adeoyo Hospital, Ibadan, Nigeria

W. E. Ike[1*], A. O. Oduleye[1], T. O. Ariom[1], C. O. Igbokwe[2], A. J. Ike[3], and J. A. Okiye[1]

[1]Department of Microbiology, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.
[2]Department of Pharmaceutical Microbiology and Biotechnology, Lead City University, Ibadan, Oyo State, Nigeria.
[3]Department of Pharmaceutical Microbiology, University of Ibadan, Ibadan, Oyo State, Nigeria.

Abstract

Hepatitis E virus (HEV) is a major public health problem in developing countries and often fatal among pregnant women. This study investigated the seroprevalence and risk factors of HEV infection in pregnant patients at Adeoyo General Hospital in Ibadan, Nigeria. The cross-sectional study was carried out from January 15, 2022 to September 30, 2022. An enzyme linked immunosorbent assay (ELISA) kit was used to assess the levels of anti-HEV IgM and IgG in 210 blood samples from pregnant women who gave their consent and joined the study. Utilizing structured questionnaires, the risk variables and sociodemographic characteristics were assessed. Out of the 210 pregnant women sampled, anti-HEV IgM/IgG seroprevalence of (1.9%/2.9%) were recorded respectively. The seroprevalence was greater (4.0%/8.0%) in the age group of 35 to 39 years and zero in age ranges 15 – 19, and >40 years. The highest prevalence for anti-HEV IgM was recorded among primary school subject (12.5%), followed by secondary (1.9%), and 4.1% among tertiary subject for anti-HEV IgG. After logistic regression, marital status, and occupation were found not to be significant risk factors for active HEV infection (p>0.05).
This study found a considerably low level of anti-HEV IgM seropositivity among pregnant women in the study group, which is indicative of emerging and non-active HEV infection. This is based on the 10.8% pooled national prevalence of HEV infection in Nigeria.

Keywords: HEV Infection, Risk factor, Seroprevalence, Ibadan-Nigeria.

***Corresponding authors, Email address:** ikewe@funaab.edu.ng

Introduction

Hepatitis E has become one of the most significant public health infectious diseases causing acute viral hepatitis (Abdullah *et al.*, 2023). The causative agent of hepatitis E is the hepatitis E virus (HEV). This virus which is a member of the genus Orthohepevirus belongs to the family Hepeviridae, a single-stranded positive-sense RNA virus (Forbes *et al.*, 2007, Levinson, 2012). As a zoonotic agent, HEV is capable of infecting vertebrates such as pigs (swine HEV), chickens (avian HEV), camel and cattle (bovine HEV), sheep, rabbits, and rodents (Woo *et al.*, 2014, Huang *et al.*, 2016, Abdullah *et al.*, 2023). Based on genotypic characterization, HEV has been classified into four genotypes. Genotypes I and II have been implicated in humans while genotypes III and IV circulate in animals which are considered reservoirs, however, they infect humans

occasionally (Huang *et al*., 2016, Abdullah *et al*., 2023).

The World Health Organization (WHO) data show that an estimated 3 million symptomatic cases of hepatitis E exist every year and there are about 44,000 hepatitis E-related deaths globally (Chandra, 2008, WHO, 2023). In Nigeria, several outbreaks of HEV have been documented. The first outbreak was reported in Port Harcourt, the capital city of Rivers State in Southern Nigeria between November 1997 and June 1998 involving 10 cases of HEV II (Folakemi *et al*., 2021). In 2017, another outbreak was reported in Bornu State in the Northeastern part of Nigeria involving approximately 1,815 cases including pregnant women harboring genotypes I and II with case fatality of 0.6% (NCDC, 2018).

The clinical manifestation of HEV disease is dependent on several variables ranging from acute to asymptomatic condition in a patient which may resolve rapidly or develop into acute-icteric condition or leads to extra-hepatic condition, ending in chronic hepatitis (WHO, 2022). Since most HEV infections are asymptomatic, any illness that results from them usually resolves on its own and lasts only a few weeks for most individuals. In acute icteric hepatitis, the characteristic phase of hepatitis E, occurrence has been recorded in 5%–30% of infected patients and this phase is marked by dark urine and jaundice (Sébastien *et al*., 2020).

HEV infections in pregnant women, particularly in their second or third trimester, are usually severe resulting in high risk of acute liver failure or fetal loss and mortality (Yadav and Kenney, 2021). Approximately 20–25% of pregnant women in developing countries of Africa, Asia and Latin America die of hepatitis E infection, especially in their third trimester (Navaneethan *et al*., 2008). Plethora of epidemiological data have shown that high incidence of HEV infection among pregnant women is associated with maternal morbidity, fulminant hepatitis, and acute liver failure (Miyashita *et al*., 2012). Transmission from mother to child has been frequently reported leading to increased risk of obstetric complications such as premature rupture of membranes, preterm labor, intrauterine growth restriction, neonatal death, and stillbirth (Miyashita *et al*., 2012, WHO, 2022). According to a study conducted in India, the mortality rates of acute liver failure linked to HEV and non-related to HEV in pregnant women were comparable, with HEV-related acute liver failure being more common during pregnancy (Sébastien *et al*., 2020). There is a lack of knowledge about HEV infection in Nigeria, particularly among the community of expectant mothers. Thus, the purpose of this study was to assess the seroprevalence of Hepatitis E virus infection among expectant mothers in Adeoyo General Hospital, Ibadan, Oyo State, Nigeria.

Materials and Methods

Study Area

The study was carried out at Adeoyo Hospital, Ibadan, Oyo State in the tropical belt of the South Western part of Nigeria (Africa). In Decimal Degrees, its coordinates are 3.9 and 7.383333, or 7º23′0″N and 3º54′0″E in DMS (Degrees Minutes Seconds) (Figure 1).

Study Population

A total of 210 consenting healthy pregnant women with no history of immuno-suppressive diseases, such as human immunodeficiency viruses (HIV), cancers, chronic liver diseases, and use of corticosteroids attending Adeoyo hospital for antenatal care, residing in different areas of Ibadan, Oyo state, in the age range 15 to >40 years were enrolled for this study.

Sample Size

According to Yamane's formula, where N (population size) =540, confidence = 95%, e =0.05 (5%). The calculated sample size for the research was 210.

Ethical Consideration

Ethical approval was obtained from the Oyo State Ministry of Health Research Ethics Committee (HREC) with NREC assigned number NHREC/OYOSHRIEC/10/11/22. In addition, individuals were provided with an informed consent form and they gave both verbal and written consent before they were included in the study. All participant data were kept private and secure, with the exception of situations in which the researcher was required by law to disclose particular instances.

Data Collection

Structured questionnaires about sociodemographic characteristics and other relevant information were considered including age, education, marital status, occupation, and trimester pregnancy. A consent form and verbal communication were also administered to

enlisted participants. Individuals who refused to give consent as well as provide necessary information on the questionnaire were excluded from the study.

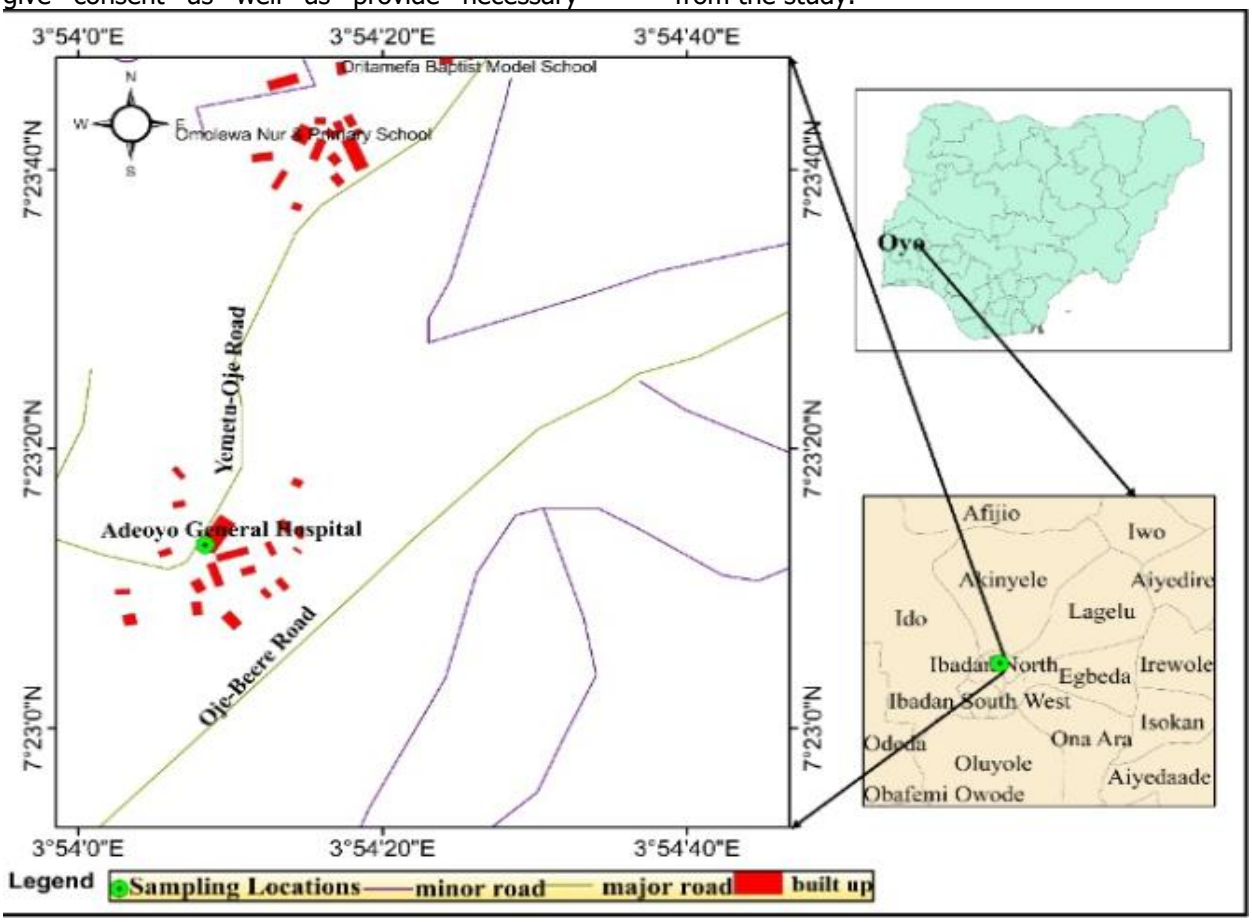

Fig 1: Geographical map showing HEV sero-survey study location of Adeoyo General Hospital, Ibadan, Nigeria.

Laboratory Analysis

Collection of Blood Samples and Extraction of Plasma

Blood samples were collected from each participant into EDTA bottles. For twenty minutes, the blood samples were centrifuged at 4000 rpm. Before being used, the plasma was separated and kept at -20°C. All the plasma was screened for anti-HEV IgM and anti-HEV IgG using Enzyme enzyme-linked immunosorbent Assay (ELISA) commercial kit (Beijing Wantai Biological) with high sensitivity and specificity. Assays were carried out in compliance with the manufacturer's guidelines. A microplate reader with an ELISA was used to read the optical density (OD). The outcomes were noted and interpreted appropriately.

Serological Analysis of the Plasma using ELISA

Ten (10) µl of Positive control, Negative control, and Specimen (plasma) were added into their respective well using different disposable pipette tips (Note: 3 negative control wells, 1 positive control well, and 92 wells containing plasma for a plate). Specimen diluent of 100µl was added into each of the wells. Following 30 minutes of incubation at 37°C, the plate's lid was placed on it. The micro-wells were left to stand for 30 to 60 seconds after each of the five wells were washed with diluted wash buffer. The surface was blotted with paper to remove excess fluid. 100µl of HRP-conjugate was added to every well. After the second incubation period, which lasted 30 minutes at 37°C, the plate was washed. To each well, 50µl of Chromogen Solution A and Chromogen Solution B were added and mixed gently. The plate was incubated at 37°C for 15 minutes in the absence of light. The appearance

of blue color indicates positive. 50µl of Stop Solution was carefully mixed into each well using a multichannel pipette. An intensive yellow color indicates positive for HEV presence. An ELISA microplate reader was used to measure the absorbance of the plates at 450 nm.

Statistical Analysis

a) The overall mean age of pregnant women was calculated.
b) The ratio of expectant mothers positive for anti-HEV IgM and anti-HEV IgG was calculated.
c) The mean age of pregnant women positive for anti-HEV IgG and anti-HEV IgG were calculated.
d) The seroprevalence of anti-HEV IgM and anti-HEV IgG were determined among positive participants in the studied population and expressed in percentages.
e) The seroprevalence of anti-HEV IgM and anti-HEV IgG were expressed in pie charts.
f) Statistical associations between sociodemographic characteristics such as age, education status, trimester, and seroprevalence of HEV infection were calculated.
g) The data obtained was analyzed using a Statistical Package for Social Sciences (SPSS version 20.0). Values were organized and summarized in terms of frequencies and the result of the study was presented in tables and figures.
h) The chi-square (x^2) test was used to determine the correlation between sociodemographic variables and HEV status.
i) At $P < 0.05$, statistical significance was established.

Results and Discussion

From the questionnaire, the sociodemography of the 210 enlisted participants (Table 1) show that the age ranges from 15 to >40 years with mean age at 28.60 ± 5.6 years. The study population for age between 15 – 19 years was 9(4.3%), 20 – 24 years was 47(22.4%), and 25 – 29 years was 70 (33.3%). Similarly, 51(24.3%) was aged between 30 – 34 years, 25(11.9%) was aged between 35 – 39 years, and 8(3.8%) was aged 40 years and above. Educational status revealed that 3.8%, 49.5%, and 46.7% obtained primary, secondary, and tertiary education respectively. In the same way, data from marital status demonstrated that 2.9%, 96.2% and 1.0% were single, married and separated correspondingly. On the basis of the occupational level, 69.5%, 13.3%, 11.4%, and 5.7% were business owners, artisans, civil servants, and students congruently. In terms of the risk factors, 33.3% have had previous miscarriage and 66.7% have never had miscarriage. Data on parity revealed 12.4% in their prime, and 87.6% as having more than one child previously. The highest seroprevalence of HEV was reported in the first trimester (58.1%) followed by the second trimester at 35.2% and the least in the third trimester at 6.0%. Regarding the ELISA result, 1.9% of the pregnant women were positive for anti-HEV IgM while 2.9% were positive for anti-HEV IgG (Figure 2 and 3).

Table 1: Socio-demographic Characterization of Pregnant Women Attending Antenatal at Adeoyo Hospital, Ibadan, Oyo State.

Variable	**Frequency**	**Percentage (%)**
Age group		
15 – 19	9	4.3
20 – 24	47	22.4
25 – 29	70	33.3
30 – 34	51	24.3
35 – 39	25	11.9
> 40	8	3.8
Educational Status		

Primary		8			3.8	
Secondary		104			49.5	
Tertiary	98			46.7		
Marital Status						
Single		6			2.9	
Married			202			96.2
Separated		2			1.0	
Occupation						
Business		146			69.5	
Artisan		28			13.3	
Civil Servant		24			11.4	
Student		12			57.0	

Mean age = 28.60 ± 5.6 years

Table 2: Risk factors Associated with the Studied Population

Variables	**Frequency**	**Percentage (%)**
Previous miscarriages		
Yes	70	33.3
No	140	66.7
Parity		
None	26	12.4
≥1	184	87.6
Gestational period/ Pregnancy trimester		
1-3 months (first trimester)	122	58.1
4-6 months (second trimester)	74	35.2
7-9 months (third trimester)	14	6.0

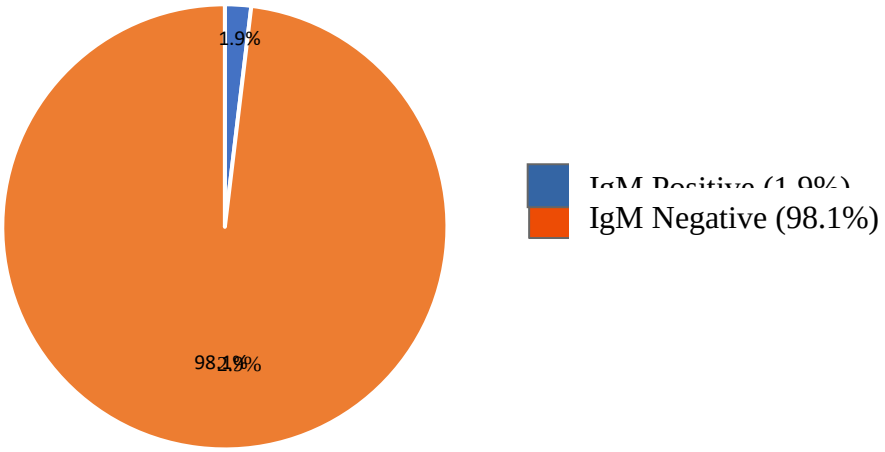

Figure 2: Seroreactivity rate of HEV IgM among pregnant women

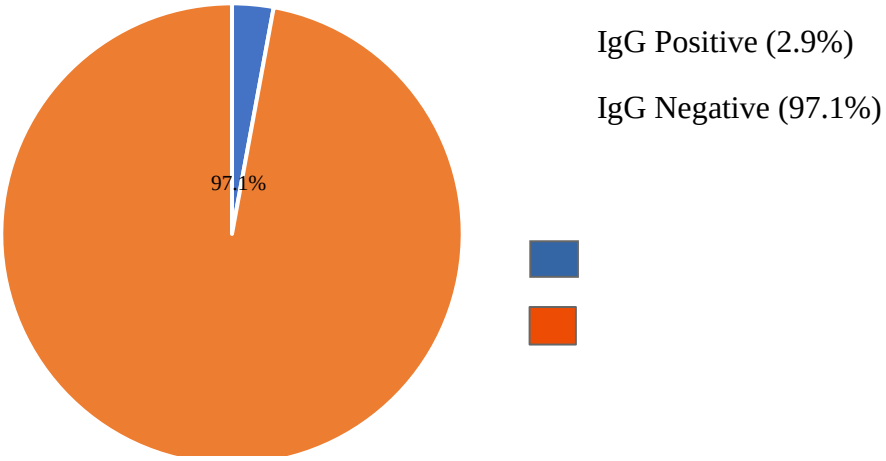

Figure 3: Seroreactivity rate of HEV IgG among pregnant women

Seroprevalence of Anti-HEV IgM and Anti-HEV IgG in Relation to Sociodemographic Variables

The distribution of seropositivity according to age, education, occupation, marital status as risk factors in this study are shown in Table 3.

Age

Prevalence of anti-HEV IgM increased with age, as 0.0% was recorded for the subjects within the age range 15 – 19 years, 2.1% for 20 – 24 years, 2.9% for 25 – 29 years, and 4.0% for 35 – 39 years. Similar pattern was observed for anti-HEV IgG where 0.0% prevalence was recorded for age 15 – 19 years, 2.1% for 20 – 24 years, 2.9% for 25 – 29 years. However, 8.0% was recorded for age range 35 – 39 years. There were no statistical association between seropositivity and age distribution as $p > 0.05$ (Table 3).

Educational Status

Considering educational status, the highest prevalence of anti-HEV IgM was recorded among those that had primary education (12.5%). This was followed by secondary education (1.9%) and tertiary education (1.0%). The result reported variability in anti-HEV IgG as the highest prevalence was recorded among those that had tertiary education (4.1%), and followed by participants that had secondary education (1.9%). No prevalence was recorded among participants that had primary education (0.0%). However, seropositivity was not significantly

associated with educational status as $p > 0.05$ (Table 3).

Marital Status

The seroprevalence of HEV according to the marital status of the studied population, the highest prevalence for anti-HEV IgM and anti-HEV IgG was recorded among those pregnant women who were married (2%) and (3%) respectively. No prevalence was recorded among single and separated pregnant women. There were no significant statistical correlation between marital status and HEV seroprevalence, ($p > 0.05$) (Table 3).

Occupation

No significant statistical correlation exists between occupation and HEV seroprevalence ($p > 0.05$). While 2.7% prevalence was reported against anti-HEV IgM among business owners (traders), zero percent prevalence was recorded against participants who were artisans, civil servants and students. The percentage of positive cases varied in anti-HEV IgG groups, highest rate was estimated to be 8.3% among students, 3.6% among artisans, and 2.7% among business owners. No positive case was documented for civil servants (Table 3).

Table 3: Association between Sociodemographic Factors and Seroprevalence of HEV

Variable	Seroprevalence IgM		Total	Seroprevalence IgG		Total
Characteristics	Positive N=4	Negative N=206		Positive N=6	Negative N=204	
Age group						
15 – 19	0(0.0%)	9(100%)	9(100.0%)	0(0.0%)	9(100.0%)	9(100.0%)
20 – 24	1(2.1%)	46(97.9%)	47(100.0%)	1(2.1%)	46(97.9%)	47(100.0%)
25 – 29	2(2.9%)	68(97.1%)	70(100.0%)	2(2.9%)	68(97.1%)	70(100.0%)
30 – 34	0(0.0%)	51(100.0%)	51(100.0%)	1(2.0%)	50(98.0%)	51(100.0%)
35 – 39	1(4.0%)	24(96.0%)	25(100.0%)	2(8.0%)	23(92.0%)	25(100.0%)
>40	0(0.0%)	8(100.0%)	8(100.0%)	0(0.0%)	8(100.0%)	8(100.0%)
Total	4(1.9%)	206(98.1 %)	210(100.0%)	6(2.9%)	204(97.1%)	210(100.0%)
P-value 0.95			0.42			
X^2	0.81			0.68		

Table 3: Association between Sociodemographic Factors and Seroprevalence of HEV

Variable	Seroprevalence IgM		Total	Seroprevalence IgG		Total
Characteristics	Positive N=4	Negative N=206		Positive N=6	Negative N=204	
Educational status						
Primary	1(12.5%)	7(87.5%)	8(100%)	0(0%)	8(100%)	8(100%)
Secondary	2(1.9%)	102(98.1%)	104(100%)	2(1.9%)	102(98.1%)	104(100%)
Tertiary	1(1.0%)	97(99.0%)	98(100%)	4(4.1%)	94(95.9%)	98(100%)
Total	4(1.9%)	206(98.1%)	210(100%)	6(2.9%)	204(97.1%)	210(100%)
P value	0.12			0.29		
X^2	0.07			0.57		
Marital Status						
Single	0(0%)	6(100%)	6(100%)	0(0%)	6(100%)	6(100%)
Married	4(2%)	198(98%)	202(100%)	6(3%)	196(97%)	202(100%)
Separated	0(0%)	2(100%)	2(100%)	0(0%)	2(100%)	2(100%)
Total	4(1.9%)	206(98.1%)	210(100%)	6(2.9%)	204(97.1%)	210(100%)
P-value	0.92	0.89				
X^2	0.16			0.25		

Table 3: Association between Sociodemographic Factors and Seroprevalence of HEV

Variable	Seroprevalence IgM		Total	Seroprevalence IgG		Total
Characteristics	Positive N=4	Negative N=206		Positive N=6	Negative N=204	
Occupation						

Business	4(2.7%)	142(97.3%)	146(100%)	4(2.7%)	142(97.3%)	146(100%)
Artisan	0(0%)	28(100%)	28(100%)	1(3.6%)	27(96.4%)	28(100%)
Civil Servant	0(0%)	24(100%)	24(100%)	0(0%)	24(100%)	24(100%)
Student	0(0%)	12(100%)	24(100%)	1(8.3%)	11(91.7%)	12(100%)
Total	4(1.9%)	206(98.1%)	210(100%)	6(2.9%)	204(97.1%)	210(100%)
P-value	0.62			0.56		
X^2	1.79			2.06		

Discussion

Hepatitis E virus is considered to a large extent one of the significant public heath diseases in many Asia and African countries including Nigeria. A town in northern Nigeria called Damasak, which borders the Niger Republic, reported the first case of HEV in May 2017. As of July 2017, 146 confirmed and suspected cases had been recorded; of these, 25 cases involved pregnant women who were infected, making up 21%. Of these, two cases resulted in death (8%) (NCDC, 2018). HEV is a widely known infectious disease that is responsible for intrauterine fetal mortality and poor outcomes for both the mother and the fetus during pregnancy (Wu *et al.,* 2020). In an endemic zone, the presence of specific anti-HEV immunoglobulin M (IgM) antibodies to the virus in a person's blood is typically sufficient for a conclusive diagnosis of HEV infection. However, rapid tests are available for field use (WHO, 2023).

The present study recorded a low seroprevalence of anti-HEV IgM (1.9%) and anti-HEV IgG (2.9%) among pregnant women in Adeoyo General Hospital, Ibadan, Oyo State. Comparatively, the seroprevalence of HEV recorded among pregnant women in this health facility was found to be lower than those reported among pregnant women in other parts of Africa and beyond, including Plateau State (42.6%) (Junaid et al., 2014), Ghana (28.66%) (Adjei *et al*., 2009), Gabon (14.1%) (Caron and Kazanji, 2008), Egypt (84.3%), Sudan (41%) and India (33.6%) (Begum *et al*., 2009, Stoszek *et al*., 2006, Al-Tayeb *et al*., 2004). The high rate in Plateau State, Ghana, Gabon, Egypt, Sudan, and India suggests endemicity in these regions which may be linked to poor sanitation and lack of potable water (Bello *et al*., 2017). However, it is similar to those reported among pregnant women in the developed world where the rate is significantly low. According to a study conducted by Lindemann *et al.* (2010) on 1040 pregnant women, the prevalence of anti-HEV IgG was 3.6%. Regarding anti-HEV IgM antibodies, 0%, 0.5%, and 0.64% have been reported in France, Spain, and China for pregnant women (Lindemann *et al*., 2010, Renou *et al*., 2014). In Nigeria, our result is similar to reports from Ifeorah et al. (2017) and Osundare et al. (2021). A close comparison with the work conducted in Plateau State, by Junaid et al. (2014), revealed that most pregnant women enlisted in that study hail from the rural settlements whereas the majority of subjects recruited in our study came from the urban centers.

In terms of risk factors associated with the acquisition of HEV, we observed in our study that HEV seroprevalence increased with age. This increase was remarkable at age >40 years for both anti-HEV IgM and anti-HEV IgG. This is in consonant with previous and recent reports by Adesina et al. (2009) and Okwara et al. (2021) but contradicts the findings reported by Junaid et al. (2014).

Considering educational status, our study revealed a significant seroprevalence rate among pregnant women who attended primary education (12.5%) as compared to secondary (1.9%) and tertiary (1.0%) education. Our result is consistence with previous studies conducted in Nasarawa state (Airiohuodion, *et al*., 2022) and Ethiopia (Abebe *et al*., 2017). This phenomenon may be associated with low literacy levels, poor hygiene, unwholesome traditional practices, and low socioeconomic status among others. Although no agreement has been demonstrated in other investigations, education may be a plausible predictor of HEV infection. Again, proximity of these married pregnant women or mothers with their children usually puts them at risk of HEV infection

In the present study, HEV seroprevalence was (2.0%) in married pregnant women. This result differs from the report of Oladipo et al. (2017) but aligns with the reports of Labrique et al. (2010) and Junaid et al. (2014) where a higher prevalence was documented among married pregnant mothers. Studies have shown that children are reservoir hosts of HEV (Kumar *et al*., 2004), therefore, married expectant mothers who are close to their kids are typically in danger of contracting HEV infection. In the case of single and separate pregnant women, zero prevalence was recorded. The statistical significance of the relationship between anti-HEV positivity and married status was not found.

From the study, no significant statistical correlation exists between occupation and acquisition of HEV. Nonetheless, a 2.7% prevalence was recorded for anti-HEV IgM among business owners (traders). This is in

disagreement with the finding made by Oladipo et al. (2017) where students (4.8%) emerged at a higher rate when compared to traders (4.3%). A zero percent prevalence was recorded for participants who were artisans, civil servants, and students. The percentage of positivity varied in anti-HEV IgG groups, the highest rate was estimated to be 8.3% among students, 3.6% among artisans, and 2.7% among business owners.

Conclusion

This study reveals the emergence of HEV infection among the population of the pregnant women analyzed in this study with age and education being the biggest potential risk factors. Although the population that had been exposed to HEV may be insignificant compared to previous studies in Oyo state, it is imperative to embrace and sustain preventive public health measures to mitigate the outbreak.

Conflicts of Interest

No conflicts of interest were declared by the authors.

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References

Abdullah, A., Ammara, K., Kshedan, L., Nami, A., Sharef, A. (2023). Seroprevalence and Potential Risk Factors of Hepatitis E Virus Infection among Pregnant Women in Tripoli, Libya. *Alqalam J.* Appl. *Med*. Sci. 6(2):707-715

Forbes, B.A., Shm, D.F., Wessfeld, A.S. (2007). Laboratory Methods in basic virology. In Bailey and Scott' diagnostic microbiology. 2007; 12th edition, Mosby Elsevier china, 718-715.

Levinson, W. (2012). Hepatitis viruses. In: Review of Medical Microbiology and Immunology. 2012; 12th edition, MC Grow Hill medical, New York, 324 – 332.

Woo, P.C., Lau, S.K., Teng, J.L., Tsang, A.K., Joseph, M., Wong, E.Y., Tang, Y., Sivakumar, S., Xie, J., Bai, R., Wernery, R., Wernery, U., Yuen, K.Y. (2014). New hepatitis E virus genotype in camels, the Middle East. *Emerg. Infect. Dis.* 20(6), 1044–1048.

Huang, F., Li, Y., Yu, W., Jing, S., Wang, J., Long, F., He, Z., Yang, C., Bi, Y., Cao, W., Liu, C., Hua, X., Pan, Q. (2016). Excretion of infectious hepatitis E virus into milk in cows imposes high risks of zoonosis. *Hepatology*. 64(2):350-359.

WHO. Hepatitis E. 2023; Available on https://www.who.int/news-room/fact sheets/detail/hepatitis-e

Chandra, V., Taneja, S., Kalia, M., Jameel, S. (2008). Molecular biology and pathogenesis of hepatitis E virus. *J. Biosci.* 33(4):451–464. https://doi.org/10.1007/s12038-008-0064-1.

Folakemi, A.O., Patrycja, K., Olusola, A.A., Bo, W., Dominik, H., Olusola, O., Moses, A.A, Emmanuel, O.B, C.-Thomas, B., Oladele, O.O. (2021). Hepatitis E virus infection in high-risk populations in Osun State, Nigeria. *One Health*. 13:2021, 100256, ISSN 2352-7714, https://doi.org/10.1016/j.onehlt.100256.

Nigeria-Centre-for-Disease-Control. (2018). Weekly Situation Report of HEV Outbreak in Borno State No. 15: Epi Weeks 05–06 2018, Borno State. (2018). Google Scholar

Sébastien, L., Olivier, M., Florence, A., Jacques, I., Nassim, K. (2020). Clinical Manifestations, Pathogenesis and Treatment of Hepatitis E Virus Infections. *J. Clin. Med.* 9:331. doi:10.3390/jcm9020331.

Yadav, K.K., and Kenney, S.P. (2021). Hepatitis E virus immune-pathogenesis. *Pathogens*, 10(9): 1180.

Navaneethan, U., Al Mohajer, M., Shata, M.T. (2008). Hepatitis E and pregnancy: Understanding the pathogenesis. *Liver Int*. 28:1190–1199, doi:10.1111/j.1478-3231.2008.01840.x.

Miyashita, K., Kang, J.H., Saga, A., Takahashi, K., Shimamura, T., Yasumoto, A., Fukushima, H., Konishi, S.K., Uchida, T., Fujinaga, A., Matsui, T., Sakurai, Y., Tsuji, K., Maguchi, H., Taniguchi, M., Abe, N., Akbar, S.M.F, Arai, M., Mishiro, S. (2012). Three cases of acute or fulminant hepatitis E caused by ingestion of pork meat and

entrails in Hokkaido, Japan: Zoonotic food-borne transmission of hepatitis E virus and public health concerns. *Hepatol Res*. 42:870– 878.

Junaid, S.A., Agina, S.E., Abubakar, K.A. (2014). Epidemiology and associated risk factors of hepatitis E virus infection in Plateau State, Nigeria. *Virol*. *Res treat*. 5:15-26.

Adjei, A.A., Tettey, Y., Aviyase, J.T., Adu-Gyamfi, C., Obed, S., Mingle, J.A.A., Adiku, T.K. (2009). Hepatitis E virus infection is highly prevalent among pregnant women in Accra, Ghana. *Virol. J*. 6:108–112.

Caron, M., Kazanji, M. (2008). Hepatitis E virus is highly prevalent among pregnant women in Gabon, central Africa, with different patterns between rural and urban areas. *Virol. J*. 5:158–161.

Begum, N., Devi, S.G., Husain, S.A., Ashok, K., Kar, P. (2009). Seroprevalence of subclinical HEV infection in pregnant women from north India: a hospital based study. *Indian J. Med. Res.* 130 (6):709–13.

Stoszek, S.K., Abdel-Hamid, M., Saleh, D.A., El Kafrawy, S., Narooz, S., Hawash, Y, Hawash, Y., Shebl, F.M., El Daly, M., Said, A., Kassem, E., Mikhail, N., Engle, R.E., Sayed, M., Sharaf, A.D.F., Emerson, S.U., Purcell, R.H., Strickland, G.T. (2006) . High prevalence of hepatitis E antibodies in pregnant Egyptian women. *R. Soc. Trop. Med. Hyg*. 100 (2):95–101.

Al-Tayeb, Z.A., Nafi, M., Yassin, M.E. (2004). Frequency of Hepatitis E Virus among Pregnant Women Attending Khartoum Hospitals. *Am. J. Res. Commun*. 2 (4).

Bello, M., Alkali, B.R., Nuhu, A., Shualbu, A.B., Faruku, N., Mustapha, U.K., Bunza, N.M., Ibrahim, A.B. (2017). Evaluation of Risk Factors Associated with Hepatitis E Virus (HEV) infection among Pregnant Women in Sokoto State, North Western Nigeria*. Int J Trop Dis Health.* 23(1): 1–8.

Lindemann, M.L.M., Gabilondo, G., Romero, B., de la Maza, O.M.S., Pérez-Gracia, M.T. (2010). Low prevalence of hepatitis E infection among pregnant women in Madrid, Spain. *J Med Virol*. 82 (10):1666–8.

Renou, C., Gobert, V., Locher, C., Moumen, A., Timbely, O., Savary, J., Roque-Afonso, A. (2014). Prospective study of Hepatitis E Virus infection among pregnant women in France. *Virol. J*. 2014; 11: 68.

Ifeorah, I.M., Faleye, T.O.C., Bakarey, A.S., Adewumi, M.O., Akere, A., Omoruyi, E.C., Ogunwale, A.O., Adeniji. J.A. (2017). Acute hepatitis E virus infection in two geographical regions of Nigeria, *J. Pathog.* 2017:1-6,

Osundare, F. A, Klink, P., Akanbi, O. A., Wang, B., Harms, D., Ojurongbe, O., Ajayi, M. A., Babaranti, E. O., Bock, C. T., Opaleye, O. O. (2021). Hepatitis E virus infection in high-risk populations in Osun State, Nigeria. *One Health,* 13: 100256

Okwara, V.C., Mbachu, I.I., Ndububa, V.I., Okpara, H.C., Mbachu, C.P. (2021). Seroprevalence, Associated Factors, and Fetomaternal Outcome in Pregnant Women That Tested Positive to Hepatitis E Antibodies in Nigeria. *Obstet*. *Gynaecol. Int.* 21; 2021: 9341974

Adesina, O.A., Japhet, M.O., Donbraye, E., Kumapayi, T.E., kudoro, A. (2009). Antihepatitis E virus antibodies in sick and healthy individuals in Ekiti State, Nigeria. *Afr. J. Microbiol. Res.* 3: 533–556.

Abebe, M., Ali, I., Ayele, S., Overbo, J., Aseffa, A., Mihret, A. (2017). Seroprevalence and risk factors of Hepatitis E Virus infection among pregnant women in Addis Ababa, Ethiopia. *PloS one* 12(6): e0180078.

Airiohuodion, P.E., Wartel, A., Yako, A.B., Mac, P.A. (2022). Seroprevalence and burden of hepatitis E viral infection among pregnant women in central Nigeria attending antenatal clinic at a Federal Medical Centre in Central Nigeria. *Front. Med* (Lausanne). 2; 9:888218.

Kumar, A., Beniwal, M., Kar, P., Sharma, J.B., Murthy, N.S. (2004). Hepatitis E in pregnancy. *Int. J Gynaecol Obstet.* 85: 240–4.

Oladipo, E.K., Awoyelu, E.H., Oloke, J.K. (2017). Human Hepatitis E Virus among Apparently Healthy Individuals in Ogbomoso, South-Western Nigeria. *J. hum. virol. Retrovirol*. 5(1):1–6.

Labrique, A.B., Zaman, K., Hossain, Z., Saha, P., Yunus, M., Hossain, A., Ticehurst, J.R., Nelson, K.E. (2010). Epidemiology and risk factors of incident hepatitis E virus infections in rural Bagladesh. *Am J Epidemiol*. 172 (8):952–961.

Junaid, S.A., Agina, S.E., Abubakar, K.A. (2014). Epidemiology and associated risk factors of hepatitis E virus infection in Plateau State, Nigeria. *Virology* (Auckland), 5:15–26.