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Serological detection of recent infections of hepatitis E virus among animal handlers in Calabar, Cross River State, Nigeria

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Abstract

One of the groups most impacted by the Hepatitis E virus is animal handlers, which raises major public health concerns that require prompt, comprehensive investigation using IgM ELISA assays. This qualitative cross-sectional study analysed plasma samples from 94 Animal handlers in different markets in Calabar Metropolis, Cross Rivers State, Nigeria. HEV-specific IgM antibodies were examined in plasma samples from 94 animal handlers. This study aimed to investigate the burden and associated factors of Hepatitis E Virus and among animal handlers in Calabar. Plasma samples were screened serologically for the presence of specific HEV IgM antibodies using ELISA kits. Sociodemographic characteristics of the participants were collected by the use of a structured questionnaire. Chi-square was used to assess the statistical significance of different socio-demographic factors to HEV. No statistical association existed between the prevalence of HEV and the socio-demographics factors studied. The IgM results yielded a prevalence of 43.62% for the animal handlers, the Highest prevalent rate for the animal handlers was found in male gender (24.47%), age group 31-40 years (23.40%), married (35.11%), secondary education (25.53%), self-employed (11.70%) and the type of water used (18.09%). This study identifies a possible danger of Hepatitis E Virus and co infections from animal handlers' transmission to the general population.

Keywords: Antibodies; Hepatitis E Virus; IgM; Calabar

1. Introduction

Globally, HEV infections have been found to be the most or second most common cause of acute viral Hepatitis among adults' resident in Africa, Asia and Middle East [1] In Africa, HEV infection is found to cause infections among human population in 28 out of 56 African countries [1]. The virus causes an estimated 20 million infections annually across the globe [2], leading to over 3 million symptomatic cases and acute cases of HEV occur globally with an estimation of 56,600 deaths [3,4,5,6]. It has been proven beyond doubt that HEV can also cause chronic hepatitis in the immunocompromised hosts and this has caught the Western world off guard [7] Hepatitis E virus (HEV) causes acute hepatitis with approximately 939 million cases worldwide [8]. The World Health Organization (WHO) estimated that 20 million new HEV infections, with more than 3 million cases of acute hepatitis and over 44,000 deaths occur annually [9]. A recent study shows a HEV outbreak in northeast Nigeria with over a thousand suspected cases that includes 8 infection-associated deaths showing that HEV poses a public health issue in Nigeria [10]. The mortality rate associated with HEV infection (1 to 4%) is higher than that of hepatitis A (0.1 to 2%) [11] and can reach up to 20% during pregnancy [12]. HEV infection in developing countries is mostly a waterborne disease associated with large epidemics due to contamination of water and water supplies, and poor sanitation conditions, [13,12] There isn't a solitary treatment that can alter the progression of

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acute hepatitis E. Hospitalization becomes necessary for pregnant women experiencing symptoms and individuals with severe hepatitis. Immunosuppressed patients dealing with chronic hepatitis E experience positive outcomes through specialized administration of the antiviral medication ribavirin. Moreover, in certain limited instances, interferon has proven effective.

2. Materials and method

2.1. Study Design and Population.

This study was cross-sectional in design. The ethical conduct of the work was approved by the Cross River State Ethics Committee. The participants' demographic information was obtained through the administration of standardised questionnaires. The 94 animal handlers included in this study were all members of the cohort of eligible participants who gave their consent. On the other hand, all subjects whose data were incomplete were excluded from the study

2.2. Laboratory Analysis

Plasma was analysed for the presence of HEV IgM at the University of Port Harcourt's Virus & Genomics Research Unit of the Department of Microbiology

2.3. HEV IgM Serological Analysis

The IgM content of plasma samples was measured using aDIA.PRO, Milano, Italy, ELISA kit. The manufacturer's instructions were followed for conducting the analysis. Interpretation was done according to the manufacturer's instructions. The results were interpreted using the sample OD450nm to cut-off value ratio and the following values: S/Co < 1.0: Negative. If the S/Co ratio calculated is below 1.0, the test outcome is regarded as negative. This suggests that the level of HEV IgM antibodies in the sample is below the required threshold for positivity. 1.0 ≤ S/Co ≤ 1.2: Equivocal - If the S/Co ratio falls between 1.0 and 1.2, the outcome is considered equivocal. This indicates that the level of HEV IgM antibodies is uncertain and cannot be definitively classified as either positive or negative. S/Co ≥ 1.2: Positive. When the S/Co ratio is equal to or exceeds 1.2, the test outcome is interpreted as positive. This signifies the presence of a notable quantity of HEV IgM antibodies in the sample.

2.4. Data analysis

Microsoft Excel version 2021 (Microsoft, USA) was utilised to evaluate the data. The statistical significance of every analysis was determined where appropriate using the Chi-square test or Fisher's exact test at a 5% significance threshold

2.5. Ethical consideration

The study was conducted after obtaining ethical clearance from the University of Port Harcourt Research Ethics Committee.

3. Results

3.1. Analysis of Study Participants' Characteristics

The socio-demographic description of the participants of the study is revealed in Table 1. The sample involved a total of 94 participants, of whom 62 (66%) were males, and 32 (34.0%) were females. With regards to age distribution, 8 (8.5%) of the participants were within the age range of 18-30 years, the majority of the participant 45 (47.9%) were in the participants were of the age range of 31-40 years, 34 (36.2%) of the participants were in the age range of 41-60 years, 7 (7.4%) of the participants were > 60 years of age. An overwhelming majority of the respondents, 93 (98.9%), resided in urban areas, while only 1 (1.1%) resided in a rural area. With regards to marital status, 20 (21.3%) of the participants were reportedly single, 73 (77.7%) of the participants were married, and 1 (1.1%) of the respondents reported being divorced. The educational attainment of the respondents was relatively high among the participants; 61 (64.9%) of the respondents reported having at least completed secondary school, 10 (10.6%) of the respondents had attained primary education, and 23 (24.5%) of the respondents had attained tertiary education. With regards to their employment status as animal handlers, 20 (21.3%) of the respondents were recorded to be unemployed, 25 (26.6%) of the respondents were reportedly employed, and 49 (52.1%) of the participants were self-employed. Furthermore, with regard to the type of animals handled by the participant, the majority, 87 (92.6%) of the participants, handle cows, and 7 (7.4%) of the participants were pig handlers.

Table 1 Socio-demographic Characteristics of Study Participants

Variables	Categories	Frequency	Percentage
Sex	Male	62	66.0
	Females	32	34.0
Age ranges	18-30 years	8	8.5
	31-40 years	45	47.9
	41-60 years	34	36.2
	> 60 years	7	7.4
Place of habitation	Urban	93	98.9
	Rural	1	1.1
Marital Status	Single	20	21.3
	Married	73	77.7
	Divorced	1	1.1
Educational Qualification	Primary	10	10.6
	Secondary	61	64.9
	Tertiary	23	24.5
Employment Status	Unemployed	20	21.3
	Employed	25	26.6
	Self employed	49	52.1
Animal Type	Pig	7	7.4
	Cow	87	92.6

3.2. Overall Seroprevalence of HEV IgG and IgM

The overall seroprevalence of HEV IgM among animal handlers is presented in **Figure 1**. 41 (43.6%) were IgM positive and 53 (56.4%) were IgM negative. The risk of HEV infection among the different types of animal handlers is reported in **Table 2**. Also, the odds of being HEV IgM +ve was 0.492 (95% CI: 0.091-2.677) times lesser among pigs compared to slaughterhouse workers handling cow, suggesting a lower likelihood of recent infection in pig handlers. This association was also not statistically significant.

3.3. Seroprevalence with Respect to Socio-demographic Characteristics

Chi-square test was used to test the relationship between socio-demographic characteristics of the animal handlers and the seroprevalence of IgM, as presented in **Table 2.** Regarding sex, seroprevalence of IgM was highest among males (56.1%), followed by females (43.9%); however, sex was not associated with the seroprevalence of IgM (χ^2 = 3.148, df = 1, p-value = 0.076). Concerning their age ranges, seroprevalence of IgM was highest among participants who were of the age range 31-40 years 22 (53.7%), followed by participants who were in the age range of 41-60 years 16 (39.0%), 18-30 years 2(4.9%) and > 60 years 1 (2.4%) however, age was not associated with the seroprevalence of IgM (χ^2 = 4.249, df = 3, p-value = 0.236). Concerning the marital status of the animal handlers, seroprevalence of IgM was highest among married participants (80.5%), followed by single participants (19.5%). However, marital status was not associated with the seroprevalence of IgM (χ^2 = 0.955, df = 2, p-value = 0.620), as indicated in Table 2.

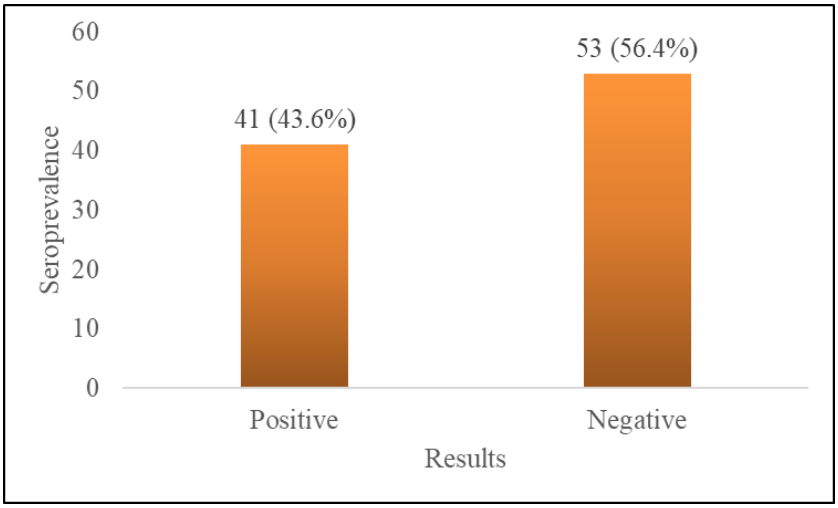

Figure 1 Seroprevalence of IgM among animal handlers

With regards to the educational qualification of the participants, seroprevalence of IgM was highest among participant with secondary school qualification (58.5%), followed by participants with tertiary education (31.7%), and then by participants with primary education (9.8%), however, educational qualification was not statistically associated with the seroprevalence of IgM (χ^2 = 2.064, df = 2, p-value = 0.356), as indicated in Table 2.

Table 2 Relationship between Socio-demographic characteristics and seroprevalence of Immunoglobulin M (IgM)

Socio-demographic Characteristics	Categories	Positive (%)	Negative (%)	χ^2	df	p-value
Sex	Male	23 (56.1)	39 (73.6)	3.148	1	0.076
	Female	18 (43.9)	14 (26.4)			
Age Ranges	18-30 yrs	2 (4.9%)	6 (11.3)	4.249	3	0.236
	31-40 yrs	22 (53.7)	23 (43.4)			
	41-60 yrs	16 (39.0)	18 (34.0)			
	> 60 yrs	1 (2.4)	6 (11.3)			
Marital Status	Single	8 (19.5)	12 (22.6)	0.955	2	0.620
	Married	33 (80.5)	40 (75.5)			
	Divorced	0 (0.0)	1 (1.9)			
Educational Qualification	Primary	4 (9.8)	6 (11.3)	2.064	2	0.356
	Secondary	24 (58.5)	37 (69.8)			
	Tertiary	13 (31.7)	10 (18.9)			
Employment Status	Unemployed	8 (19.5)	12 (22.6)	0.141	2	0.932
	Employed	11 (26.8)	14 (26.4)			
	Self employed	22 (53.7)	27 (50.9)			
Place of Residence	Urban	41 (100.0)	52 (98.1)	0.782	1	0.377
	Rural	0 (0.0)	1 (1.9)			
Type of Animal	Pig	2 (4.9)	5 (9.4)	0.696	1	0.404
	Cow	39 (95.1)	48 (90.6)			

With regards to the employment status, seroprevalence was highest among participants who were self-employed (53.7%), followed by participants who were employed (26.8%) and participants who were unemployed (19.5%).

Employment status was not associated with seroprevalence of IgM (χ^2 = 0.141, df = 2, p-value = 0.932), as indicated in Table 2. Concerning the participants' place of residence, seroprevalence of IgM, all positive IgM participants lived in urban areas (100%); however, place of residence was not associated with seroprevalence of IgM (χ^2 = 0.782, df = 1, p-value = 0.377). With regards to the type of animals handled by the participants, seroprevalence of IgM was highest among cow handlers (95.1%), followed by pig handlers (4.9%). The type of animal handled was not associated with the seroprevalence of IgM (χ^2 = 0.696, df = 1, p-value = 0.404), as indicated in Table 2.

4. Discussion

Hepatitis E virus remains an emerging endemic disease across the African continent, and is globally the leading cause of acute viral hepatitis worldwide, and transmitted through faecal contamination of drinking water as well as animal and their products of metabolism[14-15]. Hence, it is well known that the animal handlers of HEV include domestic pigs, deer, goats, sheep, rabbits, and other animals and their products. Pigs, however, are known to be reservoirs of HEV[16-17]. Therefore, certain categories of workers, such as veterinarians, farmers, hunters and slaughterhouse workers, are at risk of HEV infection[18]. This study investigated the seroprevalence of Hepatitis E virus (HEV) IgM among animal handlers in Calabar, Nigeria. The findings provide important insights into infection dynamics, risk patterns, and viral diversity within the study population.

The socio-demographic description of the participants in this study showed that 66.0% were males, and 34.0% were females. This observation agrees with that of previous studies in Nigeria. The study of Osundare et al. [19] also reported that a higher proportion (80.9%) of the animal handlers were males and 19.1% were females. Furthermore, in the study of Gidado et al. [20], which examined the prevalence of HEV among 180 participants, it was also recorded that 62.2% of the participants were males and 37.8% were females. Of the 102 classified as pig handlers, in a study by Oluremi et al. [21], 93.1% were males, and 6.9% were females. Of the 90 participants classified as butchers, 51.7% were males and 36.7% were females in the same study by Oluremi et al. [21]. However, this observation deviated from other previous studies in Nigeria. In contrast to the present study, Oluremi et al. [21] reported that 33.2% were males and 66.8% were females. Therefore, in light of other studies, we can still say that the majority of the active slaughterhouse workers were males.

With regards to age distribution in this study, the majority of the participants (47.9%) were in the age range of 31-40 years. A similar observation was made by Gidado et al. [20] in a study in Osun State, Nigeria. Furthermore, with regard to the type of animals handled by the participant, the majority (92.6%) handled cows, and 7.4% were pig handlers. In the study of Gidado et al. [20], participants included butchers, pig handlers, and non-animal handlers in Osun State. In the study by Oluremi et al. [21], 102 were pig handlers, and 90 participants were butchers.

This study reported that 41 (43.6%) of the animal handlers were IgM positive. Contrastingly, anti-HEV IgM antibody is temporary or short-lived, only lasting a few months (3-12 months)[22]. Hence, the presence of anti-HEV IgM is an indicator of a recent or current HEV infection, even though some assays have been found to have a longer persistence of anti-HEV IgM (up to 34 months), and different assays have demonstrated varied analytical efficiency [23,24]. Therefore, our result is an indicator of more recent HEV infection among the animal handlers in the slaughterhouses, not ignoring the comparatively significant indication of previous infection. Overall, the results represent evidence of frequent HEV infection inferred from the evidence of present infections. A key finding of this study is the significantly higher prevalence of HEV among animal handlers. This supports the zoonotic transmission potential of HEV, particularly genotypes 3 and 4, which are commonly associated with animal reservoirs such as pigs and cattle [14,25]. However, the 43.6% reported here differs from the 1.7% HEV IgM rate reported among HIV patients in Ibadan, Nigeria [26]. This could be due to zoonotic infection, socioeconomic status, cultural differences, hygiene, environmental and sanitation habits.

The HEV IgM rate of 43.6% found in this study disagrees with the HEV IgM rates of 0.0%, 0.4%, 0.5%, 0.6%, 0.7% and 1.1% reported in different regions of the world, including Africa [20, 27-32]. This finding is also higher than 0.0% rate reported in France [33], and 2.6% to 33.0% ranges recorded by several authors in different regions of the world [30,34-38].

Regarding sex, seroprevalence of IgM was higher among males (56.1%) than females (43.9%); however, sex was not associated with the seroprevalence of IgM (p-value = 0.076). This observation deviated from that of Gidado et al. [20] who reported a higher prevalence of HEV IgM in females than males in Osun State. Ekanem et al. [39] showed that females had a higher prevalence rate (54.8%) than males (45.2%). Adesina et al. [40] showed no significant difference between the sexes. This could be due to male subjects being more exposed to the risk factors of HEV infection, such as working in animal farms, irrigation farming using contaminated river water, and disposal of human and animal waste.

Concerning their age ranges, seroprevalence of IgM was highest among participants who were of the age range 31-40 years (53.7%), however, age was not associated with the seroprevalence of IgM (p-value = 0.236). Those below 40 years

have the highest prevalence; this observation disagrees with other studies, which reported the prevalence of anti-HEV antibodies to be highest in age groups 41-50 years of age [20], 60 years [41]. Martinson et al. [42] found the highest prevalence rate in ages 16-18 years. Ekanem et al. [39] also found anti-HEV antibodies to be highest in ages 15- 18 years. Adesina et al. [40] reported the prevalence of anti-HEV antibodies to be highest in the age group 31-40 years. An age-specific antibody profile was also reported by Fix et al. [43], working in two rural Egyptian communities. These differences could be attributed to prolonged farming, age and prolonged exposure to contaminated soil since some of the non-animal handlers were farmers [20].

Concerning the marital status of the animal handlers, seroprevalence of IgM was highest among married participants (80.5%), followed by single participants (19.5%). However, marital status was not associated with the seroprevalence of IgM (p-value = 0.620). However, in the study of Ike et al.[44], it was reported that marital status and occupation were not significant risk factors for HEV infection. This signifies that the association of sociodemographic characteristics with HEV infection is location and sample-specific.

Educational qualification also showed no statistically significant association with HEV IgM seroprevalence (p = 0.356), despite higher prevalence among individuals with secondary education (58.5%). This pattern may reflect occupational stratification rather than the direct effect of education, as individuals with secondary-level education in this setting are more likely to be engaged in animal handling or informal sector activities. Previous studies have demonstrated that knowledge alone does not necessarily translate into improved hygiene practices, particularly in resource-limited settings where environmental constraints persist [45].

Similarly, employment status was not associated with HEV infection (p = 0.932), even though self-employed participants recorded the highest seroprevalence (53.7%). This finding underscores a key limitation of broad employment categories, which may obscure task-specific exposure risks. For instance, self-employment in this context may include butchering, livestock trading, or waste handling, all of which carry varying levels of exposure to zoonotic pathogens. Evidence from Krumbholz et al. [46] highlights that direct occupational exposure to animals and contaminated biological materials is a more precise determinant of HEV risk than general employment classification. Occupational exposure to animals and contaminated environments has been identified as a major risk factor for HEV infection [46].

Although variations in HEV IgM prevalence were observed across gender, age, marital status, and employment categories, these were not statistically significant. Similar findings have been reported in previous studies, suggesting that environmental exposure may play a more critical role than individual demographic factors in HEV transmission [45]. All HEV IgM-positive cases were identified among participants residing in urban areas (100%); however, this was not statistically significant (p = 0.377). This finding should be interpreted with caution, as it likely reflects sampling bias or population clustering, rather than a true urban-specific risk. In many Nigerian urban settings, particularly in rapidly expanding cities, deficiencies in water supply, sanitation infrastructure, and waste management can facilitate fecal–oral transmission of HEV. Consistent with this, studies such as Teshale et al.[45,47] have demonstrated that environmental exposure, especially through contaminated water sources, remains the dominant driver of HEV transmission, particularly in endemic regions. Therefore, the absence of a statistically significant association does not negate the importance of environmental risk but instead highlights the limitation of using “place of residence” as a crude proxy for exposure.

The analysis of animal exposure revealed that cow handlers had the highest proportion of HEV IgM seropositivity (95.1%), compared to pig handlers (4.9%), although this difference was not statistically significant (p = 0.404). While this may suggest a potential role of cattle-associated exposure, the lack of statistical significance indicates that this observation cannot be generalised beyond the study sample. In a slight contrast, the study of Oluremi et al. [21] classified 102 participants as pig handlers and 90 participants as butchers. Gidado et al.[20] reported a higher prevalence of HEV IgM among butchers, while a zero prevalence of anti-HEV IgM antibodies was observed among pig farmers.

From an epidemiological perspective, zoonotic HEV genotypes (particularly genotypes 3 and 4) are more commonly associated with pigs as primary reservoirs[15]. Therefore, the relatively lower prevalence among pig handlers in this study contrasts with findings by Oluremi et al. [21], who reported higher seroprevalence among pig handlers and butchers. This discrepancy may be explained by differences in local husbandry practices, biosecurity measures, or sample size limitations, and suggests that zoonotic transmission pathways may vary across geographic and occupational contexts.

Overall, the findings suggest that HEV exposure in this population is likely driven more by environmental and occupational conditions than by individual demographic characteristics. A consistent pattern across all variables examined is the lack of statistically significant associations, despite observable differences in subgroup prevalence. This highlights an important epidemiological principle: descriptive differences do not equate to causal relationships in the

absence of statistical support. The findings therefore suggest that: HEV transmission in this population is likely driven by shared environmental exposures, such as contaminated water, poor sanitation, and inadequate hygiene practices. Broad sociodemographic variables are insufficiently sensitive to capture true exposure pathways. Occupational risk is better conceptualised in terms of specific activities (e.g., slaughtering, carcass handling, waste disposal) rather than general job categories.

The absence of significant associations may also reflect limited statistical power, particularly if subgroup sample sizes were small. Additionally, the cross-sectional design restricts causal inference, and the reliance on categorical variables may have masked more nuanced exposure gradients. Future studies should incorporate larger sample sizes to improve statistical power, multivariate regression models to control for confounding variables, environmental and behavioural assessments, including water sources, sanitation practices, and personal hygiene and molecular characterisation of HEV strains to better understand zoonotic transmission dynamics.

In summary, this study demonstrates that while variations in HEV IgM seroprevalence exist across sociodemographic and occupational groups, none of the examined factors was statistically significant predictors of infection. This underscores the likelihood that HEV transmission among animal handlers in Calabar is primarily driven by environmental and behavioural factors rather than individual demographic characteristics, highlighting the need for targeted public health interventions focusing on sanitation, hygiene, and occupational safety.

5. Conclusion

The seroprevalence of IgM (43.6%) among animal handlers is an indication of considerable recent exposure to Hepatitis E virus (HEV). This could be attributed to frequent contact with contaminated animal tissues and fluids. None of the socio-demographic characteristics (sex, age, place of residence, marital status, level of education, employment status, and animal type handled) was statistically associated with the seroprevalence of anti-IgM.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors claim that there are no conflicting interests.

Statement of ethical approval

All authors declare that all experiments have been examined and approved by the University of Port Harcourt Research Ethics Committee. Therefore, the study is performed following the ethical standards.

Statement of informed consent

All authors declare that informed consent was obtained from all individual participants included in the study.

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