



Hepatitis E Virus among Animal Handlers and Non-Animal Handlers in Osun State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors IOO, FAO and OOO designed the study, performed the statistical analysis and wrote the protocol. Authors IRG and FAO managed the analyses of the study. Author IOO managed the literature searches and wrote the first draft of the manuscript. Author OOO supervised the whole study which, author IRG used as part of her M.Sc. Dissertation in the Department of Medical Microbiology & Parasitology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Increase in the epidemiological information is important for effective control of hepatitis E virus (HEV). This study was conducted to determine the prevalence of HEV among butchers, pig handlers and non-animal handlers in Osun State, Nigeria.

Study Design: Cross-sectional study.

Place and Duration of Study: Molecular Biology Laboratory, College of Health Sciences, Ladoke Akintola University of Technology, Isale Osun, Osogbo, Nigeria, between June 2015 and July 2019.

Methods: A total of 180 blood samples were obtained and screened for HEV from cohorts of 90 animal handlers (69 butchers and 21 pig handlers) and 90 non-animal handlers. Questionnaires on HEV were administered to obtain a demographic characteristic of the participants. Anti-hepatitis E viruses were also screened using HEV ELISA kit.

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Results: Results showed an overall prevalence of HEV to be 21.7%. The rate of anti-HEV IgG/IgM antibodies was higher among butchers (27.5%), followed by non-animal handlers (18.9%) and was least among the pig handlers (14.3%) while the two IgM positive persons were butchers and non-animal handlers. However, 39(21.7%) of the 180 samples were positive for either anti-HEV IgG antibodies (37/180, 20.6%) or anti-HEV IgM (2/180, 1.1%). Also, the rate of anti-HEV IgG antibodies was higher among butchers (26.1%), followed by non-animal handlers (17.8%) and the pig handlers (14.3%) had the least. The two IgM positive persons were butchers (1.4%), non-animal handlers (1.1%) and pig handlers had a zero prevalence. There was no statistical significance in the prevalence of HEV IgG and HEV IgM in animal handlers as compared to non-animal handlers ($P > 0.05$). One (1.4%) of the butchers and 1 (1.1%) of non-animal handlers showed evidence of recent HEV infection by being positive to HEV IgM. Sources of drinking water were the only HEV predisposition factor for HEV ($P = 0.023$).

Conclusion: This study reported an acute HEV infection in a butcher and a non-animal handler in Osun State, Nigeria. No prevalence rates of acute HEV infection was observed among pig handlers in Osun State, Nigeria. The study also showed a low prevalence of anti-HEV IgG antibodies among these study populations. Proper hygiene is recommended for further reduction in HEV transmission in Nigeria.

Keywords: HEV; animal handlers; non-animal handlers; prevalence; Nigeria.

1. INTRODUCTION

Hepatitis E virus (HEV) infection is an important public health problem [1]. It is a major public health concern in low-income countries, yet incidence and prevalence estimates are often lacking (2). HEV infection has been identified as a major cause of enterically transmitted acute sporadic hepatitis [1] in Nigeria especially in the adult age group. Viral hepatitis is the major cause of acute/chronic liver infection and inflammation. The acute liver disease can be caused by Hepatitis A, B, C, D, E, F and G viruses [3, 4].

HEV is an enteric virus which could be transmitted through the faecal-oral route [5]. The last decade has witnessed tremendous change in our understanding of the virus in its epidemiology, clinical features, diagnostic approaches, treatment options and the need for vaccination [1]. The virus causes an estimated 20 million infections annually across the globe, leading to over 3 million symptomatic cases and acute cases of HEV occur globally with an estimation of 56,600 deaths [6-9]. It has been proved beyond doubt that HEV can also cause chronic hepatitis in the immunocompromised hosts and this has caught the Western world off guard [10]. As per the World Health Organization (WHO) estimates, there were approximately 44,000 hepatitis E-related deaths reported in 2015 [11]. In the developed countries, genetic similarities between human HEV strains and those isolated from pigs, cows, chickens, rabbits, rats, and fish have been observed [12-14].

Animals create a key reservoir for Hepatitis E infection and lead to a rise in the prevalence of the disease [14]. Populations that are at higher risk are farmers, animal butchers, veterinarians and people taking care of animal products or consumption of uncooked or raw meat from deer, wild boar and pig contaminated with HEV [15-17]. Pigs are the most important reservoir for HEV and are considered the main reservoir of zoonotic HEV [18,19]; hence contact with pigs may facilitate the possibility of zoonotic infection. But several independent studies provided recent serological and molecular evidence of HEV circulation in cattle and goats [14, 20-29]. The presence of HEV has also been established in cow products and this could also spill to the population informing the need to determine the prevalence in butchers [14]. Also, similarities in HEV RNA in pigs and that isolated from a human in some regions have been established.

Outbreaks of HEV usually occur in countries where there are limited resources such as limited access to water, poor sanitation and hygiene, and inadequate health services. Mostly during the rainy season when water that is fit for drinking most have been contaminated by flooding or with the disposal of faecal waste. In rural undeveloped areas, outbreaks commonly take place through water channel that crosses along with the soil that has been contaminated with human faeces [30].

In 2001, a vaccine based on recombinant viral proteins was developed in the 1990s and tried in

a high-risk populace in Nepal [31]. The vaccine seemed to be effective and safe, but development was stopped for the absence of cost-effectiveness since HEV is rare in advanced countries [32]. After an additional year of examination and scrutiny by China's State Food and Drug Administration (SFDA), HEV vaccine called HEV 239 was developed and made available for prevention of HEV in 2012 [33]. Owing to the absence of evidence, the World Health Organization (WHO) made no recommendation concerning its routine use as of 2015 and added that national authorities may decide to use the vaccine based on their local epidemiology [34].

In Nigeria, there exists a dearth of information on the prevalence and circulation of HEV infection in the population. This study aims to determine the prevalence of HEV among animal handlers (butchers and pig handlers) and non-animal handlers.

2. MATERIALS AND METHODS

2.1 Study Area

This study was carried out among butchers, pig handlers and the non-animal handlers in Ife, Sekona and Osogbo, Osun State, South-Western Nigeria. The laboratory analysis was carried out in the Molecular Biology Laboratory, Ladoke Akintola University of Technology, Isale Osun, Osogbo, Nigeria (Fig. 1).

2.2 Study Design

The study participants comprised of people whose occupation increased their risk of infection (both male and female). The study participants included 69 cow butchers, 21 pig handlers and 90 non-animal handlers, making a total of 180 volunteers. Extensive efforts were made to ensure high participation rates these included; advocacy visits to and sensitization talks with the head of each slab in the state, encouraging the people to participate.

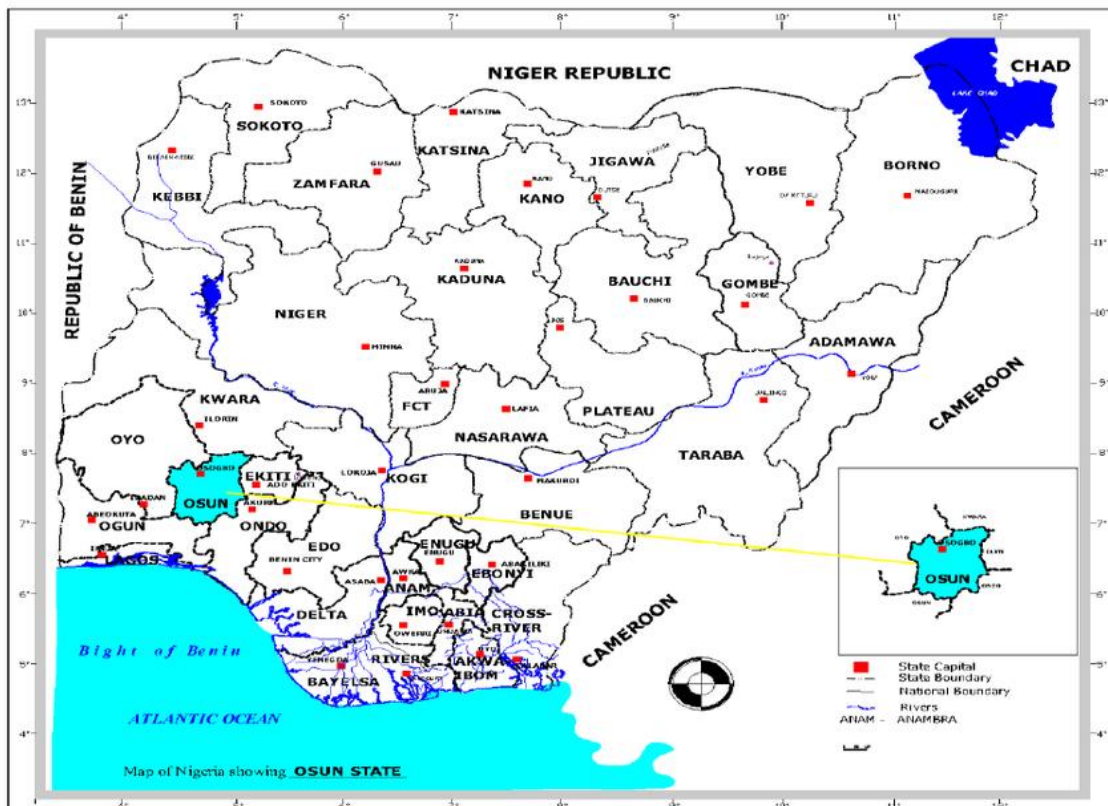


Fig. 1. Map of Nigeria showing Osun State (The Study Area)

2.3 Sample Collection

Five (5ml) of venous blood was collected from each participant into EDTA bottles. Plasma samples were separated from the freshly collected blood into Eppendorf tubes by spinning at 3000rpm. The plasma samples extracted were stored at -20°C until they are ready for analysis. Whole blood was used for serology. A well-structured questionnaire based on demographic characteristics such as age, sex, marital status, occupation, and educational level was used. Hepatitis E possible associated risk factors and behavioural characteristics such as the previous history of hepatitis, the source of drinking water, type of toilet, personal hygiene, waste disposal, multiple sexual partners, blood transfusion, interaction with animals, consumption of alcohol and past surgery were recorded.

2.4 Detection of HEV IgG and IgM Antibodies

HEV IgG and IgM antibodies were screened using the serum samples. The test was carried out using AccuDiag™ enzyme-linked immunosorbent assay (ELISA) kits for The ELISA kits were manufactured by Diagnostic Automation Inc, USA. Testing was carried out according to the manufacturer's instructions.

2.5 Statistical Analysis

Data were analyzed using SPSS version 17 to compare HBV/HEV positive and negative samples and pier-sons chi-square method was used and the level of significance was set at $p < 0.05$ at 95% confidence interval.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Social demographic characteristics of study participants

The analysis was performed on one hundred and eighty (180) blood samples from healthy individuals in Osun State, Nigeria. Sixty-nine of them was butchers, twenty-one were pig handlers and ninety were non-animal handlers. Social demographic characteristic shows that age 31-40 years had the highest number of participant, male (62.2%) were more than the female (37.8%), married people (77.2%) participated more than unmarried (21.1%), butchers (38.3%) had the highest number of

participant, and the highest level of education of the majority of the participant was secondary (37.8%). Knowledge about HEV was generally low among the participants (3.3%). Table 1 showed the social-demographic characteristics of study participants.

3.1.2 The general prevalence of HEV

The study shows the prevalence of HEV among the participant was 21.7% (Table 2). The rate of anti-HEV IgG/IgM antibodies was higher among butchers (27.5%), followed by non-animal handlers (18.9%) and was least among the pig handlers (14.3%) while the two IgM positive persons were butchers and non-animal handlers. However, 39(21.7%) of the 180 samples were positive for either anti-HEV IgG antibodies (37/180, 20.6%) or anti-HEV IgM (2/180, 1.1%) as shown in Table 2.

3.1.3 Prevalence of anti-HEV IgG and anti-HEV IgM

The overall prevalence of anti-HEV IgG and anti-HEV IgM antibodies in the study population was 20.6% and 1.1% respectively. The rate of anti-HEV IgG antibodies was higher among butchers (26.1%), followed by non-animal handlers (17.8%) and the pig handlers (14.3%) had the least. The two IgM positive persons were butchers (1.4%), non-animal handlers (1.1%) and pig handlers had a zero prevalence (Table 2). There was no statistical significance among the study groups ($p = 0.329$ and $p = 0.875$).

3.1.4 Sex and age distribution of anti-HEV antibodies

Table 3 shows the distribution of HEV among the age and sex of the participant. In the overall, higher prevalence of HEV was found among males (23.2%) than females (19.1%). However, males (22.3%) had a higher prevalence of anti-HEV IgG antibodies than their female counterparts (17.6%) while females (1.5%) had a higher prevalence of anti-HEV IgM antibodies than the males (0.9%). Age-specific prevalence of HEV revealed a higher prevalence in age groups 41-50 years (32.0%) compared to other age groups. In term of specific antibodies, higher prevalence of anti-HEV IgG antibodies occurred in age groups 51 years & above (22.3%) while the higher prevalence of anti-HEV IgM antibodies was observed in the age group 41-50 years (2.9%) than in other age groups (Table 3).

3.1.5 HEV predisposing factors among participants

The source of drinking water as a risk factor was associated with HEV seroprevalence with a highly significant difference ($p = 0.02$) with anti-HEV IgG antibodies. The results revealed that subjects who use tap and

river as a drinking water source had the highest prevalence followed by well and sachet and then all water. Although results portray no statistically significant association with, frequent washing of hands after, rearing of animal, type of toilet, eating of pork, consumption of grilled meat and cow skin ($p > 0.05$) (Table 4).

Table 1. Social demographic characteristics of study participants

	Variables	Frequency	Percentage (%)
Age groups	≤20	25	13.8
	21-30	40	22.2
	31-40	48	26.7
	41-50	25	13.9
	≥50	42	23.3
Sex	Male	112	62.2
	Female	68	37.8
Marital status	Married	139	77.2
	Single	38	21.1
	Divorce	3	1.7
Occupation	Farmers	29	16.1
	Pig handlers	21	11.7
	Butchers	69	38.3
	Civil servant	3	1.7
	Students	27	15.0
	Others	30	16.7
Education	Primary	36	20.0
	Secondary	68	37.8
	Tertiary	15	8.3
	Vocational	36	20.0
	Non	25	13.9
Knowledge on HEV	Yes	6	3.3
	No	174	96.7

Table 2. Prevalence of HEV IgG and HEV IgM among the groups

Variables		Butchers %	Pig handlers %	Non-animal handlers %	Total %	p- value
Overall	Positive	19 (27.5)	3 (14.3)	17 (18.9)	39 (21.7)	
	Negative	50 (72.5)	18 (85.7)	73 (81.1)	141 (78.3)	
HEV IgG	Positive	18 (26.1)	3 (14.3)	16 (17.8)	37 (20.6)	0.329
	Negative	51 (73.9)	18 (85.7)	74 (82.2)	143 (76.4)	
HEV IgM	Positive	1 (1.4)	0 (0.0)	1 (1.1)	2 (1.1)	0.875
	Negative	68 (98.6)	21 (100.0)	89 (98.9)	178 (98.9)	

Table 3. Sex and age distribution of HEV

Variables		No. tested (%)	No. positive (%)	HEV IgG %	HEV IgM %
Sex	Male	112(62.2)	26(23.2)	25 (22.3)	1 (0.9)
	Female	68(37.8)	13(19.1)	12 (17.6)	1 (1.5)
Age (years)	<20	25(13.8)	3(12.0)	3 (13.0)	0 (0.0)
	21-30	40(22.2)	7(17.5)	7 (17.5)	0 (0.0)
	31-40	48(26.7)	11(23.0)	10 (20.8)	1 (2.5)
	41-50	25(13.9)	8(32.0)	7 (20.0)	1 (2.9)
	51 and above	42(23.3)	10(24.0)	10 (22.3)	0 (0.0)

Table 4. HEV predisposing factors among participants

Variables		Butchers %		Pig handlers %		Non-animal handlers %		p-value
		Pos	Neg	Pos	Neg	Pos	Neg	
Type of drinking water	Tap	7 (30.4)	16(69.6)	0(0.0)	3(100.0)	1 (10.0)	9 (90.0)	0.023
	Borehole	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(20.0)	4 (80.0)	
	Well	3 (25.0)	9 (75.0)	2(16.7)	10 (8.3)	5(15.2)	28(84.8)	
	River	0(0.0)	0(0.0)	0(0.0)	1(100.0)	7(20.0)	28(80.0)	
	Spring	0(0.0)	0(0.0)	0(0.0)	1(100.0)	1(25.0)	3 (75.0)	
	Pure water	5 (23.8)	16(76.2)	1(33.3)	2 (66.7)	1(33.3)	2 (66.7)	
	All water	3 (30.0)	7 (70.0)	0(0.0)	2(100.0)	0(0.0)	0(0.0)	
Type of toilet	Water closet	6 (21.4)	22(78.6)	2(13.3)	13(86.7)	1 (5.3)	70(98.6)	0.272
	Pit	9 (52.9)	8 (47.1)	1(20.0)	4 (80.0)	0(0.0)	0(0.0)	
	Short put	3 (12.5)	21(87.5)	0(0.0)	1(100.0)	0(0.0)	19(100.0)	
Frequent washing of hand	Yes	16(25.4)	47(74.6)	3(15.0)	17(85.0)	16(17.8)	74(82.2)	0.999
	No	2 (33.3)	4 (66.7)	0(0.0)	1(100.0)	0(0.0)	0(0.0)	
Eating of pork	Yes	2 (20.0)	8 (80.0)	1 (6.3)	15(93.8)	0(0.0)	1(100.0)	0.686
	No	16(27.1)	43(72.9)	2(40.0)	3 (60.0)	16(18.0)	73(82.0)	
Rearing of animal	Yes	5 (20.0)	20(80.0)	1 (6.3)	15(93.8)	12(20.3)	47(79.7)	0.141
	No	13(29.5)	31(70.5)	2(40.0)	3 (60.0)	4 (12.9)	27(87.1)	
Consumption of grilled meat	Yes	17(41.5)	24(58.5)	0(0.0)	16(100.0)	4 (21.1)	15(78.9)	0.998
	No	1 (3.6)	27(96.4)	3(60.0)	2 (40.0)	12(16.9)	59(83.1)	
Consumption of cow skin	Yes	18(27.3)	48(72.7)	0(0.0)	3 (100.0)	4 (22.2)	14(77.8)	0.299
	No	0(0.0)	3(100.0)	3(16.7)	15 (83.3)	12(16.7)	60(83.3)	

3.2 Discussion

Research done globally on hepatitis E virus (HEV) infection is far fewer compared with other types of hepatitis virus infection [35]. Little is known on the prevalence of HEV in Nigeria. This study showed an overall prevalence of HEV to be 21.7%. This is higher than the 12.2% overall prevalence of anti-HEV reported by Odaibo and Olaleye [36] in Ibadan, Nigeria, the 15.0% and 3.8% reported previously for anti-HEV total and IgM antibodies, respectively in Osun State, Nigeria [2]. The rate of anti-HEV IgG/IgM antibodies was higher among butchers (27.5%), followed by non-animal handlers (18.9%) and the least was among the pig handlers (14.3%) while the two IgM positive persons were butchers and non-animal handlers. Diagnosis of HEV infection is based on the detection of anti-HEV IgM, anti-HEV IgG, and HEV RNA. Specifically, the presence of anti-HEV IgM is a marker of acute HEV infection [37]. Thirty-nine (21.7%) of the 180 samples were positive for either anti-HEV IgG antibodies (37/180) or anti-HEV IgM (2/180). The 37/180 positive samples reported for anti-HEV IgG antibodies in this study is higher than the 20/180 positive samples reported by Odaibo and

Olaleye [36] in Ibadan, Nigeria. The rate of anti-HEV IgG antibodies was higher among butchers (26.1%) and the two IgM positive persons were butchers and non-animal handlers. The 2/180 positive samples reported for anti-HEV IgM antibodies compared favourably with that reported by Odaibo and Olaleye [36] in Ibadan, Nigeria. Although this rate is higher than the previously reported among other populations in the industrialized countries [38-40], and some population groups HEV endemic areas of Africa [36, 41] and Asia [33], interestingly, it is lower than the previously reported rate of 44.0% among Health workers in Nigeria [42].

The anti-HEV IgG antibodies prevalence of 20.6% found in this study agrees with 17.8% reported by Meseko *et al.* [43] in Lagos but higher than 13.5% reported by Adesina *et al.* [44] in Ekiti State and lower than 28.6% reported by Adjei *et al.* [45] in Ghana and 45.5% reported by Junaid *et al.* [46] in Plateau State, Nigeria. The prevalence of anti-HEV IgG antibodies (14.3%) among pig farmers in this study was similar to 14.1% reported by Caron and Kazaji [47] in Gabon among pregnant woman. Our finding is higher than the 7.7% reported by Ekanem *et al.*

[48] in Cross Rivers State, Nigeria, the 4.6% reported by Buti et al. [49] in the Northeast of Spain, which is much lower than that obtained from this study and the 8.3% observed by Adesina et al. [44] in pregnant women in Ekiti, Nigeria.

Also, in this study, a higher prevalence of anti-HEV IgG antibodies was observed among butchers (26.1%), followed by non-animal handlers (17.8%) and pig handlers (14.3%). This is in disagreement with the 65.0% that Bansal et al. [50] reported among pig handlers in Punjab, India. It is also lower than the 50.3% reported in China [51], the 54.0% reported in Western India [52], the 96.50% reported in Lucknow, India [53]. Several other studies have reported the seropositivity for anti-HEV IgG in the range of 6.1–7.2% in Belgium, 31.0–65.0% in France, 49.8% in Germany, 68.0% in the Netherlands and 87.0% in Northern Italy [50, 54–55]. Tritz et al. [19] reported that the prevalence of anti-HEV IgG antibodies was higher in cattle farmers (59.1%) than in non-animal handlers (43.9%) in Lao People's Democratic Republic (Lao PDR). Both values were higher than the values reported in our study.

Further analysis showed that the value reported for anti-HEV IgG antibodies in this study differs from that of Ifeora et al. [56] who reported a prevalence of 10.8% and 65.7% in human and animals, respectively in Oyo and Anambra States, Nigeria. It is also higher than the prevalence of 8.0% reported by Hodges et al. [57] in Sierra Leone. It is also higher than 0.9% reported by Colak et al. [58] in Turkey. However, the 20.6% reported in this study is lower than the 78.0% reported by Goumba et al. [59] during an epidemic of HEV infection in Bangui, Central African Republic; the 84.3% that Sonia et al. [60] observed in Egypt and the 60.48% that Bansal et al. [50] reported among animal handlers in Punjab, India. This disparity could be due to a difference in personal hygiene and immunity. Turkey is a country in the European Union, and socioeconomic conditions there are better than those in Nigeria [48].

A higher prevalence of anti-HEV IgG antibodies was found in the Netherlands [61], the United States [62] and China [63] among the swine breeders, farmers and veterinarians. In Taiwan and the USA, seropositivity for anti-HEV IgG was 8.0% and 18.0%, respectively, in the general population and as high as 27.0% and 26.0% in swine handlers [62, 64]. Similarly, seropositivity

was 33.5% in South Korea and 94.1% in India for anti-HEV IgG among slaughterhouse workers and swine farmers [50, 55, 65]. Other studies outside Nigeria has reported IgG seroprevalence of 51.8% in Vientiane Capital and rates between 8.9% and 77.7% in South-East Asia [19, 66–69]. Contact with sewage without or with minimum self-protection gear could also be possible reasons for higher anti-HEV seropositivity in developing countries [50, 70].

Also, the 20.6% prevalence reported for anti-HEV IgG antibodies in this study is higher than what was reported in other populations (normal human blood donors) from developed countries such as 18.0% in the USA [62], 16.8% in Germany [71] and 10.0% in the United Kingdom [72]. This indicates that countries with hygienic potable water availability may have another route for occupational and foodborne transmission of HEV infection. It is also possible that the occupational exposure risk population could acquire this infection from HEV-infected pigs [50].

In this study, an anti-HEV IgM antibody was detected in 1.1%. The results of this present study are consistent with the 1.3% reported by Meseko et al. [43] in Lagos but lower than 32.2% reported by Nim et al. [73] in North India. The results of the present study are in agreement with previous studies where anti-HEV IgM was detected in only 0.5% subjects [55], 0.4% and 0.0% among community dwellers in two different geographical regions of Nigeria [56], 0.67% in North India [73] and 0.80% slaughterhouse workers in Punjab, India [50]. Our finding is in similarity with the rates of 0.9% reported in a study among different populations in Plateau State, Nigeria [46]. However, it differs from the 1.7% anti-HEV IgM antibodies rate reported in Ibadan, Nigeria [36]. This could be due to zoonotic infection, social-economic status, cultural differences, hygiene, environmental and sanitation habits.

In the same vein, higher prevalence of anti-HEV IgM antibodies was observed among butchers (1.4%) followed by non-animal handlers (1.1%) while a zero prevalence of anti-HEV IgM antibodies was observed among pig farmers. The anti-HEV IgM antibodies rate of 1.1% found among the study populations in this study is in disagreement with the anti-HEV IgM antibodies rates of 0.4%, 0.5%, 0.6% and 0.7% reported in different regions of the world including Africa [56, 74–76]. This finding, though higher than 0.0%

rate reported in France [77], is lower than the anti-HEV IgM antibodies rates (2.6% to 33.0% ranges) recorded by several authors in different regions of the world [45, 56, 58, 78-80].

Though varied rates of HEV IgM have been reported in other endemic regions of the world among different populations [81, 82]. The reasons for this discrepancy may range from variation in populations studied, differing cohort selection criteria, test kits used and disparities in sensitivity and specificity between commercial antibody detection kits, among others [19, 83, 84]. Variation in the performance of different ELISA kits employed in these studies may also account for the difference in rates reported [56].

Currently, there is no gold standard for the detection of anti-HEV antibodies and in particular, the results of IgM assays diverge [19, 84]. It is not surprising that our study found anti-HEV IgM antibodies in 1.1% of the participants, keeping tides with the prevalences of 0.6%– 0.9% reported in blood donors using other commercial ELISAs [85, 86] and in divergence with 17.5% reported in other participants by Tritz et al. [19]. Generally, anti-HEV IgM antibodies disappear and become undetectable 4–8 months after acute infection, but this detection period may again depend on the detection assay, as well as the immune response capacity of the host [19].

In line with previous studies [19, 87], seropositivity of anti-HEV antibodies increased significantly with age. Anti-HEV IgG and IgM antibodies-positive rates appeared to increase with age [19]. Age was significantly associated with the prevalence of the anti-HEV antibody in this study. Those above 50 years of age have the highest prevalence; this is in agreement with a similar study which reported the prevalence of anti-HEV antibodies to be highest in age 60 years [88]. This is not only because of cumulative lifetime exposure to HEV but also because animal handling is particularly popular among the elderly [19]. However, Martinson et al. [89] found the highest prevalence rate in ages 16-18 years. Ekanem et al. [48] also found anti-HEV antibodies to be highest in ages 15- 18 years. Adesina et al. [44] reported the prevalence of anti-HEV antibodies to be highest in ages 31-40 years. Age-specific antibody profile was also reported by Fix et al. [90], working in two rural Egyptian communities. These differences could be attributed to prolong farming, age and prolonged exposure to contaminated soil since

some of the non-animal handlers were farmers. Arrankalle et al. [91] speculated that age-specific antibody profile might be due to the increased exposure to HEV in young adults through exposure to high-risk environments through work and consumption of high volumes of contaminated food and water.

Likewise, the male showed a higher prevalence rate of HEV as compare to females, this is in agreement with that found in other studies [49, 19, 59]. This is because men are more likely than women to keep animals and to engage in more risk-associated activities [19]. However, this deviated from other previous studies in Nigeria. Ekanem et al. [48] showed that Females had a higher prevalence rate (54.8%) than the males (45.2%). Tritz et al. [19] reported that anti-HEV IgG seroprevalence was higher in the male gender and increased significantly with age. 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. Adesina et al. [44] showed no significant difference in both sexes. This could be because both sexes live in the same endemic environment and are exposed to the same predictors of the infection.

Interestingly, anti-HEV antibodies were also detected in non-animal handlers from the same locations where no anti-HEV IgM antibodies were detected in pig handlers. There could be several likely explanations for this discrepancy. First of all, this study may have missed out an indication of viral circulation owing to the limited size of the cohorts of pig handlers. Furthermore, besides pigs, other susceptible animals [22, 92-94] may be hosts of zoonotic HEV in Nigeria.

The results of this present study showed that only source of drinking water was significantly associated with HEV infection ($p=0.023$) while the method of human waste disposal and method of domestic waste disposal were insignificant. This could be due to consumption and contacts with contaminated surface waters [49, 95]. This finding deviated from that of Ekanem et al. [48] who reported no statistically significance with social amenities. Tritz et al. [19] also found no association between consumption of unsafe water and a higher risk of HEV infection in their study. Contaminated water was also presumed to be the main source of zoonotic HEV infection for humans in two provinces of Lao PDR [69] and elsewhere in the world [95, 96]. However, it will

be important for the community to be educated about how HEV infection is spread, about the need for improved personal hygiene, and also about boiling drinking water [48].

The limitation of this study was that this study was unable to test for the molecular characterization of HEV. The exact source of HEV infection in this state could not be ascertained but the zoonotic transmission is the high index of suspicion since anti-HEV antibodies have been detected in many animals in HEV endemic areas, and in domestic swine and rats in the United States [44]. Further studies on HEV infection is required from different cohorts such as female butchers especially the pregnant once that can transmit the virus to the foetus and also females of childbearing age to determine the rate at which animal handlers contribute to the spread of these infections. This and the knowledge gap between animal handlers and non-animal handlers should be addressed in the future.

4. CONCLUSION

Butchers were at higher risk of HEV infections irrespective of age, sex, marital status and educational status. Extensive exposure to the animals based on occupation could be responsible for the increase in zoonotic HEV infections than in population who are not exposed to contact with animals but may serve as a source of infection to others. More so, the consistent provision of contaminants-free drinking water should be available for consumption. This study was able to show that there is an acute HEV infection in a butcher and a non-animal handler Osun State, Nigeria, and reported zero prevalence rates of acute HEV infection among pig handlers in Osun State, Nigeria. The study also showed a low prevalence of anti-HEV IgG antibodies among these study populations. This can serve as a source for the transmission of these viruses among their family as well as the community. Proper hygiene is recommended for further reduction in HEV transmission in Nigeria.

CONSENT

All authors declare that written informed consent was obtained from the patient (or other approved parties) for publication of this study. The administered questionnaire was filled by all consenting individuals before sample collection; those who could neither read nor write were assisted.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the Osun State Ministry of Health, Osogbo, Nigeria (OSHREC/PRS/569T/3), and have, therefore, been performed following the ethical standards laid down in the 1964 Declaration of Helsinki. Animal Ethic committee approval has been taken to carry out this study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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