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# Serological Evidence and Risk Factors Associated With Hepatitis E Virus Infection in Pigs and Human at an Intensive Piggery Complex, Lagos Nigeria

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## Abstract

Hepatitis E virus is a leading cause of acute and chronic liver failure, and pigs are considered natural reservoir host of zoonotic genotypes 3 and 4. The zoonotic risk of HEV among occupationally exposed individuals is a public health concern especially in developing countries. This paper describes evidence and risks associated with HEV in an intensive pig farm estate in Lagos Nigeria.

In a cross sectional study, blood samples were conveniently collected from populations of pigs and pig handlers along with a questionnaire survey. Sera obtained were tested for anti-HEV IgG and IgM antibodies by two step double antigen sandwich ELISA using Hep.EV ELISA kit according to manufacturer's protocol.

Two hundred and twenty one and 73 sera were obtained from pigs and handlers respectively. Two hundred and twelve (97%) swine, and 13 (17.8%) human were positive for anti-HEV IgG. Similarly, 3 (1.4%) and 1 (1.3%) of swine and human sera were positive for anti-HEV IgM. This study shows evidence of HEV in the study populations and emphasizes its zoonotic risk among pig handlers. Improvement in biosecurity practices including sanitation and proper animal waste disposal is strongly recommended as part of control measures against HEV.

**Keywords:** Hepatitis E infection; Pig reservoir; Risk factors; Humans; Nigeria

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## Introduction

Hepatitis E virus (HEV) has been shown to cause chronic liver failure in human and was first visualised and described by Balayan and co-workers in 1983 [1]. However, the first animal strain of HEV, designated swine HEV, to be isolated and characterized was in pigs in 1997 in Illinois, United State of America [2]. Genomic sequences of HEV circulating in swine were reported to be highly similar to strains commonly infecting human hosts based on nucleotide identity, especially genotype 3 and 4 with the ability to cross interspecies barrier [3, 4]. Previous studies in Europe, Asia and the United States also demonstrated HEV infections in occupationally exposed pig farmers and farm attendants with seroprevalence rates between 1-16%. High case-fatality of about 25% has been described in pregnant women who have higher risk of infection [5-7]. In HEV infections, serological diagnoses have described prevalence at the human-animal interface particularly of genotypes 3 and 4 that are known to be zoonotic. Earlier serology and virological investigations in Nigeria described HEV prevalence in human and identified genotype 3 (human/swine strain) and I (human strain) in populations [8, 9].

Though zoonotic HEV is usually transmitted via feco-oral route and exposure to excretions from pigs in poor sanitary settings, these include pig to pig transmission as well as transmission from pig to human who had contact with pigs [4]. Nevertheless, data on the prevalence of HEV in animal reservoir hosts and at the human-animal interface in Nigeria is scanty. This is despite large scale intensive pig husbandry operations where fecal wastes are poorly managed. Evidence and data on HEV infection either through serological detection or pathogen identification that may

or not result in illness in pigs and human who are occupationally exposed in Nigeria is important because of associated zoonotic risk and for planning appropriate control measures at the human-animal interface.

# Materials and Method

## Study population

An intensive pig farm estate in Southwest Nigeria situated at latitude: 6.413207 N and longitude: 3.193127E was selected for this study. Considering the high density of pigs in this location within an urban setting with extensive human-animal contact, that may results in possible exposure to zoonotic HEV. The farm estate has over 5000 individual farms with pig population of more than 800,000 breeders, weaners and growers [10]. About 15 finisher pigs are usually selected for daily slaughter in the farm slabs whereas the bulk of the animals are traded life at local and international markets. In this location, pigs are intensively reared in closely built blockhouses with concrete floors and corrugated zinc or asbestos roofing sheets. Each pen is fitted with effluent drains that are linked to other pens and to a central water canal flowing through the city. Many years of poor maintenance resulted in dilapidated structures and broken drains such that waste water and effluents litter farm houses and its environment. The intensity of swine husbandry activities coupled with limited water supply also makes cleaning and disinfection difficult. Subsequently pig handlers, most of who do not wear personal protective clothing are exposed to pig excretion. This study was conducted between January to June 2012 for the purpose of detecting antibodies to HEV in both pigs and pig handlers in the intensive pig farm estate in Nigeria.

## Sample collection

About 5 ml of whole blood was collected from 221 pigs that were presented for slaughter at the slabs in the piggery estate. Sampling was staggered over six months and conveniently collected based on availability and cooperation from butchers. Blood samples collected in centrifuge tubes were allowed to clot at room temperature (26°C) and each sample was separated into a sterile 2 ml Eppendorf tube for storage at -20°C until ready for analyses. Human participants were recruited by voluntary consent following ethical approval obtained from the University College Hospital ethics committee. Only individuals who had regular contact with pigs by their job description in the farm estate were eligible for sampling. They were individually bled, taking 5 ml of blood into heparinised bottle. Information on demographic characteristics, biosecurity practices and other associated risk factors were collated using structured questionnaire and oral interview.

## Laboratory assay

Swine and human sera were tested for anti-HEV IgG and anti-HEV IgM by Enzyme Linked Immuno-Sorbent Assay (ELISA) kit sourced from Wantai Biological Pharmacy Enterprises Co. Ltd. China and following manufacturer's instructions. To each well of pre-coated microtitre plate containing 200 µl of diluents, 10 µl of serum sample was added and the microplate was incubated for 30 minutes at 37°C. Blank solution, non-reactive and reactive controls were included for each plate. The microtitre plate was washed six times with 300 µl of a wash solution. One hundred microliter horseradish peroxidase labeled goat anti-HEV used as conjugate was added to each well, and the micro plate incubated for 30 minutes at 37°C. Thereafter the microplate was again washed six times with 300 µl of the wash solution. Subsequently, 100 µl of substrate solution was added to each well and incubated for 15 minutes in the dark at room temperature (25-28°C). The Colour-development reaction was stopped by adding 100 µl of the stop solution to each well and the absorbance was determined at 450 nm in an ELISA plate reader.

## Interpretation of result

The test results were calculated by means of the mean OD 450 nm value of the Negative Control (NC) and a mathematical calculation using the formula: Cut-Off=NC mean OD 450 nm + 0.350. The value for each test was used for the interpretation of results as less than 0.9 (negative) 0.9 to 1.1 (equivocal) and greater than 1.1 (positive).

## Statistical analysis

Data entry, cleaning, and analysis were performed using SPSS statistical software version 16. Chi-square Tests, rates and ratios were calculated for gender, occupation, ethnicity, religion, marital status, and biosecurity practices shown in **Table 1**. The level of significance for pig farmer's characteristics was set at $P \leq 0.05$ and 96 C.I. Associations between the variables and HEV detection were compared.

## Study limitation

Considering the size of the pig farm estate, fewer pigs are slaughtered daily; most of the animals in the farm are transported live to other part of the country and neighbouring countries. Furthermore less number of samples could be collected from slaughtered pigs due to lack of cooperation from butchers. Human participants were less enthusiastic, even those that earlier consented and were recruited did not permit venepuncture despite prior sensitization for reasons of superstition.

# Results

The pig farm environment has extensive effluent drains and flows that are poorly maintained thereby exposing pigs and pig handlers to farm wastes **(Figure 1).** A total of 221 swine and 73 human sera were collected from January to June 2012. Out of 221 samples, two hundred and twelve (97%) and 13 (17.8%) of swine and human sera were positive respectively for anti-HEV IgG. Similarly 3 (1.4%) swine and 1 (1.3%) human sample were also positive for anti-HEV IgM.

The distribution of seropositive samples according to gender, job description, ethnicity, religious affiliation, and marital status of human participants are shown in **Table 1**. Statistical P value was greater than 0.05 in all observed characteristics except religious affiliation (P=0.018) at 95% confidence interval, which shows a statistically significant association.

## Discussion

The high seroprevalence of 212 (97%) HEV in swine observed in this study is comparable to observations by Cooper et al., [11], who tested 125 Mexican pigs and found 100 (80%) of them were positive for IgG anti-HEV. Similarly, in a study by Yoo et al. [12] 59.4% seropositivity was reported in Canadian pigs where 594 samples (59.4%) were found to be positive for HEV antibody with even a higher seroprevalence of 88.8 and 80.1 % within some regions. Wang et al., [13] also observed 83.3% HEV seroprevalence in swine population in China. In a previous study in Nigeria [14] a seroprevalence of 55.6% was reported in domestic pigs across the country, significantly less than is observe in this investigation that was carried out in a highly intensive pig production system. It appears farm intensification increases risk of HEV infection among pigs because of problem associated with waste management.

Findings from this study also demonstrate potential risk of Hepatitis E virus transmission from animal reservoir host to people who are involved in pig husbandry and have regular contact with secretions and excretions from these animals. The scenario may be worse by poor sanitation and hygiene, which must be considered in order to prevent zoonotic transmission of HEV at the human-animal interface [15]. Seropositive farm workers observed in this investigation suggest exposure to HEV from handling pigs that are themselves positive for HEV. Though serology alone is not sufficient to underpin the similarity of HEV strains that are detected in pigs and pig handlers because so far there is only on serotype of HEV.

The seroprevalence of 17.8% in human described in this study is higher than 13.1% reported in a similar study in Asia by Anita et al [7]. In earlier serosurveillance of randomly selected human population in Ekiti State, Nigeria, antibody to HEV was detected in 13.5% of samples analysed [9], which is lower than was observed in this study. Also, higher HEV seroprevalence of 17.8% in apparently healthy pig handlers in this investigation as against 12.2% that was observed for human immunodeficiency virus (HIV) positive individuals who are immunocompromised with underlining infection. Based on these observations, it is our hypothesis that a possible increase infection due to zoonotic HEV may be associated with extensive exposure to reservoir pigs by occupational group than populations who are not exposed or even those that may be immuno-compromised [16, 17].

Though there was no statistically significant association between age, gender and job description except religion affiliation. It is evident from observations in this study that few adherences of Islam who keep and work with pigs are less attached to the vocation as obtained from the questionnaire survey, and thus limits the frequency of contacts with the animals and subsequent degree of exposure unlike their Christian counterparts. Lack of exposure by Muslims may also be due to other underlining factors including abstinence from pork. Similarly, more pen attendants were positive for anti-HEV antibody compared to farm owners and butchers **(Table 1)** because their job requirement increases contact with pig wastes both in intensity and duration.

Pigs being the primary reservoir host of zoonotic HEV portend a greater exposure risks to occupationally exposed individual. This was also the view of World Health Organization [4] in a report on zoonotic transmission of HEV from animals to human where exposure to infectious body fluids of infected animals was a major risk factor. Globally, the highest seroprevalence rates of HEV are observed in regions where low standards of sanitation increase the risk of transmission of the virus via the feco-oral route [15]. It is therefore expedient that control and prevention of zoonotic HEV infection should target populations at the human-animal interface where there is a tendency for poor sanitary practices. This is especially important in developing countries like Nigeria that is considered endemic for zoonotic HEV especially genotype 3 that has been detected in both pigs and human in the country [4, 8, 14].

This study is one of few investigations to describe an association between HEV endemic human population and possible reservoir animals. Anti-HEV IgM antibody observed in this study also indicates some recent and active infection and that pig may continue to contribute to HEV dissemination in the environment. This may likely results in water contamination and human exposure farther from the piggery estate. Proactive measures including biosecurity and hygiene are advocated to prevent possible epidemics in the community.

**Table 1** Characteristics of pig handlers tested positive and negative for HEV-Ab.

| Variable | HEV-Ab Test | | | P-value |
|---|---|---|---|---|
| | Positive N (%) | Negative N (%) | Total | |
| **Sex** | | | | .208 |
| Female | 9 (23.1) | 30 (76.9) | 39 | |
| Male | 4 (11.8) | 30 (88.2) | 34 | |
| Total | 13 (17.8) | 60 (82.2) | 73 | |
| **Job description** | | | | .387 |
| Farm attendant | 8 (21.1) | 30 (78.9) | 38 | |
| Farmer | 3 (30) | 7 (70) | 10 | |
| Veterinary | 0 (0) | 2 (0) | 2 | |
| Butcher | 2 (8.7) | 21 (91.3) | 23 | |
| Total | 13 (17.8) | 60 (82.2) | 73 | |
| **Ethnic group** | | | | .668 |
| Yoruba | 11 (16.9) | 54 (83.1) | 65 | |
| Hausa | 0 (0) | 1 (100) | 1 | |
| Igbo | 2 (28.6) | 5 (71.4) | 7 | |
| Total | 13 (17.8) | 60 (82.2) | 73 | |
| **Religion** | | | | .018 |
| Christianity | 13 (24.1) | 41 (75.9) | 54 | |
| Islam | 0 (0) | 19 (100) | 19 | |
| Total | 13 (17.8) | 60 (82.2) | 73 | |
| **Marital status** | | | | .197 |
| Single | 2 (8.0) | 23 (92.0) | 25 | |
| Married | 11 (23.9) | 35 (76.1) | 46 | |
| Widowed | 0 (0) | 2 (100) | 2 | |
| Total | 13 (17.8) | 60 (82.2) | 73 | |

The table shows HEV positive human handlers of pigs and their attributes; there are no statistically significant association between gender, job description, and marital status and HEV infection (p>0.5) except religion affiliation (p=0.018).

**Figure 1** Study site: Closed pens housing 50-200 pigs per pen with faecal wastes spilling around farm premises.

## Conclusion

This work contributes to data on HEV in pigs and human handlers in Nigeria. It is also one of the few studies on potential occupational transmission of HEV between reservoir pigs and humans working with pigs in the country. However, further investigation using molecular technique is suggested to determine circulating genotypes in a wider study.

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