

Full Length Research Paper

Serological evidence and public health implication of hepatitis E virus infection in pigs found in Zaria, Kaduna State

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The presence of hepatitis E virus (HEV) infection has recently been demonstrated in humans, in Nigeria. Considering the zoonotic nature of the virus and its serious public health implication, it becomes imperative to determine its presence in animal reservoirs in the study area where pig's husbandry is largely practiced. The level of awareness of the disease among pig farmers in the study area was also assessed using a structured questionnaire. The study was conducted in Zaria, Kaduna state, Nigeria. A total of 168 sera collected randomly from pigs were examined by indirect enzyme linked immunosorbent assay (ELISA). Of the total number of pigs sampled, 102 were males while 66 were females. Forty one (41) out of the total samples were positive for HEV antibodies giving the overall seroprevalence rate of the infection to be 24.4%. The prevalence rates of 31.37% (32/102) and 13.64% (9/66) were observed among males and females respectively. Among the positives, males accounted for 78.05% (32/41) while females accounted for 21.95% (9/41). Chi square test further showed a significant statistical association between sex and HEV infection ($P < 0.05$). Also of the 150 structured questionnaires administered to the adult individuals in the study area, 68% (102/150) were returned. 57.8% (59/102) reported to have consumed pork meat in the last six months, while 42.2% had never consumed it. Our results show that only 10.78% (11/102) of the respondents were aware that contaminated or under cooked pork are possible source of diseases to man. Even among the pork consumers, only 11.86% (7/59) were aware of the possibility of disease transmission from pig to man, but none of the respondents was aware of hepatitis E infection in pigs and its zoonotic potential. It was concluded that the seroprevalence of HEV is high among pigs found in Zaria. There is need to carry out a detailed molecular epidemiological study of the virus not only to determine its circulating genotypes in pigs but also the genetic relatedness of the isolates from humans and animal species in the study area. Furthermore, the lack of awareness of HEV among respondents stresses the need for extensive public health enlightenment campaign on the public health significance of the disease in the study area.

Key words: Enzyme linked immunosorbent assay (ELISA), Hepatitis E Virus (HEV), Seroprevalance, Nigeria.

INTRODUCTION

Hepatitis E is the inflammation of the liver caused by hepatitis E virus (HEV), which is an enterically transmitted virus responsible for over 50% of non-A, non-

B Hepatitis. The virus is non-enveloped, single stranded, positive sense RNA virus with a size of 27-34 nm in diameter (Emerson and Purcell, 2003).

HEV was initially classified as a member of Picornaviridae but the official IOCV taxonomy now classifies the virus under the genus Hepesvirus of the family Hepeviridae (Purcell and Emerson, 2001).

There are four known genotypes of the virus that infect man; genotype 1 (Asian cluster), genotype 2 (USA genotype), genotype 3 (Mexican genotype), and genotype 4 (Chinese-Beijing genotype) (Wang et al., 1999). Genotypes 1 and 2 affect humans only while Genotypes 3 and 4 affect both humans and animals (Okamoto et al., 2007). Genotypes 5 and 6 have been variously assigned to avian and rats hepatitis E viruses respectively (Donal et al. 2014).

Hepatitis E virus originated from the stool of a volunteer orally infected with faeces from suspected cases of non-A, non-B hepatitis (Balayan et al., 1983). In 1997, it was isolated for the first time from an infected pig in the United States and was designated swine HEV (Meng, 1997). Subsequently, several other isolates from pigs were reported from different countries (Lu et al., 2006; Feagins et al., 2007). Furthermore, many evidences suggest that pigs may serve as sources of the infectious pathogens for humans. Indeed, there were reports of high genetic relatedness between HEV isolates obtained from humans and those from swine in the same geographical regions (Inoue et al., 2006). Indeed, HEV is regarded as an emerging virus with a zoonotic potential. Apart from pigs, serological evidence of HEV was detected in various other species such as rats, dogs, cows, sheep and birds (Tien et al., 1997; Favorov et al., 1998; Arankalle et al., 2001). However, their role in the transmission of the virus to humans is still unclear (Meng et al., 1997).

For several years, immune electron microscopy remains the only diagnostic tool for the confirmation of HEV. However, in early 1990s, a variety of HEV-specific antigens in the form of recombinant proteins and synthetic peptides were produced. These antigens have been used in conventional enzyme immunoassays (EIAs) as an immobilized reagent, making the test itself suitable for efficient large-scale serologic surveys (Dawson et al., 1992). Later, molecular detection assays such as RT-PCR were used to detect HEV RNA in clinical samples (Huang et al., 2002). Shortly after, real time quantitative PCR that enables viral quantitation using fluorescence assay to amplify and monitor the target nucleotide sequences had been applied for HEV detection (Patel et al., 2003).

The increasing evidence of non-A, non-B hepatitis in humans (Buisson et al., 2000) and the dearth of information on the HEV infection among pigs in the study area pose serious concern on the epidemiology of the disease. The study was therefore designed to determine

the seroprevalence of HEV infection in pigs as well as assesses the awareness of the disease among the pig farmers in Zaria, Northwestern Nigeria.

MATERIALS AND METHODS

Study area

The samples were collected from Zaria area situated on latitude 11° 12' N and longitude 07° 37' E, at an altitude of 550 – 700 m.

Samples collection and transportation

Five milliliters (5 ml) of blood samples were collected from auricular vein of each pig using 5 ml syringe and hypodermic needles. The collected blood samples were transferred into clean plain sample bottles and transported to the Veterinary Microbiology Laboratory of Ahmadu Bello University (ABU) Zaria where serum was harvested and stored at -20°C. The samples were later transported to Veterinary Microbiology Laboratory of Usmanu Danfodio University Sokoto where ELISA test was conducted. During sample collection a total of 150 structured questionnaires on the general awareness of HEV infection were administered to the adult individuals with contact to pigs in the study area. The questionnaire was initially tested for validity and reliability before the final copy was printed and used for the study.

Antibody detection

Anti HEV antibodies in the collected sera were detected using an ELISA kit (ID Screen® Hepatitis E Indirect Multi-species ELISA) obtained from ID.VET France. The components of the kit were Microtitre plate coated with a recombinant HEV capsid antigen (bi-well format), concentrated conjugate (10X), positive control, negative control, dilution buffer 2, dilution buffer 3, Concentrated wash solution (20 x), substrate solution and stop solution (H₂SO₄, 0.5 M). The test was based on specific reaction between antibodies against HEV in the sera and Recombinant genotype 3 capsid antigen of the virus that was already coated to the microtitre plates provided in the kit. The use of genotype 3 antigens makes the test ideally suited for use in swine, as genotype 3 is the genotype which infects pigs. Furthermore, the kit detects HEV-IgG antibodies in the serum.

The test was carried out according to manufacturer's instructions. Briefly, 190 µl of dilution buffer 2 was added to each well on the microplate. Then, 10 µl of the negative control was added to the wells A1, A2, B1 and B2 of the microplate. Also, 10 µl of the positive control was added to wells C1, C2, D1 and D2. Then, 10 µl of each sample was then added in its own well. The plates were then incubated for 45 min at 21°C before they were emptied and washed 3 times with 300 µl of the wash solution. Precaution was taken to avoid drying of the wells between washings. Then 100 µl of the conjugate 1x was added to each well. The plates were again incubated for 30 min at 21°C. The plates were emptied again and each well washed 3 times with 300 µl of the wash solution. Drying of the wells was also avoided between washings. Then 100 µl of the substrate solution was added to each well and incubated for 15 min at 21°C in the dark. This was followed by the addition of 100 µl

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of Stop solution to each well which brought the reaction to stop. The plates were read visually and spectrophotometrically using microplate reader at 450 nm.

For validation and interpretation of results, the net optical density (OD) was calculated using the formulae given by the manufacturer:

That is, $OD_{net} = OD_{even\ wells} - OD_{odd\ wells}$

The test was validated by considering the net value of the OD of the positive controls which was greater than 0.350. This was found to be consistent in all the test plates. $Net\ OD_{PC} > 0.350$.

The results were interpreted using the formula given by the manufacturer as shown below:

$$S/P = \frac{Net\ OD_{sample}}{OD_{PC}} \times 100$$

Where, $Net\ OD_{sample} = O.D_{even\ well} - O.D_{odd\ well}$ and $O.D_{pc}$ is given by the average OD of all odd wells of the positive control subtracted from those of corresponding even wells.

Samples presenting (S/P %) of less than or equal to 60% were considered negative; less than 70% and greater than 60% were considered doubtful and greater than or equal to 70% were considered positive.

Statistical analysis

The data obtained was subjected to chi square test of association between the seroprevalence of HEV and sex using graphad Instat software. $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

This study is probably the first seroprevalence survey for HEV antibodies in pigs in Zaria, Kaduna State, Nigeria. Results from the study indicate that out of the 168 serum samples examined for HEV antibodies, 41 tested positive spectrophotometrically. Thus, the prevalence of HEV antibodies in pigs found in Zaria as determined by our study was 24.4%. This prevalence is comparatively lower than what was reported in previous studies carried out in Nigeria (32.8%) (Junaid et al., 2014), Thailand (30.7%) (Meng et al., 1999), United States of America (34.5%) (Withers et al., 2002), Taiwan (37.1%) (Hsieh et al., 1999), Japan (93%) (Takahashi and Okamoto, 2013), Spain (98%) (Seminati et al., 2008), Laos (46%) (Blacksell et al., 2007) and Brasil (81%) (Dos Santos et al., 2009).

However, lower prevalence rates had been reported in Mexico (6.0%) (Cooper et al., 2005), Canada (18.1%) (Meng et al., 1999) and Argentina (22.7%) (Munne et al., 2006). The discrepancies in prevalence rates across various geographical areas have been attributed to socioeconomic, cultural, hygienic and climatic conditions that characterize various areas (Junaid et al., 2014).

The major implication is that HEV actively circulates in the study area and the pigs in the area have been exposed to the virus. HEV infection is a zoonosis mainly seen in humans and pigs and since HEV is capable of

Table 1. Sex distribution of HEV antibodies in pigs found in Zaria.

Sex groups	Number Positive	Number Negative	Total	P value
Males	32 (31.37%)	70	102	
Female	9 (13.64%)	57	66	
Overall	41 (24.4%)	127	168	P=0.0151

$\chi^2=5.905$. Values in parenthesis represent the prevalence rates.

cross species infection (Meng, et al., 1998a, 1998b), chances are high that human population in the study area are at risk of the infection from the pigs especially because of the close contact that exist between the pigs and the farmers. Indeed, there are growing evidences that suggest that individuals who work with swine such as pig farmers, Veterinarians and slaughter house workers are at increased risk of acquiring HEV infection (Hsieh et al., 1999).

In addition, the pigs sampled in this study were free-roaming, scavenging for food in their environment, making the transmission of the virus easy from the infected pigs to other susceptible hosts including humans. Although, some reports had identified pig's urine and meat to play some role in the transmission dynamics of hepatitis E (Bouwkneg et al., 2009) however, consumption of raw meat of infected pigs, as well as occupations involving contact with pigs or biologic pig materials have been identified as routes of pig to human HEV transmission (Junaid et al., 2014). Therefore the serological evidence of HEV in pigs found in Zaria is of serious public health concern that requires immediate attention. Moreover, infection of porcine livestock and its relationship with the human cases has been demonstrated (Pérez-Gracia et al., 2007; Ma et al., 2013; Okano et al., 2013).

The sex distribution of HEV antibodies among the pigs sampled (Table 1) revealed that 78.05% (32/41) of the positive pigs were males while 21.95% (9/41) were females. Accordingly, the prevalence rates were 31.37% (32/102) and 13.64% (9/66) for males and females respectively.

Chi square test at 95% confidence level showed a significant statistical association between sex and HEV infection ($p < 0.05$). This findings is not in agreement with what was previously reported by Sarah et al. (2013) who found no significant difference in the Seroprevalance of HEV between male and female pigs (72.1 and 70.5%, respectively, $P\ value = 0.786$). However, in order to fully understand the role of sex in the epidemiology of the disease, further researches involving large population of pigs are needed.

Out of the total of 150 structured questionnaires administered to the adult individuals in the study area, 68% (102/150) were returned. Of the 102 returned questionnaires, 57.8% (59/102) reported to have

Table 2. Awareness of contaminated / under cooked pork as source of disease to man among Adults in Zaria, Kaduna state.

Eating habit	Number sampled	Adults aware of contaminated/under cooked pork as source of disease to man	Percentage awareness	P value
Pork eaters	59	7	11.86	**
Non-Pork eaters	43	4	9.3	
overall	102	11	10.78	>0.05

** = p is considered not significant.

consumed pork meat in the last 6 months while 42.2% had never consumed it. Our results showed that only 10.78% (11/102) of the individual responded were aware of pig as a possible source of disease to man. Even among the consumers of pork, only 11.86% (7/59) are aware of the possibility of disease transmission from pig to man (Table 2). Interestingly, none of the respondents was aware of Hepatitis E infection in pigs and its possible zoonotic potential. This information is of great public health significance as Vasickova et al. (2007) has shown that consumption of undercooked liver or intestine from pigs infected with HEV could serve as a means of spreading the virus. With the increasingly popular pig offals consumption especially among some people in the Nigeria, where the virus presumably circulates, the risk of hepatitis E infection from pigs to man is therefore on the high side.

Serious awareness campaigns on the public health significance of the disease should henceforth be advocated in all pig rearing regions of the country. What is more worrisome was the total lack of awareness of the disease among the pig farmers in the study area. Indeed, with the increasing reports of the importance of the disease as a significant cause of hepatitis in man, there is urgent need for public health authorities in Nigeria to provide serious public enlightenment campaigns on many aspects of the disease. Interestingly also, since the previous reports have epidemiologically linked cases of hepatitis E infection to consumption of undercooked pig liver (Masuda et al., 2005), such enlightenment campaigns must be directed to abattoir's workers and all those whose professional callings or trade activities expose them to various risks of contracting the disease.

African human HEV isolates have been characterized from Tunisia, Morocco (Chatterjee et al., 1997), Algeria, Chad (van Cuyck-Gandre et al., 1996) and Egypt (Tsarev et al., 1999). In West African countries, the disease has also been presumed to be endemic (Krawczynski et al., 1991). The African strains were close to the Asian genetic cluster although constituting a separate subtype within genotype I (Meng et al., 1999; Tsarev et al., 1999). However, in Nigeria, the first genetic analysis of ORF1 and ORF2 regions from human HEV isolates showed evidence of a co-existence of two HEV genotypes in Africa (Buisson et al., 2000).

Furthermore, Buisson et al. (2000) have shown that

human HEV with 96% amino acid sequence homology with Mexican prototype genotype III, circulates in southern Nigeria (Buisson et al., 2000). Such epidemiological data is lacking for swine HEV in Nigeria. Therefore, there is real need for molecular epidemiological studies on HEV in man and pigs in northern Nigeria so as to determine the circulating genotypes as well as the extent of genetic relatedness between the isolates from the two species in the country.

Conflict of interests

The authors did not declare any conflict of interest.

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