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Sero-Detection of Hepatitis E Virus (HEV) in Blood Sample from Pigs, Obtained from Katsit Pig Market

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Abstract

Hepatitis E virus (HEV) is a single stranded, positive RNA virus belonging to the Hepeviridae family. HEV infection can cause an acute hepatitis that is self-limited. However, fulminant hepatic failure can occur in patients with underlying chronic liver disease, in the elderly, and in pregnant women. Genotypes 3 and 4 (HEV-3 and HEV-4) are present in humans and other animals, and are the main cause of autochthonous cases of hepatitis E in industrialized countries. It is transmitted most often via faecally contaminated water. This research work was carried out to detect the presence of HEV by analyzing for HEV antibody (IgM) in blood samples from pigs using HEV rapid test strips. A total of 200 blood samples were collected from pigs reared in Katsit community, of which 43.5% (87) and 56.5% (113) were males and females respectively. 14 blood samples were positive for the IgM antibody to HEV consisting of 57.14% males and 42.86% females, where males had the highest prevalence of 9.20% as compared to 5.31% in females. Based on the age factor, the pigs within the age of 1year had a distribution of 50%, those within the ages of 2years and 3years presented a distribution of 28.57% and 21.43% respectively. Pigs within the age of 1year presented the highest prevalence rate of 12.07%. Considering vaccination status for other diseases, 34% of the total population (200) was vaccinated prior to sampling of which only 14.29% were positive for HEV antibody. In conclusion, from this study, there was statistically no significant relationship observed between HEV and age, sex, vaccination status for other diseases, but risk of the spread of HEV in this community cannot be overlooked. It is therefore recommended that further and more intensive studies should be carried out as well as researches in order to develop competent recombinant vaccines and drugs for the prevention and treatment of HEV both in humans and animals, with as minimal side effects as possible.

Keywords: Sero-Detection; Hepatitis E Virus (HEV); Fulminant Hepatic Failure; Vaccination; Nigeria

Introduction

Hepatitis E virus (HEV) is a single stranded, positive RNA virus belonging to the Hepeviridae family. Its genome codes for three open reading frames (ORFs) and is 7.2 kb in length. HEV is considered as the most common cause of acute viral hepatitis as well as the leading cause of enterically transmitted hepatitis worldwide (Purcell *et al.* 2008). HEV infection can cause an acute hepatitis that is self-limited. However, fulminant hepatic failure can occur in patients with underlying chronic liver disease, in the elderly, and in pregnant women. Complications and extra-hepatic manifestations of hepatitis E, such as acute pancreatitis, renal failure and neurological syndromes including Guillain-Barré syndrome, neuralgic amyotrophy or encephalitis, can also occur. (Kamar *et al.* 2016) Following the identification of these novel strains, a new classification has been proposed that divides the Hepeviridae family into two genera: Orthohepevirus and Piscihepevirus (Smith, *et al.* 2014) [1,2]. Most of the HEV strains identified so far belong to the Orthohepevirus genus that is divided into four species: Orthohepevirus A, B, C and D. Four main genotypes of HEV that belong to the Orthohepevirus A species are able to infect humans (HEV-1 to-4). Genotypes 1 and 2 (HEV-1 and HEV-2) infect only humans and are associated with large waterborne epidemics in tropical and subtropical areas. Genotypes 3 and 4 (HEV-3 and HEV-4) are zoonotic strains, and are the main cause of autochthonous cases of hepatitis E in industrialized countries.

The Hepatitis E virus is also said to be a food borne virus. Food borne and enterically transmitted viruses are, due to the absence of viral envelope, very resistant to environmental stress factors such as heat, acid, freezing, drying, lipid solvents and ultrahigh hydrostatic pressure. This last one is significant for the reason that is recently used as a novel food-processing treatment (Wilkinson *et al.* 2001; Kingsley *et al.* 2002) [3].

Huge epidemics of HEV gt1 and HEV gt2 in developing countries are caused by contact with fecally contaminated water (Kmush *et al.* 2013; Mushahwar, 2008; Scobie and Dalton, 2013) [4-6]. The hepatitis E IgM virus test detects the IgM antibodies produced by the immune system in defence against the hepatitis E virus.

Materials and Methods

Study Area

This research was carried out in Katsit, a community in Kafanchan, popularly known for its pig market. Kafanchan is located in the southern part of Kaduna State, Nigeria, 9034'N 8o18'E with an elevation of 739m (2,425ft). Principally, this area experiences two types of seasons (dry and rainy seasons) with an annual temperature of 95 °F (36 °C) and humidity of 12%. Kafanchan has an estimate population of 83,092.

Sample Collection

A total of 200 blood samples were collected in plain blood vials free from anti-coagulant, using 5mls syringes, collecting the blood from the auricular vein of each pig and the sera were aseptically separated after centrifuging, before being tested.

Antibody Detection

Antibodies were detected using hepatitis E virus diagnostic test kit (cassettes), manufactured by dialab, in accordance with the manufacturer's instructions.

Method

i. Blood samples were centrifuged at 2000rmp for 5mins to obtain the serum.
ii. Serum obtained was collected in sterile plain bottles and diluted 1000times with 0.85% as instructed in the manufacturer's manual.
iii. A pouch containing the test cassette was opened and placed horizontally on the desk.
iv. Two to three drops of the serum was added into the sample well of the cassette and observed for 10-15minutes before result was read and recorded.

Principle of Test

The test is based on the principle of immunoassay, combined with conjugated colloid gold technology. The HEV test is a diagnostic device for qualitative detection of anti-HEV IgM in human serum (or plasma) samples.

Statistical Analysis

Chi square was used as a statistical tool in analysing data obtained from the research work;

$$X^2 = \sum \left(\frac{(O-E)^2}{E} \right)$$

Where
X^2 = Chi square
Σ = Sum
E = Expected
O = Observed
$P<0.05$ were considered insignificant.

Results and Discussion

Table 1 shows the distribution and prevalence of HEV in pigs from Katsit based on sex, age and vaccination status for other diseases respectively. Out of 200 samples collected, 43.5% (87) were males and 56.5% were females. 14 were tested positive for the IgM antibody for HEV. From the population consisting of positive animals, 57.14% were males and 42.86% were females, with males having the highest prevalence of 9.20% as compared to females with a prevalence of 5.31%. Based on age, those within the age of 1year had a distribution of 50%, 2years 28.57% and 3years 21.43%. Pigs within the age of 1year presented the highest prevalence of 12.07%. Based on vaccination status for other diseases, 34% of the total population (200) was vaccinated prior to sampling. Only 14.29% of the 34% of the total population turned out positive. Pigs that had no prior history of vaccination for other diseases, presented the highest prevalence rate of 9.09% as compared to 2.94% presented by those who had been vaccinated for other diseases.

Table 2 shows the distribution of pig farmers whose pigs were sampled. 25.82% of the 58 farmers were males and 74.14% were females. Out of the female population, 18.6% were pregnant.

Sex	Number positive	Number Negative	Total Sampled
Male	8 (57.14%), (9.20%)	79 (42.47%), (90.80%)	87
Female	6 (42.86%), (5.31%)	107 (57.53%), (94.69%)	113
Age (years)			
> 1	0 (0%)	0 (0%)	0
1	7 (50.00%), (12.07%)	51 (27.42%), (87.93%)	58 (29%)
2	4 (28.57%), (5.56%)	68 (36.56%), (94.44%)	72 (36%)
3	3 (21.43%), (4.29%)	67 (36.02%), (95.71%)	70 (35%)
Vaccination status			
YES	2 (14.29%), (2.94%)	66 (35.48%), (97.06%)	68 (34%)
NO	12 (85.71%), (9.09%)	120 (64.52%), (90.91%)	132 (66%)
Total	14	186	200

Table 1: Distribution and Prevalence (%) of HEV antibody in pigs from Katsit based on Sex, Age and vaccination status for other diseases, respectively

Sample Population		Total	
Number of Male pig farmers		15 (25.82%)	43 (74.14%)
Number of Female pig farmers	Non pregnant female pig farmers	35 (81.40%)	
	Pregnant female pig farmers	8 (18.60%)	
Total number of pig farmers sampled		58	

Table 2: Population distribution of pig farmers whose pigs were sampled in Katsit

HEV infection can cause an acute hepatitis that is self-limited. However, fulminant hepatic failure can occur in patients with underlying chronic liver disease, in the elderly, and in pregnant women. HEV infection is a major public health problem in many developing countries; risks of HEV in developed countries are unknown. (Vishwanathan, 1957) Presence of viral envelope, on one hand, allow the survival of enteric viruses in the harsh environment of the mammalian gut and on the other hand promote durability in acidic, marinated, pickled, frozen or lightly cooked food. It has been shown that enteric viruses are able to survive in the environment and in food products for several months without a host (Rzezutka and Cook, 2004) [7].

Results from the research conducted shows that out of 200 serum sample screened for HEV antibody (IgM), 14 tested positive for the presence of the IgM antibody, as such, the prevalence of the HEV antibodies in the pigs sampled from Katsit, as determined by the study was 7%. When considering pigs that tested positive for the presence of the HEV antibody, 57.14% of the pigs that tested positive were males while 42.86% were females. The prevalence rate was 9.20% and 5.31% for male and female respectively.

Chi square test showed no significant statistical association with sex and HEV infection (P>1.15). This agrees with what was reported previously by Sarah *et al.* 2013 who found no significant difference in the seroprevalence of HEV between male and female pigs. Though, the role of sex in the epidemiology of the disease will not be fully understood, until further researches involving larger population of pigs are carried out (Alkali *et al.* 2015) [8,9].

Among animals sampled, prevalence was observed to decrease with increase in age. From the results, pigs around the age of 1 year presented a prevalence rate of 12.07% for those positive, pigs around 2 years and 3years, had prevalence rates of 5.56% and 4.29% respectively. From this result it can be deduced that susceptibility to contacting the infection decreases with increase in age which could also say, the younger the pig, the higher the risk of contacting the infection. This is in concordance with the work done by Wang *et al.* (Wang *et al.* 2002), thus suggesting that infection may occur early in life [10]. This could be attributed to immature immune system of the young pigs, causing an inability to properly protect the animal against the disease. After birth, the immunity obtained from the mother wanes off and is not sufficient to protect the young animal from infections in the new environment. An additional form of immunity is required. This explains why younger pigs are more predisposed to the HEV infection than older pigs.

Furthermore, 68 (34%) pigs were said to have been vaccinated for other diseases. 2 (14.29%) out of this population were tested positive for HEV-IgM with a prevalence rate of 2.94%. Those animals that were vaccinated for other diseases were less susceptible to the infection, as deduced from the study, although from Chi square statistical analysis; the association between vaccination for other diseases is almost significant but not. Vaccination against other diseases and previous exposure to other diseases suffered could be a logical explanation for reduced susceptibility to HEV. There is a possibility that vaccination with other antigens or previous infection with other strains likely conferred cross protection against subsequent infections.

Method of raring could also be a predisposing factor for the spread of the HEV infection. In most communities in Kaduna State, the method of rearing widely practice is the extensive method of rearing pigs. The pigs are allowed to roam about and scavenge for food. In the process, they wallow in dirty water and filth. Since the disease is spread by faecal contamination of either water or environment, free ranging pigs are predisposed to the infection more than those reared under intensive method. Proper waste disposal also is a very important determinant in ascertaining the susceptibility of the animal to the viral infection.

From Table 1, total number of pig farmers was 58. Out of this population, 15 (25.86%) of the population of pig farmers were men, 43 (74.14%) were women and among this population of women, 8 (18.60%) were found to be pregnant. From this population breakdown, 18.60% of the women which were found to be pregnant are more at risk of contacting the infection of HEV. In studies involving pregnant women, reported by Beniwal *et al.* 2003 and Kumar *et al.* 2004, HEV accounted for 37% of cases of acute viral hepatitis and 81% of cases of fulminant hepatitis, with more than one-fourth of affected women having obstetric complications, such as premature rupture of membranes and intrauterine growth restriction [11,12]. Fulminant hepatitis or obstetric complications is usually the cause of death among pregnant women infected with HEV during the third trimester. This could probably be as a result of increased immune tolerance, which is believed to be a major contributing factor to an increased susceptibility and severity of infections in pregnancy. Besides maternal complications, HEV also causes serious consequences to foetus following transplacental transmission which varies in severity from low birth weight, prematurity, mild anicteric neonatal hepatitis or jaundice at birth with full recover to miscarriage, stillbirth etc. Foetal infection with HEV will enhance the severity of infection and risk of liver failure in the mother (El sayed *et al.* 2014), (Kumar & Begum, 2010) [13,14]. Hence, pregnant women that rear pig may be at high risk of contacting HEV.

Conclusion

In conclusion, findings of the study suggests that the HEV infection was prevalent among the males with a prevalence rate of 8 (9.20%) out of 87 males tested, as compared to the prevalence rate in females 6 (5.31%). Prevalence of was higher in animals within the age of 1year and those vaccinated against other diseases showed lower susceptibility which can be seen, as well as low prevalence rate of 2.93%. Null hypothesis is accepted in all the cases (age, sex and vaccination status) as there is no significant statistical association with sex, age and Vaccination status for other diseases and HEV infection. Precautionary measures should be taken as the presence of HEV virus could soon mean an outbreak.

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