Evaluation of Risk Factors Associated with Hepatitis E Virus (HEV) Infection among Pregnant Women in Sokoto State, North Western Nigeria

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Authors' contributions

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ABSTRACT

Hepatitis E Virus is an important cause of morbidity and mortality in humans. HEV infection has a mortality rate of about 30% among infected pregnant women. This study aimed to determine the risk factors associated with HEV infection among pregnant women attending Antenatal Clinic at Specialist Hospital, Sokoto, Nigeria. In this study, 182 serum samples from pregnant women of productive age range between 18-45 years old were screened for the presence of HEV specific IgG antibody using a commercially available ELISA kits obtained from EUROIMMUN Medical Laboratory Diagnostics AG. Socio-demographic information was obtained from the subjects using interviewer-administered Questionnaire. Data were entered into SPSS software version 20.0 and analyzed using Chi-square test. An overall prevalence of 18/182(9.9%) HEV infection was obtained. Based on blood transfusion history, prevalence rates of 10.7% and 4.3% were recorded among pregnant women without blood transfusion and those with blood transfusion history respectively. With regards to water source, prevalence rates of 37.5%, 15.4%, 10.7% and 5.4% were recorded among pregnant women that used river water, well water, tap water and borehole respectively. Prevalence rates of the infection among pregnant women in contact with sheep, goat, cow and chicken were 24.3%, 14.7%, 7.1% and 5.3% respectively. There was significant association between HEV infection with water source (p = 0.027) and animal contact (p = 0.017). However, there was no significant association between HEV infection and blood transfusion.
Our findings call for further studies to determine other domestic animals that may be reservoirs of HEV infection.

1. INTRODUCTION

Hepatitis E virus (HEV), the causative agent of hepatitis E (HE), was described for the first time using electron microscopy in 1983 as a spherical viral particle being 27 to 30 nm in size [1]. HEV has a single-stranded positive-sense RNA genome of 7.2 kb [1]. It consists of a short 5´ nontranslated region (27 to 35 nucleotides in length) followed by three partially overlapping forward open reading frames (ORFs, from 5´end ORF1, ORF3, ORF2) [1]. The virus originated from the stool of a volunteer orally infected with faeces from suspected cases of non-A and non-B hepatitis [1]. Hepatitis E virus infection is mainly transmitted by means of contaminated water, but may also be transmitted via food or blood transfusions, or vertically from mother to foetus [2].

The infection is asymptomatic in most cases, because the virus is spontaneously cleared and only a few cases develop jaundice [3,4], although this can be severe and accompanied by acute fulminant hepatitis.

In developed Western Countries, HEV seroprevalence is generally <5% [5,6], and HEV-induced acute hepatitis is sporadic and attributable to travellers from endemic areas. However, many widespread epidemics involving hundreds of thousands of people have been described in Asia, Africa, the Middle East and South America [7,8,9], with mortality rates varying from 0.2% to 4%. Mortality is more frequent in pregnant women (up to 30%), especially those in the third trimester of gestation which may probably be as a result of immunological or hormonal reason [10,11]. HEV genotypes appear to have a different geographic distribution and different clinical severity [12,13]: genotypes 1 or 2 are usually seen in developing countries and cause epidemic outbreaks.

Genotype 1 is associated with vertical transmission [14]. Genotype 3 is usually seen in developed countries; it does not cause outbreaks and the infection resolves without transmission to the infant [15]. Genotype 4 was found in sporadic cases of acute hepatitis E from China, Taiwan, Japan and Vietnam [16].

Since HEV is usually transmitted via the faecal-oral route, insufficient drinking water treatment and low standards of sanitation have been associated with major outbreaks in developing countries [1]. Contamination of drinking water with animal or human faeces is common [17].

Despite its high mortality rate in pregnant women, studies on HEV infection are scanty in Nigeria, including Sokoto State. This study aimed

to determine the risk factors of HEV infection among pregnant women in Sokoto state.

2. MATERIALS AND METHODS

2.1 Study Area and Population

Between the month of August and November, 2016, one hundred and eighty two (182) pregnant women within the age ranges 18-45 years, attending Antenatal Clinic (ANC) at Specialist Hospital, Sokoto, who willingly consented to participate in this study were recruited. Specialist Hospital, Sokoto, is a Government owned Hospital located at Sultan Abubakar Road, in Sokoto south local government area of Sokoto State. The State lies between latitude 13° 3' 490N, longitude 5° 14' 890E and at an altitude of 272 m the sea level above. Information of participants such as blood transfusion history, water source, and animal contact were obtained via administration of well structured questionnaires. A sample of the questionnaires was initially tested for validity and reliability before the final copies were printed and used for the study. Ethical approval was also obtained from Sokoto State Ministry of Health (SMH/1580/V.IV).

2.2 Samples Collection

Using a sterile syringe, 5 ml of blood was collected via vein puncture and transferred into plane vacutainer test tubes, properly labeled and transported in a cold chain to the City Campus Central Research Laboratory, Usmanu Danfodiyo University Sokoto. The blood was allowed to stand for 60 minutes in an attempt to allow coagulation.

The samples were centrifuged at 3000 rpm for 5 minutes. The supernatant (serum) was then transferred safely into 2 ml cryovial and stored at -20°C until tested.

2.3 Antibody Detection

The serum samples were screened for the presence of Hepatitis E Virus IgG antibodies using Anti-HEV ELISA kit, obtained from EUROIMMUN Medical Laboratory Diagnostics AG. The test was carried out based on the manufacturer's instructions.

Extinction value of the control or patient sample **/** extinction value of calibrator 3 = Ratio.

The results were interpreted as described by the manufacturers' kit insert.

Ratio <0.8: was considered negative.

Ratio ≥0.8 to <1.1: was considered borderline.

Ratio ≥1.1: was considered positive.

2.4 Statistical Analysis

The data obtained was subjected to chi square test of association between the seroprevalence of HEV infection and history of blood transfusion, water source and animal contact, using the Statistical Package for Social Sciences (SPSS) version 22 statistical software (SPSS, Inc., Chicago, IL, USA). P≤0.05 was considered significant.

3. RESULTS

Of the 182 serum samples analyzed, 18 (9.9%) were positive for HEV IgG antibodies.

3.1 Seroprevalence of HEV Infection in Relation to History of Blood Transfusion among Pregnant Women in Sokoto State

Out of the 182 pregnant women enrolled in this study, 23 had history of blood transfusion and 159 do not have blood transfusion history. Highest prevalence of 17(10.7%) was observed in pregnant women who do not had blood transfusion and lowest prevalence of 1(4.3%) was recorded in those that had blood transfusion (Table 1). There was no statistically significant association between HEV infection and blood transfusion (p>0.05).

3.2 Seroprevalence of HEV Infection among Pregnant Women Attending ANC at Specialist Hospital, Sokoto in Relation to Water Source

Findings from this study showed a highest prevalence of 3(37.5%) in pregnant women that used River as water source, followed by Well water and Tap water, with prevalence rates of 4(15.4%) and 8(10.7%) respectively. Lowest prevalence of 3(5.4%) was observed in pregnant women that used Borehole as water source (Table 2). There was statistically significant association between HEV infection and water source (p<0.05).

3

3.3 Seroprevalence of HEV Infection in Relation to Animal Contact among Pregnant Women Attending ANC at Specialist Hospital, Sokoto

Table 3 showed a highest prevalence of 9(24.3%) among pregnant women who had contact with Sheep, followed by those in contact with Goat 5(14.7%), Cow (6.7%), and Chicken 1(5.3%).

A lowest prevalence of 1(3.8%) was observed among pregnant women that had no contact with any animal. There was statistically significant association between HEV infection and animal contact of the studied population (p<0.05).

4. DISCUSSION

Hepatitis E is one of the important hygienic infectious problems of the world with high incidence in developing countries, mainly Asia and Africa [18]. Significant morbidity and mortality are seen in pregnant women [19].

Of the 182 serum samples from pregnant women attending ANC at Specialist Hospital, Sokoto analyzed, Anti-HEV IgG was detected in 18(9.9%). Findings from this study are comparable to that of Huang et al. [20] who reported a prevalence rate of 10.24% among pregnant women in Yunnan, China, and Mamani et al. [21] that reported 7.4% seroprevalence among pregnant women in Hamadan, Iran.

Table 1. Seroprevalence of HEV infection among pregnant women attending ANC at Specialist Hospital, Sokoto in relation to history of blood transfusion

Blood transfusion	Number examined	Positive n (%)	Negative n (%)
No	159	17(10.7)	142(89.3)
Yes	23	1(4.3)	22(95.7)
Total	182	18(9.9)	164(90.1)

Chi-Square=0.902, P value = 0.342 (p>0.05); Not significant
n=Number, %=Percentage, HEV= Hepatitis E Virus

Table 2. Seroprevalence of HEV infection among pregnant women attending ANC at Specialist Hospital, Sokoto in relation to water source

Water source	Number examined	Positive n (%)	Negative n (%)
Borehole	56	3(5.4)	53(94.6)
Tap water	75	8(10.7)	67(89.3)
Well water	26	4(15.4)	22(84.6)
Sachet water	17	0(0.0)	17(100.0)
River	8	3(37.5)	5(62.5)
Total	182	18(9.9)	164(90.1)

Chi-Square=10.93, P value = 0.027 (p<0.05); Significant
n=Number, %=Percentage, HEV= Hepatitis E Virus

Table 3. Seroprevalence of HEV infection in relation to animal contact among pregnant women in Sokoto State

Animal contact	Number examined	Positive n (%)	Negative n (%)
Cat	30	0(0.0)	30(100.0)
Dog	7	0(0.0)	7(100.0)
Sheep	37	9(24.3)	28(75.7)
Goat	34	5(14.7)	29(85.3)
Cow	28	2(7.1)	26(92.9)
Chicken	19	1(5.3)	18(94.7)
None	27	1(3.7)	26(96.3)
Total	182	18(9.9)	164(90.1)

Chi-Square=15.391, P value = 0.017 (p<0.05); Significant
n=Number, %=Percentage, HEV= Hepatitis E Virus

However, lower prevalence of 3.6% was reported by Khameneh et al. [22] in their study among pregnant women in Urmia, Iran. Higher Prevalence rate than our findings of 61.2% was reported by Musa et al. [23] among pregnant women in Khartoum, Sudan. The disparity could be due to difference in level of hygiene, water supplies, endemicity of the virus and use of different test system with varying sensitivity [24].

4.1 Seroprevalence of HEV Infection in Relation to Blood Transfusion, Water Source and Animal Contact among Pregnant Women Attending ANC at Specialist Hospital, Sokoto

4.1.1 Blood transfusion

Our findings revealed a highest prevalence rate of 10.7% among pregnant women without history of blood transfusion and lowest prevalence of 4.3% was observed among those that have blood transfusion history. There was no statistically significant association between Blood transfusion and HEV infection (p>0.05). This could be due to the fact that, in our study only 23/182 of the participants received blood previously, so it was difficult to determine the association of the variable with HEV Seropositivity with this inadequate data.

4.1.2 Water source

Based on water source of the studied subjects, a highest prevalence of 37.5% was recorded among pregnant women that used River and lowest prevalence of 5.4% was observed among pregnant women that used Borehole. The highest prevalence recorded among pregnant women that used River as water resource may be due to the disposal of human excreta into the rivers and use of water from the same river for drinking, cooking, and personal hygiene which could serve as a vehicle for HEV transmission. This study indicated that not only River, other water sources like; wells, tap water and Boreholes could also serve as vehicle for HEV transmission, and that River only increases the risk. Wells were probably contaminated because of closeness to latrines to water sources.

Findings from this study are in accordance with that of Perez et al. [25] who reported that, the rate of HEV infection is significantly higher in people having their water supply outside rather than those inside the house. Chi-Square test showed a statistically significant association between water source and HEV infection (p<0.05).

4.1.3 Animal contact

Our findings showed a highest prevalence of 24.3% among pregnant women who had contact with Sheep followed by those in contact with Goat (14.7%), Cow (6.7), and Chicken (5.6%). A lowest prevalence of 3.8% was observed among pregnant women that had no contact with any animal. This may be due to the fact that, Sheep and Goat are in close association with humans in the current study area when compared to other animals like Dog and Cat that spent most of their time usually outdoors and their contact with house holders is minimal. Results of our findings indicated that not only Sheep, Anti-HEV IgG was detected in pregnant women in contact with other animals, such as; Goat, Cow, and Chicken, suggesting that animals could be important reservoir for HEV transmission to man. Our finding agrees with that of Shuaibu et al. who argued that Sheep could serve as a reservoir of HEV infection [26]. There was statistically significant association between animal contact and HEV infection in pregnant women (p>0.05).

5. CONCLUSION AND RECOMMENDA-TION

Pregnant women attending ANC at Specialist Hospital Sokoto had been moderately exposed to Hepatitis E virus infection. Based on this study, water source and animal contact have been recognized as risk factors of HEV infection. There was significant association between Hepatitis E Virus infection among pregnant women attending ANC at Specialist Hospital, Sokoto with Animal contact and Water source (p < 0.05). However, there was no significant association between HEV infection with blood transfusion (p > 0.05). Further studies need to be carried out in the study area to determine other domestic animals that may be reservoirs of HEV infection. Public enlightenment program on Hepatitis E Virus infection and its mode of transmission should be embarked on by the appropriate authorities to help in reducing the magnitude of the infection.

CONSENT

All authors declare that written informed consent was obtained from the study subjects for

publication of this paper and accompanying images.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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QUESTIONNAIRE

My name is Muntari Bello from Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, Usmanu Danfodiyo University Sokoto, conducting a research on Evaluation of Risk Factors Associated with Hepatitis E Virus (HEV) Infection Among Pregnant Women InSokoto State, North Western Nigeria.

And wish to seek your awareness on the disease. Your response to this questionnaire will be of immense help to the study. Thank you for your cooperation.

1. Age   [ ]

2. Educational Background: Qur'anic [ ] Primary School [   ]   Secondary School [   ] Tertiary [   ]

3. Have you ever received blood transfusion before? Yes [ ] No [ ] Not sure [ ]

4. Is this your first pregnancy? Yes [ ]   No [ ] Not sure [ ]

5. If No, how many children do you have?

6. What is the age of your present pregnancy? first trimester [   ] second trimester [   ] third trimester [   ]

7. Have you ever heard of Hepatitis E disease? Yes [ ]   No [ ]   Not sure [ ]

8. If yes, do you know its mode of transmission? Yes [ ]   No [ ]   Not sure [ ]

9. What is the source of your drinking water: Tap water [   ] Sachet water [ ] Private water supply (e.g. well, Boreholes) please kindly specify…………….

10. Do you have animals in your house?

11. Did you have any contact with the animals? Yes [ ] No [ ]

12. If yes, which animals were you in contact with? (please tick all that apply) [   ] Cat [   ] Dog [ ] Sheep [ ] Goat [ ] Cow [ ] others (specify)……………

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