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Prevalence of Hepatitis E Virus (HEV) Antibodies in Sheep from Sokoto State

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Authors' contributions

This work was carried out in collaboration between all authors. Author BRA designed the study. Authors BRA, AID and ABS performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MBA and ABS managed the analyses of the study. Authors SAS, AIB, FA and MB managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Hepatitis E virus (HEV) is a cause for public health concern in many developing countries where sanitation conditions are poor. Sheep along with other species has been reported to be highly sero-positive for anti-HEV antibodies. Given the high infection rates and expanded HEV host range, increasing attention has been focused on the zoonotic nature of HEV and the close association of particular animal species with humans. This research was designed to determine the prevalence of HEV antibodies in Sheep from Sokoto Metropolis using Indirect Enzyme Linked immunosorbent assay. An overall prevalence of 31.82% (56/176) was recorded in the study. Female Sheep were found to be more exposed to Hepatitis E Virus than male Sheep with a prevalence of 39.6% and 21.3% respectively *P-value*<0.05. Sheep one year and older were more exposed to HEV infections than younger Sheep. *P-value*<0.05.It was concluded that sheep can be, and are, infected with HEV and may go about as natural reservoirs for HEV infection. Sero-prevalence may increase with age in

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sheep as a result of repeated exposure. The breed has no outcome on the prevalence of HEV infection in sheep and goats while sex had no impact on HEV infection in Sheep. There is need to carry out a detailed molecular detection and characterization of the virus to determine the epidemiological pattern of distribution of the virus in the study area.

Keywords: Antibodies; ELISA; genotype3; sero-prevalence.

1. INTRODUCTION

Hepatitis E is a disease caused by infection with hepatitis E virus (HEV), an RNA virus that exists in both enveloped and non-enveloped forms and was first recognized in the early 1980s. The virus is a member of the *Hepeviridae* family and the genus *Orthohepevirus.* It has at least 4 known mammalian genotypes (named 1 to 4), which belong to a single stereotype [1].

The viral genome contains a non-overlapping open reading frame one and partially overlapping open reading frames two and three. ORF 1 codes for the non-structural proteins and enzymes involved in viral replication, transcription and protein processing, ORF2 codes for a glycosylated protein corresponding to the structural protein of the capsid which is the target of neutralizing antibodies against HEV. ORF3 codes for the multifunctional phosphoproteins that modulate cellular activities. Genotypes 1 and 2 are found only in humans, whereas genotypes 3 and 4 have also been found in several mammalian species. The virus is relatively stable in the environment and is sensitive to heat, chlorination and ultraviolet light [2].

Clinical features of hepatitis E are indistinguishable from acute hepatitis caused by other hepatotropic viruses. The incubation period ranges from 15–60 days, with a mean of 40 days. HEV-infected persons exhibit a wide clinical spectrum, ranging from asymptomatic infection through acute icteric hepatitis to fulminant hepatitis [3]. Certain population sub-groups are at a higher risk for severe disease following HEV infection. This includes pregnant women, persons with pre-existing liver disease and persons with immunosuppression [4,5].

Several studies have shown that Pigs, wild boars, Sheep and goats amongst others can as reservoir hosts for HEV infection [1]. Antibodies against HEV infection has been detected in domestic animals all over the world. This includes:) Pigs from Brasil [6], in Sheep and goats from Egypt [7], in cows from china [8], in Goats from the United States [9], in Sheep from China [10].

Recent studies have shown that there is evidence of HEV infection in Nigeria. [11] studied the prevalence of HEV in pigs from Zaria, Kaduna state, Nigeria and recorded a prevalence of 24.4%. In another study carried out in domestic animals (Pigs, Goats and Sheep) from Jos, Plateau state, Nigeria, a prevalence rate of 24.1% was recorded [12].

In other parts of the world, varying prevalence of HEV genotype three infections have been recorded in domestic animals. In Cross-river State, Nigeria, a sero-prevalence rate of 7.7% was recorded in Children [13].

The continuous interaction between humans and the study subjects in the study area is becoming of increasing concern. The increasing evidence of non-A non-B hepatitis in humans due to HEV is of increasing concern [14]. HEV infection is a disease of public health importance, which results to 30% mortality in pregnant women [1]. No information is available on the sero-prevalence of HEV infection in sheep. Thus, establishing the existence of HEV infection in sheep in the study area will provide baseline information for further studies. The findings from this research will assist policy makers and veterinary health authorities in designing an effective and appropriate control measures in the study area. Therefore, this study was conducted to determine the prevalence of HEV infection in Sheep from Sokoto Metropolis. Also to determine the association between Hepatitis E infection and Sex, Age and Breed.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted In Sokoto metropolis Sokoto state.

Sokoto metropolis the capital city of Sokoto state, Nigeria. The state is semi-arid, located in the extreme North-western Nigeria (between

Longitudes 4°8 E and 6°54E and latitudes 12°N and 13° 58 N). It covered a total land area of about 32,000 square Km, with the estimated human population of about 2.4 million (NPC 2008) and an estimated livestock population of: 2.2 million Sheep, 2.8 million goats, 1.4 million cattle, 46,00 camels and variable species of poultry [15,16].

2.2 Study Design

Epidemiological survey.

2.3 Sampling Method

Systematic random sampling (samples were drawn systematically after each fifth animal with the first animal also being chosen at random).

2.4 Sample Collection

Blood samples were collected from animals brought to the State veterinary clinic, Aliyu Jodi road and from backyard farms within the metropolis. After properly restrain, 5 ml of blood was collected from the jugular vein of each animal sampled using a 5 ml syringe, transferred into a sterile plain vacutainer test tube transported in an ice pack to Usumanu Danfodiyo University City campus central research Laboratory, Sokoto,. Blood samples were allowed on the bench for 60 minutes in an attempt to allow coagulation and harvested by centrifugation at 3000 RPM for 10 minutes. The supernatant (sera) was carefully decanted into a 1ml serum vial, and preserved at -20℃.

2.5 Sample Size Determination

The sample size determined at 10.5% previous prevalence [12], at 95% confidence interval, with desired precision of 5%, using the formula.

$$n = \frac{z^2 x\, p^{exp}(1-p^{exp})}{d^2} \quad [17]$$

Where:

n = sample size,
z = score for a given interval which is 1.96 (SE) at 95% confidence interval,
p^{exp} = prevalence at 10.5% [12].
d^2 = precision at 0.05.

$$N = (1.96)^2 x \frac{0.105x\,(1-0.105)}{(0.05)^2},$$

$$N = \frac{0.2983}{0.0025} = 119.32$$

Thus, n=119 Sample size was adjusted to 176 to improve the chance of detection.

2.6 Antibody Detection

ID Screen® Hepatitis E Indirect Multi-species is an indirect ELISA kit, Bi-well format from Innovative diagnostics, 310 rue Louis Pasteur 34790 Grabels, France. The ELISA kit can be used to detect anti-Hepatitis E antibodies in serum and plasma of Sheep and other species. Each sample and controls were deposited in duplicate in even numbered wells and its adjacent odd numbered wells. One hundred and ninety microliters of Dilution buffer was added to each well. Ten microliters of negative control and positive control was added to wells A1, A2, B1, B2 and C1, C2, D1 D2 respectively. Ten microliters of each sample was transferred to the remaining wells. The micro-plates were incubated for 45 minutes at room temperature (26℃). Wells were emptied by inversion and washed three times with 300 µl of wash solution. Hundred microliters of Conjugate (1X) was added to each well. The plates were incubated for 30 minutes at room temperature (26℃). The wells were emptied and washed with 300 µl of wash solution. One hundred microliters of Substrate solution was added to each well and incubated in the dark for 15 minutes at room temperature (26℃). The reaction was stopped by adding 100 µl of stop solution ($H2SO_4$) to each well. The absorbance of each sample was measured using a 96 wells spectrophotometer at a wavelength of 450 nm.

2.7 Data Presentation and Analysis

Tables were used for description purpose and Chi square test was used to test for association between sero-prevalence and epidemiological variables such as Health status, breed, age and sex at 95% CI. The analysis was done using Invivo-Stat statistical software version 3.5 and SPSS version 21.0 considering Probability value <0.05 as Significant.

3. RESULTS

3.1 Sero-prevalence of Anti-HEV Antibodies in Sheep in Sokoto Metropolis

Out of 176 serum from Sheep and screened for antibodies to HEVusing ELISA, 56 samples were positive resulting in a prevalence of 31.82% (56/176).

3.2 Sex Distribution of HEV Infection in Sheep in Sokoto Metropolis

The result showed that female had a higher prevalence of 39.6% (40/101) while male had the lowest prevalence of 21.3% (16/75) as shown in Table 1. Chi square analysis shows statistical significant association between HEV infection and sex (p-value < 0.05).

Table 1. Sex distribution of HEV infection in sheep in Sokoto metropolis

Sex	Number tested	Number positive
Male	75	16 (21.3)
Female	101	40 (39.6)
Total	176	56 (31.82)

χ^2=5. 81; p-value = 0.016; p-value< 0.05

3.3 Age Distribution of HEV Infection in Sheep in Sokoto Metropolis

The result of this study showed that Sheep one year or older had a higher prevalence of 34.64% (53/153), while the younger Sheep had the lowest prevalence of 13.04% (3/23) as shown in Table 2. Chi square analysis shows statistical significant association between HEV infection and age (p-value < 0.05).

Table 2. Age distribution of HEV infection in sheep in Sokoto metropolis

Age	Number tested	Number positive
(≥1yrs)	153	53 (34.64)
(<1yrs)	23	3 (13.04)
Total	176	56 (31.82)

χ^2=4. 49; p-value = 0.0213; p-value< 0.05

3.4 Frequency of HEV Infection in Different Breed of Sheep

The result showed that Uda Breed had a prevalence of 29.13% (30/103) while Yankassa, Balami and Sudanese had a prevalence of 26.31% (5/19), 35.42% (17 /48), 66.70% (4/6) respectively as shown in Table 3. Chi square analysis did not show any statistical significant association between HEV infection and Breed (p-value >0.05).

4. DISCUSSION

HEV is a cause for public health concern in many developing countries where sanitation conditions are poor and waterborne epidemics are frequent. Both epidemic and sporadic forms of HEV infections exist in developing and under-developed countries. Pigs are the principal reservoir of HEV, but sheep and goats have also been reported to be highly seropositive for anti-HEV IgG [1].

Table 3. Frequency of HEV genotype 3 antibodies in different breed of sheep in Sokoto metropolis

Breed	Number tested	Number positive (%)
Uda	103	30 (29.13)
Yankassa	19	5 (26.31)
Balami	48	17 (35.42)
Sudanese	6	4 (66.70)
Total	176	56 (31.82)

χ^2=5. 03; p-value = 0.2843; p-value>0.05

Given the high infection rates and expanded HEV host range, increasing attention has been focused on the zoonotic nature of HEV and the close association of particular animal species with humans. In fact, numerous cases of human HEV sharing high genomic sequence identity with the virus have isolated from local animals have recently been reported [18]. These findings strongly support a zoonotic transmission, which also explains the cause of autochthonous HEV infections in developing countries. Moreover, numerous examples of human infections via consumption of contaminated raw meat or other animal products have surfaced in recent years. In Germany, autochthonous HEV infection was found to be associated with the consumption of offal and wild boar meat [19]. In Xinjiang evidence surfaced that a high level of seroconversion in sheep and suggested that the sheep liver may be a source of food borne HEV infection in humans [20].

This outcome alerted us to the necessity for surveillance of HEV transmission routes in order to prevent food borne epidemics.

Here we focused particularly on the food habits of the local inhabitants of Sokoto state, of whom the majority are Hausa-Fulani and the continuous contact with the inhabitants of the study area with sheep and goats. Pig meat is forbidden in the study area for religious reasons. Sheep meat and goat meat are common in their diet. In this study, a prevalence of 31.82% was recorded.

The result of this study corroborates with the findings of [1] who recorded a similar sero-

prevalence of 35.20% in sheep from southern Xinjiang, 28.98% in the Aksu region of Xinjiang [20]. Lower prevalence has been recorded with 10% in Plateau state [12], 4.4% from Egypt [7] and as low as 0% in China [21].

A logical explanation could be that the breed of Sheep are susceptible to HEV infection in the study area. The difference observed with previous research may be as a result of difference in time of sample collection, screening method and geographical location.

The antibodies detected in this study may be for genotype 3 or genotype 4. This may be due to the nature of the neutralizing epitopes of HEV and the existence of a single serotype, thus enabling the possible occurrence of cross-reactivity among HEV genotypes [22].

The sex specific prevalence of HEV antibodies in Sheep in this study showed that female had a higher positive rate of 71.43% with a prevalence of 39.6% (40/101) while males had the lower positive rate of 28.57% with a prevalence of 21.3% (16/75). Chi square analysis at 95% Confidence Interval showed a statistical significant association between HEV infection and sex with sheep (p-value < 0.05). This finding corroborates with [11], were 78.05% and 21.95% positive rate was recorded for male and female pigs, respectively, and contrary with what was reported by [23] who found no significant difference in the Sero-prevalence of HEV between male and female pigs (72.1 and 70.5%, respectively, *P* value = 0.786). The difference observed in the two groups may probably be because some covariates (pregnancy, lactation, history of abortion etc.) observed in female test subjects. All these factors may lead to immune-compromise of the test subject, thereby influencing susceptibility to HEV infection in Humans [1]. The same may be the case of animals.

Nevertheless, in order to fully comprehend the role of sex in the epidemiology of the disease, further investigations involving a larger population of sheep are needed.

Breed specific prevalence of HEV antibodies in Sheep showed that Uda Breed had a positive rate of 53.7% (30/56) with a prevalence of 29.13% (30/103), while Yankassa, Balami and Sudanese had a positive rate of 8.93% (5/56), 30.36% (17/56) and 7.14% (4/56), with a prevalence of 26.31% (5/19), 35.42% (17 /48), 66.70% (4/6) respectively. Chi square analysis did not show any statistical significant association between HEV infection and Breed (p-value >0.05). In a similar study carried out in china [20], a significant association between HEV infection and Sheep breed was observed p-value<0.01. This may be as a result of the Breed of Sheep in the study area is less susceptible to Hepatitis E virus infection.

5. CONCLUSION

The findings of this study show that for the first time that anti-HEV antibodies are present in sheep from Sokoto metropolis and may be a product source of food borne infection. HEV infection is influenced by Sex and Sero-prevalence increases with age.

Sheep may act as natural reservoirs for HEV infection in the study area, thus Slaughterhouse workers, veterinarians and other individuals with extensive contact with sheep and may be at risk of being infected with HEV. The breed has no consequence on the prevalence of HEV infection.

6. RECOMMENDATIONS

Our results stress the need for Molecular detection and characterization of HEV genotype 3 and 4 to clarify the distinct pattern of HEV distribution and the extent of cross-species transmission. Public enlightening by policy makers and veterinary health authorities will be required to help prevent current and future endemics. Good hygiene practices among people with extensive contact with the sheep and will help prevent future occurrence of the infection.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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