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Seroprevalence of Hepatitis E Genotype-3 in Camels Found at Antemortem Unit of Katsina Metropolitan Abattoir and Hamceta District of Mani LGA, Katsina State, Nigeria

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ABTRACT

Hepatitis E genotype-3 is an important emerging viral zoonosis reported in many developed and developing countries. The disease has been reported with life threatening conditions particularly amongst immune compromised patients and pregnant women. A cross-sectional study was carried out in Katsina Metropolitan Abattoir and Hamceta District of Mani LGA to determine the prevalence of HEV-3 antibodies and its association with demographic variables such as age, sex, species, breeds and source of drinking water, 386 camels were sampled. Blood samples were collected and screened for HEV-3 antibodies using Indirect Enzyme Linked Immune sorbent Assay (ELISA) that detects IgG produced against the capsid proteins of the virus. The prevalence rates of 3.9% (15/386) in camel was recorded and statistical analysis showed no significant association between the infection and with breeds (p=1.00), sex (p=0.77), age (p=0.99) and source of drinking water (p=0.057) in camels. This study revealed the evidence of HEV-3 infection in animals in Katsina State. This shows the need to enlighten livestock farmers and abattoir workers on the zoonotic potential of HEV-3. Agencies concerned with safeguarding animal and public health need to consider HEV-3 in their disease monitoring and surveillance programmes. Further studies need to be done on isolation and molecular characterization of the virus in livestock and humans.

Keywords: *Camels, Hepatitis E Genotype-3, Katsina, Nigeria, Seroprevalence*

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INTRODUCTION

Hepatitis E is a zoonotic, emerging enteric infectious disease caused by Hepatitis E virus (HEV) a single-stranded, positive-sense, ribonucleic acid (Tam et al., 1991). The virus is of the genus orthohepevirus A of the family hepesviridae has at least 7 different genotypes (Lhomme et al., 2016) while genotype 3 and 4 are zoonotic and infect several animal species including pigs, wild boars, avian, rabbit and deer. HEV-3 is a recognized

zoonotic disease from camel (Lee et al., 2016). The HEV-3 virus is shed in the faeces of infected animals and humans, and could be transmitted to humans via ingestion of contaminated foods and water. In Africa and Asia, HEV-3 is observed to be endemic in both human and swine (Smith et al., 2014). Hepatitis E is endemic in many developing countries including China, Sudan, India and Bangladesh. However, HEV-3 has not been diagnosed in many developing countries due to lack of public educations and research neglect. HEV-3 has been found to be endemic in Japan, United States, Europe and Australia (Colson et al., 2010). Food safety associated with HEV-3 contamination is an important public health concern, with the recent identification of infectious HEV-3 in meat and meat products and resultant sporadic cases of foodborne hepatitis E in the human population (Colson et al., 2010); (Takahashi et al., 2004); (Tei et al., 2003) and (Yazaki et al., 2003). In areas with better sanitation and water supply, hepatitis E disease is infrequent with only occasional sporadic cases (Clemente-Casares et al., 2003). Most of the cases caused by genotype 3 virus are caused through infection with virus of animal origin, through ingestion of undercooked animal meat (Meng, 2010). Serological evidence of prior exposure to the virus has been found in most areas, with higher seroprevalence rates (proportion of people who are positive for IgG antibodies to HEV-3) in regions with lower standards of sanitation and thus higher risk for transmission (Meng, 2010). HEV-3 is asymptomatic in animals and the virus is voided in the feaces (Ma et al., 2010). In humans, the infection is self-limiting and resolves within 2–6 weeks, a serious condition known as fulminant hepatitis (acute liver failure) develop which is associated with significant mortality (WHO, 2017). However, it is self-limiting in the immune competent but life-threatening in immunosuppressed individuals (Kamar et al., 2014). Other signs are jaundice and fulminating hepatitis in second or third trimester of pregnancy and with mortality of 10%-25% in pregnant women (Pillot et al., 1995; Jameel, 1999). The aim of the study is to investigate the presence Hepatitis E Genotype-3 infection in camel slaughtered at Katsina Metropolitan Abattoir using ELISA and established possible associations with age, sex, breeds and source of drinking water.

MATERIAL AND METHODS

The study was conducted in Katsina State located in North-Western Nigeria. The State lies between latitude 12°59' 26.95" N and longitude 7°36' 6.37" E. The Abattoir is located at Longitude 12°97 6.44 E and Latitude 7°59 54.52 N and Hamceta is located at Longitude 12°.88 59.85 E and Latitude 7°93 208 N (Figure 1). A cross-sectional study was conducted and convenient sampling was used to sample camel that are to be slaughtered.

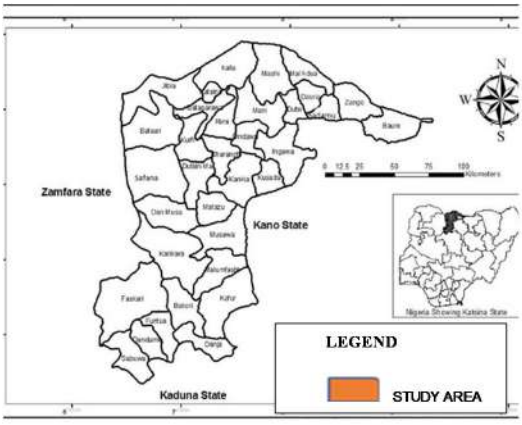

Figure 1: Map of Katsina State showing Katsina and Mani Local Govern

There is little information on HEV-3 infection in camels in Katsina State, thus an expected prevalence of 50% at 95% CI was used to determine the sample size (Thrusfield, 2010). A total of 386 samples were collected from camels.

Sample and Data Collection

Each camel was properly restrained and blood sample (5ml) was collected from the epigastric/jugular vein using 21G needle and stored in a sterile vacutainer (anti-coagulant free). Each sample was properly labeled and transported immediately in an ice pack to Haematology Laboratory at Turai Umar Yaradua Maternity and Child Hospital, Katsina State where the sera samples were harvested by centrifugation at 3000g for 10-15 minutes. The samples were transferred into 1.5ml microfuge tubes (eppendorf®) and stored at -20°C until required and later transported to Central Research Laboratory, Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto, Sokoto State, Nigeria where the serology procedure took place. Information on hypothesized risk factors such as age, sex, breed and source of drinking water were collected in a data collection form.

Serological Assay

Hepatitis E Indirect Multi-species ELISA kit (IDvet® Innovative Diagnostics, France) was used to detect seropositive samples. The kit was designed to detect anti-Hepatitis E genotype 3 antibodies in serum or plasma of Sheep and other species of animals (IDvet). This kit has a sensitivity of 98.5% and specificity of 100%.

Test Procedure

The test per sample was carried out based on the manufacturer's recommendations as follows: Each volume (serum) and control was deposited in duplicate in

even numbered wells and its adjacent odd numbered wells. One hundred and ninety microliters (190µl) of dilution buffer were added to each well. Ten microliters (10µl) of negative control and positive control were added to wells A1, A2, B1, B2 (Negative control wells) and C1, C2, D1 D2 (Positive control wells) respectively. Ten microliters (10µl) of each sample (serum) were transferred to the remaining wells. The micro-plates were incubated for 45 minutes at room temperature (26°C). The wells were emptied by inversion and washed three times with 300µl of Wash solution. Hundred microliters (100µl) of Conjugate (1X) were added to each well. The plates were incubated for 30 minutes at room temperature. The enzyme-substrate reaction was terminated by the addition of a sulphuric acid solution and the color change (to yellow) was measured using an Elisa reader (Optic System IVYMEN® 2100C, USA) at a wavelength of 450 nm.

i.Calculation of the Ratio: Calculation of the net optical density = optical density of even well – optical density of odd well.
ii.Validation: The test was validated if the net mean value of optical density of positive control was > 0.350 and also if the ratio of net mean value of the optical density of positive by optical density of negative control is > 3. I.e. net ODPC/ (net ODNC) >3.
iii. Interpretation: Sample presentation (SP) = net optical density of sample / net optical density positive control × 100. The result is positive if sample presentation S/P % > 70%.

Data Collection Form

Using data collection form, information of age, sex, breeds and source of drinking water (stream water, borehole water and tap water) was collected during the time of the collection of the blood sample.

Data Analysis

Overall seroprevalence rates was calculated at 95% confidence interval. Chi-square test was used to determine the association between the prevalence and demographic factors. Fischer's exact test was used when the Chi-square assumptions failed (Aviva and Paul, 2013). SPSS software version 16.0 was used (IBM Corporation).

RESULTS

Overall Seroprevalence of Camel Hepatis E Genotype-3 in Katsina State, Nigeria

The specie specific overall seroprevalence was 3.9% (15/386) in Camelus dromedarious (Camel) (Figure 2) in Katsina State, Nigeria.

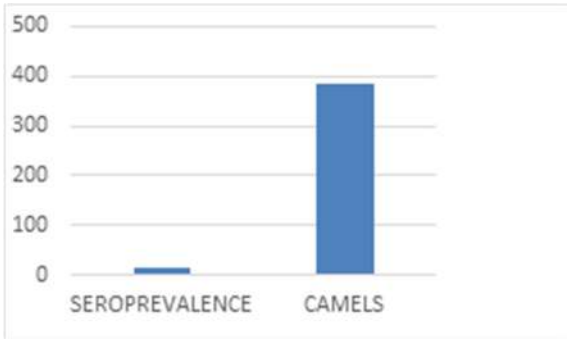

Figure 2: Overall seroprevalence of HEV-3virus in domestic animals in Katsina State

Seroprevalence of Camel Hepatitis E Genotype-3 in Katsina Metropolitan Abattoir and Hamceta District of Katsina State, Nigeria

The seroprevalence of camel sampled in Katsina Metropolitan Abattoir were 3.57% (10/280) of Camelus dromedarious and 4.7% (5/106) of those Camelus dromedarious sampled at Hamceta District of Mani LGA, Katsina State (Figure 3).

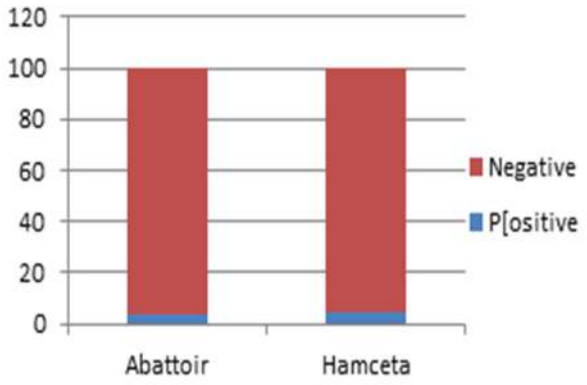

Figure 3: Overall seroprevalence of HEV-3 in camel in Katsina State

Seroprevalence of Hev-3 In Camel by Age Group at Katsina State, Nigeria

Based on age group, camels less than 3 years old had a seroprevalence of 3.88% (9/232), also has similar seroprevalence of HEV-3 with those older than 3 years had a prevalence of 3.89% (6/154), with statistically non-significant association between the infection and age (χ^2 = 0.000, p= 0.9932) (Table 1).

Table 1: Association between HEV-3 and Age Group of Camels

Age Group (years)	No. Sampled	No. Seropositive (%)
< 3	232	9 (3.88)
>3	154	6 (3.82)
Total	386	15 (3.89)

(χ^2 = 0.000, df =1, p= 0.9932)

Seroprevalence of Hev-3 in Camel by Sex in Katsina State, Nigeria

The seroprevalence of HEV-3 by sex showed female camel to had higher prevalence of 4.3% (12/282) than males 2.9% (3/104), but no statistically significant relationship between sex and seroprevalence in camels (P =0.83) was found (Table 2).

Table 2: Associations between HEV-3 and Sex in Camels

Sex (Groups)	No. Sampled	No. Seropositive (%)
Male	104	3 (2.9)
Female	282	12 (4.3)
Total	386	15 (3.9)

(P= 0.77, df= 1) by Fischer Exact Test

Seroprevalence of Hev-3 In Camels by Breeds in Katsina State, Nigeria

Seroprevalence of HEV-3 in camel by breed showed higher prevalence in brown camel with 4% (14/348) while white camel had 2.6% (1/38) positive. No statistically significant relationship between breeds and seroprevalence (P = 1.000) (Table 3).

Table 3: Association between HEV-3 and Breed of Camel

Breed	No. Sampled	No. Seropositive (%)
Brown camel	348	14 (3.6)
White camel	38	1 (2.6)
Total	**386**	**15 (3.9)**

(P = 1.000, df =1) by Fischer's Exact Test

Seroprevalence of Hev-3 in Camel by Source of Drinking Water in Katsina State, Nigeria

Seroprevalence of HEV-3 in camel based on source of drinking water showed camel that drink stream water had prevalence of 1.9% (6/316) while borehole water had 2.9% (2/70). No relationship between source of drinking water and seroprevalence (p = 0.057) was found (Table 4).

Table 4: Associations between HEV-3 in Camel and Source of Drinking Water

Source of Drinking Water	No. Sampled	No Seropositive (%)
Stream water	316	6 (1.9)
Bore-hole water	70	2 (2.9)
Total	**386**	8 (2.1)

(p= 0.057, df =1) by Fischer's Exact Test

DISCUSSION

The findings of this study showed seroprevalence of camel HEV-3 to be 3.9 % in Katsina state which is considerably less than a finding of a previous study with 22.4% of HEV-3 antibodies in Camelus dromedaroius reported in Ethiopia (Li et al., 2017). The reasons for the differences of prevalence could possibly be due to diagnostic kit, the kit use in our study is multi-species recombinant indirect ELISA (Biwell format) and kit used in their study is Dromedary Camel DcHEV-like particle. Also, the findings are less than the prevalence of 30.7% reported by (Adamu et al. 2022) in Nigeria the difference is not fully understood. Zero seroprevalence of HEV-3 antibodies in Camelus ferus (Bacterian) was reported by Li et al. (2017) in comparison to our study, the differences of seroprevalence result from different species used and the kit (IDvet multi species kit with sensitivity of 98.5% and specificity of 100%, might be the reason for detecting less Anti-HEV-3 than DcHEV-LP). Also, our result is less than 68.6% of seroprevalence reported by Bassal et al. (2019) in dromedary camel in Israel, the reason for the differences could be linked to diagnostic kit and HEV is endemic (Kamar et al., 2012).

The similarity in seroprevalence between camel age group less than 3 year (3.88%) and greater than 3 years group of (3.82%) showed that age may not be significant in the distribution of HEV-3 in Katsina and other states, as there is no previous study in camel with regard to age. The higher seroprevalence of HEV-3 observed in female camels (4.3%) compared to males (2.9%) in this study, are not statistically significant. This observation could be as a result of the adults' female tend to stay longer in the herds than their male counterpart (Lee et al., 2016), implying a possible age and sex interaction effect. Further studies need to be done in this regard. Seroprevalence in camels seems to be influenced by breeds. However, the disparity between the prevalence observed among the groups may suggest that differences in seroconversion could be breeds dependent in dromedary camels. The case of a higher prevalence observed in camel that drink bore-hole water and the less observed in Camel that drank stream water, the differences in seroprevalences are not fully understood.

Conclusion

For the first time this study demonstrated the evidence of HEV-3 infections among domestic animals in Katsina State. Specifically, the findings in this study shows only dromedary camels were found to have seroconversion of HEV-3. In line with the existing literature, this finding supports the relevance of camel in the epidemiology of HEV-3. Despite the lack of statistical evidence on the relationship between the seroprevalence of HEV-3 and demographic factors (age, sex, breeds and source of

drinking water), the zoonotic significance of the infection deserve serious attention by health professionals, researchers and government.

Recommendations

The results show the need for more sensitive and specific technique like PCR to clarify the distinct pattern of HEV-3distribution and extent of zoonotic transmission and good hygiene practice among people with contact with camel.
Public education by veterinary health authority will be required to help prevent current and future endemic. Future study should investigate the epidemiological role of camel in the transmission of HEV-3 in Northwestern Nigeria.

Ethical Approval

The research does not contain animal experimental trials. No ethical clearance obtained, but permission (consent) was obtained from the abattoir management to collect small volumes of blood samples for serological studies from the slaughtered cattle.

Conflict of Interest

The authors declare no competing interests regarding publication of this paper.

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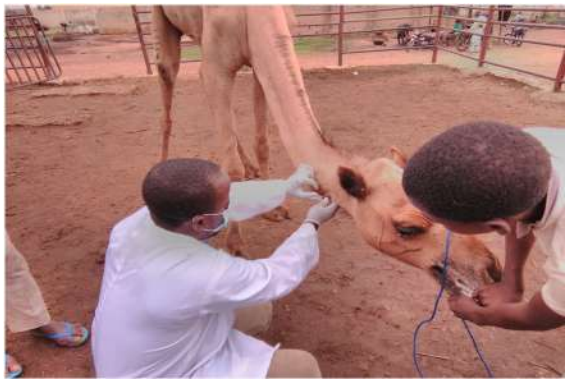
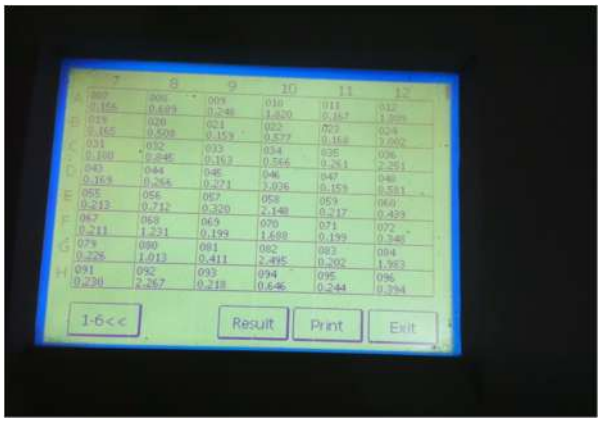
APPENDIXES



1.Sample Collection

2. Sample Collection

3. HEV-3 Elisa Reader Result of Camel Plate A



4. HEV-3 Elisa Reader Result of Camel Plate B