Original Article

Prevalence And Determinants of HBV Among Donkeys in Nkwor – Izhia Donkey Market Ohaukwu LGA, Ebonyi State, Nigeria.

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ABSTRACT

Objective: This study aimed to determine the magnitude of the prevalence of Hepatitis B virus infection among donkeys marketed in Nkwo-Izhia market, Ohaukwu Local Government Area, Ebonyi State. **Materials and methods:** A market based cross sectional study was implemented from March 1 to April 1, 2020 among 200 donkeys sold in the market lairage/garage. The animals were selected through simple random sampling method. Venous blood samples were taken from 200 donkeys and their serum were tested for hepatitis B surface antigen (HBsAg) using Enzyme Linked Immuno-sorbent Assay. **Results:** The overall prevalence of hepatitis B virus infection among donkeys was 13.5% (27) at 95% Confidence Interval. Females: non-reactive 66 (84.6%), reactive 12 (15.4%); Males: non-reactive 107 (87.7%), reactive 15 (12.3%) with overall X^2 (p-value) of 0.39 (0.53) respectively. Greater proportion of the female donkeys (15.4%) were reactive to HBV antigens compared to their male counterparts (12.3%). Among the variables; age of the donkeys, duration of stay in market, height, colour and their length, only mean difference in duration of stay in the market had statistically significant association with the HBV status of the donkeys (t= 2.15; p= 0.03).

Key words:
Donkeys,
Hepatitis B virus,
Hepatitis B surface antigens,
Nigeria

INTRODUCTION:

Hepatitis can be referred to the inflammation of liver due to infection from various sources among which are Hepatitis B virus. Hepatitis B virus is blood borne including Hepatitis C, and thus have different routes of transmission from hepatitis A, D, E which are not blood borne. Hepatitis B virus has predilection for the liver and exists asymptomatically in susceptible hosts which makes transmission to uninfected individuals very high

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because the infected individuals are unaware of their infection status, hence referred to as silent killers[1,2]. The virus also has very high infectivity due to its bio-diversity. These viruses, hepatitis A,B,C,D,E, however display similar symptoms and the potential to cause liver disease vary in degrees but differ significantly with regards to epidemiology, prevention, diagnosis, care and treatment. Knowledge of viral hepatitis remains low among Nigerians despite being a leading infectious cause of death each year. As a consequence, most of the estimated 20 million Nigerians living with viral hepatitis B or C are undiagnosed, increasing the likelihood of future transmission to others and placing them at greater risk for severe, even fatal health complications such as liver cirrhosis and liver cancer (hepatocellular carcinoma)[3,4].

Hepatitis B virus has been reported among animals with possibility of zoonosis, hence an important occupational hazards of health and slaughter house workers[5,6]. Exposure to infected blood and body fluid such as blood transfusion, accidental needle prick injuries and unprotected sexual contact with an infected person are among other major modes of transmission of HBV. However the infection can be prevented by vaccination. Over 90% of Hepatitis B virus infection exists chronically and show no signs and symptoms, unless during acute phases in which symptoms resemble malaria attack of generalized body weakness, fever, headache, joint pains and occasionally jaundice. Infection with Hepatitis B virus has been reported to have attained a silent epidemic[7,8,9]. Hepatitis D virus exists with co-infection with Hepatitis B virus infection[10]. About 1% of persons living with HBV infection (2.7 million people) are also infected with HIV[3]. Conversely, the global prevalence of HBV infection in HIV-infected persons is 7.4%[11,12].

Hepatitis B Virus transmission results in substantial morbidity and mortality from chronic HBV, liver cirrhosis, and hepatocellular carcinoma. Risk factors for transmission of HBV among donkey abattoir workers include animal slaughtering, dressing, knife cuts, sharing of knives and other related activities.

SUBJECTS, MATERIALS AND METHODS

Ebonyi State is one of the 36 states and Federal Capital Territory in Nigeria. The State Situates at the South Eastern region of the country. It lies at the coordinates of $6^{0}15^{1}$N, $8^{0}05^{1}$E, covering an area 5,533km^{2}; bounded in the north by Benue State, south by Abia, East by Cross River and West by Enugu States. The state has a population of about 3.5 million by projection of 2006 National census which recorded Ebonyi State population as 2.1 million. There are 13 local Government areas in the state, with capital at Abakaliki, largely inhabited by Igbo speaking tribes. The dialects includes Ehugbo, Edda, Izzi, Ezza, Ngbo/Izhiangbo, Ikwo, Nkalagu/Nkalaha and Ishielu, Eda, Okposi, Uburu, Onicha, Ishiagu, Etc, while Kori language speaking inhabitants are also found in some villages in Ishielu and Ohaukwu Local Government Areas. The people are mainly agrarian in nature, about 95% of the population. In 2016, the human development index (HDI) was put at 0.434 which ranked the state 24th out of 36 states of the country.

Ohaukwu Local Government Area (LGA) is the third largest LGA in the state (after Izzi and Onicha). Izhiangbo is the headquarters of Ohaukwu LGA. Nkwo-Izhia is the largest market of donkeys in the state nay South East geopolitical zone of the country. The market has well over a thousand donkey handlers (traders including dealers both local and Northerners, herders and butchers). In fact, all the donkeys slaughtered within the state and beyond are sourced from the market.

Estimation of the prevalence and determinants of HBV among the animals, which published information is lacking in the state will provide a baseline for more studies on donkeys which have become an increasing meat for people of the State and southerners in general[14,16].

Inclusion criteria: Apparently healthy donkeys in the donkey market. And Donkeys found within the market lairage

Exclusion criteria: Dead or slaughtered donkey carcass and Donkeys whose owners refused consent

Sample size determination: The following formula (Cochrane formula) was used to determine the sample size which is appropriate for cross

sectional studies[35,36]. Sample size, n = Z^2pq/d^2 Where Z = standard normal distribution at 95% confidence interval = 1.96 (constant); p = prevalence based on the local prevalence of HBV infection (10.9%) on slaughtered cows obtained from work of Kareem et al, Kano[29]; q = 1.00 minus p (1 - p); d = desired level of precision (that is, the margin of error) set at 0.05.

Sampling Technique: The animals were selected through simple random sampling method.

Study Instruments: These comprised of Laboratory Hepatitis B Surface antigen screening test kits and reagents, 5ml syringes and needles, detergents, cryovial bottles, racks, disposable gloves. The laboratory materials were used by Veterinary Doctors to collect the donkeys' venous blood, while the Laboratory Scientists (research assistants) ran HBsAg tests and obtained information of the actual number of donkeys infected with the virus under study, using Hepatitis B serological markers, in this instance, presence or absence of Hepatitis B surface antigens in the animals. During the testing, labACON rapid test kits were used. The kit is qualitative for the detection of Hepatitis B surface antigen (HBsAg) in serum or plasma. The product was manufactured by Hangzhou Biotest Biotech Co. Ltd, China. It has a relative sensitivity of more than 99.9%, specificity of 99.0% and accuracy of 99.4%. The test utilizes a combination of monoclonal and polyclonal antibodies to selectively detect elevated levels of HBsAg in serum or plasma. The test strip is a qualitative, solid phase, two-site sandwich immunoassay for the detection of HBsAg in serum or plasma.

The membrane is pre-coated with anti-HBsAg antibodies on the test line region of the test strip. During testing, the serum or plasma specimen reacts with particle coated with anti-HBsAg antibodies. The mixture migrates upwards on the membrane chromatographically by capillary action to react with HBsAg antibodies on the membrane and generate a coloured line. Positive tests were marked as R, Reactive, that is, when two distinct coloured lines appeared on both the control (C) and test regions (T). Negative tests were marked as non-reactive, that is, when one coloured line appeared in the control region (C) and no colour appeared in the test region (T).

Data analysis: Data generated was analyzed using SPSS software version 25.0. The presence or otherwise, and prevalence of HBV infection was expressed in simple proportions. Chi-square test (X^2) was used to determine the relationship between positive and negative proportions of Hepatitis B virus infection among the donkeys. Also, relationship between the dependent and independent variables was analyzed to estimate their significance. A probability value of less than (0.05) was taken as significant.

Ethical consideration: Ethical clearance for this study was obtained from the Research and Ethics Committee of Alex Ekwueme Federal University Teaching Hospital, Abakaliki (AE-FUTHA), reference number FETHA/REC/VOL.2/2019/263 and permission was obtained from Ebonyi State Ministry of Agriculture and Natural Resources, and Ministry of Health. Consent to carry out the study was also obtained from the donkey butchers and herders/traders unions of the market. Only those selected donkeys whose owners gave consent by signing the informed consent form participated in the study.

RESULTS:

Out of the 200 donkeys studied, 122 (61.0%) were males and 78 (39.0%) were females. Greatest proportion of the donkeys came from north eastern states of Yobe 50 (25.0%), Taraba 44 (22.0%) and Borno 36 (18/0%), states while the least came from Katsina State 10 (5.0%). Twenty-three (11.5%), donkeys originated from a neighboring Chad Republic. Ash 134 (67.0%), was the predominant colour among the donkeys. The prevalence of HBV infection among the studied donkeys was 13.5%. Estimated mean age of the donkeys was about 1 year 10 months with an average stay of 2 days in the market.

The study revealed that greater proportion of the female donkeys (15.4%) were reactive to HBV antigens compared to their male counterparts (12.3%). Majority of donkeys (21.7%) from Chad were tested positive to the antigen compared to those from states in Nigeria. Among the Nigerian

donkeys, those from Taraba State had a greater proportion 8 (18.2%), of reactive samples while those from Sokoto 1 (8.3%) were least reactive.

Table 1: Socio-demographic characteristics and HBV status of the donkeys

Variables	Frequency	Percent
Gender		
Males	78	39.0
Females	122	61.0
Place of origin		
Yobe	50	25.0
Taraba	44	22.0
Maiduguri	36	18.0
Chad	23	11.5
Niger	14	7.0
Sokoto	12	6.0
Adamawa	11	5.5
Katsina	10	5.0
Colour		
Ash	134	67.0
Brown	29	14.5
Black	18	9.0
White	16	8.0
Milky	3	1.5
HBV Status		
Reactive	27	13.5
Non-reactive	173	86.5
Mean Age = 1. 88 ± 0. 58 years		
Mean Duration in the market = 2. 05 ±0. 80 days		
Mean Height of Donkeys = 101. 83 ± 7. 29 cm		
Mean Length of donkeys = 135. 68 ± 9. 68 cm		

Table 2b: Association between HBV status and socio-demographic characteristics of the donkeys

Variables	Mean (s.d)	Difference in mean	t - test	p-value
Age of donkey				
Non – Reactive	1.87 (0.59)	0.06	0.46	0.65
Reactive	1.92 (0.48)			
Duration in market				
Non - Reactive	2.09 (0.90)	0.35	2.15	0.03
Reactive	1,74 (0.71)			
Height of donkey				
Non - Reactive	101.44 (7.44)	2.90	1.93	0.06
Reactive	104.33 (5.78)			
Length of donkeys				
Non - Reactive	135.43 (9.81)	1.83	0.91	0.36
Reactive	137.26 (8.83)			

There was no reactive sample from Niger State. Similarly, in terms of geographical delineation, the greatest proportion of reactive donkeys was from outside Nigeria [Chad) 5 (21.7%)], whereas, the prevalence among donkeys from the states in Nigeria was estimated at 12.4%. Disaggregation of the national prevalence along geo-political zones, showed that donkeys from the North Central Region of the Nigeria had the greatest proportion of donkeys (13.8%) reactive to HBV antigens while it was least in North West (9.1%). The findings also showed that while the greatest proportion of HBV antigen reactive donkeys were white in colour 16 (25%), all donkeys with milky colours were non - reactive. There was no statistically significant relationship between these variables on Table 2a with HBV status of the donkey.

The study also revealed that among the variables, age of the donkey, duration of stay in

Table 2a: Association between HBV status and socio-demographic characteristics of the donkeys

	HBV status N (%)		
Variables	Non-Reactive	Reactive	X2 (p-value)
Gender			
Female	66 (84.6)	12 (15.4)	0.39 (0.53)
Male	107 (87.7)	15 (12.3)	
Place of origin			5.10 (0.65)
Chad	18 (78.3)	5 (21.7)	
Taraba	36 (81.8)	8 (18.2)	
Yobe	43 (86.0)	7 (14.0)	
Maiduguri	32 (88.9)	4 (11.1)	
Katsina	9 (90.0)	1 (10.0)	
Adamawa	10 (90.9)	1 (9.1)	
Sokoto	11 (91.7)	1 (8.3)	
Niger	14 (100.0)	0 (0.0)	
Place of origin recoded, regions			
International (Chad)	18 (78.3)	5 (21.7)	
NC	50 (86.2)	8 (13.8)	
NE	85 (87.6)	12 (12.4)	
NW	20 (90.9)	2 (9.1)	
Place of origin dichotomized			
National	155 (87.6)	22 (12.4)	
International	18 (78.3)	5 (21.7)	
Colour of donkeys			2.6 (0.62)
White	12 (75.0)	4 (25.0)	
Ash	116 (86.6)	18 (13.4)	
Black	16 (88.9)	2 (11.1)	
Brown	26 (89.7)	3 (10.3)	
Milky	3 (100)	0 (0.0)	

market, height and their length, only mean difference in duration of stay in the market had statistically significant association with the HBV status of the donkeys (t= 2.15; p= 0.03).

DISCUSSION:

The study was novel because similar studies were not found on literature review both in the study area and any other place. However, related studies done on other related animals were utilized as reference point since they belong to the same genera, family and are also domesticated. It was also documented that HBV infection was found to exist among gorillas, Chimpanzees, cows and humans.[5,39] Among the 200 donkeys studied, 27 (13.5%), of them were demonstrated to have HBV antigens. This study is similar to another study conducted by professionals in collaboration,[44] in which 9 out of 62 chimpanzees (11.3%) and 2 from 11 gorillas (18%) were HBV-infected (15% combined frequency), while other Old World monkey species were negative.

Findings in this study were however at variance with result of Hepatitis B virus infection among cows in a study carried out in Kano metropolis, Nigeria by Kareem AMJ, Mohammed YI and Rago LD in which the prevalence was found to be 10.9%. [36] This could be due to herd immunity among cows who are often immunized and receive better health care than donkeys.

Though the number of female donkeys that participated during the survey was far less than that of males, the more prevalence among females to males finding could be adduced to gender inequalities among human populations as explained for HIV/AIDS. As was found in a study on HIV/AIDS ‘‘inequalities were chiefly explained by differences in the responses to risk factors; the differential effects of age, such as weaker sex, having older partners, marital status and occupation on prevalence of HIV/AIDS for women and men were among the significant contributors to this component. In Cameroon, Guinea, Malawi and Swaziland, a combination of the composition and response effects explained gender inequalities in HIV/AIDS prevalence’’.[48]

The higher HBV prevalence noted among donkeys from Chad more than those from Nigeria, is closely related to the prevalence of HBV in humans In Chad where hepatitis B virus (HBV) is of high prevalence and is consequently responsible for numerous acute and long-term complications. Though no general population-based study on the prevalence of hepatitis B has been performed in Chad, a study conducted in rural South – West Chad by Suesstrunk J and Diongali F[45] found an overall prevalence of 22.9% among the human population in the district which might not be statistically different from the result of this study presumed to be due to humans to animals and vice versa transmission. Contrasting in Nigeria, a peer review study[46], showed a much less prevalence of HBV among human population of 11.0%, which is related to a HBV prevalence of 12.4% among donkeys as found in this study, when place of origin of donkeys was dichotomized.

The difference in duration of stay in the market which had statistical significance association with the HBV status of the donkeys means that the more the donkeys were allowed to stay in the lairage/market, the less their positivity for HBV infection. This could be explained with the fact that sick donkeys may be sold and slaughtered before the healthy ones which stay longer and when screened show negative results.

All donkeys with milky colours and donkeys from Niger State were non – reactive to HBV infection. This could be due to insufficient samples or genetic constitution. Specifically for Niger State, this finding may have a reflection of the state’s low HBV infection (7.0%) seen among humans (antenatal attendees) [47]. In addition, there could be measures that lead to reduced interactions between the animals and humans such as intermittent screening for HBV infection, immunization for the animals and humans/handlers, good environmental design and care, health education for the handlers, provision of adequate social facilities in the lairage including water, drainage, clinics, medications, power supply, disposable and housing facilities.

Associations between HBV infection among age, height and length of the donkeys were statistically insignificant. This implies that HBV infection transmission could occur irrespective of the mentioned variables.

Among humans, prevalence of hepatitis B was found to be higher than the findings in this study in some regions and countries. Among the humans, one third are with varied epidemiology globally, in

regions, countries and even states in Nigeria.[1,30,31,34,35,44]

The study found the prevalence of HBV among donkeys to be 13.5%. This finding though high was comparable with results from studies conducted among related animals as stated above. This could be due to the fact that there is very poor veterinary control of the marketing due to witnessed non-cooperating attitudes of the butchers, who saw health control of their activities as trying to undermine their business. This factor severely militated against live donkeys and meat inspection with attendant selling infected donkeys; even dead ones were dressed and sold to the public. The butchers flagrantly refused to accept counselling.

CONCLUSION

Conclusively, the study has demonstrated that Hepatitis B virus infection was found among donkeys sold, slaughtered and consumed by Nigerians from Nkwor – Izhia donkey market in Ebonyi State, Nigeria. The prevalence of hepatitis B virus infection among donkeys is high (13.5%). There was the possibility of cross infectivity between the animals and humans, and human to human in the market. Donkeys from outside the country were found to be more infected with HBV infection than those within the country. Difference in mean duration of stay of donkeys in the market were also found to be statistically significant, whereas other variables such as age, gender, place of origin, length, height and colour were statistically non – significant.

We therefore recommend that;

1. There is obvious need to organize a health campaign in the markets including HBV sensitization, screening, and immunization for the donkey traders
2. Surveillance should be strengthened at Nigeria borders to track the infection for effective control of HBV among donkeys imported into the country.
3. Ebonyi State Government should enact laws to control the donkey trading in Nkwor – Izhia market and other markets in the state where the meat is sold.
4. More studies including intervention, cross – sectional among the traders become imperative.
5. Checklist should be developed to assess the environmental condition of the donkey market to design better control strategies of HBV infection.

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Duality of Interest: Nil

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