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Seroprevalence of Hepatitis B Virus Surface Antigen among Butchers and Slaughtered Cows in Kano Metropolis, Nigeria

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Abstract
Hepatitis B Virus is one of the most important human pathogens which present a significant threat to public health. The objectives of this study are to establish evidence of existence of Hepatitis B Virus (HBV) in cows and prevalence of Hepatitis B Surface Antigen (HBsAg) among butchers. A total of 138 human samples (including slaughter house workers and meat sellers) and 138 slaughtered cows' samples were collected for detection of HBsAg using ELISA. A questionnaire was administered to collect relevant information regarding risk factors. Screened result showed prevalence rate of 11.6% ($p< 0.05$) among butchers and 10.9% prevalence was detected in slaughtered cows ($p<0.05$), these were however not statistically significantly different. Occupational practices including hand cuts and sharing of knives were seen to be more common among slaughter house workers than the meat sellers thereby increasing their exposure to hepatitis B virus infection. It is concluded that both cows and butchers in Kano metropolis serve as reservoir for Hepatitis B Virus and hence there is need for urgent policy formulation (screening and use of protective clothing) in slaughter houses in Nigeria.
***Key Words**: Hepatitis B Surface Antigen, Butchers, Slaughtered Cows, Kano*

INTRODUCTION

The prevalence of HBV among humans and the nonhuman primates may speed up evolution process due to high error rate of viral reverse transcriptase, and recombination among different genotypes of hepadnavirus strains from human and non human primates (Shao *et al.,* 2004). Researchers have concentrated on prevalence of hepatitis B virus among pregnant women and health workers. Nevertheless up to the present time, no record on the prevalence of hepatitis B in non-primates in Nigeria. Slaughter house workers have been identified in occupational health studies for elevated risk of injury of which wound due to hand cut is the major form of injury reported (Din *et al.*, 2001). A large number of workers do not wear protective clothing or gloves to reduce their exposure.

It is estimated that 1-2 million people die annually from HBV related acute and chronic liver disease worldwide (Cui *et al.*, 2013). The majority of chronic carriers of HBV are found in South East Asia and sub-Saharan Africa (Musa *et al.*, 2015). Twenty five percent (25%) of carriers are at risk of chronic hepatitis and eventual death from liver cirrhosis and hepatocellular carcinoma (Sarri *et al.,* 2013). Butchers are likely to subject themselves to knife cuts and bloodletting with attendant risk of transmission of blood borne infections to co-workers with whom knives and other sharp objects are shared.

The high prevalence of the HBV in the tropics calls for the identification of groups at risk such as butchers, for targeted hepatitis B immunization. In Kano metropolis where this assessment was conducted, however, there was no data on the prevalence of hepatitis B among butchers, hence the need for the present study that aimed at determining the seroprevalence of HBV among butchers and slaughtered cows in Kano. The results of the study will be useful to stakeholders including government and NGOs (Non-governmental organizations) for policy formulation in the slaughter houses in Nigeria. It will also help to identify those that will require treatment and immunization against HBV infection. The outcome of the study would also serve as baseline data for future research endeavors and add to the existing data on the burden of HBV in Kano, other regions in Nigeria and developing countries.

MATERIALS AND METHODS

Study Area

This study was carried out on apparently healthy butchers and meat sellers from abattoir, Bachirawa and Tarauni market in Kano city, the capital of Kano State in Northwestern, Nigeria. Kano is 481metres (1,578 feet) above sea level with latitude 12° 00' N and longitude 8°31' E (Nigerian Meteorological Agency, 2017). The total area of metropolitan Kano is 499 square kilometers (193 square miles), with a population of 2,828, 861 according to Nigerian census (National Population Commission, 2006).

Study Design

The study was descriptive cross-sectional study conducted on apparently healthy butchers in abattoir and meat sellers at various meat selling points in the selected places.

Inclusion Criteria

Butchers or meat sellers in the selected places in Kano and cattle slaughtered in the abattoir. The study included only subjects that gave consent to participate in the research.

Exclusion Criteria

Butchers that did not agree to take part in the research (non- compliant) were excluded in the study.

Determination of Sample Size

A sample size for this study was determined using sample size determination in health studies, (Lwanga and Lemeshow, 1991).

$$n = \frac{Z^2 pq}{d^2}$$

Where n = minimum sample size

p = prevalence (9.4%) based on the local prevalence obtained from work of Yakubu *et al*, (2004).

Z = standard normal distribution at 95% confidence interval = 1.96 (constant)

q = 1-p

d= precision (0.05)

$$n = \frac{(1.96)^2 (0.094)(1-0.094)}{(0.05)^2}$$

$$n = \frac{(3.8416)\ \ (0.094)(0.906)}{0.0025}$$

$$n = \frac{0.3272}{0.0025}$$

$$n = 130.87$$

$$n \cong 131$$

However, 2.1% attrition rate of the calculated minimum sample size (130) which is175 samples were added to make a total of 276 for precision and accuracy……

Sampling Technique

Samples and demographic data of the subjects were collected randomly until the required sample size was obtained.

Specimen Collection

Blood specimens were collected from 138 butchers using standard blood sample collection procedure (Grizzle *et al*., 2005). Blood samples were also collected from 138 slaughtered cows. Two groups were tested. The first group consists of 138 subjects comprising 82 butchers (all males) and 56 meat sellers (46 males and 10 females). The second group comprised 138 slaughtered cows (109 females and 29 males).The age group of the human subjects was between ages 12-80 years.

Data Collection

Demographic data of the subjects which include age, sex, working history at the selected places, sharing of knives, cuts during use of knives, family, socioeconomic status, history of immunization and education on prevalence of Hepatitis B was collected through administration of questionnaires.

Ethical Clearance

Ethical clearance was obtained from Kano State Abattoir which is under Kano State Ministry of Agriculture before the commencement of the study. Consent was obtained from subjects prior to sample and data collection.

Detection of Hepatitis B Virus by Enzyme Linked Immunosorbent Assay (ELISA).

HBV infection was detected by assay of the hepatitis B surface antigen (HBsAg) using the HBsAg EIA Test Kit. It is a third generation immunoassay for the qualitative detection of the presence of Hepatitis B surface antigen in serum or plasma specimen. The test utilizes monoclonal antibodies according to manufacturer's instruction to selectively detect various subtypes of HBsAg in serum (ACON Laboratories Incorporation, San Diego, U.S.A.)

The HBsAg EIA Test Kit is a solid phase qualitative enzyme immunoassay based on a sandwich principle for the detection of HBsAg in human serum or plasma. The microwell plate was coated with monoclonal antibodies specific to various subtypes of HBsAg. During testing, the specimen and the enzyme-conjugated HBsAg antibodies were added to the antibody coated antibody microwell plate and simultaneously bind to the conjugate to form immobilized antibody-HBsAg-conjugate complexes. If the specimen does not contain HBsAg, the complexes will not be formed. After initial incubation, the microwell plate was washed to remove the unbound materials.

Substrate A and Substrate B were added and then incubated to produce blue color, indicating the amount of HBsAg present in the specimen. Sulfuric acid solution was added to the microwell plate to stop the reaction which produces a color change from blue to yellow. The color intensity, which corresponds to the amount of HBsAg present in the specimen, was measured with a microplate reader at 450nm. The test was carried out and interpreted according to the manufacturer's instruction.

Data Analysis

Data generated was analyzed using SPSS software version 20 (2011, IBM Corp). The prevalence of HBV infection was expressed in simple proportions and percentages for the study group. Chi-square (X^2) was used to determine the relationship between HBV infection and associated risk factors. A probability value of less than (0.05) was taken as significant.

RESULTS

Hepatitis B surface antigen (HBsAg) was highest among the 31-40 age (4.3%) group, followed by 21-30(2.9%) age group and 11-20(2.2%) age group respectively (Table 1). Seventy three point two percent (73.2%) of the human subjects were married while 26.8% were not married (Table 2). However, there is no any significant relationship between the age group and HBsAg status of the subjects studied ($p > 0.05$). Many risk factors for HBV transmission were examined. Risk factors like occupational practices, sexual practices, social practices and treatment practices. The results showed that majority of butchers are at risk based on the aforementioned practices. Ninety three point five percent (93.5%) of the subjects do share knife among themselves (Table 3) and 92.8% of the subjects do not go to hospital for proper medication when they fall sick but they rather go to chemists (medicine store keepers) for injections (Table 4). It was observed that butchers are at greater risk of contracting or transmitting HBV than the meat sellers. Six point five percent (6.5%) of hand cuts was recorded among meat sellers compared to 93.5% seen among butchers and 69.6% knife sharing was recorded among butchers compared to 30.4% seen among meat sellers(Table 5). It was also shown that there is a significant relationship between occupation of the subjects and HBsAg status of the subjects at p value <0.05 (Table 6). Hand cuts and wound sustained by butchers and meat sellers are not usually properly dressed (Table 7). HBsAg was detected in 11.6% (n= 138) of the human subjects, 8% in butchers and 3.6% was found among meat sellers. However 1.5% was recorded in females and 10.1% was recorded in males (Table 8). Table 9 also shows that HBsAg was detected in 10.9% (n= 138) of the animal subjects (slaughtered cows). Fifty seven point two percents (57.2%) of the samples collected were females while 42.8% of the samples were males. Seven point two percent (7.2%) of the female samples were found to be positive while 3.7% of the male samples were found to be positive.

Table 1: Age group and HBsAg Status of the Subjects

Age Group	Negative	Positive	% Positive	Total
11-20	10	3	2.2	13
21-30	42	4	2.9	46
31-40	38	6	4.3	44
41-50	22	1	0.72	23
51-60	7	1	0.72	8
61-70	2	1	0.72	3
71-80	1	0	0.00	1
Grand Total	**122**	**16**	**11.57**	**138**
$X^2 = 4.927$,		P value = 0.553		

Table 2: Marital Status in Relation to HBsAg in Subjects

Married	Negative	Positive	Total
YES	91	10	101(73)
NO	31	6	37(27)
Grand Total	**122**	**16**	**138**
$X^2 = 1.054$,		P value = 0.305	

Table 3: HBsAg Status in Relation to Sharing of Knives among the Subjects.

Sharing of Knives	Negative	Positive	Total
NO	40	2	42(30)
YES	82	14	96(70)
Grand Total	**122**	**16**	**138**
X^2 = 2.750,		P value = 0.097	

Table 4: Taking of Injection from Chemist in Relation to HBsAg Status of Subjects.

Injection By Chemist	Negative	Positive	Total
NO	8	2	10(7)
YES	114	14	128(93)
Grand Total	**122**	**16**	**138**
X^2 = 0.743,		P value = 0.389	

Table 5: HBsAg Status in Relation to Occurrence of Hand Cuts in Subjects.

Hand Cuts	Negative	Positive	Total
NO	9	0	9(7)
YES	113	16	129(93)
Grand Total	**122**	**16**	**138**
X^2 = 1.263,		P value = 0.261	

Table 6: HBsAg Status and Occupation of the Subjects.

Occupation	Negative	Positive	Total
Butchers	71	11	82(59)
Meat Sellers	51	5	56(41)
Grand Total	**122**	**16**	**138**
X^2 = 16.00,		P value = 0.039.	

Table 7: Improper Wound Dressing in Relation HBsAg Status of the Subjects.

Improper Wound Dressing	Negative	Positive	Total
NO	61	7	68(49)
YES	61	9	70(51)
Grand Total	**122**	**16**	**138**
X^2 = 0.221,		P value = 0.638	

Table 8: HBsAg Status in Relation to Sex of the Subjects.

Sex	Negative	Positive	Total
Female	8	2	10(7)
Male	114	14	128(93)
Grand Total	**122**	**16**	**138**
X^2 = 0.743,		P value = 0.389	

Table 9: HBsAg Status of Animal Samples Collected

Sex	Negative	Positive	Total
Female	69	10	79(57)
Male	54	5	59(43)
Grand Total	**123**	**15**	**138(**

DISCUSSION

Majority of the previous studies on HBV infection amongst possible high risk groups in Nigeria were targeted on healthcare workers and pregnant women. It is remarkable that the 11.6% prevalence gotten from this study is higher than the 8% cut-off value stipulated by the World Health Organization for HBV hyper-endemic regions. A study from University of Ibadan has shown that butchers from Ibadan, Nigeria have a prevalence rate of HBV infection at 9.3% which may lead to the spread of infection in the community (Ola *et al.*, 2002).

Outbreak of Hepatitis B in a butchery was reported in Jerusalem and the result showed that at working sites, HBsAg positive workers, 26% (14 out of 54) of the butchers had evidence of Hepatitis B infection while in other sites that each had a single HBsAg- positive worker, 57% (16 out of 28) of butchers had hepatitis B exposure (Mevorach *et al.*, 2012).

It is therefore clear from the finding of this study that butchers constitute a high risk group and serve as reservoir for HBV. Hence, slaughter-houses in Nigeria and possibly in other developing countries seem to serve as reservoirs for HBV infection. Slaughter house workers have been identified in occupational health studies for elevated risk of injury of which wound due to hand cut is the major form of injury reported (Din *et al.*, 2001). A large number of workers do not wear protective clothing or gloves to reduce their exposure. Hence, this group should be targeted when introducing control measures to reduce the transmission of infection.

Researchers have concentrated on prevalence of hepatitis B virus among pregnant women and health workers and up to the present time, no record on the prevalence of hepatitis B in non-primates in Nigeria. The prevalence observed in this study (10.9%) from slaughtered cows showed that there is also HBV in cows. The 10.9% is less than the 24.8% prevalence gotten from swine from a farm in China ((Liu *et al.*, 2010). Therefore, presence of HBV among humans and the nonhuman primates may speed up evolution process in the nearest future due to high error rate of viral reverse transcriptase, and recombination among different genotypes or hepadnavirus strains from human and non human primates (Shao *et al.,* 2004). Research on HBV-like viruses in domestic animals has been carried out since 1985 (Din *et al.,* 2001). Liver of captive swine and chickens were found to be naturally infected with HBV in China and these findings together with known ability of HBV to cross species barriers, suggested that human and non-human HBV variants might share hosts in nature (Yang *et al.*, 2007). Evidence of the existence of a novel member of the hepadnavirus family endemic in swine has also being established and temporarily this virus was designated as swine hepatitis B virus (SHBV) (Liu *et al.*, 2010). This SHBV can be detected by using human hepatitis B virus diagnostic kits including ELISA, immunohistochemical staining, and transmission electron microscopy (TEM). Screened result showed that overall prevalence of HBsAg was 24.8% out of 416 samples of swine serum collected from 5 randomly selected farms in Beijing, China (Liu *et al.*, 2010).

A study was also carried out in China to determine the presence of HBV in serum and liver of chickens and the overall prevalence of HBsAg, anti-HBs, anti-HBc was 28.68%, 53.49%, 17.05%, respectively, whereas HBeAg, anti-HBe were barely detectable (Wengui *et al.*, 2012) . The viral DNA sequence identified in two of the chicken livers shared 92.2% of one known HBV strain and 97.9% nucleotide sequence of another HBV strain (Wengui *et al.*, 2012). Serological data from several samples of swine from Brazil and partial genome sequencing (252-365bp) of three of these samples confirmed evidence that human populations have been so far infected with HBV variants of animals used for food and therefore, animal source food deserves closer attention (Salassa *et al.,* 1991).

CONCLUSION AND RECOMMENDATIONS

The current study shows the prevalence of HBV among human subjects (butchers and meat sellers) and animal subjects (slaughtered cows) in Kano metropolis was found to be 11.6 % and respectively10.9%. There is a significant relationship ($p < 0.05$) between the occupation of the human subjects and their HBsAg status. Hence butchers and cows in Kano serve as reservoir for HBV and this call for urgent policy formulation (screening and use of protective clothing) in slaughter houses in Nigeria. It is recommended that further studies at molecular level should be carried out to fully understand the structure of HBV found in cows. The discovery of HBV in cow may unveil novel evolutionary aspects of hepatitis and provides new information for further hepadnavirus research since none of the currently available animal models are ideal. It is hoped that the development of additional experimental animal models may provide answers for many HBV research questions like what are the ways by which HBV can spread.

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