



Circulating HBV Genotypes among Animal and Non-Animal Handlers in Osun State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors IOO and OOO designed the study and wrote the protocol. Authors IRG and BUA managed the analyses of the study and performed the statistical analysis. Author IOO managed the literature searches and wrote the first draft of the manuscript. Author OOO supervised the whole study which, author IRG used as part of her M.Sc. Dissertation in the Department of Medical Microbiology & Parasitology, Ladoké Akintola University of Technology, Ogbomoso, Nigeria. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Hepatitis B virus (HBV) is not uncommon among animal and non-animal handlers. The brutality of HBV infection and the outcome of treatment is linked with exact HBV genotypes. No study on the circulation of HBV genotypes has been reported among animal and non-animal handlers in Nigeria. This study was intended to evaluate the genotypic distribution among animal and non-animal handlers in Osun State, Nigeria.

Study Design: Cross-sectional study.

Place and Duration of Study: Ladoké Akintola University of Technology (LAUTECH), Nigeria, between June 2015 and July 2019.

Methods: Blood samples were obtained from HBsAg positive individuals and screened for HBV-DNA from cohorts of animal and non-animal handlers. HBV-DNA was extracted, amplified and genotyped using a multiplex PCR technique with primers specific for six genotypes of HBV (Genotype A, B, C, D, E and F).

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Results: Results showed that a total of 11 (6.1%) of the 180 animal and non-animal handlers evaluated were positive to HBsAg and 4.4% were positive for HBV-DNA by a semi-nested PCR using HBV specific primer pairs. The molecular analysis of the sera of 11 HBsAg positive animal and non-animal handlers showed that 72.7% of them had a true HBV infection. Results further show that genotype E (75.0%) was predominant over genotype A (12.5%) and mix genotypes (D and E) with 12.5% prevalence. Other genotypes were not detected. Of the 8 positive HBV-DNA samples, 7 (87.5%) were males and one (12.5%) was a female. All animal and non-animal handlers with true HBV infection were found to harbour HBV genotype E predominantly.

Conclusion: The molecular analysis of HBV-DNA and genotypes circulating among animal and non-animal handlers shows that the majority of the subjects with true HBV infection were found to predominantly harbour HBV genotype E in Osun state, Nigeria. The study further highlights the predominance HBV genotype E in Nigeria.

Keywords: HBV genotypes; HBV-DNA; Nigeria.

1. INTRODUCTION

Globally, the Hepatitis B virus (HBV) is ranked among the deadliest infectious diseases with increasing public health consequences [1, 2]. HBV is not uncommon among animal and non-animal handlers. The brutality of HBV infection and the outcome of treatment is linked with exact HBV genotypes [3]. HBV infection is endemic in Sub-Saharan Africa [3, 4]. So far, ten different HBV genotypes have been reported based on sequence diversity [5]. Based on sequence divergences in the entire genome of >8 %, HBV genomes have been classified into ten genotypes designated as A to J, according to their genetic variability [6-10]. Factors such as genotypes, viral load, presence of HBeAg, and escape mutations have been reported to affect the consequence of HBV infection [3, 5].

HBV genotypes display a distinct geographic and ethnic distributions among different countries, the genotype dependence of pathogenesis and clinical sequences have been shown [10-13]. For instance, authentic genotype (GT)-B (designated GT-Bj) is related to a high rate of HBe antigen seroconversion and slow disease progression [13-15]. On the other hand, infection with GT-A results in a higher chronicity rate than infection with other genotypes [13, 16-17].

In Nigeria, detection of HBV infection is typically done with the assessment of a serological marker (HBsAg) alone [2, 18, 19]. The appearance of variants of HBV and the necessity for early detection of HBV infection necessitates enhancement of currently existing immunoassays [20]. At present, there is a gap in knowledge on the circulation of HBV genotypes among HBV-infected animal and non-animal

handlers in Nigeria. In light of the above, this study aimed at determining the prevalence of HBV-DNA and HBV genotypes among HBV-infected animal and non-animal handlers in Osun State, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

This study was carried out among butchers, pig handlers and the non-animal handlers in Ife, Sekona and Osogbo, Osun State, South-Western Nigeria. The laboratory analysis was carried out in the Molecular Biology Laboratory, Ladoke Akintola University of Technology, Isale Osun, Osogbo, Nigeria (Fig. 1).

2.2 Study Design

This is a cross-sectional study involving people whose occupation increased their risk of infection (both males and females). Ethical approval was obtained by the Osun State Ministry of Health, Osogbo. The protocol number was OSHREC/PRS/569T/3.

2.3 Study Population

The study participants comprised of people whose occupation increased their risk of HBV infection. The study participants included 90 animal and 90 non-animal handlers, making a total of 180 volunteers.

2.4 Sample Collection

Five (5 ml) of venous blood was collected from each participant into EDTA bottles. Whole blood was used for HBV DNA detection.

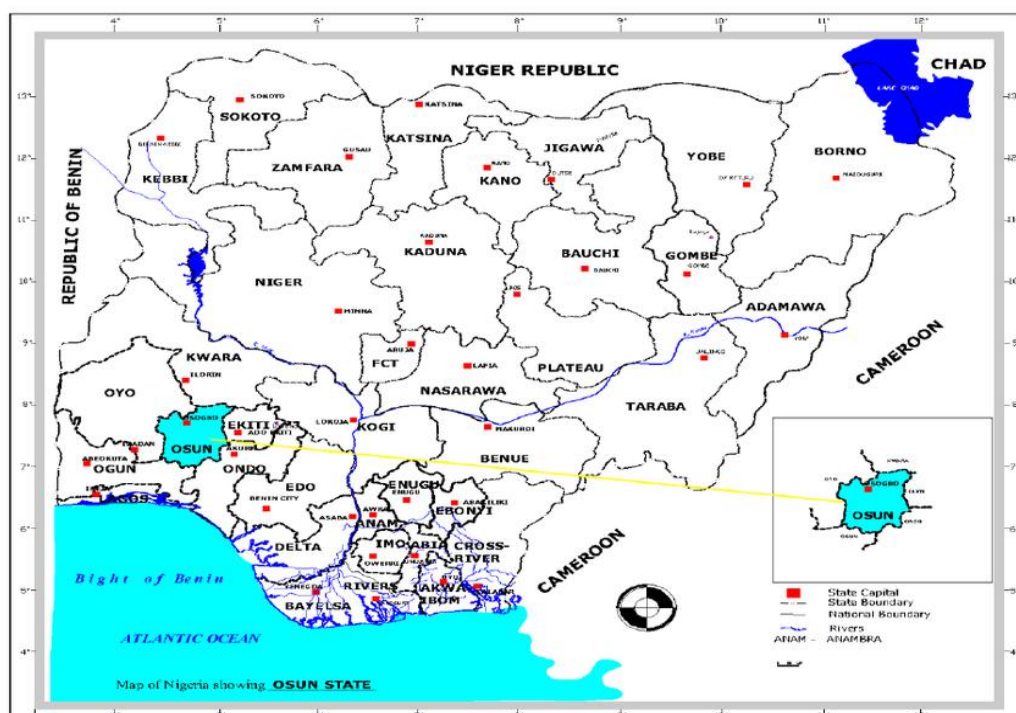


Fig. 1. Map of Nigeria showing Osun State (the study area)

2.5 HBV DNA Extraction

Genomic DNA extraction kit (Qiagen diagnostics, Germany) was used for HBV DNA extraction. The initial steps included the preparation of lysis buffer, wash buffer and proteinase K, after which elution buffer was preheated to 70°C. Blood lysis: 25 µl of proteinase k, buffer PR, and 200 µl of the sample were added into 1.5 ml microcentrifuge tube; 200 µl of lysis buffer was also added to the mixture which was then vortex vigorously for about 10-20 sec. The samples were incubated at 70°C for 10-15 min, after which 210 µl of ethanol (96-100%) was added and vortex. For each preparation, one spin column was placed in a collection tube and the samples were loaded onto the column which was centrifuged for 1min at 11000 rpm. The column was placed in a new collecting tube of 2ml and 500 µl of wash buffer was added. It was centrifuged for 1min at 11000 rpm, the flow-through was discarded and the collecting tube was reused. The wash step was repeated once. The residual ethanol was removed by centrifuging for 1min at 11000 rpm. The Genomic DNA spin column was placed in a 1.5 ml microcentrifuge tube, 100 µl preheated elution buffer (70°C) was added onto the silica membrane which was incubated at room temperature for 1min and later 1min at 11000 g then the DNA was eluted and stored at -20°C.

2.6 HBV Genotyping (PCR)

Naito et al. [21] method were used for genotypic identification of HBV using multiplex PCR nested reaction under the following conditions in the first reaction, initial denaturation at 95°C for 10 mins, followed by 40 cycles at 94°C for 20mins, 55°C for 20 mins, 72°C for 1 min and final extension was done at 72°C for 10 mins. In the second reaction initial denaturation 95°C for 10 mins, followed by 20 cycles of 94°C for 20 secs, 58°C for 20 secs, 72°C for 30 secs and another 20 cycles 94°C for 20 secs, 60°C for 20 secs, 72°C for 30 secs and a final extension at 72°C for 10 mins [21]. The primer set (forward primer 5'-TCACCATATTCTTGGGAACAAGA-3' and reverse primer 5'-CGAACCACTGAACAAATGGC-3') used for the amplification of the target gene has been described in a previous study [3]. The second-round PCR products from the sample were separated by gel- electrophoresis on an agarose gel, stained with cyber green, and was evaluated under the UV light. The size of the PCR product was assessed by migration pattern of a 100-bp DNA ladder. The positive controls were HBV-DNA obtained from the Robert Koch Institute in Germany. It is a specific HBV genotype E.

2.7 Data Analysis

Data generated were analyzed using SPSS version 20.0 to compare HBV-DNA positive and negative samples and Pearson's chi-square method were used and the level of significance was set at $P < 0.05$ at 95% confidence interval.

3. RESULTS AND DISCUSSION

3.1 Results

Eleven (6.1%) of the 180 samples were positive for HBsAg while 8 (4.4%) were HBV-DNA positive by a semi-nested PCR using HBV specific primer pairs and of the 11 HBsAg positive samples (Tables 1 and 2). Molecular analysis of the sera of all 11 animal and non-animal handlers that were seropositive for HBV showed that 8 (72.7%) of them had a true HBV infection (Table 1). Of the 8 positive HBV-DNA samples, 7 (87.5%) were males and one (12.5%) was a female. There was no significant association between HBV-DNA and gender (Table 1).

Results further show that genotype E was the predominant followed by genotype A and mixed genotypes D and E. Other genotypes were not detected as shown in Tables 1 and 2.

Fig. 2. shows the gel picture of PCR products with the expected band size of 68 bp of the fragments amplified. Genotyping of the HBsAg samples using genotype-specific primers with detectable HBV-DNA showed that 1 (12.5%) of the HBV-DNA positive samples were HBV genotype A (Table 2).

Fig. 3 shows the gel pictures of product of PCR amplification fragment of HBV-DNA for the genotypes E and mixed HBV genotypes (D and E) of HBV respectively. Genotyping of the HBsAg samples using genotype-specific primers with detectable HBV-DNA showed that 6 (75.5%) of the HBV-DNA positive samples were HBV genotype E (band size of 167bp of the fragments amplified) while one (12.5%) sample had mixed HBV genotypes (genotype D with a band size of 119bp of the fragments amplified and genotype E) (Fig. 3. and Table 2).

3.2 Discussion

Treatment outcome of HBV infections is associated with specific genotypes [3, 22]. Reports on the circulating HBV genotypes in

Nigeria are sparse. Indeed, there is no report on the genotypic prevalence of HBV among animal and non-animal handlers in Nigeria. This gap informed the present study.

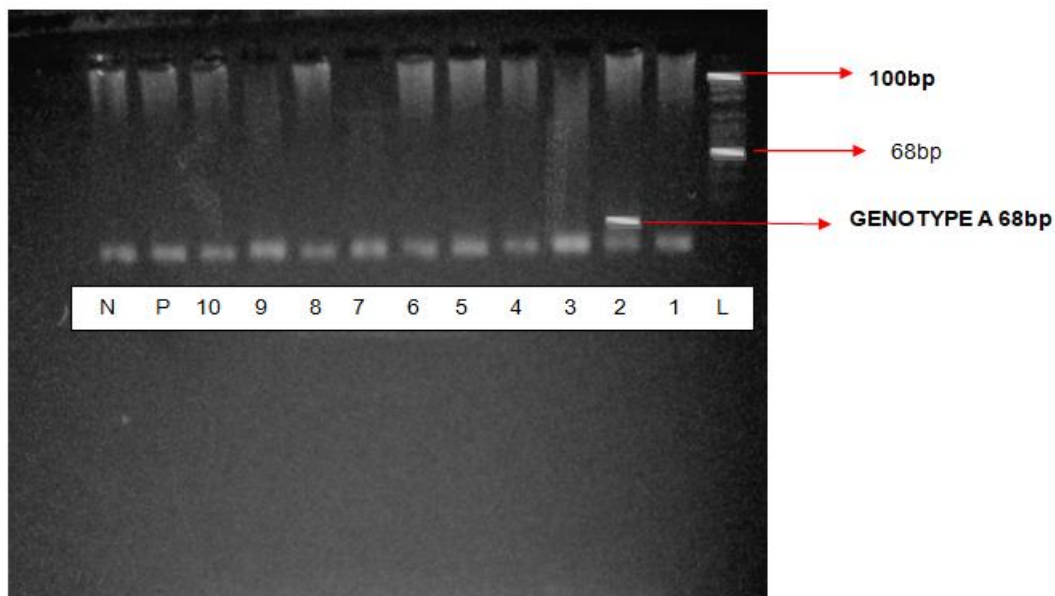
A total of 11 (6.1%) of the 180 animal and non-animal handlers were positive for HBsAg and 4.4% were positive for HBV-DNA. Molecular analysis of the sera of 11 HBsAg positive animal and non-animal handlers showed that 72.7% of them had a true HBV infection. Similar higher prevalence of true HBV infection has been reported by other African studies [23-26] with a lower value documented in one study conducted outside Africa [27]. The HBV infection outcome is believed to have been linked to specific HBV genotype [3, 5]. Thus, the observed variations in this study population might be as a result of the changes in the immune status and HBV genotypes. In line with the assertions by Oladeinde et al. [3], this discrepancy could be owing to variances in HBV infection severity, different molecular diagnostic protocols adapted for the study, and the targeted HBV genotypes.

HBV genotype A is the most frequently disseminated globally and is foremost genotype in Africa, Europe, India and North America, while B and C remain the leading genotypes in East and Southeast Asia [10, 28, 29]. Genotype D is predominant in the India, Middle East and Mediterranean region, and E is frequently distributed in sub-Saharan Africa, among other continents [10, 30-32]. Also, the most frequently detected genotypes in Asia are B and C except for India where the genotypes A and D are most predominant [10, 33-34]. Outside the Central and South America, genotypes F and H are seldomly found [35, 36], and G is mostly found in France, Germany, Mexico and the USA [10, 37]. This genotype G is usually existing as a co-infection with other HBV genotypes, most frequently with genotype A. The genotype I has been found in China, Laos and Vietnam [10, 38-39], while the newest genotype J was discovered in the Ryukyu Islands in Japan [8, 10]. Therefore, the genotype dependency of the strength and/or manner of host-immune responses during HBV infection should be considered [13].

Also, in this study, majority of the HBsAg positive individuals with true HBV infection were found to predominantly harbour HBV genotype E. HBV genotype E has been reported to account for a majority of HBV infection in West Africa [3, 30, 31]. This predominance of HBV genotype E has been reported among other study populations in several African studies [3, 25, 26, 40]. However,

Table 1. HBV markers detection among animal and non-animal handlers by gender in Osun State, Nigeria

Results	Total (%)	Gender (%)	
		Male	Female
ELISA test			
Positive (%)	11(6.1)	10 (8.9)	1 (1.5)
Negative (%)	169(93.9)	102 (91.1)	67 (98.5)
PCR test			
Positive (%)	8(4.4)	7 (6.3)	1 (1.5)
Negative (%)	172(95.6)	105 (93.7)	67 (98.5)
Genotype			
True infection	8/11(72.7)	7/8(87.5)	1/8(12.5)
E	6(75.0)	6 (75.0)	0 (0.0)
A	1(12.5)	0 (0.0)	1 (12.5)
D/E	1(12.5)	1 (12.5)	0 (0.0)

**Fig. 2. The gel picture of PCR products with the expected band size of 68 bp of the fragments amplified (PCR representation of the Mix A of the Nested reaction for HBV genotyping)**

Lane 2 was positive for Genotype A, Ladder =100 bp, Genotype A= 68 bp, N= Negative control, P= Positive control

some discrepancies existed in other previous studies in Africa. No HBV genotype E was detected among in a previous study in South Africa [24], Columbia [27] and Pakistan [41]. Several studies have shown that HBV genotype E is essentially found in Africa and not otherwise [3, 30]. HBV genotype E also predominates even among African-American populations especially those with West African origin [30]. Thus, suggesting that HBV genotype E is probably a recently evolved genotype [3, 30].

Table 2. HBV Genotypes among HBsAg Positive animal and non-animal handlers

Genotype	Positive sample (%)
A	1 (12.5)
B	0 (0.0)
C	0 (0.0)
D	0 (0.0)
E	0 (0.0)
F	0 (0.0)
D and E	1 (12.5)

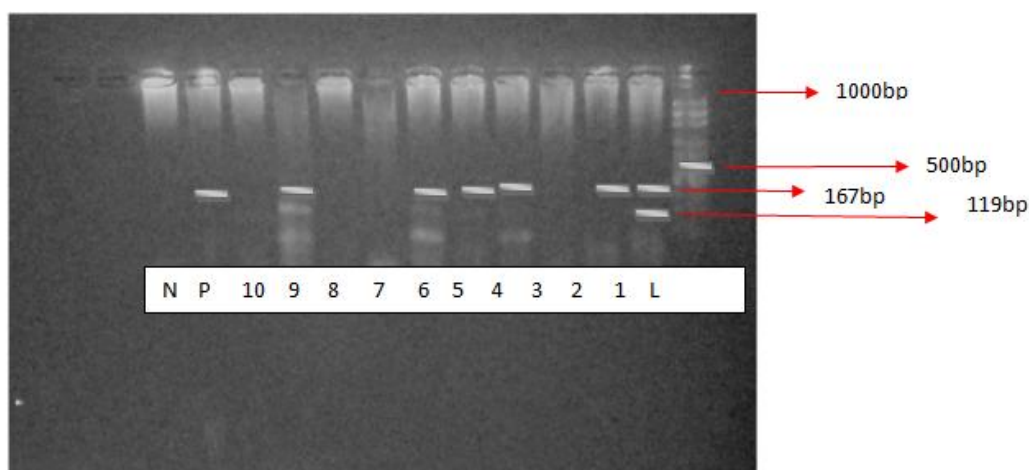


Fig. 3. The gel pictures of product of PCR amplification fragment of HBV-DNA for the genotypes E and mixed HBV genotypes (D and E) of HBV respectively (PCR representation of Mix B of the Nested reaction for HBV Genotyping)

Lane 1, 2, 4, 5, 6, 9 were positive for Genotype E while Lane 1 shows a genotype of D and E. Ladder = 100bp, Genotype E = 167bp, Genotype D = 119bp, P = Positive control, N= Negative control.

Although no HBV genotypes B, C, D and F was found in this study, their presence has been reported in other previous studies outside of Africa. HBV subtype B was reported in a previous French study [42]. Another study carried out in the United States of America reported a predominance of HBV genotype C [43] while a study in Venezuela [44] reported marked differences in HBV genotype distribution among different study populations. These studies deviated from the findings of this present study with the predominance of genotype E. The observed differences may be attributed to variations in geographical location and possibly other predisposing factors such as genetic factor. However, further in-depth study to authenticate this factor is highly advocated.

One of the limitations of this study is that we did not sequence the HBV genome in this study. Another limitation is that though the predominant HBV genotype was genotype E, we did not explore to know the variation in the disease outcome from one person to the other since they are principally genotyped E. Truly sequencing seems to be the gold standard for genotyping however there are several other methods of genotyping which are equally valid other than sequencing and multiplex PCR is one. However, we believe that these limitations did not significantly affect the final interpretation of study findings. We used the multiplex PCR method which is a good and proven alternative to sequencing in a resource-limited country like Nigeria.

4. CONCLUSION

The molecular analysis of HBV-DNA and genotypes circulating among animal and non-animal handlers shows that the majority of the subjects with true HBV infection were found to predominantly harbour HBV genotype E in Osun state, Nigeria. The study further highlights the predominance HBV genotype E in Nigeria. The impact of these findings is to highlight the fact that there could be mixed genotypes other than the genotype E that has been known, but the impact is a question for another study.

CONSENT

All authors declare that written informed consent was obtained from the patient (or other approved parties) for publication of this study.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the Osun State Ministry of Health, Osogbo, Nigeria (OSHREC/PRS/569T/3), and have, therefore, been performed following the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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