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Molecular detection and genotyping of Hepatitis B Virus on the island of Ngazidja, Comoros

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ABSTRACT— Africa bears a disproportionate share of the global hepatitis burden. The Comoros are classified among the countries with intermediate prevalence of hepatitis B virus (HBV) infection. The aim of this study was to determine the diagnostic circumstances and the genotypes of circulating HBV among HBV-infected patients on the island of Ngazidja, Comoros. Genotyping was performed using specific primers in multiplex PCR (Polymerase Chain Reaction) targeting the S region. 60 patients with chronic hepatitis B were included in the study, comprising forty chronic patients and thirty randomly selected samples from in the medical biology laboratory of Elmaarouf National Hospital Center (CHN) in Moroni. The average age of the patients in this study was 34.4 years. The circumstances of HBV diagnosis were primarily related to transmission prevention, notably through blood donations and prenatal screening. Genotype distribution revealed a predominance of genotype D (100%) patients and 1 patient presented with a co-infection of genotypes D and E. This study underscores the importance of monitoring medical practices that risk HBV transmission and shows that genotype D predominates among patients on the island of Ngazidja. Further research is needed to enhance prevention and treatment strategies.

KEYWORDS: chronic, HBV, genotype, Comoros

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1. Introduction

Hepatitis B infection represents a significant global public health challenge. This viral infection has affected nearly a billion people, with more than 254 million individuals serving as chronic carriers. By 2022, it is estimated that the disease will cause more than a million deaths [30]. Additionally, HBV is a major cause of severe liver complications, including cirrhosis and 80% of cases of hepatocellular carcinoma [7], [23]. Africa is the most endemic region, with approximately 70% of infections, and the infection can be contracted at any age.

HBV is classified into 10 genotypes, designated from A to J, each having a narrow geographic distribution that evolved in parallel with human movements and anthropological activities. Genotypes A and D are ubiquitous, primarily present in North America, Northern Europe, India, and Africa [11], [14], [13]. Genotypes B and C predominantly occur in Asia [6]. Genotype E is endemic to Africa and Madagascar [3], [15] with some cases reported in other regions of the world among individuals of African origin [5], [8], and sporadically in Colombia [2] and India [27]. Genotypes F and H are mainly observed in the Americas, dominating among the indigenous populations [19], [24]. Genotype G is observed in the United States and Europe. Genotype I is a recombinant of genotype C and another unknown genotype, circulating in Laos and China [32]. Genotype J has been putatively described in a Japanese patient with carcinoma [28]. Ngazidja Island, the largest island of the Comoros archipelago, is located at the crossroads of the Mozambique Channel, northeast of Madagascar and Africa, in the Indian Ocean. The archipelago has a resident population estimated at less than one million individuals, with more than 50% under the age of 25. According to the WHO, the Comoros exhibit an intermediate prevalence (5%) of HBV infection. In Ngazidja, our study conducted from 2018 to 2024 among the specific population of blood donors revealed a prevalence of hepatitis B surface antigen (HBsAg) of 2.53%. However, the geographic distribution of HBV genotypes in the Union of the Comoros remains incomplete. The objective of this study is to identify the diagnostic circumstances of the infection and the genotypes currently in circulation on the island of Ngazidja.

2. Materials and Methods

2.1 Study population

This study involved 30 adults with chronic HBV infection and 30 patients from the serum Bank of the CHN Elmaarouf laboratory. All study participants were over 17 years of age and had tested positive of HBsAg.

2.2 Laboratory Analysis

2.3 Sampling and viral DNA extraction

Each patient donated 5 ml of blood, collected aseptically via venipuncture into standard red-capped tubes. The whole blood was allowed to clot at room temperature for approximately 15 minutes. Subsequently, the clot was removed by centrifugation at 2,000 × g for 10 minutes. The resulting supernatant, referred to as "serum," was immediately stored at -40°C in the medical biology laboratory of Elmaarouf CHN in Moroni, Comoros. It was then transported to Morocco and stored at -80°C until further use.

2.4 DNA Extraction and Genotyping

The COBAS AmpliPrep/COBAS TaqMan HBV Test, v2.0 was utilized for total DNA extraction from serum samples, following the manufacturer's instructions. Viral DNA amplification was performed using real-time PCR on the COBAS z480, an automated system for nucleic acid amplification and detection by real-time PCR in the X region, based on an open plate format, according to the method described by [16].

Viral DNAs were amplified using Green Taq Mix via a multiplex PCR approach. Primers were designed by to distinguish genotypes A through F by restriction fragment polymorphism of the S gene amplicon from the first cycle [12] (Table 1). PCR products were analyzed by loading 5.0 µL of the PCR product onto a 2.5% agarose gel in 1× TBE buffer. Electrophoresis was conducted at 150 volts for 30 minutes using an electrophoresis system. The 100 bp DNA ladder was employed to examine the PCR amplicons.

The DNA extraction, quatification and genotyping were performed at the Institut Pasteur molecular biology laboratory of the Institut Pasteur du Maroc (IPM) (Casablanca, Morocco).

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Tableau 1: Primers for different genotypes and their respective amplicon sizes [12]

Génotype	Primer	Size of amplificate (bp)
A	5'-CGGAAA CTA CTG TTG TTA GAC GAC GGG AC-3'; 5'-AAT TCC TTT GTC TAA GGG CAA ATA TTT AGT GTG GG-3'	**370**
B	5'-CCG CTT GGG GCT CTA CCG CCC G-3'; 5'-CTC TTA TGC AAG ACC TTG GGC AGG TTC C-3'	**190**
C	5'-CCT GAA CAT GCA GTT AAT CAT TAC TTC AAA ACT AGG-3'; 5- AGC AGG GGT CCT AGG AAT CCT GAT GTT G-3'	**701**
D	5-ACA GCA TGG GGC AGA ATC TTT CCA CCA G-3'; 5'-CCT ACC TTG TTG GCG TCT GGC CAG G-3'	**147**
E	5'-CTA ATG ACT CTA GCT ACC TGG GTG GGT GTA-3'; 5'-CCA TTC GAG AGG GAC CGT CCA AGA AAG C-3'	**787**
F	5'-ACA GCA TGG GAG CAC CTC TCT CAA CGA CA-3'; 5'-AGA GGC AAT AGT CGG AGC AGG GTT CTG-3'	**481**

3. Results

3.1 Demographic characteristics

The median age of the patients was 34.4 years, with an age range of 17 to 65 years, and 33% of the patients were in the age group of 33 to 40 years. There was a male predominance among the studied subjects, with 61.43% being men (n = 43) compared to 38.3% being women (n = 27). The primary circumstances under which the condition was discovered varied by sex: it was frequently identified during a blood donation for men and during a prenatal screening for women.

Tableau 2: Demographic Characteristics and Diagnostic Circumstances of Patients

Demographic characteristics	Frequency	%
Gender		
Male	43	**61.43**
female	27	**38.57**
Age		
[18-25]	14	**20.29**
[26-32]	15	**21.74**
[33-40]	23	**33.33**
>=41	17	**24.64**
Emplyoment*		
students	3	**10**
Formal work	11	**36.67**
Informal work	16	**53.33**
Marial status *		
Married	21	**75**
Single	7	**25**
Discovery circumstances		
Healthy Chekup	3	**5.17**
Prenatal visit	15	**25.86**
Blood donation	21	**36.21**
Medical prescription	19	**32.76**

Domicile		
Moroni (Capital of Comoros)	26	**43.33**
Peripheral zone	44	**56.67**

(*) only of chronic HBV patients who responded to the questionnaire

3.2 Genotyping

We are pleased to report that 70 patients were genotyped using specific multiplex PCR, with primers targeting each HBV genotype (Table 2). We are delighted to announce that 47/60 of these patients tested positive: 46 had genotype D only, and 1 patient had co-infection of genotypes D and E (Fig. 1).

4. Discussion

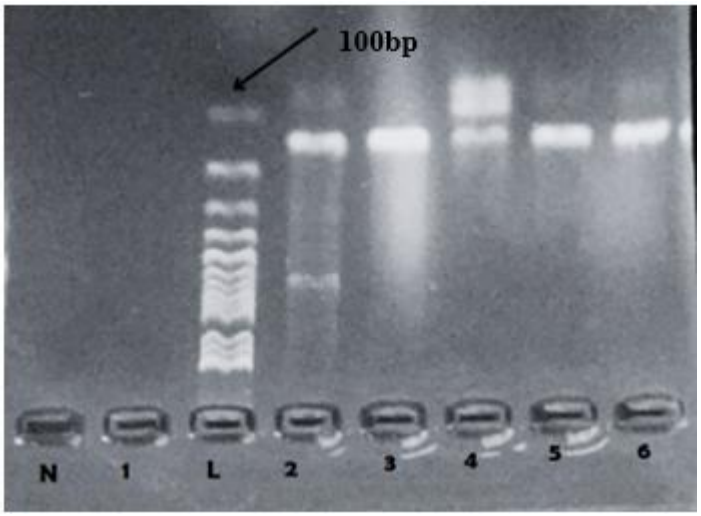

Figure 1: Agarose gel electrophoresis shows the results of amplification of the different genotypes by multiplex HBV PCR Lane L: 100 bp DNA ladder, lanes 3-7: approximately 147 bp HBV amplicon of HBV genotype D, lane 1: negative N: negative control and Lane 2: co-infection (D & E)

In sub-Saharan Africa, HBV infection accounts for 70% of global cases [31]. In Comoros, the prevalence in the general population is not well documented. The WHO estimates that HBV endemicity is intermediate, and a study of specific populations, including donors and students, reported a prevalence of 2.3% [20], [18]. Understanding the circulating genotype is crucial for elucidating infection pathways, which is essential for improving prevention strategies and management practices [25].

In this study we included a sample of 70 HBsAg positive patients. Of these, 56.25% were male and 43.75% were female. The mean age of the patients was 34.4 years, with more than 70% under the age of 40, reflecting a young and dynamic population. This distribution may be explained by the conditions for HBsAg screening in our population, which is mainly made up of blood donors and pregnant women, accounting for over 60% of cases [21]. This average age corresponds to that reported in the literature for HBsAg carriage. These diagnostic circumstances are comparable to those reported in Cameroon, thereby underscoring the incidental nature of the diagnosis and the asymptomatic nature of the infection. However, studies show that as we age, our risk of infection increases due to the accumulation of risk factors, one of which is sexual transmission [17].

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This study identifies genotype D as the most prevalent among patients with infection, followed by genotype E (Figure 1). Our findings are consistent with those reported in Africa, where researchers frequently detect genotypes D and E [11]. Genotype E is more dominant in West Africa and Madagascar but ranks third in East Africa, which aligns with our results [11].

However, our findings diverge from those of a prior study conducted on the neighboring island of Mayotte, which did not document the presence of genotype E [9]. The presence of genotype E in our study can be explained by several hypotheses, notably the existence of a historical and growing migratory dynamic between the population of Ngazidja, Madagascar and the African continent, where genotype E is endemic, in contrasting with the island of Mayotte, where exchanges are very limited to date due to the status of French territory.

In this study, only one HBV sample examined showed mixed infection of genotypes D and E. This type of co-infection is frequently reported in areas where both genotypes circulate, such as reported in Egypt [33], Ghana [22], Ouganda [34].

Our study finds a predominance of genotype D, consistent with studies conducted on the continent, particularly in Morocco [4], [26] and Tunisia [10] also on the word Irak [1]. However, in sub-Saharan Africa, this genotype is generally second only to genotype A in terms of prevalence. Globally, genotype D is ubiquitous, affecting about 22.1% of people fully infected with HBV, with a distribution of 61.9% in Asia, 22% in Africa and 13.5% in Europe [29].

Limits of the study
The main limitations of this study are the small sample size and the lack of sequencing of the samples. Additionally, the absence of data on HBeAg, anti-HBe antibodies, and viral load represents another significant limitation.

5. Conclusion

Our study reveals that hepatitis B virus infection on the island of Ngazidja is often discovered fortuitously. The predominant circulating genotype is genotype D, followed by genotype E. However, further sequencing analysis is required to identify circulating subgenotypes within the island.

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Abreviation

CHN: National Hospital Center
HBsAg: Hepatitis B Surface Antigen
HBV: Hepatitis B Virus
HBeAg: Hepatitis B e Antigen
PCR: Polymerase Chain Reaction

Data availability

The dataset utilized in the present study can be obtained from the corresponding author upon a reasonable

request.

Competing interests

The authors declare that they have no competing interests.

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6. References

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