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Seroprevalence and Biomolecular Study of the Hepatitis E Virus in Brazzaville

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Abstract

Objectives: *To describe the serological and biomolecular profile of hepatitis E virus (HEV) in Brazzaville.*

Population and methods: *This was a descriptive cross-sectional study conducted from January 1 to September 30, 2024, at blood collection centers of the National Blood Transfusion Center (CNTS) and pig farms in the city of Brazzaville. Blood donors and pig farmers aged 18 years and older, regardless of sex, who agreed to participate in the study were included. Sociodemographic characteristics, risk factors for HEV transmission, IgM, IgG, and viral RNA were the variables studied. The statistical tests used were Pearson's chi-squared test and odds ratio with adjustment. The significance level was set at 0.05.*

Results: *98 people were recruited for our study. Men represented 53% and women 47%. The mean age was 39.18 ± 14.15 years, with a range of 18 to 77 years. More than half of the population had contact with animals, and in 43% of cases, this contact was with pigs. The overall frequency of HEV was 19.3% (n=19/98). The frequencies of anti-HEV IgG and IgM antibodies were 84% (n=16/19) and 16% (n=3/19), respectively. The frequency of HEV was 17.6% (n=12/68) among blood donors and 23.33% (n=7/38) among pig farmers. RNA was detectable in 26.32% of cases.*

Conclusion: *This study constitutes the first seroprevalence and molecular biology survey of HEV in Congo-Brazzaville. The results indicate that HEV infection is present in Brazzaville, particularly among blood donors and pig farmers, with a high seroprevalence.*

Keywords: frequency, hepatitis E virus, Brazzaville.

Introduction

The hepatitis E virus (HEV) is a hepatotropic virus responsible for an infectious disease called hepatitis E [1]. It is the leading cause of acute viral hepatitis worldwide and poses a significant public health problem [2]. Indeed, the WHO estimated 20 million HEV infections in 2017, with more than 3.3 million cases of acute viral hepatitis E and 44,000 deaths related to this infection [3]. It is a fecal-oral virus transmitted through ingestion of contaminated water or food, transfusion of labile blood products, and direct human-animal contact [4–6]. The potential severity of this infection lies in the risk of fulminant hepatitis, with a case fatality rate reaching 4% [7]. The groups of people at risk are infants, the elderly, pregnant women, those with liver failure and immunocompromised individuals [8].

Hepatitis E virus (HEV) frequently occurs in epidemics, with the most significant outbreak occurring in the Ouaddaï province of eastern Chad since April 2023 [9].

In Congo, the serological and molecular profile of HEV is unknown. Our research hypothesis focused on the presence or absence of HEV in Congo. Therefore, we undertook this study, which aimed to describe the serological and biomolecular profile of HEV in Brazzaville and, more specifically, to:

- Describe the sociodemographic characteristics of HEV carriers in Brazzaville;
- Determine the frequency of HEV in Brazzaville;
- Identify the factors associated with anti-HEV antibody positivity.

Population Et Methodes

We conducted a descriptive cross-sectional study between January and September 2024 at CNTS blood collection points and pig farms. Blood donors and pig farmers aged 18 years and older, regardless of sex, who agreed to participate in the study were included. Sociodemographic characteristics, risk factors for HEV transmission, IgM, IgG, and viral RNA were the variables studied.

Epidemiological data were collected using a survey form. Blood samples were collected in ETDA tubes, then packaged and transported according to the regulations set by the European Agreement concerning the International Carriage of Dangerous Goods by Road (ADR). They were then centrifuged for 5 minutes. After centrifugation, plasma volumes ranging from 1000 to 2000 µl were extracted and placed in 2 ml graduated cryotubes. The cryotubes were then stored at -20°C until analysis.

Plasma samples were serologically tested by detecting and differentiating anti-HEV immunoglobulins G and M using the immunochromatographic method. The kit used was the HEV IgG/IgM Rapid Test Cassette from Biopanda (England).

Molecular analyses were performed using the Hepatitis E Virus RT-qPCR Kit (Nzytech Portugal). RNA extraction from blood samples was performed using the Total RNA Purification Kit Insert PI12200-37 (Norgen Biotek Corp, Canada) in three steps: lysate preparation, purification, elution, and RNA storage.

The extracted total RNA underwent RT-PCR amplification using the Hepatitis E Virus RT-qPCR Kit from NZYtech Ltd. Statistical analyses were performed using Epi Info 7.2.6 software.

Resultats

Of these, 98 were included in the study, and 15 were excluded. The study population consisted of 52 men (53.06%) and 46 women (46.94%), for a sex ratio of 1.13. The mean age of the participants was 39.18 ± 14.15 years, with a range from 18 to 77 years. The table shows the distribution of participants by occupation.

	n	%
Executives	29	29,59
Self-employed worker	18	18,37
Employee, worker	37	37,76
Unemployed	14	14,28
Total	98	100,00

Table I: Distribution of participants by profession

In our study, the overall frequency of HEV was 19.39% (n=19/98), and the frequencies of anti-HEV IgG and IgM antibodies were 84.21% (n=16/19) and 15.79% (n=3/19), respectively. HEV RNA was quantified by real-time RT-PCR in 5 samples, resulting in an RNA detection rate of 26.32%.

	n	%				
Anti-HEV antibodies (N=98)			DS (N=60)		EP (N=30)	
			n	%	n	%
Negatives	79	80,61	48	80,00	31	81,58
Positives	19	19,39	12	20	7	18,42
Antibody type (N=19)						
IgG	16	84,21	10	83,33	6	85,71
IgM	3	15,79	2	16,67	1	14,29
ARN viral (N=19)						
Detected	5	26,32	4	80,00	1	20,00
Not detected	14	73,68	1	20,00	4	80,00

Table II: Population distribution according to the frequency of anti-HEV antibodies, the type of antibody, and the presence of viral RNA

DS: blood donors
EP: pig farmers

The main risk factors were education level, contact with pork, consumption of raw vegetables, pork consumption, hand hygiene, drinking water, and travel to an endemic country. However, education level and contact with pork were the two factors that were statistically associated with a high frequency of hepatitis E virus antibodies.

	Ac anti-VHE		OR [IC95%]	p-value
	Negative	Positive		
	n (%)	n (%)		
Contact with pigs				
Non	20(66,7)	10(33,3)	Réf	-
Oui	59(86,8)	9(13,2)	5,380[0,109-0,858]	**0,023**
Good hand hygiene				
Non	16(76,2)	5(23,8)	0,334[0,563-0,756]	0,382
Oui	63(81,8)	14(18,2)	Réf	-
Drinking water				
Non potable	23(76,7)	7(23,3)	4,31[0,246-2,014]	0,512
Potable	56(82,4)	12(17,6)	Réf	-
Consumption of raw vegetables				
Non	18(90)	2(10)	1,417[0,529-11,896]	0,234
Oui	61(78,2)	17(21,8)	Réf	-
Journey to an endemic country				
Non	74(80,4)	18(19,6)	0,030[0,90-7,480]	0,862
Oui	5(83,3)	1(16,7)	Réf	-
Pork consumption				
Non	20(80)	5(20)	0,008[0,304-2,968	0,569
Oui	59(80)	14(19,2)	Réf	-

Table III: Frequency of anti-HEV antibodies according to the main risk factors

Discussion

In our study, the overall frequency of HEV was 19.39% (n=19/98), and the frequencies of anti-HEV IgG and IgM antibodies were 84.21% (n=16/19) and 15.79% (n=3/19), respectively. Our results differ from data in the African literature. Indeed, in 2002, a study conducted by Josep Escribá et al. in the Central African Republic reported a frequency of HEV of 63% in the northern districts of Bangui [10]. This difference could be explained not only by the size of their sample, which was 351 patients compared to ours of 98, but also, and more importantly, by the fact that their patients presented with acute jaundice and significant physical asthenia related to probable acute hepatitis E. Given this high frequency among symptomatic patients, the WHO declared the first epidemic of viral hepatitis E in Central Africa in 2002.

Our frequency is higher than that published by Bivigou Mboumba et al. in Gabon and Feldt et al. in Cameroon, which were 3.5% and 14.2%, respectively [11, 12].

The frequency of 19.39% that we obtained in this initial study places Congo-Brazzaville in an endemic zone according to the WHO. Indeed, Congo is ranked 149th in the 2023-2024 Human Development Index of the United Nations Development Programme [13].

This ranking reflects the political instability, the

geographical situation, the deterioration of the healthcare system, and a very poor epidemiological surveillance system, all of which contribute to the country's vulnerability to the risk of epidemics, even though this virus is still relatively unknown in our country. It should also be noted that this previously unknown high prevalence could be explained by the migration of people from the Central African Republic (CAR) to Congo, a consequence of the armed conflicts that the country has experienced in recent decades. Depending on the population group, our study reports a frequency of 17.65% (n=12/68) among blood donors and 23.33% (n=7/38) among pig farmers. This suggests that, despite the absence of an epidemic, HEV is circulating in our population, likely asymptomatically. Our results differ from data in the literature. Indeed, a study of HEV seroprevalence among blood donors in Casablanca reported a frequency of 11%. This difference could be explained by their large sample size of 1,000 blood donors. Furthermore, direct comparisons between the two studies may be limited by the use of different diagnostic tests. The test kit used in this study is more sensitive than previously available HEV diagnostic serology kits [14].

Conversely, in Europe and America, prevalence rates are low in some countries, such as Italy, Germany, and the United States (California), with respective prevalence rates of 2.6% in Italy, 2.1% in Germany, and 1.2% in the United States [15–17].

Among pig farmers, the frequency of HEV in our study was 23.33%. It should be noted that few studies have focused on HEV seroprevalence among pig farmers worldwide. Our results are comparable to those of Lee Jiane et al. in Taiwan, who reported a frequency of 29.5% of HEV among pig farmers in 2013 [18].

In univariate analysis, the factors significantly associated with anti-HEV antibody positivity were educational level and contact with pigs.

Anti-HEV IgG seroprevalence data represent a marker of prior exposure to the virus, while IgM seroprevalence data indicate recent infection.

In our study, 19 samples positive for anti-HEV antibodies were used for molecular characterization. These samples included 12 blood donors and 7 pig farmers. HEV RNA was quantified by real-time RT -PCR in 5 samples, representing an RNA detection rate of 26.32%. These results are similar to those found by Modiyinji et al. [19] in Cameroon, where the RNA detection rate was 19.1% (22/115) in patients positive for anti-HEV IgM antibodies.

However, in Gabon, in all patients with positive serology, no detection of HEV RNA was noted [20]

Conclusion

This study constitutes the first seroprevalence and molecular biology survey of the hepatitis E virus in Congo-Brazzaville. The results indicate that HEV infection is present in Brazzaville with a high seroprevalence, placing Congo in an endemic zone according to the WHO. The risk of HEV infection was higher in males under 30 years of age with a secondary education. Educational attainment and contact with pigs were the two factors statistically associated with this high frequency. These unexpected data suggest that, despite the absence of an epidemic, HEV is circulating asymptomatically in our country, hence the need to conduct studies on a large population to obtain sufficient statistical po-

wer to better extrapolate the seroprevalence of this virus in our country.

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