

Short Communication

Hepatitis E virus infection among rural Afro-descendant communities from the eastern Brazilian Amazon

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Abstract

Introduction: The prevalence, public health impact, and epidemiological characteristics of hepatitis E virus (HEV) are poorly understood in Brazil. **Methods:** Serum samples from 535 individuals from three rural Afro-descendant communities located in eastern Brazilian Amazon were collected in October 2015 and tested for presence of anti-HEV IgM and IgG antibodies. Serologically positive samples were also tested for HEV-RNA. **Results:** Two cases were confirmed for anti-HEV IgM (0.3 %) and two cases for anti-HEV IgG (0.3 %). No sample was positive for HEV-RNA. **Conclusions:** Results indicated low prevalence of HEV infection in Afro-descendant rural communities from the eastern Brazilian Amazon.

Keywords: Hepatitis E. Quilombola. Zoonosis.

Hepatitis E virus (HEV) is primarily transmitted via contaminated food and water and can cause asymptomatic infections, self-limited or acute fulminant hepatitis in immunocompetent individuals, and chronic hepatitis in immunosuppressed patients (transplant recipients or HIV-positive individuals)¹.

HEV is currently classified into the *Hepeviridae* family, *Orthohepevirus A* species, which is subdivided into seven genotypes (numerically identified as 1-7)². Data on the serological and molecular prevalence, genotypes, and epidemiological characteristics of HEV are limited in many countries, populations, and geographic regions in Latin America³. In general, hepatitis E diagnosis is not performed routinely in many diagnostic centers in Brazil and other Latin American countries, because of which the circulation status of HEV is poorly understood and/or possibly underdiagnosed³.

Seroepidemiological studies have indicated a prevalence of HEV infection ranging from 1.9 % - 12.9 % in different rural populations in the Brazilian Amazon suggesting the occurrence of possible intra-regional epidemiological differences in virus circulation in the northern region of the country⁴⁻⁸.

It is estimated that around two to three thousand Afro-descendant rural communities - also called "quilombola

communities" - exist throughout the Brazilian territory, with a large density in the eastern Amazon⁹. These communities are historically characterized by their black ancestry and are predominantly located in isolated or semi-isolated rural areas originally formed as resistance during periods of slavery or after the formal abolition of black slavery in Brazil⁹.

The prevalence and clinical-epidemiological characteristics of HEV are still poorly understood in the Brazilian Amazon, including among Afro-descendant rural populations. The occurrence of HEV infection in these rural communities has not yet been reported.

The circulation of the zoonotic HEV genotype 3 has been previously described in pigs from the eastern Brazilian Amazon¹⁰. The present study aimed to evaluate the serological prevalence of hepatitis E virus (HEV) infection and to describe the demographic and epidemiological characteristics of three Afro-descendant rural communities from the eastern Brazilian Amazon.

A cross-sectional seroepidemiological study was carried out in October 2015. It included the evaluation of 535 individuals living in the Afro-descendant rural communities of Bela Aurora (n = 118), Camiranga (n = 327), and Itamoari (n = 90), located in the Municipality of Cachoeira do Piriá in the Pará State (**Figure 1**).

Individuals who agreed to participate in the study, without predilection for sex or age, signed an informed consent form (TCLE) or a term of assent (individuals under 18 years old), filled out individual questionnaires for the collection of demographic and epidemiological data (sex; age; occupation; hepatitis history;

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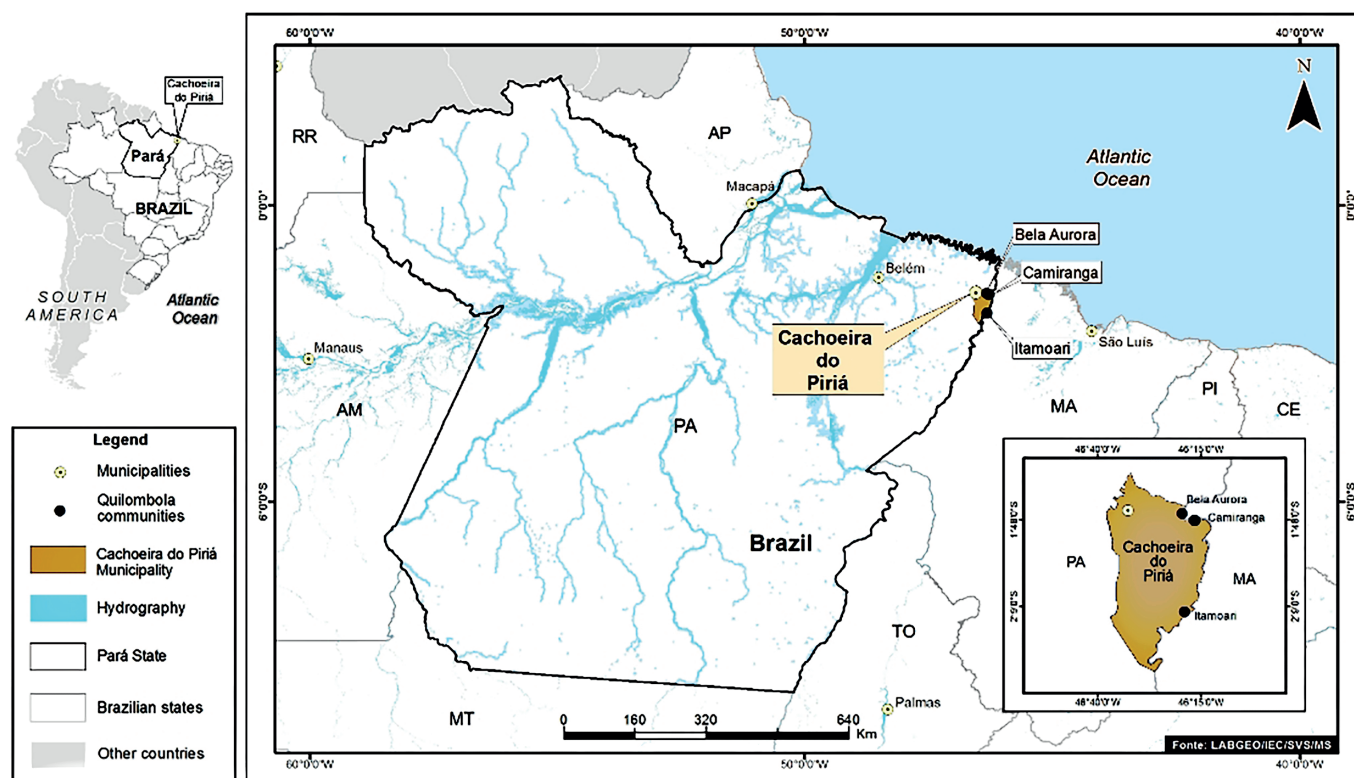


FIGURE 1: Geographical location of the rural Afro-descendant communities of Bela Aurora, Camiranga, and Itamoari in the Cachoeira do Piriá Municipality, Pará State, in eastern Brazilian Amazon.

house location; contact with animals at or around home; game meat consumption; source of water for consumption; destination of drinking water, waste, and garbage), and were recruited for blood collection via intravenous puncture (5 mL). Samples were then centrifuged to obtain serum, which was used for hepatitis E detection. The execution of this project was approved by Research Ethics Committee involving Human beings of the Evandro Institute Chagas (IEC/SVS/MS) under protocol No. 1,601,607.

Serum samples of 535 subjects were tested for anti-HEV IgM and IgG antibodies by ELISA using the RecomWell® HEV IgM and RecomWell® HEV IgG (Mikrogen) commercial kits, respectively. Samples with positive or inconclusive results from ELISA were submitted for confirmatory recombinant immunoblot tests (RIBT) using RecomLine® HEV IgM/IgG (Mikrogen). All serological assays were carried out according to the manufacturer's instructions.

Serum samples that were positive for anti-HEV antibodies were submitted for extraction of viral nucleic acids with QIAamp UltraSens Virus Kit (QIAGEN) using 1 mL of serum eluted in a final volume of 60 µL/sample, and stored at -70°C until further use.

Total nucleic acids from each sample were individually tested in duplicates for HEV-RNA by quantitative RT-PCR (RT-qPCR). These assays were performed using previously described primers, probe, and thermocycling conditions¹¹, using a standard curve containing 10⁹-10¹⁰ copies/mL, positive (swine HEV-RNA genotype 3 isolate)¹⁰, negative, and no template controls.

Individuals with anti-HEV antibodies were also tested for hepatitis A virus (HAV) infection by analyzing the total

anti-HAV by ELISA (anti-HAV Sym, Symbiosys) and anti-HAV IgM antibodies (anti-HAV IgM, Cobas e411, Roche) by electro-chemiluminescence immunoassay (ECLIA), according to the manufacturer's guidelines.

The overall prevalence of anti-HEV antibodies (IgM or IgG) was 1.6 % (9/535). Anti-HEV IgM was detected in 1.1 % (6/535) of individuals (three from the Camiranga and three from Itamoari), and anti-HEV IgG was detected in 0.5 % (3/535) of the evaluated cases (two subjects from Camiranga and one from Bela Aurora). Only four of the nine positive cases by ELISA were confirmed by RIBT. Two cases were positive for IgM (0.3 %) and two cases for IgG (0.3 %).

Total anti-HAV antibodies were detected in four of the six samples with anti-HEV IgM positivity and in all three samples positive for anti-HEV IgG. None of the samples were concomitantly positive for anti-HEV IgM and anti-HAV IgM antibodies or anti-HEV IgM and IgG antibodies.

The RT-qPCR assays showed adequate amplification of the standard curve and positive controls. However, none of the nine serologically positive samples were positive for HEV-RNA.

Demographic data from the three communities indicated that most of the participants were female (53.1 %; 284/535), the predominant age group was 0-15 years (42.7 %; 228/535), and the mean age was 24.9 years (95% CI: 23.3 – 26.6) (range= 0-88; SD=19.6).

In the three evaluated communities, the main occupation of the individuals was student (33.4 %; 179/535), followed by domestic (18.1 %; 97/535), and rural activities on small family

farms (15.5 %; 83/535). The most frequently observed levels of education were incomplete elementary education (64.4 %; 345/535), complete elementary education (9.9 %; 53/535), and incomplete high school education (4.1 %; 22/535). None of the persons presented clinical signs of liver disease at the time of sample collection, although 4.1 % (22/535) of individuals declared a history of hepatitis.

The houses of the three communities were predominantly located in areas near the forest (73 %; 391/535). Poor sanitary conditions were observed, and 39.7 % (212/535) of the subjects declared that human excreta was deposited in a dry pit, while 38.8 % (208/535) declared an open-air destination of the excreta, and only 21.5 % (115/535) declared that excreta were deposited in a septic tank.

The lack of a sewage system was observed in all three communities, and domestic sewage was discarded in open air by 99 % (530/535) of the individuals, while only 1 % (5/535) used a sewage system. The water source for more than 90 % of the individuals was a well (92.8 %; 497/535), 5.8 % (31/535) used water from an artesian well, 0.4 % (2/535) from a public system, 0.2 % (1/535) from rivers, and 0.8 % (4/354) from other sources.

The habit of breeding animals (dogs, cats, birds, pigs, or others) was observed for a high number of individuals (89.5%; 479/535). Dogs and birds were the most frequently

bred animals (both dogs and birds 67.6 %; 362/535), and pig farming was reported by only 6.1 % (33/535). A high frequency of individuals reported the consumption of game meat (68.9 %; 369/535), including animals like armadillo, paca, agouti, deer, and caititu. The demographic, epidemiological, and laboratory results of the nine anti-HEV IgM or IgG positive cases are summarized in **Table 1**.

The present study was the first to detect the circulation of HEV in Afro-descendant rural communities of Brazil and the results of serological analysis indicated a low prevalence of HEV among individuals from Bela Aurora, Camiranga, and Itamoari communities.

In the Brazilian Amazon, the seroprevalence of HEV is still poorly described. However, in comparison to other studies, our results indicated a lower prevalence of HEV than that detected in other populations in the Amazon such as miners (6.1 %; 6/97)⁴; riverine communities located between the Acre and Amazonas States (4.0 %; 14/349)⁵; children in the Amazon Basin of Mato Grosso State (4.5 %; 22/487)⁶; and individuals from rural communities in the Pará state (1.9 %; 9/463)⁸. However, these differences may be attributed to the sensitivity and specificity of the serological tests used and/or to intraregional epidemiological variability.

Since commercial kits for the serological detection of HEV have variable sensitivity and specificity¹², after using RIBT, we

TABLE 1: Demographic, epidemiological, and laboratory findings of individuals who were serologically positive for HEV from the rural Afro-descendant communities in the Cachoeira do Piriá municipality, Pará State, Brazil, October 2015.

Age	Sex*	Occupation	Community	Anti-HEV				HEV RNA	Anti-HAV		Game meat	Contact with pigs	Hepatitis History
				ELISA [§]		RIBT [‡]			ELISA	ECLIA [¶]			
				IgM	IgG	IgM	IgG		Total	IgM			
33	M	NR ^a	Camiranga	+	-	+	NT ^b	-	+	-	Yes	No	No
49	M	Small farmer	Camiranga	+	-	-	NT	-	+	-	Yes	No	Yes
12	F	Student	Camiranga	+	-	+	NT	-	+	-	Yes	No	No
11	F	Student	Itamoari	+	-	-	NT	-	-	-	Yes	No	No
13	F	Student	Itamoari	+	-	-	NT	-	-	-	Yes	No	No
55	M	Small farmer	Itamoari	+	-	-	NT	-	+	-	Yes	No	No
41	M	Small farmer	Bela Aurora	-	+	NT	+	-	+	-	Yes	No	No
16	F	Student	Camiranga	-	+	NT	-	-	+	-	Yes	Yes	No
80	M	Retired	Camiranga	-	+	NT	+	-	+	-	No	No	NR ^a

Anti-HAV: Anti-Hepatitis A virus antibodies; **Anti-HEV:** Anti-Hepatitis E virus antibodies; **IgM:** IgM immunoglobulin; **IgG:** IgG immunoglobulin. ***M:** Male; **F:** Female; ***NR:** Not reported; **^bNT:** Not tested; **[§]ELISA:** enzyme-linked immunosorbent assay; **[‡]RIBT:** recombinant immunoblot test; **[¶]ECLIA:** electrochemiluminescence assay.

observed a reduction in the number of ELISA-positive cases because RIBT can reduce the frequency of false-positive cases and the use of RIBT in non-endemic HEV regions may help to better characterize the real seroprevalence of HEV under these circumstances¹³.

The absence of HEV-RNA in serologically positive samples is possibly related to the course of infection, since acute HEV infection presents a short period of viremia, and viral clearance occurs soon after seroconversion¹. Therefore, at the time of sample collection, individuals who were asymptomatic for infection may have exhibited anti-HEV antibodies associated with the absence of serum HEV-RNA.

The highest prevalence of anti-HEV recorded in the Brazilian Amazon has been in individuals from the rural area "Ramal do Granada" in the Acre State in the Brazilian western Amazon. Among these individuals, 12.9 % (50/388) exhibited anti-HEV IgG and 9.3 % (4/43) exhibited anti-HEV IgM antibodies and increase in age was the only factor associated with seroprevalence⁹.

The limited number of serologically confirmed cases detected in our study did not allow a broad statistical analysis to assess risk factors associated with anti-HEV antibody positivity, but the observation of a low serological frequency of HEV infection and sanitary conditions that, in theory, could favor the dissemination of pathogens transmitted by the fecal-oral route¹⁴, may suggest low distribution of HEV among individuals from the three communities.

The four serologically confirmed cases of HEV infection among Afro-descendant individuals exhibited total anti-HAV antibodies, reinforcing the notion that these individuals were previously exposed to enterically transmitted viral pathogens¹⁴. Since we detected a high frequency of sanitary and epidemiological evidence that would favor infection by HEV and other pathogens¹⁴, additional environmental studies may be developed for monitoring the HEV infection risk in these communities.

Passos-Castilho et al¹⁵ identified a prevalence of 10 % (30/300) anti-HEV IgG among blood donors in the region of the Itajaí Valley in southern Brazil. Due to German immigration history in this population, there is significant industrial swine breeding and a substantial cultural habit of consumption of swine meat. Although isolates of swine HEV genotype 3 have already been revealed in the eastern Brazilian Amazon¹⁰, a low frequency of swine breeding was identified among the three rural Afro-descendant communities evaluated (developed on familiar small-scale farms). Therefore, low frequency of anti-HEV antibodies observed among quilombola communities may also be related to a low exposure to swine sources of HEV infection. Additional studies to assess the prevalence of HEV in swine (and other possible animal sources of HEV infection) are necessary to evaluate the hypothesis of low prevalence of HEV among pigs, in environmental sources, and/or the existence of wild reservoirs of HEV infection in these communities.

A low serological frequency of HEV infection was detected in the communities of Bela Aurora, Camiranga, and Itamoari, suggesting that HEV has low endemicity in Afro-

descendant rural communities in eastern Brazilian Amazon as well as in other populations from Brazil. However, despite low prevalence, additional seroepidemiological evaluation in Afro-descendant rural communities and other Amazonian populations is necessary to better characterize the distribution and epidemiological characteristics of HEV in the Amazon region and in Brazil.

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Conflict of interest

The authors declare that there is no conflict of interest.

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