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Short Communication

Hepatitis E seroprevalence and associated factors in rural settlers in Central Brazil

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Abstract

Introduction: Prevalence of hepatitis E virus (HEV) infection and associated factors were investigated in rural settlements in Central Brazil. **Methods:** A total of 464 settlers were interviewed, and serum samples were tested for anti-HEV IgG/IgM. Positive samples were tested for HEV RNA. **Results:** Sixteen participants (3.4%; 95% CI 2.0-5.7) were positive for anti-HEV IgG. None was positive for anti-HEV IgM. HEV RNA was not detected. Dwelling in a rural settlement for >5 years was associated with HEV seropositivity. **Conclusions:** The results revealed the absence of acute infection and a low prevalence of previous exposure to HEV.

Keywords: Hepatitis E virus. Epidemiology. Rural settlement.

Hepatitis E, caused by the hepatitis E virus (HEV), has a worldwide distribution and represents a significant public health problem. Approximately 20 million cases of HEV infection are reported annually worldwide, causing 3.3 million acute symptomatic cases and 56,600 deaths[1]. Different epidemiological patterns are shown in developing and developed regions. In developing areas, HEV is mainly transmitted through the fecal-oral route, and in developed countries, sporadic cases can occur through the consumption of raw or undercooked meat or liver, as well as by direct contact with animal reservoirs. Therefore, both sporadic cases and epidemic outbreaks have been commonly related to poor hygiene and sanitary conditions[2].

Some studies have shown that rural populations have a high HEV prevalence[3,4]. An association between this infection and living in a rural area was also reported[5]. In Brazil, about 30 million people live in rural areas, representing around 16% of the Brazilian population. Of these, an increasing portion resides in rural settlements[6]. However, these areas have adverse conditions for life and health, such as inadequate disposal of waste, inadequate access to drinkable water, and overcrowding at home, favoring the emergence of diseases transmitted by the fecal-oral route. Besides, very little is known about the seroepidemiology of HEV infection in rural residents in Brazil[3,7], with just one study conducted on agricultural settlers in the Amazon Region[4]. Until recently, only HEV genotype 3 (zoonotic) has been reported in Brazil[8,9]. This study aimed to investigate the prevalence of HEV infection and associated factors in rural settlers in Central Brazil.

A cross-sectional study was conducted in rural settlements in the southwest of the State of Goiás (about 300km from Goiânia, the capital of this state), Central Brazil. Individuals from seven rural settlement projects (Lagoa do Bonfim and Três Pontes in Perolândia County and Santa Rita, Rio Claro, Nossa Senhora de Guadalupe, Terra e Liberdade 3T, and Rômulo Souza Pereira in Jataí County) were studied. These settlements were established between 1998 and 2007 by the Brazilian government for agricultural reform, which was designed to encourage landless rural workers on unproductive properties to relocate to potentially productive areas through a system of land grants. These settlements were administered for subsistence agriculture by the *Núcleo de Estudos em Agricultura Familiar* [NEAF, Campus Jataí, Federal University of Goiás (UFG)]. Activities related to cattle ranching have also been carried out in some of these settlements. Until these settlements were established, the landless rural residents were arranged in canvas or plastic tents and lived in this precarious condition for many years.

The study sample was estimated according to the population size (approximately 2,500 residents) and based on an expected HEV prevalence of 2.1%[7], alpha and beta errors of 5% and

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20%, respectively, precision of 1.5%, and design effect of 1.5%. The minimum sample size necessary would be 462 individuals. Therefore, this study included 464 residents of the abovementioned rural settlements. During the recruitment period (May to July 2011), informed consent was obtained from all participants (or their parents in the case of children). They were interviewed using a standard questionnaire to record their sociodemographic and behavioral characteristics. Blood was collected (10mL) from all participants, and serum samples were stored at -20°C for serological tests and -80ºC for molecular analysis.

All serum samples were tested by an enzyme-linked immunosorbent assay (ELISA) for the detection of hepatitis E markers [anti-HEV immunoglobulin G (IgG) and immunoglobulin M (IgM)] using commercial reagents (*recom*Well HEV IgG/IgM, Mikrogen GmBH, Neuried, Germany). Positive samples were confirmed using an immunoblot assay (*recom*Line HEV IgG/IgM, Mikrogen GmbH).

Anti-HEV IgG- and IgM-positive or indeterminate samples were tested for the presence of hepatitis E virus ribonucleic acid (HEV RNA), which was extracted from 200µL of serum using the High Pure Viral Nucleic Acid Kit (Roche Diagnostics GmbH, Mannheim, Germany), following the manufacturer's instructions. Real-time polymerase chain reaction (RT-PCR) was performed for the detection (limit of four copies of HEV RNA per reaction) and quantification of HEV RNA using TaqMan real-time PCR technology[10].

Prevalence rates and 95% confidence intervals (CIs) were calculated. Factors associated with HEV seropositivity (defined as positive for anti-HEV IgG and/or IgM) were initially estimated by univariate analysis. Variables that presented $p < 0.10$ were included in a multivariate logistic regression model. A p-value of <0.05 was defined as statistically significant. The chi-square test or Fisher's exact test were used to compare categorical variables. Data were analyzed using the statistical package for the social sciences (SPSS), version 20 (SPSS Inc., Chicago, USA).

Table 1 shows the sociodemographic characteristics of 464 residents of rural settlements studied in Central Brazil. The mean age was 37.7 years (standard deviation, 19.9). Most of the

TABLE 1

Sociodemographic characteristics of 464 residents of rural settlements in Central Brazil.

Characteristics	Number	Percentage
Age (years) (mean ± SD, 37.7 ± 19.9)		
<10	62	13.4
11-20	62	13.4
21-30	41	8.8
31-40	61	13.1
41-50	86	18.5
>50	152	32.8
Sex		
male	242	52.2
female	222	47.8
Marital status*		
married	269	73.3
single	74	20.2
divorced/widowed	24	6.5
Race/ethnicity		
brown/*pardo*	281	60.6
white	109	23.5
black	71	15.3
other	3	0.6
Monthly income (US dollars)		
<330	252	54.8
330-660	159	34.6
>660	49	10.6
Schooling (years)**		
<5	206	46.6
5-8	161	36.4
>8	75	17.0

SD: standard deviation; **US dollars:** United States dollar. *Corresponding to individuals above 14 years of age. **School-age individuals: >6 years of age.

TABLE 2

Factors associated with hepatitis E virus among rural settlers in Central Brazil.

Variables	HEV positive/ total*	Pecentage	OR (95% CI)	p	Adjusted OR (95% CI)**	p
Age (years)						
≤30	5/165	3.0	1.0		1.0	
>30	11/298	3.7	1.2 (0.4-3.6)	0.796	1.6 (0.5-5.1)	0.400
Sex						
female	4/222	1.8	1.0		1.0	
male	12/241	5.0	2.8 (0.9-9.0)	0.076	2.9 (0.9-9.3)	0.072
Schooling (years)						
>8	1/75	1.4	1.0			
5-8	3/161	1.9	1.4 (0.1-13.7)	0.770		
<5	11/206	5.3	4.2 (0.5-32.9)	0.175		
Family income (US dollars)						
≥661	1/48	2.1	1.0			
331-660	5/159	3.1	1.5 (0.2-13.4)	0.703		
≤330	10/252	4.0	1.9 (0.2-15.3)	0.531		
History of incarceration						
no	9/303	3.0	1.0			
yes	2/51	3.9	1.3 (0.3-6.4)	0.663		
Time of dwelling in a rural settlement (years)						
≤5	6/207	2.9	1.0		1.0	
>5	10/118	8.5	3.1 (1.1-8.8)	0.025	3.4 (1.2-9.6)	0.023
Treated water (filtrated or boiled)						
yes	8/320	2.5	1.0		1.0	
no	8/127	6.3	2.6 (1.0-7.1)	0.051	2.3 (0.8-6.4)	0.118
Number of inhabitants/home						
≤4	10/320	3.1	1.0			
>4	6/139	4.3	1.4 (0.5-3.9)	0.523		
Ever used illicit drug						
no	11/353	3.1	1.0			
yes	1/17	5.9	1.9 (0.2-16.0)	0.436		
Previous blood transfusion						
no	13/404	3.2	1.0			
yes	2/54	3.7	1.2 (0.2-5.3)	0.693		

HEV: hepatitis E virus; **OR**: odds ratio; **CI**: confidence interval. *The denominator represents the number of settlers who answered the question. **Adjusted for age, time of dwelling in a rural settlement, and use of treated water (filtrated or boiled).

participants were male (52.2%), married (73.3%), and brown/*pardo* (mixed race) (60.6%) and reported a family monthly income below the minimum wage (US$330) (54.8%). Almost half of the study group (46.6%) had received <5 years of formal education (elementary level in Brazil).

Of 464 samples from the rural settlers, 19 (4.1%) were positive for anti-HEV IgG by ELISA. Of these, 16 were confirmed positive, 2 were negative, and 1 was indeterminate by immunoblot, resulting in a prevalence of 3.4% (95% CI, 2.0-5.7) among rural settlers. For anti-HEV IgM, three samples were positive by ELISA. However, none was confirmed positive by immunoblot (two were negative and one was indeterminate). HEV RNA was not detected in any sample positive or indeterminate for anti-HEV.

Factors associated with HEV seropositivity in rural settlers studied, with their respective 95% CIs, are shown in **Table 2**. Following multivariate analysis, the variable *dwelling in a rural settlement for >5 years* [odds ratio (OR), 3.4; 95% CI, 1.2-9.6] was associated with HEV seropositivity. Male sex (OR, 2.9; 95% CI, 0.9-9.3) was also associated with HEV seropositivity, with a borderline p-value.

The prevalence of anti-HEV IgG (3.4%; 95% CI, 2.0-5.7) found in this study was lower than those observed in other rural populations in Brazil, such as agricultural settlers in the Amazon Region (12.9%; 95% CI, 9.5-16.2)[4] and rural workers who handled swine in Mato Grosso (8.4%; 95% CI, 5.7-12.2)[3]. Meanwhile, residents in rural areas in Rio de Janeiro (Sumidouro) had a similar seroprevalence (2.1%; 95% CI, 0.5-6.4)[7]. Regarding studies conducted in rural populations in Latin America, similar anti-HEV IgG prevalence rates were also reported in Bolivia (6.3%; 95% CI, 3.6-10.3)[11] and Venezuela (5.4%; 95% CI, 2.9-9.4)[12], but a higher rate was reported in Mexico (36.6%; 95% CI, 31.0-42.7)[13]. Differences in regional endemicity, risk characteristics, and the performance of diagnostic assays for hepatitis E are the possible reasons for these differences in the observed rates.

Non-detection of anti-HEV IgM antibodies and HEV RNA in the tested samples reveals the absence of acute infection in this population during the study period. Other studies conducted in Brazil in rural[4] and urban[14,15] populations also reported a low prevalence for anti-HEV IgM antibodies and the absence of HEV RNA.

Here, only dwelling in a rural settlement for >5 years was associated with HEV seropositivity. The long time living under unfavorable infrastructure, socioeconomic disadvantages, and unsanitary conditions may indicate that over time, there is a cumulative risk of acquiring HEV infection. Male sex was marginally associated. A larger sample is possibly needed to identify statistical difference. Our results may be justified by the predominant circulation of HEV genotype 3 in Brazil[8,9]. However, besides the poor local sanitary and hygiene conditions, the epidemiological criteria to zoonotic HEV infection were not fully attended to.

This study has some limitations. Firstly, this is a cross-sectional study; therefore, no follow-up of the study population was performed. Secondly, the collection of data on contact with animals eating habits was not systematic, making it impossible to analyze the results. Thirdly, an *in house* real-time PCR was used to detect HEV RNA in the serum samples of the participants; moreover, HEV RNA was not examined in their stool samples. Nonetheless, given the very few data on the epidemiology of HEV infection among rural populations in Brazil, this study provided data on HEV prevalence and associated factors in rural settlers.

In conclusion, our findings revealed the absence of acute infection and a low prevalence of previous exposure to HEV, compatible with the reported pattern in other areas of low endemicity. However, the association of dwelling in a rural settlement for >5 years with HEV seropositivity highlights the unsafe conditions in their environment.

Ethical considerations

This study was approved by the Research and Ethics Committee of Hospital das Clínicas, *Universidade Federal de Goiás* (protocol no. 127/2010).

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Conflict of interest

The authors declare that there is no conflict of interest.

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