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Declining prevalence of hepatitis A and silent circulation of hepatitis E virus infection in southeastern Brazil

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ABSTRACT

Objectives: Hepatitis A virus (HAV) infection is considered highly endemic in Brazil, especially in low-income areas. In contrast, only a few human cases of hepatitis E have been reported. This study aimed to estimate the prevalence and potential risk factors of HAV and hepatitis E virus (HEV) infections in an adult population from a rural township of southeastern Brazil.
Methods: We conducted a cross-sectional survey using serum samples from urban and rural residents of Cássia dos Coqueiros, São Paulo state. A total of 990 samples were tested for anti-HAV IgG by chemiluminescent microparticle immunoassay, and a subset of 248 samples tested for anti-HEV IgG, using two commercial ELISA. Variables associated with anti-HAV and anti-HEV positivity were assessed by a multivariate analysis using a binomial logistic regression model.
Results: Seroprevalence of HAV and HEV was 89.1% and 20.7%, respectively. Age was significantly associated with HAV infection. Wantai and Mikrogen ELISA yielded comparable HEV seroprevalence results.
Conclusions: Anti-HAV seroprevalence has declined and correlates with age, whereas anti-HEV was significantly higher than that found in previous population-based studies. These results indicate a need for further investigations of the magnitude of HEV infection in Brazil using the currently available, more sensitive diagnostic methods.

Introduction

Hepatitis A and E virus infections occur worldwide, with hundreds of thousands of infections each year, thousands of cases of acute infection, and about 100,000 deaths from hepatitis A and 57,000 deaths from hepatitis E (Cuthbert, 2001; Kamar et al., 2014; Lozano et al., 2012). Over the past two decades, several studies have shown changes in the epidemiological pattern of HAV infections, with a progressive decrease in prevalence, especially in younger age groups, probably due to improvements in socioeconomic and sanitary conditions (Aggarwal and Goel, 2015; Jacobsen and Wiersma, 2010). However, autochthonous cases of human hepatitis E virus (HEV) infection have increased not only in industrialized countries, but also in certain developing countries (Sooryanarain and Meng, 2019).

Hepatitis A virus (HAV) belongs to the *Picornaviridae* family and *Hepatovirus* genus. HAV has one serotype and seven genotypes, including four genotypes that infect humans and three genotypes that infect non-human primates (Cuthbert, 2001). HEV belongs to

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the *Hepeviridae* family, *Orthohepevirus* genus, and the *Orthohepevirus A* species, that comprises seven genotypes (Smith et al., 2016). HEV genotypes 1 and 2 circulate among humans and are transmitted through contaminated water and food, as occurs with HAV. Genotypes 3 and 4 are zoonotically transmitted to humans primarily by contaminated meat consumption and handling of domestic pigs, wild boars, deer, and rabbits. These genotypes occur worldwide, but the zoonotic transmission of HEV3 accounts for most of hepatitis E human cases in developed countries (Kamar et al., 2014).

Besides the differences in sample representativeness, the comparison between different types of populations concerning HEV endemicity is hindered by the sensitivity and specificity discrepancies of the serological assays used for detecting HEV markers (Kamar et al., 2014). Given the decline in HAV endemicity and restricted data about HEV epidemiology and genotype distribution in Brazil, we performed a seroepidemiological study on the adult population of a rural township in the southeastern region aiming to estimate the prevalence and determine factors potentially associated with HAV and HEV past infections. Cássia dos Coqueiros is located in northwestern area of São Paulo State, about 80 km from Ribeirão Preto city. In 2019, a 2523 inhabitants (30% in rural areas) population was estimated, with a City Human Development Index (MHDI-2010) of 0.734, according to the 2010 Census information ("Cássia dos Coqueiros (SP) | Cidades e Estados | IBGE," n.d.).

Methods

This study is part of a research project developed by the School of Medicine of Ribeirão Preto in the town of Cássia dos Coqueiros to evaluate serological markers of different diseases, including viral hepatitis, toxoplasmosis, cysticercosis, hantavirus, and dengue. Between 2011 and 2013, a serum bank included 1001 samples obtained from urban and rural residents (18-year or older) during visits to the local health center and routine home visits by family health professionals. Serum samples were aliquoted, labeled, and stored at −20 °C.

Data were collected using a standardized questionnaire developed by the Department of Social Medicine in 2015 for studying hepatitis B and C virus infections. Most interviews (>90%) were carried out by a single researcher, and the other interviews were conducted by a well-trained community health agent of the Family Health Program of Brazil (Melo et al., 2015). Data from 990 individuals who had completed the questionnaire were entered into a database and used in all investigations underway in Cássia dos Coqueiros. Data on socioeconomic strata were categorized following the Brazilian Economic Classification Criterion ("Critério Brasil - ABEP, " n.d.), where the levels "A" and "E" represent the highest and the lowest socioeconomic status respectively. A total of 990 serum samples were tested using the ARCHITECT HAVAb-IgG assay (Abbott Diagnostics) for the detection of anti-HAV IgG antibodies. Considering that anti-HEV assays were available for only 250 samples testing, we opted for a systematic sampling approach to select one of every four persons from the 990 participants. Accordingly, we obtained a subsample comprising 248 individuals to detect anti-HEV IgG antibodies, using the Wantai HEV-IgG ELISA (Beijing Wantai Biological Pharmacy Enterprise, Beijing, China).

The Wantai assay is widely used, with the specificity and sensitivity for HEV IgG of 97.96% and 99.6%. Some studies suggested that non-Wantai assays may underestimate HEV seroprevalence (Capai et al., 2019). Therefore, frozen aliquots of 224 available serum samples were also tested by the *recom*Well HEV IgG ELISA (Mikrogen Diagnostik, Neuried, Germany). Those samples with discrepant results between Wantai and *recom*Well ELISA were retested in duplicate and submitted to the *recom*Line HEV IgG/IgM immunoblot assay (Mikrogen Diagnostik, Neuried, Germany).

Frozen preserved aliquots of 244 available serum samples were investigate for the presence of HEV RNA by quantitative one-step reverse transcription-polymerase chain reaction (qRT-PCR) (Jothikumar et al., 2006) using the AgPath-ID one-step RT-PCR kit (Applied Biosystems, USA) and primers and probe previously described (Bottino, 2018). The RT-qPCR process's quality control included negative (nuclease-free water) and positive (serum

Table 1
Sociodemographic characteristics of the total sample and sub-sample of participants from the population-based study. (Cássia dos Coqueiros - SP, Brazil, 2013).

Parameter	Total (n = 990)		Sample (n = 248)	
	n	%	n	%
Gender				
Male	392	39.6	93	37.5
Female	598	60.4	155	62.5
Age (years)				
18–21	47	4.8	8	3.2
22–29	136	13.7	27	10.9
30–49	384	38.8	106	42.7
50 and above	423	42.7	107	43.2
Household area				
Urban	684	69.1	172	69.3
Rural	306	30.9	76	30.6
Original Federal Unit				
São Paulo	743	75.1	187	75.4
Other	247	24.9	61	24.6
Education				
Up to incomplete elementary	521	52.6	140	56.4
Complete elementary and incomplete high school	112	11.3	19	7.7
Complete high school and incomplete higher education	260	26.3	59	23.8
Complete higher education	97	9.8	30	12.1
Socioeconomic stratum				
A_2	12	1.2	5	2.0
B_1 or B_2	315	31.8	80	32.2
C_1 or C_2	577	58.3	144	58.1
D	83	8.4	19	7.7
E	3	0.3	–	–

samples obtained from experimentally infected cynomolgus monkeys) controls added to each PCR plate. All RNA samples were analyzed in duplicate.

Laboratory results for HAV and HEV serological markers were entered into a database previously used for analyzing hepatitis B and C virus infection and were evaluated using statistical package SAS/STAT version 9 (2003). The results considered inconclusive for hepatitis E were excluded from the analysis.

The prevalence of HAV and HEV antibodies and 95% confidence intervals were determined by age group. Association of HAV and HEV markers with potential risk factors were analyzed by univariate analysis using the chi-square test. Variables with $p \leq 0.20$ were subjected to multivariate analysis using a binomial regression model for each viral hepatitis type. A possible association between risk factors and the presence of serological markers was assessed by calculating prevalence ratios and respective 95% confidence intervals.

The study was approved by the Research Ethics Committee of HCFMRP in February 2016.

Results

Table 1 shows sociodemographic characteristics of the total sample and the subsample. There was a predominance of women, individuals aged ≥30 years, urban dwellers, born in the state of São Paulo, with low education and belonging to middle socioeconomic strata (classes B and C). There were no significant intergroup differences in these parameters, demonstrating good representativeness of the subsample relative to the total sample.

The overall prevalence of anti-HAV IgG in the population older than 18 years was 89.1% (95% CI, 87.1–90.9) and varied considerably between age groups, ranging from 36.2% in the age group 18–21 years to 100% in the age group ≥50 years. Figure 1 displays the seroprevalence of HAV in different age groups, urban and rural residents, and total population. Prevalence was close to 100% among individuals born from 1937 to 1969, regardless of the area, but lower among subjects born in the 1970s, further decreasing among those born after the mid-1980s.

The distribution of anti-HAV IgG antibodies according to the variables of interest is shown in Table 2. Univariate analysis showed that the prevalence increased as the age group increased and reached 2.637 (95% CI, 1.803–3.857) in individuals older than 30 years compared to baseline. There was a significant association between the presence of anti-HAV IgG and the place of birth, level of education, socioeconomic stratum, history of hepatitis, household contact with infected people, and history of elimination of *Taenia* sp. proglottids in the stool. There was no significant

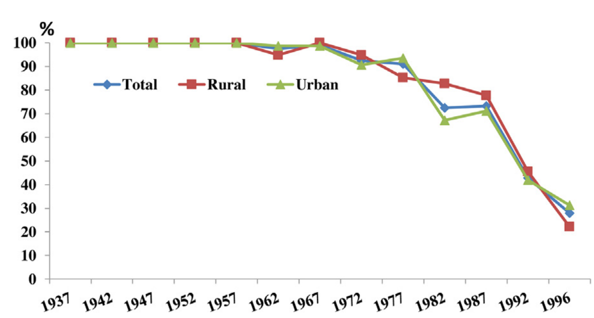

Figure 1. Hepatitis A seroprevalence as a function of household area and year of birth, Cássia dos Coqueiros - SP, Brazil, 2013.
Note: the information in this figure were based on the following age groups 18–24, 25–29, 30–34, 35–39, 40–44, 45–49, 50–54, 55–59, 60–64, 65–69, 70–74, 75–79, 80, and above. The markers represent the average year of birth for each age group, except for 1937, which stands for all those born previously.

association between the seroprevalence of HAV and gender, which was similar between men and women, corresponding to 89.8% and 88.6%, respectively. There were no significant differences in seroprevalence between urban and rural residents, corresponding to 88.9% and 89.5%, respectively (data not shown). Multivariate analysis showed that only age was independently associated with the presence of hepatitis A IgG antibodies (Table 3). As all individuals older than 49 years harbored anti-HAV IgG antibodies, data from 567 participants aged 18–49 years were analyzed to verify specificities in this age group for the presence of serological markers. The findings were very similar to those in the total sample.

Among the 248 individuals tested by the Wantai HEV-IgG ELISA, the overall HEV seroprevalence was 20.7% (95% CI, 15.5–25.7), and increased as age increased, corresponding to 14.3% and 25.0% in the age group 18–29 years and ≥30 years, respectively (Table 4). Table 4 shows the distribution of anti-HEV IgG antibodies and potential risk factors. Only low education was significantly associated with HEV infection, and the prevalence ratio of subjects with low education compared to the other groups was 1.758 (95% CI, 1.015–3.044). Only gender, age, education level, household contact with hepatitis patients, and regular raw-meat consumption were included in the multivariate analysis model, and none of these variables was associated with the risk of HEV infection (Table 5).

From 224 samples tested by two different ELISA, the percentages of HEV IgG positive results were 21.4% (Wantai HEV-IgG) and 237% (Mikrogen *recom*Well HEV IgG). Sixteen samples (7,1%) had discordant results between these two assays; from those, 12 available samples were tested by the Mikrogen *recom*Line immunoblot assay, which is considered a golden pattern test for HEV diagnosis. According to the immunoblot assay score, the ELISA resulted true-positive in two (Wantai) vs. six (Mikrogen) out of the 12 samples tested, and true-negative in two (Wantai) vs. one (Mikrogen) (data not shown).

None of the 244 samples tested by qRT-PCR had HEV RNA detectable by qRT-PCR.

Discussion

Many studies on the seroprevalence of hepatitis A and E have been carried out in Brazil and abroad. However, differences in sampling method, age groups, and serological assays' sensitivity and specificity to antibody detection (particularly anti-HEV testing) restrain comparisons among different studies. To increase sample representativeness, in this study, we sampled above 50% of the adult residents in Cássia dos Coqueiros. However, individuals younger than 18 years were excluded because of the greater difficulty obtaining informed consent from parents or guardians and a higher likelihood of non-compliance due to a need for venipuncture. Even so, the significant decrease in positivity for hepatitis A in individuals born before 1995 indicates that this township can be classified as having low endemicity, just as the capital cities in southeastern Brazil, according to the National Viral Hepatitis Survey ("Estudo de prevalência de base populacional das infecções pelos vírus das hepatites A, B e C nas capitais do Brasil," 2007). It is worth noting that water chlorination and fluoridation was implemented in Cássia dos Coqueiros in 1976, and a sewage treatment plant started operating in 1989, coinciding with the sharp decrease in positivity among individuals born in these two time intervals. Such a decrease in hepatitis A prevalence has been a worldwide trend and recorded on all continents (Jacobsen and Wiersma, 2010). Several studies found a positive correlation between age and HAV infection, and this result may be due to the cohort effect because infection usually occurs at an early age and

Table 2
Distribution of participants positive for hepatitis A IgG antibody (anti-HAV) as a function of sociodemographic characteristics and potential risk factors. (Cássia dos Coqueiros – SP, Brazil, 2013).

Parameter	Total	Anti-HAV + n	%	PR	95% CI
Gender					
Male	392	352	89.8	1.013	0.969–1.058
Female	598	530	88.6	1.000	
Age group					
18–21	47	17	36.2	1.000	
22–29	136	95	69.9	1.931	1.300–2.868
30 and above	807	770	95.4	2.637	1.803–3.857
Household area					
Rural	306	274	89.5	1.007	0.961 –1.055
Urban	684	608	88.9	1.000	
Original Federal Unit					
São Paulo	743	654	88.0	1.000	
Other	247	228	92.3	1.048	1.002–1.096
Education					
Up to incomplete elementary	521	511	98.1	1.239	1.181–1.300
Complete elementary or more	469	371	79.1	1.000	
Socioeconomic stratum					
A2 to C2	904	799	88.4	1.000	
D and E	86	83	96.5	1.091	1.042–1.144
History of hepatitis					
Yes	63	61	96.8	1.084	1.031–1.140
No	856	764	89.2	1.000	
Household contact with hepatitis cases					
Yes	157	150	95.5	1.085	1.040–1.131
No	812	715	88.0	1.000	
***Taenia* sp. proglottids**					
Yes	168	159	94.6	1.074	1.028–1.123
No	779	686	88.1	1.000	
Raw vegetable consumption					
Yes	946	848	89.6	1.160	0.986–1.363
No	44	34	77.3	1.000	

PR = prevalence ratio.

Table 3
Results of log-binomial regression to test for association with the presence of hepatitis A IgG antibodies (anti-HAV). Cássia dos Coqueiros - SP, Brazil, 2013.

	PR	95% CI
Gender		
Male	1.002	0.931–1.080
Female	1.000	
Age group (years)		
18–21	1.000	
22–29	1.221	1.012–1.474
30 e +	1.365	1.141–1.634
Original Federal Unit		
São Paulo	1.000	
Other	1.040	0.914–1.102
Education		
Up to incomplete elementary	1.052	0.962–1.150
Complete elementary or more	1.000	
Socioeconomic stratum		
A2 to C2	1.000	
D and E	1.008	0.885–1.147
History of hepatitis		
Yes	1.028	0.879–1.201
No	1.000	
Household contact with hepatitis cases		
Yes	1.025	0.925–1.136
No	1.000	
***Taenia* sp. proglottids**		
Yes	1.001	0.918–1.091
No	1.000	
Raw vegetable consumption		
Yes	1.066	0.945–1.204
No	1.000	

PR = prevalence ratio.

HAV antibodies are produced throughout life (Focaccia et al., 1998; Vitral et al., 2014; Carrillo-Santisteve et al., 2017).

The association of HAV infection markers with low socioeconomic status and lack of infrastructure and basic sanitation is well established because these factors increase the risk of exposure to diseases transmitted via the fecal–oral route (Aggarwal and Goel, 2015). In this respect, the contamination of water and food with human feces strongly contributes to the transmission of HAV and intestinal parasites. It is of note that the univariate analysis revealed an association with the self-reported elimination of proglottids of *Taenia* sp., and this variable was included in the questionnaire. Migration from areas of high endemicity may also contribute to the increase in disease prevalence in areas inhabited by immigrants, and this result was demonstrated in Cássia dos Coqueiros for hepatitis B in the 1980s (Passos et al., 1993). However, these results were not confirmed in the multivariate analysis.

The absence of a strong association with low socioeconomic status, low education, history of hepatitis, household contact with hepatitis cases, migration from other states (especially Minas Gerais), consumption of raw vegetables, and the presence of *Taenia* sp. proglottids in the stool using the log-binomial model might be due to the long time elapsed from exposition to HAV markers detection, consequently limiting the recall of events potentially associated with the risk of infection. Furthermore, the economic conditions during the study period may not reflect the conditions present at the time of infection because of a significant improvement in the quality of life in the past few decades across Brazil, including a reduction in environmental contamination and, consequently, higher quality of fresh foods. Moreover, improvements in water treatment and sewage collection in Cássia dos

Table 4
Distribution of participants positive for hepatitis E IgG antibody (anti-HEV) as a function of sociodemographic characteristics and potential risk factors. (Cássia dos Coqueiros – SP, Brazil, 2013).

Parameter	Total	HEV + n	%	PR	95% CI
Gender					
Male	90	19	21.1	1.035	0.628–1.720
Female	152	31	20.4		
Age group					
18–29	34	5	14.3		
30 and above	208	45	21.6	1.471	0.629–3.441
Household area					
Urban	169	37	21.9	1.229	0.695–2.172
Rural	73	13	17.8		
Original Federal Unit					
São Paulo	181	39	21.5		
Other	61	11	18.0	1.195	0.653–2.183
Education					
Up to incomplete elementary	138	35	25.3	1.758	1.015–3.044
Complete elementary or more	104	15	14.4		
Socioeconomic stratum					
A2 to C2	224	45	20.1		
D and E	18	5	27.7	1.382	0.628–3.044
History of hepatitis					
Yes	16	3	18.7	1.102	0.384–3.164
No	208	43	20.7		
Household contact with hepatitis cases					
Yes	40	4	10.0	2.284	0.870–5.992
No	197	45	22.2		
***Taenia* sp. proglottids**					
Yes	44	10	22.7	1.136	0.614–2.101
No	190	38	20.0		
Raw vegetable consumption					
Yes	232	49	21.1	2.112	0.323–13.800
No	10	1	10.0		
Raw meat consumption					
Yes	31	3	9.7	2.301	0.762–6.946
No	211	47	22.3		
Hepatitis A positivity					
Yes	224	45	20.1	1.382	0.627–3.044
No	18	5	27.7		

*HEV-IgG ELISA (Beijing Wantai Biological Pharmacy Enterprise, Beijing, China); PR = prevalence ratio.

Table 5
Results of log-binomial regression to test for association with the presence of hepatitis E IgG antibodies (anti-HEV)*. Cássia dos Coqueiros – SP, Brazil, 2013.

	PR	95% CI
Gender		
Male	1.049	0.636–1.729
Female	1.000	
Age group (years)		
18–29	1.000	
30 e +	1.074	0.405–2.846
Education		
Up to incomplete elementary	1.736	0.925–3.056
Complete elementary or more	1.000	
Household contact with hepatitis cases		
Yes	1.000	
No	2.360	0.902–6.171
Raw meat consumption		
Yes	1.000	
No	2.529	0.841–7.604

* HEV-IgG ELISA (Beijing Wantai Biological Pharmacy Enterprise, Beijing, China); (PR = prevalence ratio.

Coqueiros might have contributed to decreasing the risk of infection in the population, regardless of their socioeconomic status.

In addition to the vaccination of children included in the Brazilian National Immunization Program, in 2014 (Brito et al., 2020), special attention should be paid to young adults due to the lower prevalence of antibodies, and consequently, their higher susceptibility to HAV infection and progression to severe disease. In this respect, the introduction of the catch-up strategy for this age group should be evaluated by public health authorities, given the risk of traveling to areas of higher endemicity in Brazil and the increase in transmission between men who have sex with men (MSM) ("Boletim-Hepatites-2018.pdf," n.d.).

Two distinct epidemiological patterns of HEV infection occur worldwide. Outbreaks are frequent in countries with a predominance of genotypes 1 and 2 and are usually associated with contaminated water, affecting thousands of people. The prevalence of these genotypes is high, and infection is associated with poor sanitary conditions (as is the case with hepatitis A). This pattern is typical in Southeast Asia countries, especially India and China, and all African countries (Echevarría, 2014). There is no report of HEV outbreaks in Brazil, besides the high percentage of hepatitis A notified cases associated with food/waterborne transmission, in young adults, from 2013 to 2018 ("Boletim Epidemiológico de Hepatites Virais (2019)," n.d.).

In contrast, genotypes 3 and 4 (especially HEV3) occur on all continents, but most cases are asymptomatic or subclinical, and the incidence of acute hepatitis is low. Transmission is mainly zoonotic, and some studies have detected high levels of HEV RNA in sausages and other pork products and livestock (Meng, 2011). Outbreaks are related to the consumption of raw or undercooked pork meat (sausages made with meat, blood, or offal), game meat, oysters and, possibly vegetables contaminated with HEV (Mansuy et al., 2016).

Direct contact with feral pigs, such as *Sus scrofa* (wild boar) is also a risk factor for infection, given that the prevalence of HEV is high in these animals. Therefore, the increase in the population of wild boars may also contribute to the zoonotic transmission due to meat consumption and fecal contamination of soil and water (Meng, 2011). In Brazil, wild boar invasion occurred in the 1990s, imported from Europe and Canada from swine farmers, who bred them with domestic pigs resulting in a fattest pig, known as "javaporco." What followed was a widespread (intentional or unintentional) release of half-bred feral pigs, and São Paulo is the most affected state. Several municipalities have reported an increasing invasion of feral pigs (Pedrosa et al., 2015), including some locations neighboring the rural region of Cássia dos Coqueiros. Since 2013, the Brazilian Institute of Environmental and Renewable Nature Resources (IBAMA) authorizes the persecution and slaughter of feral pigs aiming at controlling their population size. However, neither hunting nor the consumption of game meat was addressed in the studied population of Cássia dos Coqueiros.

Other limitations should be considered when analyzing and comparing the studies on hepatitis E. In contrast to anti-HAV IgG antibodies, which remain throughout life, the persistence of anti-HEV antibodies in the blood after viral infections is unknown, which may underestimate the circulation of the virus in a community. Besides, the accuracy of different tests is variable, especially the sensitivity to detect IgG antibodies. Currently, the Wantai diagnostic test is one of the most commonly used. Bendal et al. (2010) have argued that, depending on the chosen laboratory test, several serological surveys using non-Wantai assays could underestimate HEV3 infection prevalence. These authors found that the seroprevalence was 3.6% in blood donors from England and increased to 16.2% using the Wantai test. In southeastern France, the seroprevalence in blood donors varied from 16.6% to 52.5% using the test with the highest sensitivity (Mansuy et al., 2011). Another study from France found that the prevalence of anti-HEV IgG antibodies in the stored sera of blood donors increased from 10.9% to 31.3% using the Wantai test (Rossi-Tamisier et al., 2013). Such a discrepancy was not observed in the present study, since the Wantai HEV-IgG ELISA was compared with another commercially available test (Mikrogen *recom*Well HEV IgG) and proved to yield a comparable seroprevalence (21.4% vs. 23.7%, data not shown). Discordant results between these two assays could be partly solved using the Mikrogen *recom*Line immunoblot assay, which allows us to discriminate between false and true results.

A meta-analysis of anti-HEV prevalence using the Wantai assay classified industrialized countries into high, medium, and low risk based on a 19% cut-off prevalence rate. Accordingly, in our study, the overall seroprevalence of 20.7% could allow us to classify Cássia dos Coqueiros into a medium risk, as are classified the USA, UK, Spain, Austria, Norway, and Denmark (Capai et al., 2019). According to a meta-analysis including 20 studies published between 1995 and 2018, the estimated overall seroprevalence of HEV infection in the Brazilian adult population was 6.0% (95% CI: 5.0–7.0), and ranged from 0.4% to 20.9% among various studies from the five Brazilian regions (Tengan et al., 2019).

Differences in seroprevalence according to geographic region were observed in Europe. A study showed that the average prevalence of anti-HEV antibodies in blood donors in France was 22.4% and ranged from less than 10% in some regions to 60–80% in the south, where endemicity and pork consumption were higher, and the wild boar population was large (Mansuy et al., 2011). In Brazil, HEV3 has been the single HEV genotype identified in humans and pigs. This genotype was found in pigs in south, southeast, northeast, and mid-west regions, and also in slaughterhouse's wastewater in the state of Rio de Janeiro (dos Santos et al., 2011, 2009; Passos-Castilho and Granato, 2017; de Oliveira-Filho et al., 2019). The first human case of HEV3 acute infection in Brazil was confirmed in Rio de Janeiro, and associated with zoonotic transmission (Lopes Dos Santos et al., 2010). Furthermore, there were reported cases of chronic HEV3 infection in solid organ transplant recipients in São Paulo and an asymptomatic blood donor from southern Brazil. All these human isolates showed a high similarity with swine isolates from the same Brazilian regions (Passos et al., 2013; Passos-Castilho et al., 2014; Moss da Silva et al., 2019).

More recent studies conducted in Brazil using sensitive diagnostic tests found that the prevalence of HEV was higher than those reported in previous studies. Vitral et al. (2014) found a 12.9% prevalence of anti-HEV IgG in Amazonia's rural. Among blood donors, HEV seroprevalence was 7.1%, (Moss da Silva et al., 2019), 9.8%, and 10% in southern and southeastern townships (Passos-Castilho et al., 2017). A study evaluated 708 blood donors and found that the prevalence of HEV in southern states, which are the most prominent region for pork livestock in Brazil, was 40.25% using an in-house ELISA test (Pandolfi et al., 2017).

Several studies showed the presence of HEV IgG antibodies in association with age but not gender (Mansuy et al., 2011, 2016; Vitral et al., 2014; Passos-Castilho et al., 2014, Passos-Castilho et al., 2016, Passos-Castilho et al., 2017). In Cássia dos Coqueiros, there was no significant association between these variables, although HEV prevalence was higher in older age groups. Furthermore, there was no significant association between the serological markers of HEV and HAV infections, which reinforces the hypothesis that the most common genotype in Brazil is HEV3, whose transmission is predominantly zoonotic.

Studies in France and Spain found that the risk of infection was higher in rural areas than urban areas, which agrees with a high circulation of HEV among pig farms (Mansuy et al., 2011). In our study, the absence of correlation may be due to a poor distinction between urban and rural areas in Cássia dos Coqueiros, where many individuals live in the city but work on small rural properties. However, the fact that data were collected using a questionnaire not initially designed for hepatitis A and E should be considered. Therefore, the kind of livestock consumed could not be addressed.

This study presents limitations. Children and adolescents were not included in the analysis; the participation of men was lower than that of women; none of the sample had detectable HEV RNA and, genotypes circulating in this community keep unconfirmed. Besides, the questionnaire was designed primarily for assessing hepatitis B and C virus infections, and not infections transmitted via the fecal–oral route; some relevant risk factors could not be analyzed, including sanitary conditions and contact with farm animals, especially pigs. However, other large-scale population studies on viral hepatitis have not included smaller communities located far from large urban centers, which underscores the importance of the present study. Furthermore, the present findings evidence a high seroprevalence of HEV amongst the general population of a rural township located in the southwestern, the richest region of the country. Further epidemiological surveys may elucidate epidemiologic aspects of HEV endemicity in Brazil.

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