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## Original article

# Seroprevalence of hepatitis E virus in chronic hepatitis C in Brazil

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### ABSTRACT

**Background and aims:** Hepatitis E virus infection in patients with underlying chronic liver disease is associated with liver decompensation and increased lethality. The seroprevalence of hepatitis E virus in patients with chronic hepatitis C in Brazil is unknown. This study aims to estimate the seroprevalence of hepatitis E virus in patients with chronic hepatitis C and to describe associated risk factors.

**Methods:** A total of 618 patients chronically infected with hepatitis C virus from three reference centers of São Paulo, Brazil were included. Presence of anti-HEV IgG was assessed by enzyme-linked immunosorbent assay (WANTAI HEV-IgG ELISA).

**Results:** Out of the 618 patients tested, 10.2% turned out positive for anti-HEV IgG (95% CI 8.0–12.8%). Higher seroprevalence was found independently associated with age over 60 years (OR = 2.04;  $p = 0.02$ ) and previous contact with pigs (OR = 1.99;  $p = 0.03$ ).

**Conclusions:** Patients with chronic hepatitis C are under risk of hepatitis E virus superinfection in São Paulo. Contact with pigs is a risk factor for the infection, suggesting a possible zoonosis with oral transmission.

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## Introduction

Hepatitis E virus (HEV) is the most common hepatotropic virus in the world.<sup>1–3</sup> The World Health Organization estimates the

occurrence of 20 million infections per year worldwide, with around 3.3 million symptomatic infections, and 44,000 deaths in 2015.<sup>4</sup>

HEV includes four main genotypes that infect humans with different epidemiological characteristics. Genotypes 1 and 2, described only in humans, is the etiologic agent of large fecal-oral transmission epidemics, typical of overpopulated regions and with poor sanitary conditions. Genotype 1 has

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been described in Asia and Africa, and genotype 2 in Mexico and Africa. Genotype 3, described in Europe, the Americas and Japan, and genotype 4, described in Asia, infect a large variety of mammals and occur in the form of sporadic cases. Ingestion of undercooked pork meat is implicated in the transmission of such zoonosis. HEV can also be transmitted alternatively by parenteral route, such as transfusion of blood products.<sup>2</sup>

Brazil exhibits an intermediate HEV seroprevalence, with rates of 2–9.8% reported in blood donors,<sup>5,6</sup> 12% in intravenous drug users,<sup>7</sup> and 15% in renal transplant patients.<sup>8</sup> Previous study of HEV seroprevalence among pigs in Brazil detected seropositivity in 97.3% of the animals older than 25 weeks of age.<sup>9</sup> Genotype 3 was identified in humans and pigs in this country.<sup>8,10,11</sup>

It is estimated that 1.6–3.2 million people are chronically infected with hepatitis C virus (HCV) in Brazil.<sup>12,13</sup> A previous study found high morbidity and mortality rates of HEV infection in patients with underlying liver disease.<sup>14</sup>

HEV seroprevalence in patients with chronic hepatitis C virus infection is unknown in Brazil. The objective of this study was to estimate the prevalence of anti-HEV antibodies in chronic HCV infected patients in the State of São Paulo, as well as to evaluate associated risk factors.

## Material and methods

### Study design

A cross-sectional study of seroprevalence was conducted at the hepatitis outpatient services of Universidade Federal de São Paulo, Hospital de Transplante Euryclides Jesus Zerbini, and Instituto de Vacinação e Infectologia de Piracicaba, in the State of São Paulo, Brazil.

Between October 2015 and December 2016 patients of both sexes, older than 18 years, and with chronic HCV infection confirmed by RT-PCR RNA were included.

### Sample size calculation

Assuming an expected prevalence of HEV infection of 8%, a confidence level of 99%, and a total width of confidence interval (CI) around the expected proportion of 6%, a total of 576 HCV infected patients would be necessary.

### Anti-HEV antibodies detection

The presence of anti-HEV IgG was assessed by enzyme-linked immunosorbent assay (WANTAI HEV-IgG ELISA), strictly according to the manufacturer's recommendations. Patients positive for HEV IgG were further tested for the presence of HEV IgM antibodies using a specific kit from the same manufacturer.

### Data collection

The following variables possibly associated with HEV infection were assessed: sex, age, socioeconomic class, HIV co-infection, previous history of contact with pigs (have ever lived in a place where pigs were raised), consumption of pork

meat (never, seldom, often), previous use of intravenous drugs, prior surgery, transfusion of blood products, presence of tattoos, and hemodialysis.

For the characterization of the socioeconomic class the validated questionnaire of economic classification developed by the Brazilian Association of Research Companies (ABEP), version 2015<sup>15</sup> was used.

### Statistical analysis

Seroprevalence of IgG antibodies is presented with the relevant 95% confidence interval, calculated according to the Wilson method.

Student's t-test was used to compare means. Proportions were compared by the Chi-square test with Yates correction, or by the Fisher's exact test, when appropriate.

Associations with significance level with  $p < 0.20$  in univariate analyses were subsequently included in multiple logistic regression models. Statistically significant independent association was considered if  $p < 0.05$  after multivariate analysis.

All the analyses and charts were drawn using program R, version 3.3.2 (The R Foundation for Statistical Computing).

### Ethical aspects

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a prior approval by the Institutional Ethics Review Board of the Federal University of São Paulo (0350/2015). A written informed consent was obtained from each patient included.

## Results

A total of 618 patients with chronic HCV infection was included. Table 1 summarizes the distribution of the variables evaluated. Fifty-three percent of the sample were males, mean age of 53.8 years, minimum 22 and maximum of 86 years. History of previous contact with pigs was reported by 30.4% of the assessed patients. Twenty-six cases of HIV infection (4.2%), five hemophiliacs, three pregnant women, and two transplanted women were included.

A total of 63 cases were anti-HEV IgG reactive, determining seroprevalence of 10.2% (95% CI 8.0–12.8%). Three cases (0.5%) had an indeterminate result and the information was considered as negative for hypothesis testing. Anti-HEV IgM was screened in all reactive and indeterminate cases for IgG and all of them turned out non-reactive.

Table 2 summarizes the results of univariate analyses for the dependent variable anti-HEV IgG and the assessed variables. Only the variables age and history of home contact with pigs were significantly associated with anti-HEV IgG. Hemophilia exhibited a level of borderline significance (Fisher's exact test  $p = 0.09$ ).

Fig. 1 illustrates the conditional density curve of anti-HEV IgG associated with age, showing a seemingly linear increase from 30 to 70 years, with prevalence ranging from zero up to 20% between these two points. The mean age of patients with positive and negative anti-HEV IgG was 57.98 and 53.36 years (Student's t-test with  $p < 0.001$ ) and medians 59 and 54 years,

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