A cross-sectional study was carried out among 996 volunteer blood donors enrolled from May 1999 to December 1999 to determine the seroprevalence of hepatitis E virus (HEV) infection among volunteer blood donors of the Regional Blood Bank of Londrina, State of Paraná, Brazil, and to evaluate whether the rate of seroprevalence of IgG anti-HEV antibodies is associated with sociodemographic variables and with seropositivity for hepatitis A virus (HAV) infection. All participants answered the questionnaire regarding the sociodemographic characteristics. Serum samples were tested for IgG antibodies to HEV (anti-HEV) by an enzyme linked immunosassay (ELISA). All serum samples positive for anti-HEV IgG and 237 serum samples negative for anti-HEV were also assayed for IgG anti-HAV antibodies by ELISA. Anti-HEV IgG was confirmed in 23/996 samples, resulting in a seroprevalence of 2.3% for HEV infection, similar to previous results obtained in developed countries. No significant association was found between the presence of anti-HEV IgG antibodies and the sociodemographic variables including gender, age, educational level, rural or urban areas, source of water, and sewer system (p > 0.05). Also, no association with seropositivity for anti-HAV IgG antibodies was observed (p > 0.05). Although this study revealed a low seroprevalence of HEV infection in the population evaluated, the results showed that this virus is circulating among the population from Londrina, South Brazil, and point out the need of further studies to define the clinical and epidemiological importance of HEV infection and to identify additional risk factors involved in the epidemiology and pathogenesis of this infection in this population.

KEYWORDS: Hepatitis E virus; Hepatitis A virus; Epidemiology; Seropositivity; Blood donors.
women considered at risk for human immunodeficiency virus type 1 (HIV-1) in the Southeast, and 12% among intravenous drug users in the Southeast. In the State of Paraná, South Brazil, no studies of the HEV infection have been reported.

HEV infection usually presents an acute and benign clinical course and does not provoke chronic infection. However, among all the responsible hepatitis viruses, HEV is associated with the largest number of fulminant hepatitis cases (3.0%) in the general population, and up to 20% in pregnant women.

Hepatitis A virus (HAV) is an extremely stable virus which is spread by the fecal-oral route, either directly from person to person or by contaminated food or water, with the prevalence being closely correlated with the degree of environmental sanitation and the prevailing socioeconomic and hygienic conditions. Considering that the HEV and HAV infections share the same transmission routes, it is possible that the local seroprevalence of both infections may also be similar. However, few studies have examined the epidemiology of the association between HEV and HAV infections.

The aim of this study was to determine the seroprevalence of HEV infection among volunteer blood donors of the Regional Blood Bank of Londrina, State of Paraná, Brazil, and to evaluate whether the rate of seropositivity of anti-HEV IgG antibodies is associated with the sociodemographic characteristics and the seropositivity for HAV infection. These data can contribute to define the epidemiological importance of the HEV infection in this district and to elaborate rational clinical and epidemiological strategies in the investigation of cases classified as non-A, non-B, and non-C hepatitis.

**MATERIALS AND METHODS**

A cross-sectional study was carried out to determine the seroprevalence of HEV infection among volunteer blood donors of the Regional Blood Bank of Londrina, State of Paraná, Brazil, and to evaluate the socio-demographic characteristics of the blood donors enrolled according to seropositivity for anti-HEV IgG and anti-HAV IgG antibodies and the variables evaluated. A questionnaire was applied to the participants to obtain information about sociodemographic data including gender, age, educational level, place of residence (rural or urban area), source of water, and the sanitary conditions of their homes (sewer or pit), in order to evaluate possible risk factors for HEV infection. On the basis of the standard clinical screening of the blood donors, all individuals enrolled in this study were also questioned about a past clinical history of symptomatic hepatitis.

The results obtained for the blood donors with positive anti-HEV IgG were compared with those obtained for blood donors with negative anti-HEV IgG.

**Serological assays for anti-HEV IgG and anti-HAV IgG:** All serum samples were stored at -20 °C until used. Serum samples were tested for specific anti-HEV IgG antibodies by enzyme linked immunosorbent assay (ELISA), according to the method described elsewhere using commercially available reagents (HEV EIA, ABBOTT Laboratories, IL, USA). According to the manufacturer, this assay presents 98.5% sensitivity and 99.5% specificity. The results were scored as positive or negative according to standard procedures recommended by the manufacturers. The individuals were considered to be seropositive when they showed two repeated positive reactions. Positive and negative controls were included in all the ELISA microplates assayed. All serum samples from blood donors positive for anti-HEV IgG and 237 samples from blood donors negative for anti-HEV IgG were also assayed for anti-HAV IgG antibodies by ELISA, as described elsewhere, using commercially available products (ABBOTT Laboratories, HAV EIA). According to the manufacturer, the assay presents 99.0% sensitivity and 99.5% specificity.

**Statistical analysis:** A database was set up using the EPI INFO software version 6.04d. Descriptive statistical analysis was used to calculate the mean, median, standard deviation (SD), and percentage of the sociodemographic variables of the blood donors, and 95% confidence intervals (95% CI) of the proportion of seropositivity for anti-HEV IgG and anti-HAV IgG antibodies and the variables evaluated. The statistical analysis was performed using the Chi-square (χ²) and Exact Fisher tests. The level of significance adopted for all tests was 5% (p < 0.05).

**RESULTS**

The age of the volunteer blood donors ranged from 18 to 60 years (mean: 29.9 ± 9.8 years; median: 26.9 years) and 605 (60.7%) were males. Table 1 shows that 23 blood donors showed positive anti-HEV IgG antibodies, corresponding to an overall seroprevalence rate of 2.3% (95% CI = 1.5 - 3.5). The sociodemographic characteristics of the blood donors enrolled according to seropositivity for anti-HEV IgG are shown in Table 2. No association was observed (p > 0.05) between seropositivity and the variables evaluated including gender, age (≤ 35 years old and > 35 years old), educational level (incomplete elementary school and ≥ elementary school), origin (urban or rural area), source of water (public or other) and sewer (public service or pit). Most subjects lived under adequate sanitary housing conditions in view of the high

<table>
<thead>
<tr>
<th>ANTI-HEV IgG *</th>
<th>Number</th>
<th>%</th>
<th>CI (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>23</td>
<td>2.3</td>
<td>1.5 – 3.5</td>
</tr>
<tr>
<td>Negative</td>
<td>973</td>
<td>97.7</td>
<td>96.5 – 98.5</td>
</tr>
<tr>
<td>Total</td>
<td>996</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

* Anti-hepatitis virus E IgG antibodies evaluated by enzyme immunoassay (ELISA); †:confidence interval.
rate of presence of a sewage system (73.2%) and public water (98.6%). All the 23 (100.0%) subjects with positive results for anti-HEV IgG and 959 (98.6%) with negative results had public piped water available. Therefore, there was no statistically significant difference in anti-HEV prevalence when the presence of a sewage system and the source of water were taken into consideration.

The results obtained from the 23 blood donors with positive anti-HEV IgG were compared with those obtained from 237 blood donors with negative anti-HEV IgG. Among the 260 serum samples assayed for anti-HAV IgG, 211 were positive for anti-HAV IgG, corresponding to a seropositivity of 81.1%. Among the positive samples for anti-HEV IgG, 19 (82.6%) were also positive for anti-HAV IgG. Regarding the samples that were negative for anti-HEV IgG, 192 (81.0%) were also positive for anti-HEV IgG. No association between the two rates of seropositivity (anti-HEV and anti-HAV) was observed (p = 1.000), as shown in Table 3.

### DISCUSSION

Viral hepatitis is considered to be a worldwide public health problem, representing a significant cause of morbidity and mortality. On most occasions, the etiologic agent of the infection is not defined, generating problems in the handling of those cases and in the decision about the most adequate prophylactic measures.

The areas of the world with a high incidence of HEV infection are mainly developing countries, mainly in Asia and in Africa. In Brazil, different patterns of hepatitis virus distribution are found throughout the country. Outbreaks of HEV infection have not yet been described. However, the possibility of the occurrence of such outbreaks exists since in our country there are great social differences and sanitary conditions are quite precarious in many areas. Besides, the disease surveillance is deficient in the country as a whole, with the consequent serious problem of undernotification of cases.

### Table 2
Sociodemographic characteristics of 996 volunteer blood donors of the Regional Blood Bank of Londrina, Paraná, Brazil, distributed according to seropositivity for anti-hepatitis virus E (anti-HEV IgG) antibodies

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Anti-HEV positive n (%)</th>
<th>Anti-HEV negative n (%)</th>
<th>Total n (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>15 (65.2)</td>
<td>590 (60.6)</td>
<td>605 (60.7)</td>
<td>0.8190* NS</td>
</tr>
<tr>
<td>Female</td>
<td>8 (34.8)</td>
<td>383 (39.4)</td>
<td>391 (39.3)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 35</td>
<td>17 (73.9)</td>
<td>692 (71.1)</td>
<td>709 (71.2)</td>
<td>0.9526* NS</td>
</tr>
<tr>
<td>&gt; 35</td>
<td>6 (26.1)</td>
<td>281 (28.9)</td>
<td>287 (28.8)</td>
<td></td>
</tr>
<tr>
<td>Place of residence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural area</td>
<td>2 (8.7)</td>
<td>58 (6.0)</td>
<td>60 (6.0)</td>
<td>0.6442† NS</td>
</tr>
<tr>
<td>Urban area</td>
<td>21 (91.3)</td>
<td>915 (94.0)</td>
<td>936 (94.0)</td>
<td></td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elementary school</td>
<td>5 (21.7)</td>
<td>175 (18.0)</td>
<td>180 (18.1)</td>
<td>0.5886† NS</td>
</tr>
<tr>
<td>≥ Elementary school</td>
<td>18 (78.3)</td>
<td>798 (82.0)</td>
<td>816 (81.9)</td>
<td></td>
</tr>
<tr>
<td>Source of water</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Public</td>
<td>23 (100.0)</td>
<td>959 (98.6)</td>
<td>982 (98.6)</td>
<td>1.000† NS</td>
</tr>
<tr>
<td>Other (mine, shaft)</td>
<td>0 (0.0)</td>
<td>14 (1.4)</td>
<td>14 (1.4)</td>
<td></td>
</tr>
<tr>
<td>Sanitary conditions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sewer</td>
<td>14 (60.9)</td>
<td>715 (73.5)</td>
<td>729 (73.2)</td>
<td>0.2660* NS</td>
</tr>
<tr>
<td>Pit (closed and open)</td>
<td>9 (39.1)</td>
<td>258 (26.5)</td>
<td>267 (26.8)</td>
<td></td>
</tr>
</tbody>
</table>

* Chi-square test ($\chi^2$); † Exact Fisher Test; n: number of samples; NS: not significant.

### Table 3
Seropositivity for anti-hepatitis A virus (anti-HAV) IgG antibodies obtained for volunteer blood donors of the Regional Blood Center of Londrina, Paraná, Brazil, distributed according to the seropositivity for anti-hepatitis E virus (anti-HEV) IgG antibodies

<table>
<thead>
<tr>
<th>Serologic result</th>
<th>Anti-HEV*Positive n (%)</th>
<th>Anti-HEV*Negative n (%)</th>
<th>Total n (%)</th>
<th>CI† (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HAV+ positive</td>
<td>19 (82.6)</td>
<td>192 (81.0)</td>
<td>211 (81.1)</td>
<td>75.75-85.61</td>
</tr>
<tr>
<td>Anti-HAV+ negative</td>
<td>4 (17.4)</td>
<td>45 (19.0)</td>
<td>49 (18.8)</td>
<td>14.39-24.25</td>
</tr>
<tr>
<td>Total</td>
<td>23 (100.0)</td>
<td>237 (100.0)</td>
<td>260 (100.0)</td>
<td></td>
</tr>
</tbody>
</table>

*: Anti-hepatitis E virus IgG antibodies evaluated by enzyme immunoassay (ELISA); †: Anti-hepatitis A virus IgG antibodies evaluated by enzyme immunoassay (ELISA); Exact Fisher Test: p = 1.000 (not significant); †CI: confidence interval.
HA V infection is highly prevalent in Brazil, as demonstrated in the individuals' life including housing, drinking water, and sewer infection is the fecal-oral one, which is also the route of transmission of anti-HEV IgG among the different groups and among blood donors. A study conducted on selected populations revealed a 4.3% prevalence of HEV infection. Unlike HA V infection, HEV infection appears to affect mainly distinct epidemiological features that differentiate it from HA V infection. In Brazil, the seroprevalence of anti-HEV IgG among blood donors was 2.0%. In a study carried out in the city of São Paulo, the overall prevalence observed (4.0%) was considered to be low.

As mentioned previously, the main route of transmission of HEV infection is the fecal-oral one, which is also the route of transmission of HAV infection, whose prevalence is associated with the quality of the individuals' life including housing, drinking water, and sewer service. HAV infection is highly prevalent in Brazil, as demonstrated in a study carried out in the city of São Paulo, State of São Paulo, on individuals ranging in age from 16 to 30 years, in which the prevalence of anti-HAV was 91.9% among individuals of middle socioeconomic condition and 100% among those of low socioeconomic condition.

Regarding the similarity of the transmission mechanisms, there is a trend to evaluate the association between these two infections (HAV and HEV infections). A study carried out in Israel on hemophiliac patients detected a strong association between the viruses. In Brazil, the same association was found in a study carried out in Salvador, State of Bahia, and the authors concluded that similarities exist between the epidemiology of the two infections, and that individuals exposed to HAV have a higher probability to be also exposed to HEV.

In the present study, no association was found between these two infections, a result similar to that reported in a study that evaluated the prevalence of these viruses in the general population of the city of São Paulo, State of São Paulo. Also, no association was found in the urban population of Burundi, Africa, and the authors concluded that, although the two viruses are transmitted by the fecal-oral route, the epidemiological models of the two infections are different. HEV infection has a few distinct epidemiological features that differentiate it from HAV infection. Unlike HAV infection, HEV infection appears to affect mainly young adults, which is unusual for a virus transmitted via the fecal-oral route. Also unusual is that secondary cases of HEV infection are much less frequent than HAV infection, and finally a major difference is the high mortality rate observed among pregnant women infected with HEV.

In California (USA), a group of candidates to blood donors was evaluated and the results did not show an association between those infections and the authors concluded that, possibly, different patterns of transmission of the two viruses may exist and the risk factors for HEV infection in the sporadic cases that occur in non-endemic areas have not been defined.

Similarly, in this study, since the association of the type of water consumed, including the use of untreated water, with the seroprevalence of IgG anti-HEV antibodies was not demonstrated, other possible transmission routes should be suggested.

HEV infection, unlike HAV infection, presents a very low rate of inter-human transmission, as demonstrated by several authors. According to these authors, this route of transmission of HEV infection should not be the only one responsible for the sporadic cases that occur in non-endemic areas. Other transmission mechanisms, not yet clarified, may play an important role in the epidemiology of HEV infection in these areas.

According to some authors, HEV infection could be classified as a zoonosis because some animals may act as reservoirs of the virus, a fact justifying the circulation of the virus in non-endemic areas. Occasionally, HEV may infect humans through direct contact with domestic or farm animals or with contaminated water supplies.

The analysis of the genome of the HEV isolated from swine and from individuals with HEV infection suggests that these animals can serve as a reservoir of the virus. The existence of cross-infection with HEV between humans and swine is a possibility that cannot be ruled out. The high prevalence of HEV antibodies among apparently healthy humans (20.0%) and farm pigs (72.0%) in Bali, Indonesia, whose population is mostly Hindu and has a habit of consuming pork, indicates that HEV infection may occur as a zoonosis.

In a study, related by authors as the first report that showed evidences of HEV circulation in Brazilian animal species and pig handlers, was detected the soroprevalences of HEV infection in cows (1.42%), dogs (6.97%), chickens (20%), swines (24.3%) and rodents (50.0%), as well in pig handlers (6.3%). The recognition of swine HEV infection in pigs in many countries of the world led these authors to investigate a larger sample of pigs of same Brazilian region that demonstrated a total seroprevalence of 63.6%. However, as the seroprevalence reached 97.3% of animals older than 25 weeks, the authors concluded that swine HEV infection seems to be almost universal within this Brazilian pig population.

The true participation of the animals in the transmission of HEV is still unclear, with many points that still require elucidation. Similarly, the true meaning of the seroprevalence of the anti-HEV IgG antibodies detected in the present study it is still to be defined.

Taken together, although showing a low prevalence of HEV infection among blood donors, the results obtained in the present study demonstrate that HEV circulates in the district of Londrina. However, further studies are necessary to define the clinical and epidemiologic importance of the HEV infection in this area and to identify additional risk factors involved in the epidemiology and pathogenesis of HEV infection. The results obtained open the possibility of other routes of
spreading different from that previously recognized ones such as fecal-oral contamination, mainly the possible participation of animals in the transmission of HEV.

RESUMO

Soroprevalência da infecção pelo vírus da hepatite E (VHE) em candidatos a doadores de sangue voluntários do Hemocentro Regional de Londrina, Londrina, Estado do Paraná, Brasil

Os objetivos do estudo foram determinar a soroprevalência da infecção pelo vírus da hepatite E (VHE) em candidatos a doadores de sangue voluntários do Hemocentro Regional de Londrina, Paraná, e avaliar se essa soroprevalência está associada com variáveis sociodemográficas e com o índice de sorosoropositividade para hepatite por vírus A (VHA). Foi realizado estudo transversal para determinar a taxa de soroprevaência da infecção pelo VHE em 996 candidatos a doadores de sangue. Todos os participantes responderam um questionário sobre características sociodemográficas. A pesquisa de anticorpos específicos IgG anti-VHE foi realizada por método de enzimaimunoensaio (ELISA). Todas as amostras soropositivas para IgG anti-VHE e 237 amostras soronegativas para IgG anti-VHE foram submetidas também à pesquisa de IgG anti-VHA por ELISA. Entre as 996 amostras de soro analisadas, anticorpos IgG anti-VHE foram detectados em 23 amostras, resultando em uma soroprevalência de 2,3% (IC 95%: 1,5 - 3,5), semelhante aos resultados de estudos realizados em países desenvolvidos. Não houve associação entre o índice de sorosoropositividade de IgG anti-VHE e as variáveis sociodemográficas (sexo, idade, escolaridade, procedência, fonte de água e origem dos dejetos); também não se detectou associação entre sorosoropositividade para IgG anti-VHE com sorosoropositividade para IgG anti-VHA. Os resultados obtidos sugerem que, embora tenha se observado uma baixa prevalência da infecção pelo VHE na população analisada, verificou-se que existe circulação do VHE no município de Londrina e que estudos posteriores são necessários para definir a importância clínica e epidemiológica da infecção pelo VHE nesse município bem como identificar outros possíveis fatores de risco envolvidos na epidemiologia da infecção pelo VHE.

REFERENCES


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