Seroprevalence of hepatitis E virus infection in domestic pigs in the Federal District, Brazil


INTRODUCTION

Hepatitis E is an important public-health concern and is a major cause of enterically transmitted hepatitis worldwide. The disease is caused by the hepatitis E virus (HEV) which is currently known to be a zoonotic pathogen transmitted by pigs (Tam et al., 1991; Meng, 2006). Humans and pigs share the HEV genotypes 3 and 4 (HEV-3 and HEV-4) that seem to circulate in many countries such as Brazil (Paiva et al., 2007; Lopes dos Santos et al., 2010; Smith et al., 2014).

Swine transmit the disease to humans through the consumption of raw or undercooked meat, the directly exposure to the animals or their feces and the environmental contamination of water sources (Yugo and Meng, 2013). The infection in pigs is asymptomatic and mostly occurs at the age of 2–4 months (Meng et al., 1997).
In Brazil, human hepatitis E is a notifiable disease (Brasil, 2005). Outbreaks of the disease have never been reported, despite the fact that environmental conditions and sanitation in some regions favor the transmission (Brasil, 2014). However, autochthonous cases have already been reported (Lopes dos Santos et al., 2010; Brasil, 2012).

According to data from Brazilian Ministry of Health, from 1999 to 2011 there were 967 confirmed cases and 86 deaths by hepatitis E reported in the country (Brasil, 2012). The Ministry of Health recognizes that many cases may not have been registered leading to high underreporting. This could be happening due to the broad spectrum of this disease and the considerable proportion of asymptomatic cases that remain unknown to the surveillance system (Brasil, 2012). Therefore, the lack of a national epidemiological study about the disease hinders the knowledge of its real situation in the country.

The swine production has an important role in Brazilian livestock economy. The country is today the fourth largest producer and exporter of pork (Livestock…, 2015). However, there are few studies about the prevalence of HEV in its domestic swine herd. Moreover, in two of these studies conducted in the states of Mato Grosso and Rio de Janeiro, a high seroprevalence of HEV was found in pigs (Guimarães et al., 2005; Vitral et al., 2005).

Thus, a cross-sectional study was performed in order to estimate the seroprevalence of hepatitis E virus infection among domestic pigs from different localities in the Federal District, Brazil.

**MATERIALS AND METHODS**

The sample size calculation to estimate the true prevalence considered an assumed prevalence of 0.75 (based on the results of similar studies conducted in other states of the country), a population size of 163,985 (provided by the Secretary of Agriculture of the Federal District and related to the year of 2014), an assumed sensitivity of 0.9096 and an assumed specificity of 0.9404 (HEV…, 2015), a confidence level of 0.95 and a desired precision of 0.5.

As a result, 449 serum samples were required. The samples were randomly selected from a previous epidemiological survey carried out by the Secretary of Agriculture of the Federal District for classical swine fever. After the fulfillment of the purpose of this investigation the serum samples were used in this study. The 449 selected samples belonged to 234 subsistence farms situated in 12 localities (Brazlândia, Ceilândia, Gama, Paranoá, Planaltina, Recanto das Emas, Riacho Fundo, Samambaia, Santa Maria, São Sebastião, Sobradinho, and Taguatinga) (Figure 1).

The samples were collected from June to September 2014 and they were stored in microtubes at -18°C until the time of analysis. Each sample was collected from the jugular vein of male and female domestic pigs that were born at their respective farms. These animals were between 6 and 48 months of age and there was no information about their breed. In order to compare the ages, pigs were subdivided in two categories: young animals (6-10 months) and adult animals (11-48 months).

The standard gathering data consisted in a questionnaire, which included information such as contact with wild swine, contact with pigs from other establishments, if there was the arrival of new animals, the supply of food waste and the proximity to nature reserves, protected areas, or national parks with wild swine. This information was used to evaluate possible risk factors.

Swine IgG antibodies against HEV were detected in sera samples by the commercial enzyme-linked immunosorbent assay PrioCHECK® HEV Ab porcine in accordance with the manufacturer’s instructions. This kit is the only commercially available ELISA based on recombinant ORF2- and 3-derived antigens of HEV genotypes 1 and 3, which significantly improves the specificity and sensitivity of the test (HEV…, 2015).
EpiTools® program was used to calculate the sample size and to estimate true prevalence, whereas the chi-square test ($\chi^2$) was used to compare the seroprevalence between genders and ages (Sergeant, 2015). A $p$ value $<0.05$ was considered statistically significant. Odds ratio with a 95% confidence interval was used to evaluate possible risk factors.

All procedures of this work were approved by the University of Brasilia Ethics Committee on Animal Use (CEUA-UnB) (UnBDoC n° 43447/2014).

**RESULTS**

Of the 449 animals tested, 304 showed anti-HEV positive reactions (67.7%, 95% CI = 63.2%, 71.9%). There was no difference of seroprevalence by gender ($p=0.1$) or age ($p=0.58$) (Table 1 and 2). The seroprevalence among farms ranged from 0.0% to 85.7% (Table 3).

<table>
<thead>
<tr>
<th>Anti-HEV IgG</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positieve</td>
<td>(%)</td>
</tr>
<tr>
<td>Male</td>
<td>98</td>
</tr>
<tr>
<td>Female</td>
<td>206</td>
</tr>
<tr>
<td>Total</td>
<td>304</td>
</tr>
</tbody>
</table>

Table 1. Frequency of anti-HEV IgG by gender among pigs of the Federal District, Brazil

<table>
<thead>
<tr>
<th>Anti-HEV IgG</th>
<th>Age group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positieve</td>
<td>(%)</td>
</tr>
<tr>
<td>Young</td>
<td>85</td>
</tr>
<tr>
<td>Adult</td>
<td>219</td>
</tr>
<tr>
<td>Total</td>
<td>304</td>
</tr>
</tbody>
</table>

Table 2. Frequency of anti-HEV IgG by age classes among pigs of the Federal District, Brazil
Table 3. Prevalence of anti-HEV IgG in swine sera by regions of the Federal District, Brazil

<table>
<thead>
<tr>
<th>Localities</th>
<th>Farms</th>
<th>Animals</th>
<th>Positive</th>
<th>Prevalence (%) [CI = 95%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brazlândia</td>
<td>18</td>
<td>26</td>
<td>16</td>
<td>61.5 [42.5 – 77.6]</td>
</tr>
<tr>
<td>Ceilândia</td>
<td>20</td>
<td>38</td>
<td>30</td>
<td>78.9 [63.7 – 88.9]</td>
</tr>
<tr>
<td>Gama</td>
<td>22</td>
<td>35</td>
<td>30</td>
<td>85.7 [70.6 – 93.7]</td>
</tr>
<tr>
<td>Paranoá</td>
<td>29</td>
<td>43</td>
<td>35</td>
<td>81.4 [67.4 – 90.3]</td>
</tr>
<tr>
<td>Planaltina</td>
<td>85</td>
<td>211</td>
<td>123</td>
<td>58.3 [51.6 – 64.7]</td>
</tr>
<tr>
<td>Recanto das Emas</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>50.0 [9.5 – 90.5]</td>
</tr>
<tr>
<td>Riacho Fundo</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>20.0 [3.6 – 62.4]</td>
</tr>
<tr>
<td>Samambaia</td>
<td>20</td>
<td>35</td>
<td>27</td>
<td>77.1 [61.0 – 87.9]</td>
</tr>
<tr>
<td>Santa Maria</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.0 [0.0 – 79.3]</td>
</tr>
<tr>
<td>São Sebastião</td>
<td>12</td>
<td>20</td>
<td>17</td>
<td>85.0 [64.0 – 94.8]</td>
</tr>
<tr>
<td>Sobradinho</td>
<td>16</td>
<td>23</td>
<td>17</td>
<td>73.9 [53.5 – 87.5]</td>
</tr>
<tr>
<td>Taguatinga</td>
<td>6</td>
<td>10</td>
<td>7</td>
<td>70.0 [39.7 – 89.2]</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>234</td>
<td>449</td>
<td>304</td>
<td><strong>67.7 [63.2 – 71.9]</strong></td>
</tr>
</tbody>
</table>

DISCUSSION

A high prevalence of anti-HEV IgG was detected in domestic pigs in the Federal District. A high prevalence of swine anti-HEV antibodies was also detected by Guimarães et al. (2005), in Mato Grosso state, and Vitral et al. (2005), in Rio de Janeiro state. Guimarães et al. (2005) tested 260 animals from 13 different counties and found a prevalence of anti-HEV IgG of 81.2%. Vitral et al. (2005) analyzed 357 swine sera and found a prevalence of 63.6%. On the other hand, in Pará state, only 13 of 151 samples of swine sera (8.6%) were positive for anti-HEV IgG (Souza et al., 2012).

Throughout the world there are a number of studies that have reported the detection of anti-HEV IgG antibodies in pigs. The seroprevalence of anti-HEV antibodies in swine in European countries varies from 27% to 92.8% (Grierson et al., 2015; O‘Connor et al., 2015). In Asia, the prevalence is still significant and varies from 14.8% to 64.7% (Choi et al., 2003; Liang et al., 2014). In the Americas, the seroprevalence of swine anti-HEV antibodies also ranges widely from 22.7% to 80% (Cooper et al., 2005; Munné et al., 2006). However, in Brazil, these serologic studies are still scarce.

This wide variability on swine HEV seroprevalence results can be due to different specificity and sensibility of the serological assays used (Khudyakov and Kamili, 2011). Besides, some studies have shown that HEV prevalence is directly associated with differences in the hygiene and sanitary management of the rearing facilities (Casas et al., 2011; Walachowski et al., 2014).

There was no difference in prevalence between genders (p=0.1) and age classes (p=0.58), once again concurring with Guimarães et al. (2005). This can be explained because both studies analyzed only young and adult samples and the disease appears to have no preference among genders. The exclusion of younger pigs was due to the presence of maternal antibodies (Santos et al., 2009).

The most common form of HEV transmission among pigs is fecal-oral, such as with humans. Pigs become infected when a direct contact with other infected pigs occurs randomly or through the ingestion of water or food contaminated with feces (Kasorndorkbuad et al., 2004).

It was not possible to establish risk factors because of the small number of animals exposed to the majority of investigated conditions, although it is known that the introduction of new animals in the herd could facilitate the transmission of several diseases and the contact with wild boars was shown to be another source...
Seroprevalence of hepatitis...

of transmission to pigs (Schlosser et al., 2014). Besides that, most of the pigs received food waste, but the proportion of positive animals was very similar in both exposed and non-exposed groups. Therefore, any association between the consumption of food waste and the presence of antibodies to the disease was not found, although it is known that the supply of food waste could be a risk factor due to its easy contamination with animal feces.

CONCLUSIONS

Hepatitis E virus circulates among domestic pigs in the Federal District, Brazil, and it serves as a warning to the local public health system due to a possible involvement of this animal in human infections. Moreover, the high seroprevalence found in the region helps to better understand the epidemiology of this disease. It is also important to obtain more information in order to prevent a zoonotic transmission at an early stage.

ACKNOWLEDGEMENTS

The authors would like to thank the veterinarians from the Secretary of Agriculture of the Federal District for their support and assistance and CAPES for the financial support.

REFERENCES


