

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

[*Soroprevalência da infecção pelo vírus da hepatite E em suínos domésticos no Distrito Federal, Brasil*]

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ABSTRACT

Hepatitis E is caused by the hepatitis E virus (HEV) which is currently known to be a zoonotic pathogen transmitted by pigs. In Brazil, there is no information about the circulation of HEV in the swine herd of the Federal District. Therefore, a cross-sectional study was performed with sera from 449 domestic pigs, provided by the Secretary of Agriculture of the Federal District. Blood samples were collected between June and September 2014. A commercially available ELISA kit was used for the detection of IgG antibodies. High seroprevalence of antibodies to HEV was found, since 304 animals showed anti-HEV positive reactions (67.7%; 95% CI = 63.2%, 71.9%). The seropositivity presented no difference by gender or age. The results suggest that HEV circulates among domestic pigs in the Federal District and it can serve as a warning to the local public health system due to their possible involvement in human infections.

Keywords: hepatitis E, swine, Federal District, Brazil

RESUMO

A hepatite E é causada pelo vírus da hepatite E (HEV), considerado um patógeno de transmissão zoonótica que tem suínos como reservatórios. No Brasil, não há informações a respeito da circulação do HEV no rebanho suíno do Distrito Federal. Por isso, foi conduzido um estudo transversal com amostras de soro de 449 suínos domésticos provenientes de 234 propriedades, cedidas pela Secretaria de Agricultura do Distrito Federal. As amostras sanguíneas foram coletadas entre junho e setembro de 2014. Um kit de ELISA comercialmente disponível foi utilizado para a detecção sorológica de anticorpos IgG contra o HEV. Foi encontrada uma alta soroprevalência de anticorpos contra o HEV, uma vez que 304 animais apresentaram amostras reagentes (67,7%, IC 95% = 63,2%, 71,9%). A soropositividade não variou com relação ao gênero ou à idade. Os resultados sugerem que o HEV circula entre os suínos domésticos no Distrito Federal, e isso pode servir como um alerta para o sistema de saúde pública da região devido ao possível envolvimento desses animais em infecções humanas.

Palavras-chave: hepatite E, suínos, Distrito Federal, Brasil

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).

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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).

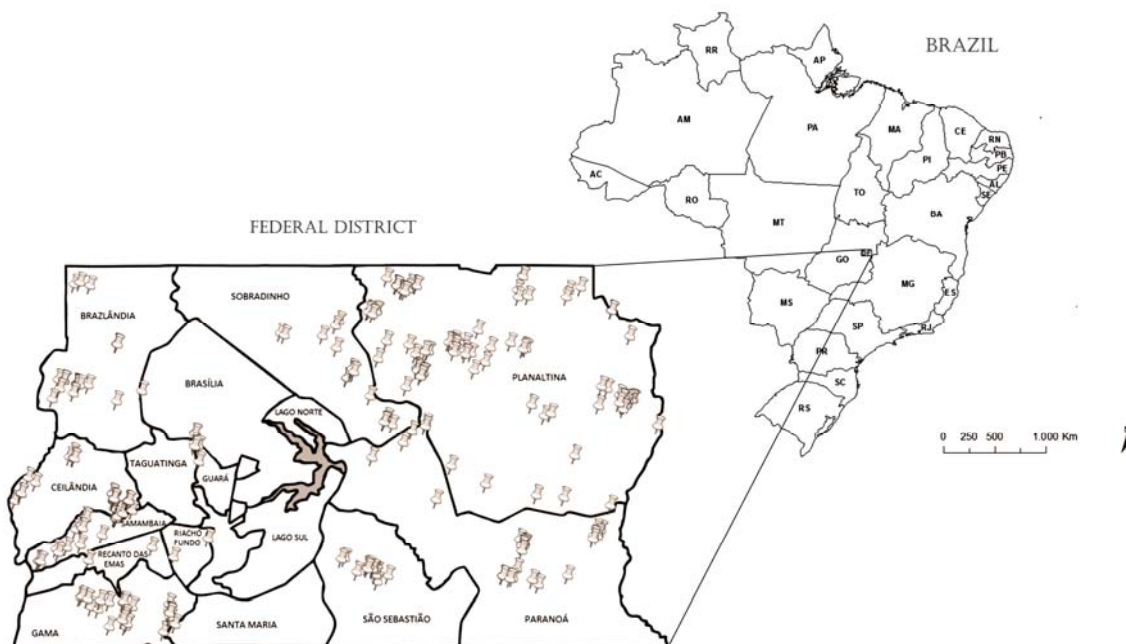


Figure 1. Approximate location of the subsistence farms from which the samples were collected, Federal District, Brazil.

EpiTools® program was used to calculate the sample size and to estimate true prevalence, whereas the chi-square test (χ^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 1. Frequency of anti-HEV IgG by gender among pigs of the Federal District, Brazil

Gender	Anti-HEV IgG				Total	(%)
	Positive	(%)	Negative	(%)		
Male	98	62.8	58	37.2	156	34.7
Female	206	70.3	87	29.7	293	65.3
Total	304	67.7	145	32.3	449	100

Table 2. Frequency of anti-HEV IgG by age classes among pigs of the Federal District, Brazil

Age group	Anti-HEV IgG				Total	(%)
	Positive	(%)	Negative	(%)		
Young	85	69.7	37	30.3	122	27.2
Adult	219	67.0	108	33.0	327	72.8
Total	304	67.7	145	32.3	449	100

Table 3. Prevalence of anti-HEV IgG in swine sera by regions of the Federal District, Brazil

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

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 (Kasorndorkbua *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

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.

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