

## Short Report: Occupational Exposure to Hepatitis E Virus (HEV) in Swine Workers

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**Abstract.** The aim of this work was to study the prevalence of hepatitis E virus (HEV) and the risk factors for the acquisition of the virus in a population in contact with swine and unexposed to swine. A total of 198 individuals, 97 unexposed (49%) and 101 exposed (51%) to swine, were tested for the presence of HEV infection. The prevalence of anti-HEV IgG in the exposed group was 18.8% versus 4.1% in the unexposed to swine group. People exposed to swine were observed to be 5.4 times ( $P = 0.03$ ) at risk of having anti-HEV IgG. Ten (52.6%) of the IgG-positive individuals showed two concomitant risk factors: untreated water consumption and exposure to swine. These data support that HEV infection should be treated as a vocational illness in swine workers. Therefore, systematic application of hygiene measures in this collective is highly recommended to avoid the exposition to this virus.

Hepatitis E virus (HEV) is the main causative agent of enterically transmitted non-A non-B hepatitis and self-limiting clinical presentation in humans.<sup>1</sup> It is a non-enveloped virus with a positive-sense, single-stranded RNA genome of ~7,200 nucleotides in length and contains three open reading frames (ORFs). Nowadays, HEV is classified into the family *Hepeviridae*, genus *Hepevirus*. Regarding the phylogeny, HEV has been divided into four genotypes,<sup>2</sup> although only one serotype of HEV is recognized.<sup>3</sup> Transmission of HEV infection primarily occurs through contaminated water, although person to person transmission and sexual transmission occur infrequently.

Hepatitis E has been considered an infectious endemic in developing areas such as India, Africa, and Southeast Asia, because of poor sanitary conditions in drinking water.<sup>4</sup> The mortality rate of hepatitis E in the normal population is generally < 1%, but it can be as high as 20–25% among pregnant women.

In industrialized countries, HEV has been found mainly in individuals who had traveled to endemic zones. Actually, the increasing number of autochthonous cases of hepatitis E<sup>5</sup> and the recent findings of HEV in domestic animals such as swine give rise to the suspicion that HEV is underdetected in idiopathic non-A non-B hepatitis. Therefore, the transmission pathways from animals to humans remain obscure. However, in developed countries, seroprevalence ranges varying from 1–18% have been reported. In the last years, several studies have been published describing differences in the prevalence of anti-HEV antibodies between people exposed and not exposed to swine,<sup>6–12</sup> but the risk factors for the acquisition of the virus have not been studied.

Accordingly, the aim of this work was to study the prevalence of HEV and the risk factors for the acquisition of the virus in healthy Spanish people distributed in exposed and unexposed to swine groups.

A retrospective study was carried out to determine the prevalence of HEV during the period from October 2004 to July 2007 in Spain.

A total number of 198 healthy individuals, 101 (51%) men

and 97 (49%) women, were included in this study to detect the prevalence of HEV. Participants filled out an epidemiologic questionnaire including name, age, area of residence, travel abroad, exposure to swine, and consumption of raw vegetables, raw shellfish, and untreated water. Informed approval was obtained from all participants. Individuals were divided into two separate groups taking into consideration exposition to swine: 97 unexposed (NE; 27 men and 70 women) and 101 exposed (E; 74 men and 27 women). Individuals included in the E group were made up of swine farmers, pig handlers, and swine veterinarians, whereas the NE group was made up of volunteers with no contact with swine.

Blood samples were obtained from all the participants by venipuncture, and sera were obtained and frozen at  $-20^{\circ}\text{C}$  until used. RNA was extracted from 140  $\mu\text{L}$  of each serum using a commercial kit following the manufacturer's instructions (QIampViral RNA Kit; Qiagen, Valencia, CA). Two pairs of degenerate oligonucleotide primers<sup>13</sup> were used to amplify a 348-bp fragment of ORF-2 of HEV using a reverse transcriptase-nested polymerase chain reaction (PCR).<sup>14</sup> These primers were based on 18 human HEV sequences and the swine HEV prototype strain from the United States. A positive control from a naturally infected pig (GenBank accession number AY323506) was included in each procedure. Different stages of assay were performed in different places to avoid the possibility of cross-contamination. The PCR products were separated by electrophoresis in 2% agarose and were detected by staining with ethidium bromide.

Sera from all individuals were tested for the presence of HEV antibodies (anti-HEV IgG and IgM) using a commercial ELISA (Fortress Diagnostics, Antrim, UK) according to the manufacturer's instructions. This kit used polystyrene microwell strips precoated with recombinant HEV antigens (HEV-Ag) corresponding to structural proteins ORF2, derived from genotype 1. The sensitivity and specificity of the ELISA assay use in this study were determined by the manufacturer as 92% and 88%, respectively. Positive results obtained using this assay were confirmed by means of an HEV immunoblot test (Recomblot HEV IgG/IgM; Mikrogen, Martinsried, Germany). Antigens used in this kit were the N-terminal part of the capsid antigen (GST fusion protein O2N, 50 kd), the C-terminal part of the capsid antigen (triple band; O2C 38–41 kd), the middle part of the capsid antigen (O2M; 28 kd), and the ORF3 protein (O3; 15 kd) of genotypes 1 and 2.

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Liver function tests, including transaminase levels (aspartate aminotransferase [ALT] and alanine aminotransferase [AST]) in serum were determined using a Thermo Spectronic spectrophotometer (Helios, Barcelona, Spain).

To determine the correlation between the data obtained from the questionnaire and the laboratory results, odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) were calculated using binary logistic regression analysis by means of SPSS version 15.0 statistical software. For the statistical comparison of the seroprevalence obtained in the E and NE groups, the Pearson  $\chi^2$  test and Student *t* test were applied.

All individuals tested negative for the presence of HEV RNA in serum. The overall prevalence of anti-HEV IgG confirmed by immunoblotting was 11.6% (23/198). The seroprevalence of anti-HEV IgG in the E group and in NE group was 18.8% (19/101) and 4.1% (4/97), respectively (Table 1). Values of transaminase enzymes were located within the normal range (ALT: men < 45 IU/L, women < 36 IU/L; AST: < 34 IU/L for men and women) in all individuals. No significant differences in the levels of transaminases were observed between the anti-HEV IgG-positive group (ALT:  $22 \pm 14$ ; AST:  $12 \pm 7.5$ ) and the anti-HEV IgG-negative group (ALT:  $15 \pm 12.2$ ; AST:  $11 \pm 6.8$ ). The statistical analysis showed a significant association ( $P < 0.05$ ) between the presence of anti-HEV IgG and the consumption of untreated water with an OR value of 5.6 ( $P = 0.01$ ). Additionally, people exposed to swine were observed to be 5.4 times ( $P = 0.03$ ) at risk of having anti-HEV IgG antibodies. Ten (52.6%) of the IgG-positive individuals showed two concomitant risk factors: untreated water consumption and exposure to swine. The  $\chi^2$  goodness-of-fit test showed a good fit with the observed and expected frequencies in the E and NE groups ( $\chi^2 = 10.4$ ,  $P = 0.01$ ) and consumption of untreated water ( $\chi^2 = 12.9$ ,  $P = 0.01$ ). No significant differences were observed between the rest of the study parameters.

This is the first study in Spain reporting the prevalence of IgG anti-HEV antibodies in swine workers (18.8%) and in

people unexposed to swine (4.1%). The increased risk (5.4 times at risk) of having IgG anti-HEV observed in swine workers in this work is not surprising, taking into account the high number of farms (76%) and pigs (23%) testing positive for HEV RNA in the same area.<sup>14</sup> This datum is higher than the OR (1.46) reported by Meng and others<sup>6</sup> in 2002 in the only study that calculated the risk for a veterinarian to be positive for IgG anti-HEV. The fact that the values of transaminases were similar between positive and negative individuals suggests that HEV might be responsible for subclinical infections, because none of the participants reported any past clinical signs of acute hepatitis. The factors triggering the development of an acute or a subclinical hepatitis E infection remain obscure in industrialized countries. Some authors point to several contributing factors such as age,<sup>15</sup> pre-existing hepatopathy,<sup>16</sup> and the genotype of the strain.<sup>17</sup>

It has been reported for autochthonous hepatitis E in developed regions that swine isolates from genotype 3 are more related to human strains from the same geographic region than to swine strains from different areas. Moreover, HEV strains circulating in Spanish swine farms are highly homologous with Spanish human strains, which raises the possibility of HEV transmission from swine to humans.<sup>18</sup> HEV has been suggested to be a zoonotic infection where pigs play an important role in the spreading of the disease. HEV is capable of crossing the species barrier, as has been shown by means of experimental infections in pigs with a human HEV strain and in non-human primates with a swine HEV strain.<sup>19</sup>

The results obtained in this study support the link between the presence of anti-HEV antibodies and direct contact with swine, as reported by several authors. Thus, in the United States,<sup>6,7</sup> significant prevalences between veterinarians working with swine (26% and 10.9%, respectively) and unexposed people (18% and 2.4%, respectively) were reported. Similar results were described in The Netherlands, Moldova, and Taiwan,<sup>8–10</sup> with values for those exposed to swine of 11%, 51%, and 27% versus 2%, 24.7%, and 2.4%, respectively. In contrast, studies in Sweden<sup>11</sup> found no significant differences be-

TABLE 1  
Characteristics and risk factors of the studied population according to the presence or absence of anti-HEV IgG

	Anti-HEV IgG positive	Anti-HEV IgG negative	<i>P</i>	OR	95% CI
Sex					
Male	21 (20.8%)	80 (79.2%)	0.01	0.08	0–0.3
Female	2 (2%)	95 (97.9%)			
Age (years)	$38.2 \pm 10.4$	$26 \pm 9.0$			
ALT	$22 \pm 14$	$15 \pm 12.2$			
AST	$12 \pm 7.5$	$11 \pm 6.8$			
RNA-HEV	0 (0%)	0 (0%)			
Consume raw vegetables			0.46	1.75	0.3–7.9
No	2 (7.4%)	25 (92.6%)			
Yes	21 (12.3%)	150 (87.7%)			
Consume raw shellfish			0	0	0
No	23 (11.6%)	175 (88.4%)			
Yes	0 (0%)	0 (0%)			
Consume untreated water			0.01	5.6	12.2–14.5
No	13 (7.8%)	154 (92.2%)			
Yes	10 (32.2%)	21 (67.8%)			
Travel abroad			0.29	0.6	0.2–1.6
No	18 (13.2%)	118 (86.8%)			
Yes	5 (8%)	57 (92%)			
Exposure to swine			0.03	5.4	1.7–16.5
No	4 (4.1%)	93 (95.9%)			
Yes	19 (18.8%)	82 (81.2%)			

OR = odds ratio; CI = confidence interval; ALT = alanine aminotransferase; AST = aspartate aminotransferase.

tween those exposed (13%) and unexposed to swine (9.3%), and in Italy,<sup>12</sup> prevalences of 3.3% in swine farmers and 2.9% in people without occupational exposure to swine were reported. The high variation among the prevalences described above might be caused by differences in sample size, country of origin, and the diagnostic assay used. In this context, it has been described that there are significant sensitivity variations in developed countries depending on the type of ELISA kit used, as well as immunoblotting confirmation of the ELISA-positive samples. The data obtained by Herremans and others<sup>20</sup> in 2007 suggest that there are few differences in the sensitivity of ELISAs based in genotype 1 or 3 antigens. Therefore, the number of false negatives in the healthy population is expected to be low. In our study, to minimize the possibility of false positives and yield more accurate prevalence results, positive samples were confirmed by means of an immunoblot assay (Recomblot HEV; Mikrogen).

Regarding other risk factors studied in this work, an elevated prevalence (32.2%) and risk (OR = 5.6) in people who reported consumption of untreated water from water fountains in the countryside was recorded. The relationship between untreated water consumption and exposure to swine in swine workers is not surprising because the farms are located in the countryside where untreated water fountains are numerous. Additionally, it is very common among farmers to fertilize cultivated fields with manure from swine farms, which could infiltrate down through the ground, contaminating subterranean water and reaching to the water fountains. However, this hypothesis needs to be confirmed by further studies detecting HEV in water fountains.

The seroprevalence observed in other industrialized countries such as the United Kingdom,<sup>19</sup> Italy,<sup>21</sup> France,<sup>22</sup> New Zealand,<sup>23</sup> and Brazil,<sup>24</sup> with 6.3%, 2.6%, 3.2%, 4%, and 2.3%, respectively, was lower than the value reported in our study. The overall percentage found in this study (11.6%) is also higher than the one observed by Mateos and others<sup>25</sup> (2.8%) and the rate obtained by Buti and others (7.3%)<sup>26</sup> in a normal Spanish population. These cannot be properly compared with the data obtained in this study because of the high number of exposed people (50%). These high prevalences suggest that autochthonous HEV is circulating in Spain, and the infection is underdiagnosed. Although transfusion-transmitted HEV is probably much too rare to sustain HEV transmission, it should be taken into account that HEV is spread through uncertain routes, and the potential risk of transfusion-transmitted HEV infection should be considered.<sup>27</sup>

In conclusion, this is the first study in Spain reporting a high prevalence of IgG anti-HEV antibodies in swine workers. These data support that HEV infection should be treated as a vocational illness in swine workers. Therefore, systematic application of hygiene measures in this group is highly recommended to avoid the exposition to this virus.

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