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1 **EPIDEMIOLOGICAL STUDY OF HEPATITIS E VIRUS INFECTION IN**
2 **EUROPEAN WILD BOARS (*Sus scrofa*) IN SPAIN**

3

4

Short communication

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23

24 **Running title:** HEV in European wild boar

25

26 The GenBank/EMBL/DDBJ accession numbers of the sequences reported in this paper are
27 from EF429174 to EF429194.

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33 **Summary**

34 Evidence of Hepatitis E virus (HEV) infection in Spanish domestic pig has been reported and
35 hence it was advisable to search for this zoonotic pathogen in wild boar populations. A total
36 of 150 wild boar serum samples from 8 geographic areas from South-Central Spain were used
37 to investigate HEV infection in European wild boar (*Sus scrofa*) in Spain by means of
38 serology and PCR and its distribution by age, region and management system. Anti-HEV IgG,
39 IgM and IgA were determined by an in-house ELISA. The overall seroprevalence was 42.7%
40 (range 30.63%-55.65%) and 19.6% (range 13.53%-27.40%) of the animals tested positive for
41 HEV RNA. Wild boar sequences were clustered within the genotype 3. This is the first
42 description of HEV infection in Spanish wild boar and the results obtained may suggest a
43 possible role of wild boar as a HEV reservoir for both domestic animals and humans.

44
45 Key words: Wild boar, HEV, serology, ELISA, RT-PCR, phylogenetic analyses.

46

47

48 1. Introduction

49 Hepatitis E (HE) is an important public health problem caused by hepatitis E virus
50 (HEV), the only member of the *Hepeviridae* family (Panda et al., 2007). HEV is a small, non-
51 enveloped virus, which contains a single strand positive RNA genome of approximately 7.2
52 Kb (Emerson and Purcell, 2003). Humans are natural hosts of HEV and the associated disease
53 is characterized by an acute hepatitis with jaundice, anorexia, hepatomegaly, abdominal pain,
54 nausea, vomiting and fever (Panda et al., 2007). The disease is endemic in many tropical and
55 subtropical regions and sporadic in developed countries (Emerson and Purcell, 2003).

56 First HEV strain of animal origin was described in swine in 1997 and it was
57 genetically closely related to human HEV (Meng et al., 1997). Since then, serologic evidence
58 of contact with HEV has been described in sheep, goat, cow, dog, chicken, rodents,
59 mongoose, deer and wild boar (Panda et al., 2007; Takahashi et al., 2004). Interspecies
60 transmission of HEV has been experimentally demonstrated by infecting pigs with the human
61 HEV strain US-1 (Halbur et al., 2001) and non-human primates with swine HEV (Meng et al.,
62 1998). Evidence of potential zoonotic transmission was further supported by the human
63 outbreaks of HEV infection in Japan following ingestion of uncooked or undercooked deer
64 and wild boar meat (Matsuda et al., 2003; Tamada et al., 2004). Moreover, identified human,
65 deer and wild boar HEV strains in the same area have been demonstrated to be genetically
66 closely related (Takahashi et al., 2004). These evidences support the hypothesis that wild and
67 domestic animals could act as HEV reservoirs for humans.

68 In Spain, HEV has been detected in humans, slaughterhouse sewage and different aged
69 and farm bred domestic pigs (de Deus et al., 2007; Pina et al., 2000). The relatively high HEV
70 seroprevalence in domestic pigs in Spain suggest that HEV infection is probably widespread
71 (Seminati et al., 2007) and that the virus could also be present in wild boar populations, as
72 commonly found for other pathogens (Ruiz-Fons et al., 2006). The expanding scenario of

73 both wild boar distribution and density in Spain (Acevedo et al., 2006; Gortazar et al., 2000)
74 stresses the need of a better understanding of the epidemiology of pathogens shared or in
75 common between wild boars and other domestic animals and/or humans, including swine
76 HEV (Ewald, 2004). Therefore, our main goals were to search for HEV in wild boar
77 populations in Spain by means of serology and PCR and to describe basic epidemiological
78 features of HEV in this wild specie. Also, we aimed to compare HEV strains from Spanish
79 wild boars with other available strains.

80

81 **2. Material and methods**

82 2.1. Wild boar samples

83 Sera from 150 wild boars were collected between 2000 and 2005, from animals
84 located in eight geographic areas in south-central Spain (Table 1) during the hunting period.
85 Sex (n=71 males, n=74 females; sex unknown for 5 animals) and age were recorded. Wild
86 boar age was estimated by means of tooth eruption patterns according to Saenz de Buruaga et
87 al. (1991). Thus, animals between 7 and 12 months were classified as juveniles (n=38),
88 between 12 and 24 months as sub-adults (n=53) and over 24 months as adults (n= 59).
89 Management conditions of wild boar in the sampled areas was estimated by means of
90 interviews to gamekeepers (Vicente et al., 2004). According to data obtained through
91 interviews, estates were classified as open (no fencing and no management), fenced (fencing
92 and artificial feeding) and intensive (livestock-like management).

93

94 2.2. Serological analyses

95 Serum samples were tested for specific anti-HEV IgG, IgA and IgM antibodies by
96 means of an in-house ELISA as described elsewhere (Seminati et al., 2007). A purified 55 kD
97 truncated ORF2 protein of human Sar-55 HEV strain (Genotype 1) was used as antigen. The

98 capsid antigen of this protein reacts both with human and swine antibodies (Engle et al., 2002;
99 Meng et al., 1997). Plates were coated with the antigen diluted in 50 mM pH 9.6 carbonate-
100 bicarbonate buffer (0.25 µg/ml) for 18 h at room temperature. After coating, plates were
101 washed with PBS, blocked for 1h at 37°C with 0.5% gelatine in PBS and sera were added at a
102 1:100 dilution in Tris/BSA (100 µl). Samples were tested in duplicate in coated and uncoated
103 wells and two positive controls, two negative controls and a blank were included in each
104 plate. Plates were incubated 1h at 37°C and then washed four times before addition of HPR-
105 conjugate goat (100 µl/well) anti-swine IgG, IgM or IgA as secondary antibody (Serotec Ltd,
106 Oxford, UK). After 30 min incubation at 37°C, plates were washed four times with carbonate-
107 bicarbonate buffer. The reaction was revealed by adding 100 µl of TMB (Sigma Chemical, St.
108 Louis, Mo, USA) and stopped with 2M H₂SO₄. The optical density (OD) value of each
109 sample was read at 450 nm. The specific absorbance value of each serum sample was
110 calculated by subtracting the optical density of coated wells from the absorbance values of
111 uncoated wells. Cut-off values were determined using 43 domestic swine serum samples
112 previously described as HEV RT-PCR and serology negative and 34 sera from caesarean-
113 derived, colostrum-deprived (CDCD) animals. Cut-off values of 0.300, 0.380 and 0.320
114 (optical densities mean + 4SD) were considered for IgG, IgM and IgA, respectively. Serum
115 samples with values equal to or greater than cut-off values were considered positive.

116

117 2.3. Reverse transcription-polymerase chain reaction (RT-PCR)

118 For the RT-PCR, 138 samples were available. Viral RNA was extracted from 150 µl of serum
119 with Nucleospin® RNA virus kit (Macherey-Nagel GmbH & Co, Düren, Germany), following
120 manufacturer's instructions. HEV detection was done by means of a semi-nested RT-PCR as
121 previously described (de Deus et al., 2007).

122

123 2.4. Sequencing and phylogenetic analysis

124 All positive sequences to PCR were sequenced to confirm the presence of HEV. Therefore,
125 resulting PCR products were excised from the agarose gel and purified by using the
126 NucleoSpin® Extract II (Macherey-Nagel GmbH & Co, Düren, Germany), following
127 manufacturer's instructions. Both strands of the purified DNA amplicon were sequenced
128 using the ABI PRISM 3700 DNA analyzer (Applied Biosystems, Foster City, USA). HEV
129 sequences obtained in this study were compared with those from genotype 3 either from
130 humans, domestic swine and wild boar available at the GenBank and the European Molecular
131 Biology Library by using the BLAST utility (available from:
132 <http://www.ncbi.nlm.nih.gov/BLAST>). Obtained HEV sequences were also compared with
133 sequences of other HEV genotypes (fig. 1). Alignments were carried out by using ClustalX
134 1.8 program (available from: <ftp://ftp-igbmc.u-stras/pub/clustalX>) and the alignments were
135 analyzed with MEGA version 3.1 (Kumar et al., 2004) to calculate the distances between
136 sequences. A phylogenetic tree was constructed by the neighbour-joining method using a
137 1000-bootstrap procedure, based on the partial nucleotide sequence of the ORF2 region (168
138 bp). Bootstrap values greater than 70% were considered to provide significant evidence for
139 phylogenetic grouping (Baldauf, 2003). The sequences reported in this study have been
140 deposited in the GenBank database under accession numbers EF429174 to EF429194.

141 From the 27 positive sequences, 21 were used for phylogenetic studies and deposited in the
142 GenBank database under accession numbers EF429174 to EF429194.

143

144 2.5. Statistical analyses

145 For statistical evaluation, SAS 9.1 software was used (SAS institute Inc., Cary, North
146 Carolina, USA). We analyzed the association of sex (categorical; male vs. female), age
147 (categorical; juvenile, sub-adult and adult), geographic area and management (categorical;

148 open, fenced and intensive) with serological and PCR results by means of Chi-square tests.
149 Relationship between seropositivity and presence of HEV RNA in the serum was also
150 analyzed by means of a Pearson's chi-square test. An analysis of variance according to the
151 general linear model procedure (SAS) and LSMEANS follow-up test was used to compare
152 mean ELISA OD values of wild boars of different ages. Differences were considered
153 statistically significant when $p < 0.05$.

154

155 **3. Results**

156 3.1. Prevalence of anti-HEV antibodies

157 Overall, 64 animals (42.7%, 95% confidence intervals [CI] =30.63-55.65) were
158 positive for at least one of the tested immunoglobulin (Table 1). Specifically, serum samples
159 from 42 wild boars (28%, 95% CI=15.78-44.21) were positive for anti-HEV IgG, 34 (22.6%,
160 95% CI=10.73-40.58) for IgA and 32 (21.3 %, 95% CI=9.55-39.82) for IgM (Table 1). The
161 presence of IgA in the wild boars was associated to IgM ($\chi^2=7.48$ df=1 $p < 0.05$) and IgG
162 ($\chi^2=51.24$ df=1, $p < 0.001$). No significant differences in HEV seroprevalence were observed
163 between sexes and age classes, but IgG prevalence, which was significantly higher in
164 juveniles (100%, 5/5) under intensive management conditions (exact Fischer test: $p < 0.05$,
165 Table 2).

166 IgG seroprevalence ranged from 0 (95% CI =0.84-32.15%) in Cuenca and Ruidera to
167 64.3% (95% CI =33.44-87.30) in Montes Toledo. Statistical significant differences were
168 found for IgG seroprevalence considering management types ($\chi^2= 7.17$ df = 2 $p < 0.05$), with
169 higher values in intensively managed (40.5%, 95% CI=26.04-56.68) than in open areas
170 (15.7%, 95% CI=7.49-29.15). No statistical significant differences were observed between
171 intensive and fenced neither between fenced and open management types.

172 Anti-HEV IgM seroprevalence by region ranged from 7.7% (95% CI=2.01-21.98) in

173 Sierra Morena to 60% in Guadalajara (95% CI=17.04-92.74). Statistical significant higher
174 IgM seroprevalence values (35.5%, 95% CI=21.82-51.80) were evidenced for wild boars
175 under intensive conditions when compared to those from open (13.7%, 95% CI=6.13-26.84;
176 $\chi^2= 3.92$ df= 1 $p<0.05$) or fenced estates (14%, 95% CI=6.65-26.31; $\chi^2= 6.37$ df= 1 and
177 $p<0.05$). Nevertheless, no significant differences were observed considering open and fenced
178 areas neither between age classes.

179 Anti-HEV IgA seroprevalence by region ranged from 7.7% (95% CI=2.01-21.98) in
180 Sierra Morena to 42.9% (95% CI=27.59-59.58) in Montes Toledo and it did not statistically
181 differ between management types. When anti-HEV IgA seroprevalence was plotted against
182 age a decreasing (although not statistically significant) tendency was observed. IgA
183 prevalence were significantly higher in juveniles (100%, 95% CI=46.29-98.13) kept under
184 intensive management conditions (Fisher exact test, $p<0.001$; Table 2).

185 Statistically significant ($p<0.05$) higher IgG mean OD value was observed in juvenile
186 (1.29 ± 0.22) than in adult (0.89 ± 0.4) wild boars. Mean IgA juvenile OD value (1.11 ± 0.6)
187 was higher than in sub-adult (0.73 ± 0.36) and adult wild boars (0.82 ± 0.53), but the difference
188 was not statistically significant. Finally, mean IgM OD value did not statistically differ
189 between juvenile (0.590 ± 0.187), sub-adult (0.445 ± 0.065) and adult (0.445 ± 0.07) wild
190 boars.

191

192 3.2. HEV RT-PCR

193 Twenty-seven out of 138 samples 19.6% (95% CI=13.53-27.40) were RT-PCR
194 positive. Such viral prevalence ranged from 7.7% (95% CI=0.40-37.92) in Toledo to 40%
195 (95% CI=7.26-82.96) in Guadalajara (Table 1). No statistically significant differences in HEV
196 prevalence were observed among geographic areas.

197 Twenty-one out of 27 positive samples (77.8%, 95% CI=57.29-90.64) were positive to

198 at least one immunoglobulin. Nine HEV infected wild boars were positive to IgG (33.3 %,
199 95% CI=17.21-53.95), 10 to IgA (37%, 95% CI=20.05-57.50) and 15 to IgM (55.6%, 95%
200 CI=35.68-74.00). No differences among antibody prevalences to HEV were found between
201 PCR positive and negative wild boars. However, the proportion of positive IgM animals was
202 higher in RT-PCR positive animals ($\chi^2= 19.74$ df= 1 $p<0.001$). The prevalence of HEV
203 positive animals did not differ statistically by management system, age and sex. However
204 intensively reared wild boars showed higher percentage of HEV infection (22.6%, 95% CI
205 =10.29-41.56) than those from open (19.6%, 95% CI =10.28-33.54) and fenced systems
206 (17.9%, 95% CI=9.37-30.89).

207 Moreover, juveniles were more frequently RT-PCR positive (26.3%, 95% CI =13.96-
208 43.37) in comparison to sub-adult (22.2%, 95% CI =11.70-37.45) and adult wild boars
209 (12.7%, 95% CI =5.68-25.06). When juveniles and sub-adults were grouped together, non-
210 adult wild boar had a higher probability to be positive than adults (OR=2.17, 95% CI =0.85-
211 5.56, $p=0.09$). HEV prevalence was higher in females (16.2%, 95% CI =9.00-26.99) than in
212 males (14.1%, 95% CI =7.33-24.86) although no statistical differences were observed.

213 A significantly higher proportion of RT-PCR positive juvenile wild boars (41.2%,
214 95% CI =19.44-66.57) were present in fenced systems compared to sub-adults (10.5%, 95%
215 CI=1.83-34.51) and adults (5%, 95% CI =0.26-26.94) (Fisher exact test, df=1, $p<0.05$; Table
216 2). Although not significant, a higher proportion of positive sub-adults were found in open
217 and intensive conditions (33 and 27% respectively) compared to fenced estates (10.5%).

218

219 3.3. Sequence and phylogenetic analysis

220 Wild boar HEV analyzed sequences showed high percentage of homology between
221 them (77-100%). Moreover, 76.1-99.4% and 78.5-95.8% identity was found between wild
222 boar HEV strains and domestic swine and human VH2 strains, respectively. On that score,
223 wild boar strains were 76.7-91% identical to other domestic swine strains from genotype 3.

224 Obtained wild boar sequences were also compared with wild boar sequences from Japan and
225 shared 77.9 to 86.9% nucleotide identity. Nucleotide identity among different sequences was
226 not associated with sex, age, geographic region or management condition of the wild boar.

227 In the phylogenetic analysis, wild boar HEV strains from the present study were
228 clustered within genotype 3 (fig. 1). Wild boar sequences were closely related with those of
229 domestic swine strains from Spain. However, some strains (WBSPJ04-35, WBSPJ04-57,
230 WBSPJ04-60, WBSPJ04-66, WBSPJ04-67 and WBSPJ04-344) were clustered apart.

231

232 **4. Discussion**

233 First evidences of wild ungulates as HEV reservoirs for humans were reported as a
234 consequence of HE cases in humans in Japan after consumption of uncooked or undercooked
235 deer and wild boar meat (Li et al., 2005; Tei et al., 2003). Therefore, the relevance of the wild
236 boar as a potential HEV reservoir for both domestic animals and humans is of concern.
237 Moreover, serological analyses evidenced high seroprevalence rates in domestic pig in Spain
238 (Seminati et al., 2007). Hence, the possibility of wild boar being in contact with HEV was
239 supposed to be high, as reported for other shared diseases between domestic and wild suids
240 (Ruiz-Fons et al., 2007). Studies on HEV in wild boars are nowadays limited to Japan,
241 Australia and Italy (Chandler et al., 1999; Martelli et al., 2007; Sonoda et al., 2004; Takahashi
242 et al., 2004).

243 More than 40% of the studied wild boars had anti-HEV IgG, IgA or IgM antibodies
244 and around 20% of them were PCR positive. Nevertheless, the mean HEV seroprevalence
245 found was higher when compared to that reported for feral pigs in Australia (25.0%) and for
246 Japanese wild boars (9.0-27.1%) (Chandler et al., 1999; Michitaka et al., 2007; Sonoda et al.,
247 2004). On the other hand, Martelli et al. (2007) detected similar percentage of infected
248 animals (25%) by analyzing bile, which is considered a more sensitive sample when studying

249 swine HEV infection (de Deus et al., 2007). Wild boar under intensive management showed
250 higher IgG and IgM seroprevalences, while juveniles from fenced estates were more
251 frequently viraemic. Taking into account that HEV in humans and domestic swine is
252 transmitted by fecal-oral route (Kasorndorkbua et al., 2004; Panda et al., 2007), it is
253 expectable the same transmission route in the wild boar. Wild boar densities are higher in
254 managed than in open estates (Acevedo et al., 2007) and, therefore, the risk of HEV
255 transmission between animals is presumably increased under these conditions as shown for
256 other diseases (Ruiz-Fons et al., 2006; Vicente et al., 2004). Our results are in accordance
257 with Michitaka et al., (2007) who reported a positive rate in bread boar, significantly higher
258 than in the wild-caught boar.

259 HEV was detected in animals from all age classes and both seroprevalence and viral
260 infection prevalence did not significantly differ between age classes. Nevertheless, non-adult
261 (juveniles plus sub-adults) wild boars were more prone to get infected than adults. These
262 results roughly describe HEV infection dynamics in the wild boar. In general, serologic OD
263 mean values from wild boars decreased with age with the exception of IgA that decreased
264 from juveniles to sub-adults and then increased in adults. The presence of HEV RNA in
265 serum was more consistently related to the concomitant presence of IgM (15/27) than to the
266 presence of IgG (9/27) or IgA (10/27). Although IgA has been consistently associated to HEV
267 viraemia (Takahashi et al., 2005; Tian et al., 2006), our results did not show IgA to be as a
268 useful tool for recent HEV infection assessment in wild boars.

269 The studied wild boar HEV sequences showed high similarities. Sequences clustered
270 within the genotype 3 together with other Spanish HEV sequences, although a group of them
271 formed an isolated cluster. This may be indicative of the existence of heterogeneity among
272 HEV strains in wild boars, as it has been suggested for domestic swine (Takahashi et al.,
273 2003). An interesting finding was the high similarity found between wild boar and domestic

274 pig HEV strains (76.1-99.4%), which could relate to HEV transmission between both suids.
275 Nevertheless, the full genomic sequence of both wild boar and domestic pig HEV strains
276 should be carried out to test this hypothesis. Some evidences of interspecies HEV
277 transmission have accumulated over the last 10 years. Sonoda et al. (2004) reported that
278 Japanese wild boar HEV sequences were closely related to those from humans and domestic
279 pigs, showing 82.9 to 93.9% identity. Later studies in the same country showed 99.7%
280 similarity between wild boar and deer entire HEV genomes, thus suggesting HEV interspecies
281 transmission (Takahashi et al., 2004). It is also known that HEV from domestic pig origin is
282 able to infect other species such as the chimpanzee (*Pan troglodytes*) and the rhesus monkey
283 (*Macaca mulatta*) (Meng et al., 1998), further supporting the potential interspecies
284 transmission of the virus. HEV sequences in this study did not cluster according to region, age
285 or management conditions, which could relate to the common practice of wild boar
286 translocations for hunting purposes in the studied populations.

287

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292

293 6. References

- 294 Acevedo, P., Escudero, M.A., Muñoz, R., Gortázar, C., 2006, Factors affecting wild boar
295 abundance across an environmental gradient in Spain. *Acta Theriol* 51, 327-336.
- 296 Acevedo, P., Vicente, J., Hofle, U., Cassinello, J., Ruiz-Fons, F., Gortazar, C., 2007,
297 Estimation of European wild boar relative abundance and aggregation: a novel method
298 in epidemiological risk assessment. *Epidemiol Infect* 135, 519-527.

- 299 Baldauf, S.L., 2003, Phylogeny for the faint of heart: a tutorial. *Trends Genet* 19, 345-351.
- 300 Chandler, J.D., Riddell, M.A., Li, F., Love, R.J., Anderson, D.A., 1999, Serological evidence
301 for swine hepatitis E virus infection in Australian pig herds. *Vet Microbiol* 68, 95-105.
- 302 de Deus, N., Seminati, C., Pina, S., Mateu, E., Martin, M., Segales, J., 2007, Detection of
303 hepatitis E virus in liver, mesenteric lymph node, serum, bile and faeces of naturally
304 infected pigs affected by different pathological conditions. *Vet Microbiol* 119, 105-
305 114.
- 306 Emerson, S.U., Purcell, R.H., 2003, Hepatitis E virus. *Rev Med Virol* 13, 145-154.
- 307 Engle, R.E., Yu, C., Emerson, S.U., Meng, X.J., Purcell, R.H., 2002, Hepatitis E virus (HEV)
308 capsid antigens derived from viruses of human and swine origin are equally efficient
309 for detecting anti-HEV by enzyme immunoassay. *J Clin Microbiol* 40, 4576-4580.
- 310 Ewald, P.W., 2004, Evolution of virulence. *Infect Dis Clin North Am* 18, 1-15.
- 311 Gortazar, C., Herrero, J., Villafuerte, R., Marco, J., 2000, Historical examination of the status
312 of large mammals in Aragon, Spain. *Mammalia* 64, 411-422.
- 313 Halbur, P.G., Kasorndorkbua, C., Gilbert, C., Guenette, D., Potters, M.B., Purcell, R.H.,
314 Emerson, S.U., Toth, T.E., Meng, X.J., 2001, Comparative pathogenesis of infection
315 of pigs with hepatitis E viruses recovered from a pig and a human. *J Clin Microbiol*
316 39, 918-923.
- 317 Kasorndorkbua, C., Guenette, D.K., Huang, F.F., Thomas, P.J., Meng, X.J., Halbur, P.G.,
318 2004, Routes of transmission of swine hepatitis E virus in pigs. *J Clin Microbiol* 42,
319 5047-5052.
- 320 Kumar, S., Tamura, K., Nei, M., 2004, MEGA3: Integrated software for Molecular
321 Evolutionary Genetics Analysis and sequence alignment. *Brief Bioinform* 5, 150-163.
- 322 Li, T.C., Chijiwa, K., Sera, N., Ishibashi, T., Etoh, Y., Shinohara, Y., Kurata, Y., Ishida, M.,
323 Sakamoto, S., Takeda, N., Miyamura, T., 2005, Hepatitis E virus transmission from

- 324 wild boar meat. *Emerg Infect Dis* 11, 1958-1960.
- 325 Martelli, F., Caprioli, A., Zengarini, M., Marata, A., Fiegna, C., Di Bartolo, I., Ruggeri, F.M.,
326 Delogu, M., Ostanello, F., 2007, Detection of Hepatitis E virus (HEV) in a
327 demographic managed wild boar (*Sus scrofa scrofa*) population in Italy. *Vet*
328 *Microbiol.* doi:10.1016/j.vetmic.2007.07.004.
- 329 Matsuda, H., Okada, K., Takahashi, K., Mishiro, S., 2003, Severe hepatitis E virus infection
330 after ingestion of uncooked liver from a wild boar. *J Infect Dis* 188, 944.
- 331 Meng, X.J., Halbur, P.G., Shapiro, M.S., Govindarajan, S., Bruna, J.D., Mushahwar, I.K.,
332 Purcell, R.H., Emerson, S.U., 1998, Genetic and experimental evidence for cross-
333 species infection by swine hepatitis E virus. *J Virol* 72, 9714-9721.
- 334 Meng, X.J., Purcell, R.H., Halbur, P.G., Lehman, J.R., Webb, D.M., Tsareva, T.S., Haynes,
335 J.S., Thacker, B.J., Emerson, S.U., 1997, A novel virus in swine is closely related to
336 the human hepatitis E virus. *Proc Natl Acad Sci U S A* 94, 9860-9865.
- 337 Michitaka, K., Takahashi, K., Furukawa, S., Inoue, G., Hiasa, Y., Horiike, N., Onji, M., Abe,
338 N., Mishiro, S., 2007, Prevalence of hepatitis E virus among wild boar in the Ehime
339 area of western Japan. *Hepato Res* 37, 214-220.
- 340 Panda, S.K., Thakral, D., Rehman, S., 2007, Hepatitis E virus. *Rev Med Virol* 17, 151-180.
- 341 Pina, S., Buti, M., Cotrina, M., Piella, J., Girones, R., 2000, HEV identified in serum from
342 humans with acute hepatitis and in sewage of animal origin in Spain. *J Hepatol* 33,
343 826-833.
- 344 Ruiz-Fons, F., Segalés, J., Gortázar, C., 2007, A review of viral diseases of the European wild
345 boar: Effects of population dynamics and reservoir rôle. *Vet J* in press.
- 346 Ruiz-Fons, F., Vicente, J., Vidal, D., Hofle, U., Villanua, D., Gauss, C., Segales, J., Almeria,
347 S., Montoro, V., Gortazar, C., 2006, Seroprevalence of six reproductive pathogens in
348 European wild boar (*Sus scrofa*) from Spain: the effect on wild boar female

- 349 reproductive performance. *Theriogenology* 65, 731-743.
- 350 Saenz de Buruaga, M., Lucio, A.J., Purroy, J., 1991, Reconocimiento de sexo y edad en
351 especies cinegéticas. Vitoria, Diputación Foral de Alava.
- 352 Seminati, C., Mateu, E., Peralta, B., de Deus, N., Martin, M., 2007, Distribution of hepatitis E
353 virus infection and its prevalence in pigs on commercial farms in Spain. *Vet J.*
354 doi:10.1016/j.tvjl.2006.11.018.
- 355 Sonoda, H., Abe, M., Sugimoto, T., Sato, Y., Bando, M., Fukui, E., Mizuo, H., Takahashi,
356 M., Nishizawa, T., Okamoto, H., 2004, Prevalence of hepatitis E virus (HEV)
357 Infection in wild boars and deer and genetic identification of a genotype 3 HEV from
358 a boar in Japan. *J Clin Microbiol* 42, 5371-5374.
- 359 Takahashi, K., Kitajima, N., Abe, N., Mishiro, S., 2004, Complete or near-complete
360 nucleotide sequences of hepatitis E virus genome recovered from a wild boar, a deer,
361 and four patients who ate the deer. *Virology* 330, 501-505.
- 362 Takahashi, M., Kusakai, S., Mizuo, H., Suzuki, K., Fujimura, K., Masuko, K., Sugai, Y.,
363 Aikawa, T., Nishizawa, T., Okamoto, H., 2005, Simultaneous detection of
364 immunoglobulin A (IgA) and IgM antibodies against hepatitis E virus (HEV) Is highly
365 specific for diagnosis of acute HEV infection. *J Clin Microbiol* 43, 49-56.
- 366 Takahashi, M., Nishizawa, T., Miyajima, H., Gotanda, Y., Iita, T., Tsuda, F., Okamoto, H.,
367 2003, Swine hepatitis E virus strains in Japan form four phylogenetic clusters
368 comparable with those of Japanese isolates of human hepatitis E virus. *J Gen Virol* 84,
369 851-862.
- 370 Tamada, Y., Yano, K., Yatsuhashi, H., Inoue, O., Mawatari, F., Ishibashi, H., 2004,
371 Consumption of wild boar linked to cases of hepatitis E. *J Hepatol* 40, 869-870.
- 372 Tei, S., Kitajima, N., Takahashi, K., Mishiro, S., 2003, Zoonotic transmission of hepatitis E
373 virus from deer to human beings. *Lancet* 362, 371-373.

- 374 Tian, D.Y., Chen, Y., Xia, N.S., 2006, Significance of serum IgA in patients with acute
375 hepatitis E virus infection. *World J Gastroenterol* 12, 3919-3923.
- 376 Vicente, J., Segales, J., Hofle, U., Balasch, M., Plana-Duran, J., Domingo, M., Gortazar, C.,
377 2004, Epidemiological study on porcine circovirus type 2 (PCV2) infection in the
378 European wild boar (*Sus scrofa*). *Vet Res* 35, 243-253.
- 379
380

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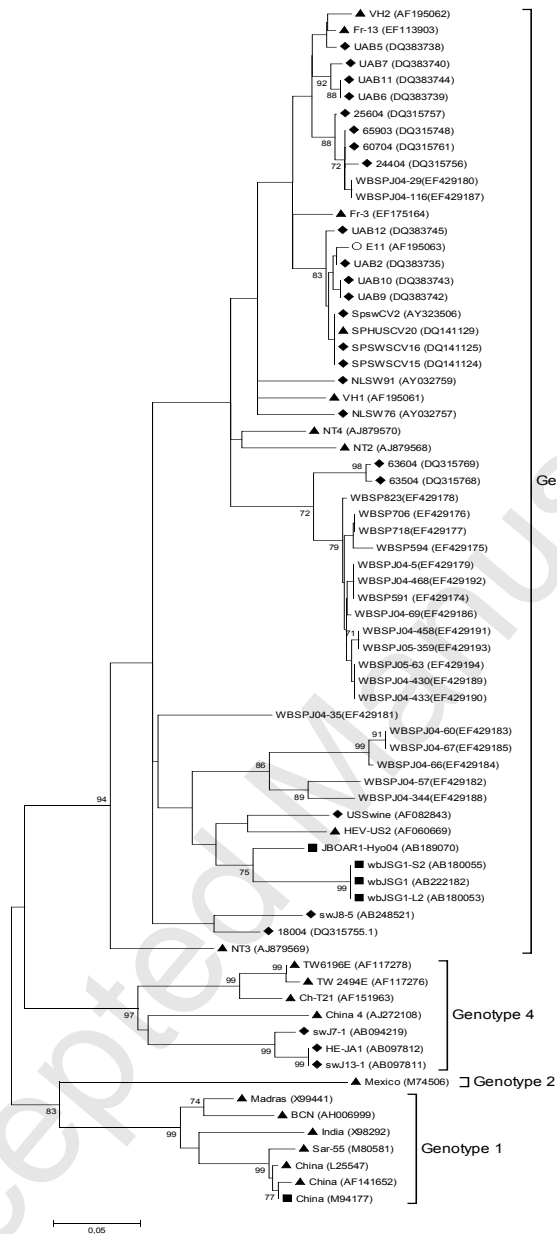
1 **Table 1.** IgG, IgM and IgA serology to HEV and RT-PCR results in different Spanish
 2 regions (percentage in parentheses).

Geographic area	IgG	IgM	IgA	IgG, IgM or IgA	RT-PCR
Albacete	1/11 (9.1) *	1/11(9.1)	1/11(9.1)	2/11 (18.2)	1/11(9.1)
Cuenca	0/11 (0)	2/11 (18.2)	3/11 (27.3)	5/11 (45.5)	3/11 (27.3)
Guadalajara	1/5 (20)	3/5 (60)	1/5 (20)	4/5 (80)	2/5 (40)
Guadiana	12/31 (38.7)	6/31 (19.3)	9/31 (29)	15/31 (48.4)	6/27 (22)
Montes de Toledo	18/28 (64.3)	13/28 (34.2)	12/28 (42.9)	21/28 (75)	8/28 (28.6)
Ruidera	0/12 (0)	2/12 (16.6)	1/12 (8.3)	2/12 (16.7)	1/5 (20)
Sierra Morena	7/39 (17.9)	3/39 (7.7)	3/39 (7.7)	11/39 (28.2)	5/38 (13.2)
Toledo	3/13(23.1)	2/13 (15.4)	4/13 (30.8)	4/13 (30.8)	1/13(7.7)
Total	42/150 (28)	32/150 (21.3)	34/150 (22.6)	64/150 (42.7)	27/138 (19.6)

3 * Number of positive cases/number of cases tested in each region.

Table 2. IgG, IgM and IgA serology to HEV and RT-PCR results stratified by age [juveniles (J), sub-adults (SA) and adults (A)] and management systems.

	Open			Fenced			Intensive			Total age			Total management			Total
	Juvenile	Sub-adults	Adults	Juvenile	Sub-adults	Adults	Juvenile	Sub-adults	Adults	Juvenile	Sub-adults	Adults	Open	Fenced	Intensive	
IgG (%)	2/16 (12.5)	3/15 (20)	3/20 (15)	3/17 (17.6)	6/20 (30)	8/20 (40)	5/5 (100)	5/18 (27.8)	7/19 (36.8)	10/38 (26.3)	14/53 (26.4)	18/59 (30.5)	8/51 (15.7)	17/57 (29.8)	17/42 (40.5)	42/150 (28)
IgM (%)	3/16 (18.7)	2/15 (13.3)	4/20 (20)	4/17 (23.5)	1/20 (5)	3/20 (15)	1/5 (20)	6/18 (33.3)	8/19 (42.1)	8/38 (21.1)	9/53 (16.9)	15/59 (25.4)	9/51 (17.6)	8/57 (14)	15/42 (35.7)	32/150 (21.3)
IgA (%)	5/16 (31.2)	4/15 (26.7)	4/20 (20)	2/17 (11.8)	5/20 (25)	2/20 (10)	5/5 (100)	3/18 (16.7)	4/19 (21.1)	12/38 (31.6)	12/53 (22.6)	10/59 (16.9)	13/51 (25.5)	9/57 (15.8)	12/42 (28.6)	34/150 (22.6)
Total Prevalence	5/16 (31.2)	7/15 (46.7)	8/20 (40)	6/17 (35.3)	7/20 (35)	9/20 (45)	5/5 (100)	8/18 (44.4)	9/19 (47.4)	16/38 (42.1)	22/53 (41.5)	26/59 (44.1)	20/51 (39.2)	22/57 (38.6)	22/42 (52.4)	64/150 (42.7)
PCR positive	2/16 (12.5)	5/15 (33.3)	3/20 (15)	7/17 (41.2)	2/19 (10.5)	1/20 (5)	1/5 (20)	3/11 (27.3)	3/15 (20)	10/38 (26.3)	10/45 (22.2)	7/55 (12.7)	10/51 (19.6)	10/56 (17.9)	7/31 (22.6)	27/138 (19.6)



1

2 **Fig. 3.** Phylogenetic tree constructed by neighbour-joining method based on the
 3 nucleotide sequences (as bootstrap search option) with 1000 replications (mega 3.1
 4 program). Bootstrap values higher than 70% are indicated for the major nodes as a
 5 percentage of the data obtained. Human (▲), wild boar (■), swine (◆) and
 6 slaughterhouse (○) HEV strains from previous studies were included in the phylogenetic
 7 tree. Wild boar strains from present study are indicated as W BSP followed by the
 8 reference of the strain.