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1 **Widespread Diffusion of Genotype 3 Hepatitis E Virus among Farming Swine in**
2 **Northern Italy**

3

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25 **Abstract**

26 Hepatitis E virus (HEV) causes acute hepatitis in humans, and infects several animal species,
27 mostly asymptotically. Swine and human HEV strains are genetically related suggesting
28 both a zoonotic and a possible foodborne transmission. The prevalence of swine HEV was
29 investigated in 274 randomly selected pigs from 6 different swine farms of Northern Italy,
30 testing viral RNA in stools by nested reverse-transcription-polymerase chain reaction. HEV
31 genome was detected in 115 stools (42%). All farms resulted positive for HEV, with a
32 prevalence ranging between 12.8% and 72.5%. HEV positive pigs were detected in all age
33 groups and production stages tested, although infection was more prevalent in weaners than in
34 the older fatteners (42.2% vs. 27.0%). Genetic characterization of swine strains identified was
35 performed by sequencing and database alignment. Phylogenetic analysis on the nucleotide
36 sequences from 16 positive PCR products indicated that all strains belonged to genotype 3. In
37 particular, one group of seven Italian strains clustered close (91.6% to 96.2% identity) to
38 human and swine European HEV strains.

39
40 **Key words:** Hepatitis E (HEV); single-stranded RNA virus; prevalence; infection; swine;
41 genotype 3; zoonosis.

42

43 1. Introduction

44 Hepatitis E virus (HEV; genus *Hepevirus*, family *Hepeviridae*) is a small non-enveloped
45 virus with a single-stranded positive sense RNA genome. The virus genome is approximately
46 7.2 kb, and contains three open reading frames (ORF). ORF1 encodes non-structural proteins
47 including an RNA-dependent RNA polymerase, ORF2 the capsid protein, and ORF3 a small
48 protein with unclear function (Li et al., 2006; Lu et al., 2006).

49 HEV causes human hepatitis E (Huang et al., 2002), generally associated to a low (<1%)
50 mortality rate, except for pregnant women (20-25% mortality) (Emerson and Purcell, 2003).
51 The disease is endemic in many developing countries of Asia and Africa, and in Mexico,
52 where it usually occurs as epidemics (Okamoto, 2007). The natural transmission of HEV is
53 fecal-oral, and contaminated drinking water following heavy rains or flooding is a major
54 source of infection (Emerson and Purcell, 2003; Okamoto, 2007). Sporadic cases of disease
55 have also been reported in US, Japan and Europe (Meng et al., 1998b; Zanetti et al., 1999;
56 Clemente-Casares et al., 2003; Li et al., 2006; Reuter et al., 2006; Renou et al., 2007). Many
57 of these are associated with traveling through endemic countries (Clemente-Casares et al.,
58 2003; Renou et al., 2007), although an increasing number of cases have been reported among
59 patients without history of traveling abroad (Emerson and Purcell, 2003). In industrialized
60 countries, anti-HEV antibodies have also been detected in a significant proportion of healthy
61 individuals (Mast et al., 1997; Meng et al., 2002).

62 There is increasing evidence that HEV may represent a zoonotic agent (Meng et al.,
63 1997; Tei et al., 2003; Yazaki et al., 2003; Sonoda et al., 2004; Banks et al. 2007). Different
64 species of domestic and wild mammals have been reported to have anti-HEV antibodies (Lu
65 et al., 2006; Okamoto, 2007), and/or to be infected with viruses closely related to human HEV
66 (Tei et al., 2003; Banks et al., 2004). Conversely, avian hepatitis E viruses are genetically
67 distant from human viruses (Huang et al., 2004). HEV encompasses at least 4 genotypes,
68 which differ with respect to geographic distribution, host range and pattern of infection,

69 although they are similar serologically (Tei et al., 2003; Banks et al., 2004; Lu et al., 2006;
70 Okamoto, 2007).

71 Genotype 1, associated with large waterborne outbreaks, and genotype 2 are prevalent
72 in Asia and Africa, and in Chad, Mexico and Nigeria, respectively, infecting mostly humans
73 (Zheng et al., 2006; Caron et al., 2006). In addition to infecting humans, genotype 3 is also
74 widespread in swine herds in industrialized countries including North America, Europe, and
75 Japan. Genotype 4 is mainly distributed in Asia, and is associated with sporadic cases and
76 foodborne outbreaks (Lu et al., 2006).

77 Swine HEV (Meng et al., 1997) usually infect pigs subclinically, and the swine may
78 be an important reservoir of the virus (Li et al., 2006; Lu et al., 2006). In fact, increasing
79 evidence of autochthonous cases of hepatitis E in industrialized regions suggests a zoonotic
80 transmission of HEV from pigs (Drobeniuc et al., 2001; Lu et al., 2006). HEV strains
81 identified in swine show a close genetic relatedness to human genotypes 3 and 4 strains
82 (Emerson and Purcell, 2003; Clemente-Casares et al., 2003; Banks et al., 2004; Lu et al.,
83 2006). Experimental interspecies transmission of HEV between non-human primates and pigs
84 has been demonstrated (Meng et al., 1998a), and seroepidemiological studies have shown that
85 pig handlers have an increased risk of HEV infection (Meng et al., 2002). Finally, studies in
86 Japan have linked human HEV cases to consumption of raw or undercooked meat from deer,
87 wild boars and pigs (Tei et al., 2003; Yazaki et al., 2003; Masuda et al. 2005). In France a
88 direct zoonotic transmission of genotype 3 HEV from a pet pig to its owner was also
89 described (Renou et al., 2007).

90 In the Mediterranean Area, hepatitis E cases in humans have been reported
91 sporadically, including Italy (Zanetti et al., 1999), and seroprevalence appears to be around
92 5%. To date very little information exists on the prevalence, epidemiology and genetic
93 characteristics of swine HEV in Italy (Caprioli et al., 2007).

94 In this study we evaluated the prevalence of swine HEV in asymptomatic pigs from six farms
 95 of Northern Italy, and compared the genome sequences identified . with HEV sequences of
 96 different origin reported worldwide.

97 **2. Materials and Methods**

98 *2.1 Samples*

99 From January-June 2006, 274 fecal samples were collected from randomly selected pigs of 3–
 100 4 (weaners) or 8–9 months of age (fatteners), gilts (0 parities), young sows (1-2 parities) and
 101 old sows (>2 parities) belonging to 6 different farms of Northern Italy. The farm size ranged
 102 from 500-1,100 sows. All pigs sampled appeared to be clinically healthy. From each farm, at
 103 least 10 animals were sampled for each production stage, except for the 2 farrow-to-weaning
 104 farms where fatteners were not sampled. This scheme allows systematic sampling of different
 105 pig classes within the herds, estimating with a 95% probability, the prevalence of HEV-
 106 positive animals with an expected prevalence of 30% and an accepted error of 25%.
 107 Information on herd typology (farrow-to-finish or farrow-to-weaning) and herd size (number
 108 of sows) was also collected. Feces from rectal swabs were suspended in 10% DEPC water and
 109 stored at -70°C until processing.

110 *2.2 RNA extraction and HEV RT-Nested-PCR*

111 RNA was extracted from 170 µl of fecal suspension into a 40 µl elution volume, using a
 112 QIAamp Viral kit (Qiagen, Hilden, Germany).

113 An HEV-specific RT-nested-PCR was performed using SuperScript One-Step RT-PCR with
 114 Platinum Taq (Invitrogen; Carlsbad, CA, USA), and two degenerate primer sets targeting the

115 ORF2 region. RT-PCR primers were HEVORF2con-s1—5'
 116 GACAGAATTRATTTTCGTCGGCTGG-3' and HEVORF2con-a1—5'
 117 CTTGTTTCRTGYTGGTTRTCATAATC-3'. For the nested-PCR step, HEVORF2con-s2—5'
 118 GTYGTCTCRGCCAATGGCGAGC-3' and HEVORF2con-a2—5'

119 GTTCRTGYTGGTTRTCATAATCCTG-3' primers were used, yielding a final product of
120 145 bp (Erker et al., 1999).

121 Four microliters of RNA were added to 2X Buffer RT-PCR Master Mix (Invitrogen),
122 dNTP mix (0.2mM each nucleotide) and 0.3 μ M of primers HEVORF2con-A1 and
123 HEVORF2con-S1, in a final volume of 15 μ l. RT-PCR was performed by: a reverse
124 transcription cycle at 45°C for 30 min, followed by denaturation at 94°C for 2 min, and 40
125 cycles at 94°C for 45 s, 49°C for 45 s, and 72°C for 1 min, followed by a 7 min elongation
126 step at 72°C .

127 Samples were then subjected to nested PCR using internal primers HEVORF2con-A2
128 and HEVORF2con-S2 (Erker et al., 1999) (template amount corresponding to 10% of the
129 final volume). The PCR mix was set-up with the following reagents (final concentration):
130 1XPCR buffer (Applied Biosystem, ABI, Foster City, CA, USA), 2.5mM MgCl₂, 0.2 μ M of
131 each primers, 2 units of AmpliTaq DNA Polymerase. The PCR was performed as follows: 1
132 cycle at 94°C for 9 min, followed by 35 cycles at 94°C for 45 s, 49°C for 45 s, and 72°C for 1
133 min, and 7 min elongation at 72°C. Negative and positive swine stools were included as
134 control in each assay. Amplified products were stained with ethidium bromide in a 2%
135 agarose gel. To control cross-contamination, each phase of diagnostic routine (from sample
136 preparation throughout nested PCR) was completed in separate rooms, inside biohazard
137 cabinets or PCR hoods, and using dedicated equipment and material.

138 *2.3 Sequencing and phylogenetic analysis*

139 Nested RT-PCR products of the expected size from 16 samples selected randomly
140 from different farms were excised from agarose gel. DNA was purified with a Qiaquick gel
141 extraction kit (Qiagen, Hilden, Germany), and sequenced (ABI Prism 310 DNA sequencer,
142 Applied Biosystems, Foster City, CA, USA) with the BigDye Terminator Cycle Sequencing
143 Ready Reaction Kit version 3.1 (Perkin Elmer, Applied Biosystems, Foster City, CA, USA)
144 using PCR primers.

145 The sequences obtained were aligned in the NCBI GenBank (<http://www.ncbi.nlm.nih.gov/>)
146 and edited using the DNASIS Max software (Hitachisoft). Phylogenetic analyses were carried
147 out with the Bionumerics software packages (Applied Maths, Kortrijk, Belgium), and the
148 dendrogram was obtained with the UPGMA method. Representative human and swine HEV
149 (HuHEV and SwHEV) strains included: genotype 1 (HuHEV, Mya86-Li strain, accession no.
150 DQ079624); genotype 2 (HuHEV, Mexico strain, accession no. M74506); genotype 3
151 (SwHEV and HuHEV) (United States, accession no. AF082843 and AF060669), SwHEV
152 swJ570 (Japan, accession no. AB073912), SwHEV NLSw15 and HuHEV NLSw20 (The
153 Netherland, accession no. AF332620 and DQ200292), HuHEV (Hungary, accession no.
154 AY940427.1), SwHEV (Hungary, accession no. EF530672), SwHEV P354 (United Kingdom,
155 accession no. AF503511), SwHEV NLSW28 (The Netherlands, accession no. AF336292),
156 sewage sample HEV BCN13 (Spain, AF490994), Italian swine HEV strains accession no.:
157 MO/9_3/06/IT, EF681107; MO/36_4/06/IT, EF682083; HEVBO/01, EF681109; HEVPI/01,
158 EF681110; Italian human HEV strain accession no. AF110390; genotype 4 (HuHEV, JKK-
159 SAP strain, accession no. AB074917 and SwHEV, swCH31 strain, accession no. DQ450072);
160 avian HEV (United States, accession no. AY535004) (Huang et al., 2004).

161 *2.4 Statistical analyses*

162 A binary logistic regression was performed to analyze HEV prevalence against the
163 animal production stage and the herd dimension. To evaluate the effect of herd size, farms
164 were arbitrarily divided into two categories based on the median of sows present: large
165 farms(> 1000 sows), and small farms (<1000sows). The proportion of positive animals was
166 evaluated using the χ^2 test, and factors screening through $p < 0.15$ were then evaluated using
167 binary logistic regression. The model was based on the simultaneous entry of all variables,
168 and its efficacy was assessed based on the likelihood-ratio and the Hosmer–Lemeshow
169 statistic. The odds ratio (OR) and 95% confidence intervals (95% CI) were calculated from
170 the final binary logistic model.

171 All statistical analyses were performed using the software SPSS 12.0.0 (SPSS Inc., Chicago,
172 IL, USA).

173 **3. Results**

174 *3.1 HEV Detection in Swine Fecal Samples*

175 HEV RNA was detected in 115 of 274 pigs tested (42%). All six farms resulted positive for
176 HEV, with a prevalence ranging between 12.8% and 72.5% (Table 1). HEV positive pigs
177 were detected and in all age groups and production stages tested (Table 2). In the fattening
178 stage, a higher HEV prevalence was observed between weaners (90 to 120-day-old), with 27
179 positives out of 64 tested (42.2%), while in fatteners (>120-day-old) the prevalence was 27%.
180 Among breeding animals, 43.1% of gilts tested positive. The prevalence decreased slightly in
181 young sows (38.6%), being higher in old sows (53.4%). To investigate risk factors for HEV
182 infection further, a logistic regression analysis was performed (Table 2). HEV prevalence was
183 significantly higher (54.2%) in herds with >1000 sows (OR 4.98, 95% CI 2.73-9.09, $p =$
184 0.000). The odds of virus shedding was also 2.54 times higher in old sows (>2 parities), close
185 to statistical significance (95% CI 0.98-6.55, $p = 0.054$). The Hosmer–Lemeshow statistic
186 showed a good fit for the final model ($\chi^2 = 4.03$, $p = 0.777$).

187 *3.2 Phylogenetic analysis and geographic clustering of swine HEV strains*

188 The 121 bp ORF2 region amplified by nested RT-PCR was sequenced for a total of 16
189 swine HEV strains, selected randomly from each farm. Due to the insufficient amount of
190 DNA, only one readable sequence was obtained from farm No. 6. The sequences obtained (80
191 bp) were compared with sequences from the NCBI database representing human and swine
192 HEV strains circulating worldwide and belonging to the four known genotypes (Figure 1).
193 The Italian swine HEV showed a sequence homology lower than 79% with respect to
194 genotype 1, 2, and 4, and between 87.6% and 96.2% with the genotype 3 reference strains.
195 The human genotype 3 HEV/Hu/It1/99 reported by Zanetti in 1999 (Zanetti et al., 1999)
196 showed only a 79.6% identity with the swine viruses of this study, which were all assigned to

197 genotype 3. Between the Italian swine strains, nucleotide sequence identity ranged from 90.5
198 to 100%, whereas the amino acid sequence was identical in all cases due to synonymous
199 mutations. In particular, one group of seven strains clustered close (91.6% to 96.2% identity)
200 to a human HEV strain reported in The Netherlands in 2005 (DQ200292). Nine other swine
201 strains appeared to be related (93.1%) to both a swine HEV strain from The Netherlands
202 (AF336292) and a Spanish HEV obtained from sewage (AF490994). The latter two HEV
203 strains are classified into the G3 subtype f (Lu et al., 2006).

204 **4. Discussion**

205 This study represents the first extended survey on swine HEV prevalence conducted in
206 Italy, and demonstrates that HEV infection is largely widespread in Italian pig farms. The
207 overall rate of virus shedding observed (42% of 276 fecal samples tested) is markedly higher
208 than we detected (5.9%) previously in farms of the same area (Caprioli et al., 2007). At least
209 partially, this may be due to the individual stools examined in the present study versus fecal
210 pools in our earlier investigation, where viral load is predictably lower. Also, the limited
211 number of samples (35 from 5 farms) analyzed in the earlier study implies large variation
212 weakening comparisons. However, our data are in line with recent reports from Spain
213 (Fernandez-Barredo et al., 2006; Seminati et al., 2007; Fernandez-Barredo et al., 2007) and
214 the Netherlands (Rutjes et al., 2007) which suggest that HEV infection is probably more
215 frequent in European intensively reared swine herds than previously thought (van der Poel et
216 al., 2001; Banks et al., 2004). This could be at least partially due to the implementation of
217 diagnostic methods for HEV (Rutjes et al., 2007), even though a change in the epidemiology
218 of infection cannot be ruled out.

219 HEV ranged from 12.8% to 72.5% between farms, independent of farm typology, with
220 a higher prevalence in herds with a number of sows >1000. This latter finding is common for
221 most swine infectious diseases and, in addition to the number of susceptible animals, may also
222 be related to the density and closeness of individuals, as suggested by Nakai et al. (2006), and

223 the increased HEV shedding in pregnant sows (Fernandez-Barredo et al., 2006). Other
224 variables such as the frequency of introduction of animals, the number of suppliers, etc, could
225 also influence virus spread inside bigger farms. HEV prevalence was also higher in farrow-to-
226 weaning (66.7%) than in farrow-to-finish farms (32.1%), but the low number of farrow-to-
227 weaning farms examined limits possible inferences.

228 Within the herd, virus shedding was observed in pigs of all age categories, although
229 prevalence of HEV in fatteners (older than 120 days) was lower than in the younger weaners
230 (27.0% vs. 42.2%). Previous studies on serum and fecal samples demonstrated that HEV
231 RNA can be primarily detected in pigs of 2-5 months of age, while animals younger than 2
232 months are generally negative (Meng et al., 1997; Meng et al., 1998b). This, together with the
233 knowledge that maternal immunity lasts approximately two months, makes occurrence of
234 natural infection more likely at approximately 2-3 months of age (Nakai et al., 2006; Satou
235 and Nishiura, 2007). Our findings seem to confirm this pattern of infection. Viremia has been
236 reported to last for 1-2 weeks after infection, and virus excretion for about 3-4 weeks
237 followed by seroconversion (Meng et al., 1998a; Nakai et al., 2006). Therefore, animals older
238 than 6-8 months are expected to have cleared HEV infection. However, in our study we
239 detected HEV RNA in feces of animals of all age categories, from weaners of 3-4 months
240 (42.2%) to old sows (53.4%) well beyond 6-8 months of age. Fairly high HEV prevalence
241 has also been reported recently among breeders in Spain (Fernandez-Barredo et al., 2006;
242 Fernandez-Barredo et al., 2007), and to a lesser extent (7%) in 5 to 6 month-old swine in
243 Japan (Nakai et al., 2006). In particular, Leblanc and colleagues (Leblanc et al., 2007) have
244 found fecal HEV in 41.2% of slaughtered swine at an age of 22-29 weeks, suggesting that the
245 swine may retain its susceptibility to HEV infection at any age. These and our present
246 findings are consistent with a recent report from USA (Feagins et al., 2007) showing that 11%
247 of commercial pig livers from grocery store contained HEV RNA of genotype 3. Infection in
248 pigs could be more prolonged than previously thought or might become chronic, possibly

249 sustained by an incomplete protective immunity, but particularly in a virus loaded
250 environment (Fernandez-Barredo et al., 2006) the possibility of continuous re-infection
251 favored by a short-lasting immunity may not be excluded. The latter hypothesis may fit with
252 the finding that the high HEV prevalence among weaners and gilts in our study decreased in
253 fatteners and young sows, but was higher in old sows than in other age groups. Our findings
254 could otherwise be explained by a recent introduction of HEV into previously *naïve* herds.
255 However, both the simultaneous occurrence in six distinct farms and the identification of a
256 variety of HEV strains make such an event very unlikely. It is more reasonable to assume the
257 hypothesis that new different viruses can be continuously introduced into farms, where pigs
258 may be incompletely protected against novel strains. Although newly introduced animals are
259 the most probable vehicle of novel viral strains into farms, contaminated feed and
260 environment and humans might also play a role, in particular in farrow-to-finish or farrow-to-
261 weaning farms. Biosecurity measures, and control of animal import may thus be important to
262 prevent new virus introduction. Although all farms had in place basic measures such as access
263 control, boundary fencing, and all-in/all-out production systems, our investigation was not
264 designed to examine biosecurity and risks in detail, and we cannot add any conclusive
265 evidence on these aspects.

266 In our study, all pigs sampled looked clinically healthy, as reported in previous studies
267 (Clemente-Casares et al., 2003; Banks et al., 2004; Zheng et al., 2006; Fernandez-Barredo et
268 al., 2006). However, further studies should desirably evaluate HEV pathogenesis in more
269 detail in particular when associated to other viral infectious agents such as Porcine Circovirus
270 2 (PCV2) and porcine reproductive and respiratory syndrome virus (PRRSV) (Cho and Dee
271 2006; Martin et al., 2007).

272 Phylogenetic analysis showed that all HEV strains sequenced in this study belonged to
273 genotype 3, sharing a 100% aminoacid identity. The Italian swine strains presented closer
274 homology with either human or swine HEV strains circulating in Europe than with other

275 genotype 3 swine isolates from more distant geographic areas (van der Poel et al., 2001;
276 Banks et al., 2004). Interestingly, a human genotype 3 HEV strain identified earlier in Italy
277 (Zanetti et al., 1999) showed a lower phylogenetic correlation (79% nucleotide identity) with
278 our present strains, which were only 86-88% identical with Italian swine strains from 2001
279 (Caprioli et al. 2007), suggesting continuous evolution of this virus. Within the 80 bp ORF2
280 fragment analyzed, the 16 swine HEV genomes, representing the different farms tested,
281 showed a nucleotide identity ranging from 90.5 to 100%, and five strains from 4 farms proved
282 identical. These findings suggest that at least 12 different virus variants/strains were
283 circulating in the pig farms of Northern Italy at the time of investigation, that more strains can
284 be present in a same farm, and that a same strain of HEV can be spread in farms separated
285 geographically, implying a a common source or origin of infecting virus.

286 The enzoonotic nature of HEV infection in pigs and its ability to infect across species
287 raise potential concern for zoonotic transmission and public health (Banks et al., 2007; Satou
288 and Nishiura, 2007; Feagins et al., 2007). The finding of a high HEV prevalence in all
289 examined farms, together with the observation that infection may be sub-clinical and affect
290 animals at slaughtering age, highlights the possible risk of transmission of HEV to humans by
291 contact with either infected pigs or with environment and working instruments contaminated
292 with pig feces, or via ingestion of contaminated undercooked meat.

293

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412 **Figure 1.** Dendrogram, drawn using UPGMA and an avian HEV strain (United States,
413 accession no. AY535004) as outgroup, was based on 80 bp of the ORF2 fragment. GenBank
414 accession no., origin, country, and genotype are reported. Animals are indicated as: YS (0
415 parities), Young sow 7-10 months; YS (1-2 parities), Young sow 11-15 months; OS (>2
416 parities), Old sows 1-5 years; Weaners, < 120 days; Fatteners, >120 days. Strains identified
417 in this study are indicated with code MO/individual no._farm no./06/IT (**boldface type**).
418

Figure 1

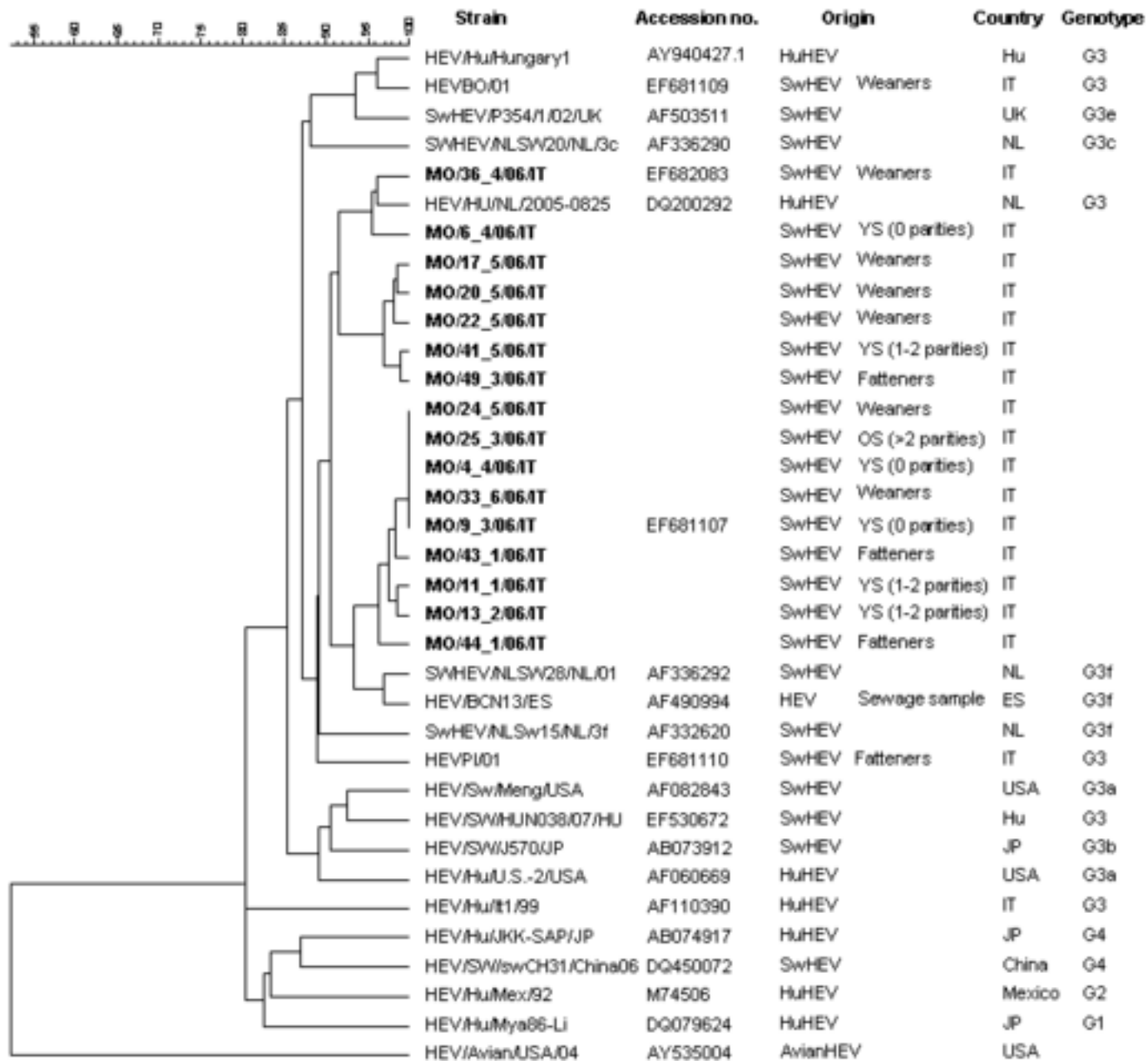


Table 1. Detection of swine HEV RNA in stools, by type of herd and production stage.

		Pigs positive/tested by production stage (%)											
Type of farm*	Farm no.	Gilts (0 parities ^a)		Young sows (1-2 parities ^b)		Old sows (> 2 parities ^c)		Weaners (< 120 days old)		Fatteners (> 120 days old)		Total	
F-W	2	7/10	(70.0)	7/10	(70.0)	7/10	(70.0)	8/10	(80.0)	-	-	29/40	(72.5)
F-W	6	4/9	(44.4)	4/10	(40.0)	8/10	(80.0)	7/9	(77.8)	-	-	23/38	(60.5)
Total F-W		11/19	(57.9)	11/20	(55.0)	15/20	(75.0)	15/19	(78.9)	-	-	52/78	(66.7)
F-F	1	3/10	(30.0)	5/10	(50.0)	9/10	(90.0)	1/10	(10.0)	6/10	(60.0)	24/50	(48.0)
F-F	3	4/9	(44.4)	2/10	(20.0)	2/9	(22.2)	2/10	(20.0)	2/10	(20.0)	12/48	(25.0)
F-F	4	2/10	(20.0)	2/10	(20.0)	1/10	(10.0)	1/7	(14.3)	0/10	(0.0)	6/47	(12.8)
F-F	5	5/10	(50.0)	2/7	(28.6)	4/9	(44.4)	8/18	(44.4)	2/7	(28.6)	21/51	(41.2)
Total F-F		14/39	(35.9)	11/37	(29.7)	16/38	(42.1)	12/45	(26.7)	10/37	(27.0)	63/196	(32.1)
All farms		25/58	(43.1)	22/57	(38.6)	31/58	(53.4)	27/64	(42.2)	10/37	(27.0)	115/274	(42.0)

* F-W = farrow-to-weaning; F-F farrow-to-finish

^aAge range: 7-10 months^bAge range: 11-15 months^cAge range: 1-5 years

Table 2. Logistic regression analysis of HEV shedding according to swine age, production stage and herd size

		HEV positive pigs/ total (%)	Odds ratio	95.0% C.I. for OR	p
Production stage	gilts (0 parities) ^a	25/58 (43.1)	1.58	0.61-4.09	0.345
	young sows (1-2 parities) ^b	22/57 (38.6)	1.33	0.51-3.47	0.559
	Old sows (>2 parities) ^c	31/58 (53.4)	2.54	0.98-6.55	0.054
	Weaners (<120 day-old)	27/64 (42.2)	1.38	0.54-3.51	0.504
	Fatteners (> 120 day-old)	10/37 (27.0)	-	-	-
Herd size	> 1000 sows	97/179 (54.2)	4.98	2.73-9.09	0.000
	< 1000 sows	18/95 (18.9)	-	-	-
	Constant	-	0.15		0.000

^aAge range: 7-10 months

^bAge range: 11-15 months

^cAge range: 1-5 years