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Detection of Rat Hepatitis E Virus in Pigs, Spain, 2023

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We identified rat hepatitis E virus (HEV) RNA in farmed pigs from Spain. Our results indicate that pigs might be susceptible to rat HEV and could serve as viral intermediaries between rodents and humans. Europe should evaluate the prevalence of rat HEV in farmed pigs to assess the risk to public health.

Hepatitis E virus (HEV) is a major cause of acute viral hepatitis in Europe. HEV is classified into 8 major genotypes, but zoonotic genotype 3 is the most prevalent on the continent (*1*). HEV was considered the only zoonotic species in the Hepeviridae family until rat HEV (*Rocahepevirus ratti*) was identified. Rat HEV was the causal agent of chronic hepatitis in a transplant recipient from Hong Kong in 2018 (*2*). Since that discovery, nearly 30 cases of chronic and acute hepatitis have been reported in America, Asia, and Europe (*3*–*6*), affecting both immunosuppressed and immunocompetent persons. Those cases highlight the zoonotic potential of rat HEV, emphasizing its growing concern to public health.

Rodents are the main host of rat HEV, but its transmission routes remain unclear. Although direct and indirect contact with rodents have been suggested as potential transmission routes, only 1 registered case has involved such contact (*6*). Thus, alternative sources of infection seem possible, potentially from an alternate host with which humans have more contact (*7*). Because domestic pigs (*Sus scrofa domestica*) are highly susceptible to HEV and constitute the main natural viral reservoir, they could also be susceptible to rat HEV and potentially serve as hosts. Confirming that hypothesis could have major implications for public health. We aimed to assess the presence of rat HEV in a population of farmed pigs in Spain.

During May–June 2023, we randomly selected and prospectively sampled domestic pigs from 5 intensive breeding system farms in Cordoba, southern Spain. We collected rectal fecal samples from each pig and stored samples at −80°C until RNA extraction (Appendix, https://wwwnc.cdc.gov/EID/article/30/4/23-1629-App1.pdf).

We included a total of 387 pigs in the study and found rat HEV in 44 pigs, an individual prevalence of 11.4% (95% CI 8.6%–14.9%) (Table). Sequencing confirmed the identity as rat HEV (species *R. ratti)* (GenBank accession nos. OR977681–7 and OR977689–7711) (Appendix Figures 1, 2). Among the 5 farms, 2 (40%) had ≥1 rat HEV–positive pig. Of note, 93.2% (41/44) of positive animals were from the same farm (Figure; Appendix Table 3). HEV RNA was detected in 6 pigs, indicating a prevalence of 1.6% (95% CI 0.6%–3.4%). All HEV-positive pigs were from the same farm and had sequences consistent with HEV genotype 3f (GenBank accession nos. OR818554–60), but rat HEV was not detected in that farm.

The hypothesis that pigs are not susceptible to rat HEV was formed on the basis of experimental in vivo studies (*8*). Because animals in that study were not infected after challenge with rat HEV strains (*8*), it

Table. Demographic data of pigs and characteristics of farms in a study of detection of rat HEV in pigs, Spain, 2023*

Characteristics	No. (%), n = 387
Age range	
Adult	188 (48.6)
Subadult	169 (43.7)
Unknown	30 (7.8)
Breed	
Iberian	159 (41.1)
White	148 (38.2)
Iberian cross	80 (20.7)
Aptitude	
Reproductive	188 (48.6)
Fattening	199 (51.4)
Farm HEV status	
Rat HEV–positive	44 (11.4)
HEV-positive	6 (1.6)

*HEV, hepatitis E virus.

appeared that pigs were resistant to rat HEV infection. However, our study detected rat HEV RNA in pigs, suggesting the possible role of pigs in rat HEV epidemiology. That finding increases the range of species susceptible to rat HEV, suggesting that its transmission might not be restricted to rodents. The number of positive animals we found suggests that rat HEV is widespread among pig populations in the study area. That observation might be linked to the elevated positivity rate (55%) discovered in rodents from the same region (*9*), implying that the lack of rodent control measures might increase the risk for rat HEV transmission.

The presence of rat HEV in farmed pigs is of public health concern, especially considering global pork consumption. Our study highlights the possibility that pigs intended for human consumption could contribute to rat HEV transmission. The European Food Safety Authority (EFSA) recommends monitoring HEV in pigs to identify alterations in virus distribution and prevent its spread to new farms, aiming to reduce human cases (*10*). Our results suggest that a preliminary evaluation of rat HEV in farmed pigs should be also conducted in Europe, which could confirm our results and increase our understanding of virus transmission.

The first limitation of our study is that because of its exploratory nature, the sampling area was restricted to a single region, but our findings underscore the need to extend the evaluation of rat HEV to determine its magnitude. Second, because no serologic assays

Figure. Geographic locations of farms included in a study of rat hepatitis E virus in pigs, Spain, 2023. Triangles indicate farms with ≥1 pig positive for rat HEV RNA are marked, circles farms with no positive pigs. Inset shows shaded area in Spain where the sampling occurred.

are available for detecting rat HEV antibodies in pigs, our analysis was limited to molecular testing on fecal samples; thus, we cannot confirm rat HEV infection. However, our study justifies the design of new studies to evaluate the presence of rat HEV in blood and tissues samples. Finally, implementation of serologic analysis on rat HEV might enhance our comprehension of the pathogenesis of both HEV and rat HEV and assist in future investigations into risk factors.

In conclusion, our study shows the possibility that pigs are susceptible to rat HEV infection, challenging previous assumptions. Further studies are warranted to determine the role of pigs in rat HEV epidemiology and to assess the risk for direct or indirect zoonotic transmission from pigs. In addition, Europe should conduct an evaluation of rat HEV in farmed pigs to assess the overall risk to public health.

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Author contributions: L.R.-M., A.R.-J., and A.R. were involved in the study design and conception, interpretation of the data, drafting of the manuscript, study supervision, and funding obtention. M.G., S.C.-S., T.F., and I.A.-R. were involved in sampling design, collection and storage. L.R.-M., P.L.-L., M.C.-J., and J.C.-G. performed RNA extraction and molecular determinations, phylogenetic analysis and GenBank submission. All authors have revised the manuscript and approved its publication.

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Seroprevalence of Avian Influenza A(H5N6) Virus Infection, Guangdong Province, China, 2022

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In 2022, we assessed avian influenza A virus subtype H5N6 seroprevalence among the general population in Guangdong Province, China, amid rising numbers of human infections. Among the tested samples, we found 1 to be seropositive, suggesting that the virus poses a low but present risk to the general population.

The highly pathogenic avian influenza A virus subtype H5, identified in Guangdong Province, China, in 1996, has evolved into multiple distinct phylogenetic clades and undergone reassortment events (*1*). In 2014, a new clade (2.3.4.4) that included influenza A(H5N6) virus emerged in Asia and has caused both epizootic and zoonotic cases worldwide (*2*). As of August 1, 2023, a total of 86 human cases of H5N6 infection have been reported globally; 40 (46.5%) have resulted in death (*3*). Most cases were reported in China, and 1 case was reported in Laos (*3*). An increase in the number of H5N6 human infections during 2021 and 2022 has been observed, reaching a total of 55 cases, exceeding the cumulative total number of the reported H5N6 human infections in the preceding years (Figure 1, panel A). This sudden upsurge has consequently raised concerns over a higher risk for H5N6 transmission.

Previous studies have indicated a higher prevalence of human infections with H5 viruses, according to serologic evidence, compared with the number of World Health Organization–confirmed cases (*4*). A shortage of serologic surveillance studies focusing on human H5N6 infections in the general population exists (*5*,*6*). To better assess the risk for H5N6 infections during the 2021–22 wave, we conducted a cross-sectional serologic study during January–March 2022 (Figure 1, panel A) in Dongguan and Huizhou cities in Guangdong Province. The cities were the epicenters of human H5N6 infections in 2021 (Figure 1, panel B). Given the unclear seroprevalence of H5N6 virus in the general population, we used an estimated H5N1 seropositivity rate of 1.2% (*4*) for our sample size calculation, targeting a 95% CI and a precision of 0.006. Assuming a dropout rate of 15%, we calculated that a sample size of 6,012 in the general population would be required. This study was approved by the ethics committee of the First Affiliated Hospital of Guangzhou Medical University (ethics approval no. 2016-78).

We excluded poultry workers and patients with oncologic diseases, hematologic malignancies, or immunocompromising conditions from our study. The patients who reported respiratory symptoms or diseases were not excluded and represented a small fraction of the sample pool (46 [0.72%]). We collected serum samples from 6,363 participants at 72 local hospitals and physical examination centers across Dongguan and Huizhou cities, ensuring a broad regional representation. Of the participants, most were outpatients (4,284 [67.33%]); the remaining participants were hospitalized patients (699 [10.99%]) or persons undergoing routine physical examinations (1,380 [21.69]). The median age of participants was 41 years (25th–75th percentile 29–55 years). Of the 6,363 samples, 42.2% (2,685) were from men and 57.8% (3,678) from women; 53.4% (3,401) of samples were from Huizhou (Table). We screened the residual serum samples by using a hemagglutination inhibition (HI) assay against a recombinant H5N6 virus

[1]These authors contributed equally to this article.

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Appendix

Animal and Farm Sampling

Intensive pig farms included in the present study were selected by simple random sampling from official flock registers obtained from the Regional Government of Andalusia. The sample size was calculated assuming a herd prevalence of 50%, which provides the highest sample size in studies based on unknown prevalence, with a 95% CI and accepted error of 5%, giving 385 animals to be sampled (*1*). Within each farm, a mean of 75 (range 71–80) animals were sampled by systematic random sampling, with the objective of detecting rat hepatitis E virus (rat HEV) infection with a probability of 95% and a minimum expected prevalence of 4% (*2*).

Rat HEV Molecular Evaluation

Viral RNA extraction from 0.25 mg of feces was performed by diluting feces in 300 µL of PBS and processed using the IndiSpin Pathogen Kit (formerly known as QIAamp Cador Pathogen Mini Kit) using the QIAcube (QIAGEN, Hilden, Germany) automatic procedure. RNA was then eluted in 50 µL.

All individuals underwent real-time quantitative PCR (qPCR) testing for HEV and rat HEV. For HEV evaluation, a qPCR assay previously developed and validated by our group was employed (*3*), using the 1st WHO standard for acid nucleic amplification–based HEV RNA assays (supplied by the Paul-Ehrlich-Institut under the code PEI 6219/10) as a positive control. To detect rat HEV RNA, we used a PCR targeting the 5′ untranslated region (5′UTR) (*4*). A rat liver sample from a rodent identified in our lab (GenBank accession no. OR282813) was used as

positive control. Samples positive for HEV, rat HEV, or both, underwent sequencing. In cases of HEV-positive samples, a nested PCR targeting a 420 nt segment of ORF2 was conducted (*3*). The approach for sequencing rat HEV–positive samples involved three nested PCRs, targeting three regions located on the ORF1. The regions had lengths of 880 bp (*5*), 220 bp, and 230 bp, respectively.

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Appendix Table 1. Primer and probe sets used for the detection of rat hepatitis E virus RNA*

HEV	PCR type	Forward primer, 5′→3′	Reverse primer, 5′→3′	Probe, 5′→3′	Ref.
HEV_qPCR	qPCR	RGTRGTTTCTGGGGTGAC	AKGGRTTGGTTGGRTGA	5′-FAM-TGAYTCYCARCCCT TCGC-TAMRA-3′	(*3*)
HEV_ORF2	Nested	CAAGGHTGGCGYTCKGTTG AGAC	CCCTTRTCCTGCTGAGCRT TCTC		(*3*)
		GYTCKGTTGAGACCWCBGG BGT	TTMACCWGTCRGCTCGCC ATTGGC		
RatHEV					
RatHEV_Parraud	qPCR	CCACGGGGTTAATACTGC	CGGATGCGACCAAGAAAC AG	5′-6FAM-CGGCTACCGCCTTT GCTAATGC-BBQ-3′	(*4*)
RatHEV_Mulyanto	Nested	CCTYTGCAGCTTGTCTTTGA	ATGCGTGCTCATGGHATG		(*5*)
		CTGTTTCTTGGTCGCATCC	CTGATCTTTCCTTTTGCAC		
RatHEV_B	Nested	TTTGCTAATGCTCAGGTGGT	ATGCGTGCTCATGGHATG		This study
		CTGTTTCTTGGTCGCATCCG	AACATCCGCCGTTGCATTC TT		
RatHEV_E	Nested	CCTYTGCAGCTTGTCTTTGA	ATGCGTGCTCATGGHATG		This study
		CTGTTTCTTGGTCGCATCC	CTGATCTTTCCTTTTGCAC		

*Underlined letters indicate degenerate primers. qPCR, quantitative PCR; Ref., reference.

Appendix Table 2. Thermocycle conditions

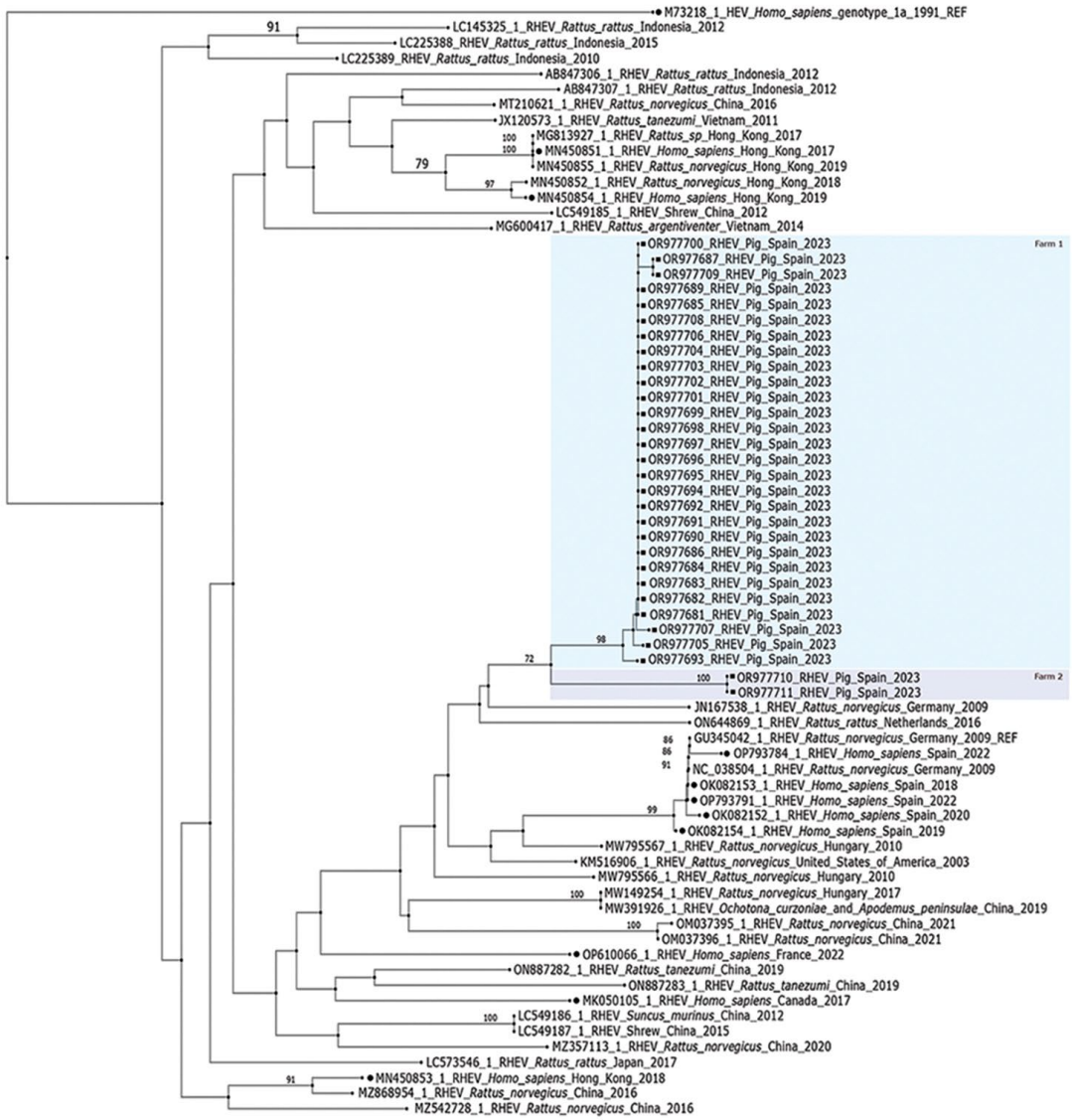
Step	No. cycles	Time, s	Temperature					
			HEV qPCR	HEV ORF2	RatHEV Parraud	RatHEV Mulyanto	RatHEV B	RatHEV E
1st PCR								
UNG Activity		600	25°C	25°C	25°C	25°C	25°C	25°C
Retrotranscription		300	52°C	52°C	52°C	52°C	52°C	52°C
Denaturalization		10	95°C	95°C	95°C	95°C	95°C	95°C
Denaturalization	x45	5	95°C	95°C	95°C	95°C	95°C	95°C
Annealing		30	58°C	51°C	58°C	58°C	58°C	58°C
2nd PCR								
Denaturalization		120		95°C		95°C	95°C	95°C
Denaturalization	x45	60		95°C		95°C	95°C	95°C
Annealing		60		52°C		58°C	58°C	58°C
Extension		60		72°C		72°C	72°C	72°C
Final extension		300		72°C		72°C	72°C	72°C

*HEV, hepatitis E virus; ORF, open reading frame; qPCR, quantitative PCR; ratHEV, rat hepatitis E virus.

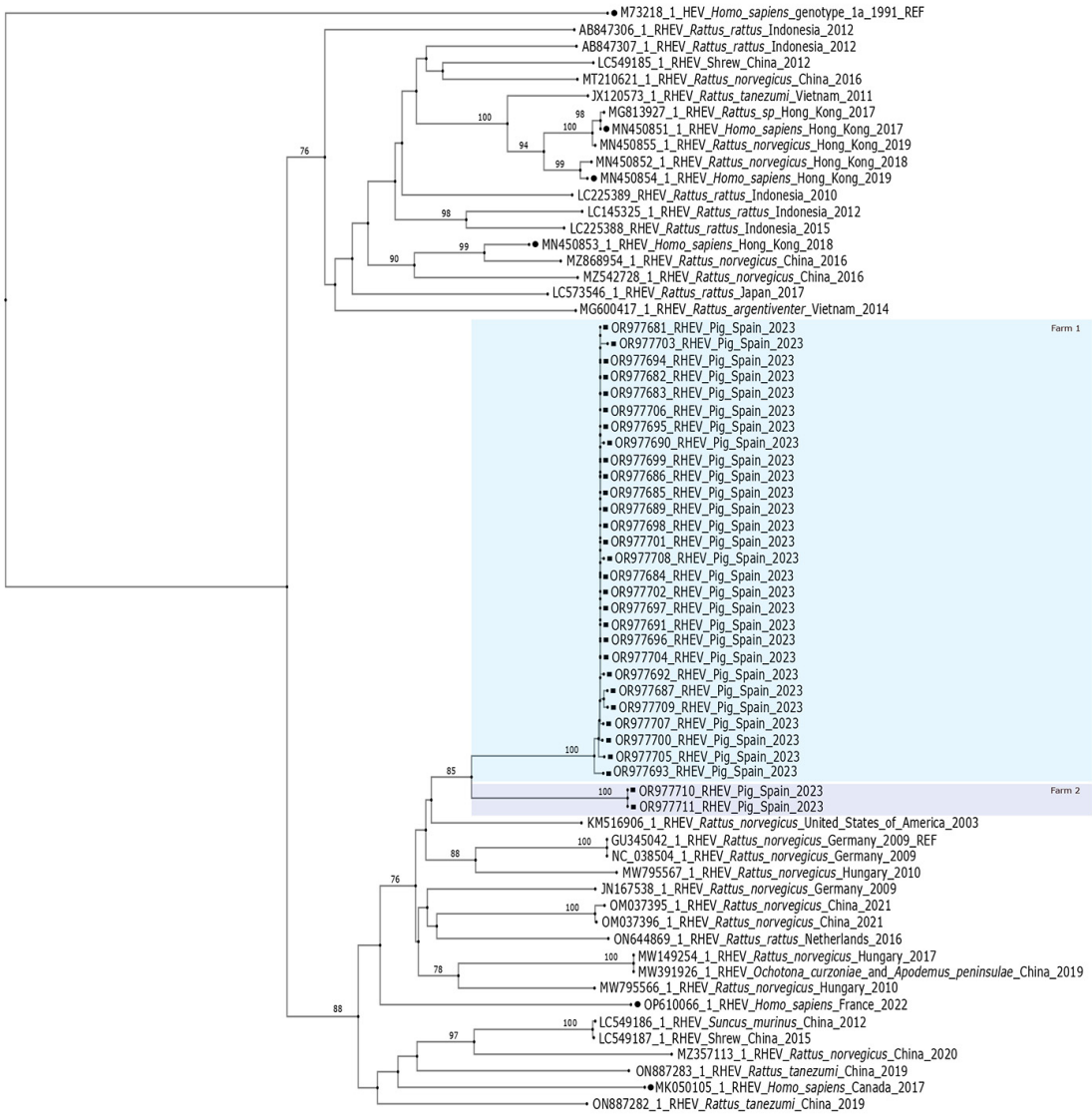
Appendix Table 3. Characteristics of pigs that tested positive for rat hepatitis E virus*

ID no.	Farm code	Aptitude	Breed	Ct value	GenBank accession no.
158	017CO00036	Reproductive	White	33.22	OR977681
159	017CO00036	Reproductive	White	41.32	OR827969
167	017CO00036	Reproductive	White	39.60	OR827970
170	017CO00036	Reproductive	White	39.29	OR977682
171	017CO00036	Reproductive	White	4120	OR827971
175	017CO00036	Reproductive	White	40.91	OR827972
176	017CO00036	Reproductive	White	41.84	OR827973
186	017CO00036	Fattening	White	38.88	OR977683
187	017CO00036	Fattening	White	37.88	OR977684
188	017CO00036	Fattening	White	38.82	OR977685
190	017CO00036	Fattening	White	36.00	OR977686
191	017CO00036	Fattening	White	37.60	OR977687
192	017CO00036	Fattening	White	37.50	OR977688
193	017CO00036	Fattening	White	37.18	OR977689
195	017CO00036	Fattening	White	38.63	OR977690
196	017CO00036	Fattening	White	37.47	OR977691
198	017CO00036	Fattening	White	35.53	OR977692
199	017CO00036	Fattening	White	38.85	OR977693
200	017CO00036	Fattening	White	37.90	OR977694
201	017CO00036	Fattening	White	32.43	OR977695
202	017CO00036	Fattening	White	37.08	OR827964
203	017CO00036	Fattening	White	30.57	OR977696
204	017CO00036	Fattening	White	34.48	OR977697
205	017CO00036	Fattening	White	39.02	OR827965
207	017CO00036	Fattening	White	33.54	OR977698
208	017CO00036	Fattening	White	35.08	OR977699
210	017CO00036	Fattening	White	38.66	OR827976
211	017CO00036	Fattening	White	33.82	OR977700
212	017CO00036	Fattening	White	31.85	OR977701
213	017CO00036	Fattening	White	35.13	OR977702
214	017CO00036	Fattening	White	36.29	OR977703
215	017CO00036	Fattening	White	40.04	OR827977
216	017CO00036	Fattening	White	31.32	OR977704
217	017CO00036	Fattening	White	35.27	OR827978
218	017CO00036	Fattening	White	34.00	OR977705
219	017CO00036	Fattening	White	34.36	OR977706
220	017CO00036	Fattening	White	36.95	OR977707
221	017CO00036	Fattening	White	35.93	OR977708
223	017CO00036	Fattening	White	38.76	OR977709
224	017CO00036	Fattening	White	38.47	OR827979
234	ES140050000005	Reproductive	Iberian Cross	37.06	OR827980
248	ES140050000005	Reproductive	Iberian Cross	37.41	OR827981
265	ES140050000005	Fattening	Iberian Cross	34.70	OR977710
302	ES140050000005	Fattening	Iberian Cross	38.00	OR977711

*Ct, cycle threshold; ID, identification.



Appendix Figure 1. Phylogenetic analysis of 65 hepatitis E sequences identified in the study. Sequences were 788 nt in length. Squares (■) indicate sequences of pigs identified in this study; circles (●) indicate previously identified human cases. In color is highlight the farm of origin of positive pigs. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model. The bootstrap consensus tree inferred from 1,000 replicates is taken to represent the evolutionary history of the taxa analyzed.



Appendix Figure 2. Phylogenetic analysis of 70 hepatitis E sequences identified in the study. Sequences were 285 nt in length. Squares (■) indicate sequences of pigs identified in this study; circles (●) indicate previously identified human cases. In color is highlight the farm of origin of positive pigs. The evolutionary history was inferred by using the maximum-likelihood method based on the Tamura-Nei model. The bootstrap consensus tree inferred from 1,000 replicates is taken to represent the evolutionary history of the taxa analyzed.