Occurrence of Hepatitis E virus (HEV) in Calabrian wild boars

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ABSTRACT

Hepatitis E virus (HEV) is an emerging pathogen in industrialized countries. HEV infections in humans are mainly related to the HEV-3 genotype, predominant in Europe and widespread in wild boars' food products. However, there are little relevant data around HEV prevalence in wild boars, although they are considered the main HEV reservoir and used for typical food products such as liver sausages. Our study aimed to assess HEV occurrence and genetic variability in Calabrian wild boars hunted in the central and ionic area of Catanzaro's province. A total of 86 wild boar liver samples were analyzed showing an overall HEV RNA prevalence of 26.7% (23/86). All positive samples were characterized molecularly as genotype 3 and predicted as HEV-3c subtype despite the shortness of fragment employed for the molecular analysis. This data is in line with previous studies conducted in Europe highlighting the public health concern of these results.

Biomolecular methods performed in our study detected only the HEV RNA positivity of analyzed samples without information about the virus viability. Consequently, it is not possible to fully estimate the risk related to the consumption of wild boar's liver sausages or wild boar meat products. Our results highlight the need for further studies in order to investigate the virus viability and to link wild boar's meat consumption with HEV human seroprevalence in Italian regions (Abruzzo, Lazio, Campania and Calabria) where typical wild boar's products are consumed. In this way, the Competent Authorities could perform a complete risk assessment, implement risk management and establish proper measures to ensure the public health and prevent relative human disease.

Keywords

Foodborne disease; Risk assessment; Zoonosis; Molecular methods

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1. INTRODUCTION

Hepatitis E virus (HEV) belongs to the family *Hepeviridae*, genus *Orthohepevirus*, species *Orthohepevirus A* and it is a hepatotropic, non -enveloped, and single-stranded RNA virus. Five different genotypes of Orthohepevirus A can infect humans (Johne et al., 2014). Of them, genotypes HEV-3 and HEV-4 can infect both humans and animals (pigs, wild boar, deer, cows, goats, rabbits) and are the main cause of hepatitis E in humans in several developed countries (Huang et al., 2016; Kamar et al., 2017; La Bella et al., 2021; Treagus et al., 2021). HEV is the etiological agent of self-limiting human acute hepatitis and it can also produce chronic hepatitis in immunocompromised or with chronic liver disease patients (Adlhoch et al., 2016).

It has been estimated that 20 million people contract HEV, 3.3 million develop an symptomatic form and 70.000 die every year all over the world (La Bella et al., 2021; WHO, 2021).

During the last decade, in the European Union (EU) there were more than 21,000 cases of HEV, with 28 deaths. The major infection route was the zoonotic transmission, in particular, the foodborne pathway due to the consumption of uncooked or undercooked infected meat or derivatives (Di Cola et al., 2021; Ricci et al., 2017).

In Europe, human cases are mainly associated with HEV-3 that is the predominant genotype and widespread in animals used in food products such as domestic pigs, wild boar, and deer (Doceul et al., 2016; Montone et al., 2019). Foodborne zoonotic transmission of HEV-3 was frequently reported in sporadic and clustered human cases and associated with the consumption of liver, offal products, sausages and raw and undercooked meat of pork, wild boar and deer (De Sabato et al., 2020; Ricci et al., 2017).

Moreover, other potential transmission pathways for this virus can be the consumption of vegetable and shellfish due to a cross-contamination with animal and human faeces (King et al., 2018; La Rosa et al., 2018; O'Hara et al., 2018; Terio et al., 2017). In Italy, wild boars have been mainly infected by 3c, 3e and 3f HEV-3 subtypes (De Sabato et al., 2020; Di Pasquale et al., 2019; Zecchin et al., 2019). These are the most common subtypes in Europe (Nicot et al., 2021), although the predominance of variants within genotypes can change during the time (Nicot et al., 2018; Oeser et al., 2019).

Until now, different studies showed that HEV detection in pig and wild boar muscles is rare (Di Bartolo et al., 2012) but a recent German report highlighted the HEV presence in wild boar livers and muscles equally (Anheyer-Behmenburg et al., 2017).

The wild boar is the natural wildlife reservoir of HEV (Rivero-Juarez et al., 2018) and, consequently, the health surveillance in this species is important to reduce the potential effect of wild boar meat consumption on human health and to increase the information on the epidemiology of HEV. Furthermore, the risk for human health is associated with the hunted wild boars' meat that can be employed to produce wild boar sausages, a traditional Calabrian product consumed mainly in this region.

During the last years, in the central and ionic area of Catanzaro's province, there was an uncontrolled proliferation of wild boars causing a lot of damage (Coldiretti-CALABRIA, 2021). It was necessary to prolong the hunting period leading to a possible increase in the consumption of wild boars' products. Moreover, there were very few studies mapping the HEV spread in Calabrian wild boars.

Our study aimed to evaluate the presence and the genetic variability of HEV in livers of Calabrian wild boars hunted in the central and ionic area of Catanzaro's province to provide epidemiological HEV updated data.

2. MATERIALS AND METHODS

2.1 Sampling

Eighty-six liver samples were collected from wild boars during the hunting season in 9 Catanzaro's municipality (Calabria, Italy) from October 2019 to January 2020 (Table 1). During the inspection activity by the local health authority, livers (30-40 cm^3) were collected and transported under refrigerated conditions to the laboratory and stored at -80 °C until use.

2.2 Sample preparation and nucleic acid extraction

Virus extraction was performed following the method described by Di Pasquale et al. (2019) with appropriate modifications. Briefly, 1 g of liver was shredded with sterile blade and was added to a tube containing 3.5 ml of TRIZOL Reagent (Life Technologies, Monza, Italy) and 1 sterile tungsten carbide bead (3mm diameter). Samples were homogenized for 5 min at 25 hz/s in Tissue Lyser II (Qiagen, GmbH, Hilden, Germany). After mechanical disruption of the livers, samples were incubated at Room Temperature (RT) for 15 min and centrifugated at 8000 x g for 20 min at 4 °C. Then the supernatant was added with 0.7 ml of chloroform and vortexed for 15 sec and incubated for 15 min at RT. The aqueous phase obtained by centrifugation at 8000 x g for 15 min at 4°C, was recovered and stored at – 80°C until use.

Nucleic acid extraction was performed using Nuclisens MiniMag extraction system (bioMerieux, France) following the manufacturer's instruction. The RNA extracted was stored at -80°C.

2.3 Touchdown RT-PCR

The touchdown RT-PCR (TD/RT-PCR) assay was carried out in a single step using SuperScript™ One Step RT-PCR with Platinum Taq (ThermoFisher scientific, Milan, Italy). All components for TD/RT-PCR were assembled in a 25 µl reaction with primers described by Drexler et al. (2012) that amplify

121 a part of ORF 2 region of HEV genome since, as described by the authors, the test is able to amplify
122 all members of the family Hepeviridae. The reaction contained 12.5 µl of 2X Reaction Mix, 0.5 µl of
123 RT/Platinum™Taq Mix, 1µM of forward primer 1853 (5'-ACYTTYTGTGCYYT[I]TTTGGTCC[I]TGGTT-3'),
124 1 µM of reverse primer 1854 (5'-GCCATGTTCCAGAYGGTGTTCCA-3') and 2 µl of viral RNA. The
125 thermocycling conditions were 30 min at 50 °C, 2 min at 94°C, followed by touchdown step included
126 10 cycles of 15 sec at 94°C, 15 sec from 60°C to 50°C with a decrease of 1°C for cycle and 30 sec at
127 68 °C. The amplification step consisted of 40 cycles of 15 sec at 94°C, 30 sec at 52°C, 30 sec at 68°C
128 and final elongation for 10 min at 68°C.

129 **2.4 Touchdown hemi-nested PCR**

130 Touchdown hemi-nested PCR (TD/hn-PCR) amplified a part of the ORF 2 region of HEV genome to
131 increase the sensibility of methods . The reaction was performed in a final volume of 25 µl using
132 12.5 µl of HotStarTaq Master Mix (Qiagen, GmbH, Hilden, Germany), 1 µM of forward primer 1853
133 (5'-ACYTTYTGTGCYYT[I]TTTGGTCC[I]TGGTT-3'), 1 µM of reverse primer 1855 (5'-
134 CCGGGTTCRCC[I]GAGTGTTTCTTCCA-3'). TD/hn-PCR was performed with 2,5 µl of RT-PCR product,
135 previously diluted 1:100. The thermocycling conditions were 3 min at 94°C, followed by touchdown
136 step included 10 cycles of 15 sec at 94°C, 15 sec from 60°C to 50°C with a decrease of 1°C for cycle
137 and 30 sec at 68 °C. The amplification step consisted of 40 cycles of 15 sec at 94°C, 13 sec at 50°C,
138 30 sec at 68°C and final elongation for 10 min at 68°C. Amplifications were carried out using standard
139 pre- cautions followed to prevent PCR contamination. All positive results were confirmed by
140 replicating the analysis in a second, independent, PCR amplification.

141 **2.5 Detection of amplified products**

142 TD/hn-PCR-amplified products were displayed by electrophoresis on 1.5% (w/v) agarose NA
143 (Pharmacia, Uppsala, Sweden) gel in 1X TBE buffer containing 0.089 M Tris, 0.089 M boric acid, 0.002
144 M EDTA, pH 8.0 (USB, Cleveland, OH, USA), and stained with Green Gel Safe 10000X Nucleic Acid

145 Stain (5 ll/100 ml) (Fisher Molecular Biology, USA). The Gene Ruler™ 100 bp DNA Ladder molecular
146 weight marker (MBI Fermentas, Vilnius, Lithuania) was used.
147 Image acquisition was performed with Gel Doc™ EZ imager Bio-rad.
148
149 **2.6 Sequencing and analysis**
150 Cycle sequencing was carried out using BigDye Terminator Cycle chemistry (Applied Biosystems,
151 Foster City, California, US). Consensus sequences were obtained using the Geneious software
152 version 10.0.5 (Biomatters Ltd, New Zealand). Redundancy of the sequences generated was
153 collapsed grouping identical sequences (unique haplotypes) by using mothur v 1.45.3 (Schloss et al.,
154 2009).
155 Taxonomic assignment to genotypes and subtypes of HEV sequences was performed using the
156 online tool HEVnet ("Hepatitis E Virus Genotyping Tool,"; Mulder et al., 2019) that uses phylogenetic
157 method in order to identify the Hepatitis E virus genotype and subtypes also using subgenomic
158 nucleotide sequences.
159 Moreover, our haplotypes were pooled with the reference sequences for hepatitis E virus subtypes
160 generated and listed by Smith et al. in Table 1 (2020). The sequences were multialigned using MAFFT
161 (Katoh et al., 2018) and the alignment was manually checked using SeaView v.4.0 (Gouy et al., 2010).
162 The final alignment was used to infer an haplotype network using Minimum spanning network
163 (Bandelt et al., 1999) implemented in PopART v1.7 (Bandelt et al., 1999; Clement et al., 2000; Leigh
164 and Bryant, 2015). Relationships with other HEV 3 sequences of the same study area were also
165 explored. Molecular data, including the RNA-dependent RNA polymerase fragment sampled in the
166 Italian regions from several hosts, were downloaded from GenBank (February 2022). Data were
167 merged with our haplotypes in addition to representative sequences from other geographic areas
168 or hosts. Sequences were aligned using MAFFT (Katoh et al., 2018) and the alignment was manually

checked using SeaView v.4.0 (Gouy et al., 2010). A Maximum Likelihood tree was built in FastTree (Price et al., 2010) using GTR gamma model and HEV-4 strain was used as outgroup (AB197673). Visualization and annotation of the tree was performed in iTOL ("iTOL: Interactive Tree Of Life," n.d.) (Letunic and Bork, 2019).

Map of sampling was built using leaflet R package ("leafletR: Interactive Web-Maps Based on the Leaflet JavaScript Library. R package version 0.4-0").

3. RESULTS

The presence of HEV was detected in 23/86 (26.7%) of samples by the amplification of a fragment of 306 bp. Generation of unique sequences reduced the dataset from 23 sequences to 4 non redundant unique sequences (haplotypes) (Table 2), one of them (Hap_1) including sequences sampled from three different geographic sites (Figures 1,2). Molecular identification using HEVnet identified all haplotypes belonged to hepatitis E virus with high support (>90%) and the subtype HEV-3c was inferred with low support (about 70%) for three haplotypes (Hap_1, Hap_3, Hap_4), whereas no subtype was inferred for Hap_2 (Table 2) (Suppl. Trees Files 1-4). Evolutionary prediction by HEVnet showed all haplotypes matched FJ705359 as closest reference sequence (Hap_1 296/305 nt (97%); Hap_2 293/302 nt (97%); Hap_3 294/305 nt (96%); Hap_4 295/305 nt (97%)) that was previously found both in human and wild boar and in several geographical areas (France, Germany, Netherlands, Sweden, UK, Thailand, Canada). A final multi alignment including 65 sequences with 306 nucleotides (data not provided) was used to generate haplotype network that highlighted our haplotypes as distinct respect the other HEV-3 subtypes (supplementary figure). In particular, the most abundant haplotype (Hap_1) was linked with the closest reference FJ705359 (nine nucleotides of differences), while the other haplotypes (Hap_2, Hap_4, Hap_3) diverged from it. Based on a total of 103 sequences, the tree-building was selected, and removing redundant sequences, the final alignment included 87 sequences of 291 bp (including the four haplotypes and the outgroup) (figure

193 3). In the tree, our haplotypes were grouped together in a clade and the Germany reference sampled
194 in wild boar (FJ705359) was confirmed to be the most related with the Calabrian haplotypes. Sister
195 sequences to the clade also included two references, one collected in Central Italy from wild boar
196 (MT840360) by Aprea et al., 2020 and a second sampled in Bulgaria from swine (MZ519907) by
197 Palombieri et al., 2021 . In the Supplementary Table 1 were reported the sequence's details used in
198 the tree, including Accession number and all the features extracted from the entries GenBank and
199 the HEV3 subtypes summarized or predicted by Nicot et al., 2021.

200 **4. DISCUSSION**

201 Our results confirmed the circulation of HEV-3 in wild boar populations and an overall prevalence
202 of 26.7%. These results were in line with Italian studies highlighting a high HEV circulation in wild
203 boars as described by Martelli et al., (2008) and De Sabato et al., (2018b) with an overall prevalence
204 of 25% and 52.2%, respectively. These data are higher if compared to results obtained in the
205 previous studies conducted in animals hunted in Italy, with an HEV positivity ranging from 2.45 % to
206 16.3 % (Aprea et al., 2020; Di Pasquale et al., 2019; Forzan et al., 2021; Martelli et al., 2008; Pierini
207 et al., 2021). Furthermore, in Europe, several epidemiological investigations reported an HEV RNA
208 prevalence in retail liver and liver products of pigs ranging from 4% to 11.8% (Treagus et al., 2021).
209 In detail, it has been reported that 4% of retail livers in France and Germany, 3% of slaughterhouse
210 livers in Spain and 11.8% of raw liver sausages in Switzerland, tested positive for HEV RNA (Di Bartolo
211 et al., 2012; Moor et al., 2018; Rose et al., 2011; Wenzel et al., 2011). Instead, the prevalence of HEV
212 RNA in wild boars liver samples was detected in percentage ranging from 1.9% to 33.5% (Ricci et al.,
213 2017).

214 Our results could be justified by the wild boar population density, the sampling strategy, and the
215 hunting area. In detail, in our study, the sampling area is restricted; every municipality is at a

maximum of 60 Km away from each other but, the geographical barriers between Catanzaro's municipalities could explain the detection of different haplotypes.

The analyses of positive samples obtained from wild boars at Calabria municipalities, revealed that all the haplotypes belong to the HEV-3 genotype which is the prevalent genotype in European countries. All the haplotypes found the same best hit, previously isolated both in human and wild boar and in several geographic areas (Smith et al., 2020), suggesting in the industrialized countries a transmission by food or close contact with animals (Brayne et al., 2017). Subtype inference showed that three haplotypes could belong to HEV 3c subtype. However, the nomenclature of HEV subtypes isn't stable making difficult the comparison among subtypes from different studies.

Moreover, HEV-3 strains are known to be highly divergent (Okamoto, 2007; Smith et al., 2020) and some of them do not fit the subtype definitions remaining unassigned as in recent studies detecting unclassified strains from wild boar in Central and southern Italy (De Sabato et al., 2018a; Pierini et al., 2021). In our study, despite the shortness and the highly conserved nature of the fragment used, analysis of the haplotypes suggest they could be distinct or diverging variants within the 3c subtype. Interestingly, in the phylogenetic tree built, the molecular data of HEV 3c generated from wild boars or other hosts sampled in Italian areas, seem to be poor related with our Calabrian sequences. These findings could be related to the different management applied on wild boar host populations during the time and in different Italian areas also including National parks. The different pressures could have generated cycles of restriction and expansion in the host population size of the wild boars and a consequent bottleneck in HEV genotype variants. This suggests that the marked divergence among Italian HEV 3c haplotypes could be a signal related to a founder effect and it highlights the need for a closer link between epidemiology of HEV, history and management of the host population. This data is in line with other studies conducted in other countries confirming general HEV 3 genotypes's high divergence reported by Takahashi et al., 2011 in Japan wild boar and with the multiple

subtypes' circulation in German wild boars described by Adlhoch et al., 2009; Oliveira-Filho et al., 2014.

However, a limitation of the present study is the relatively short length of the fragment used for the evolutionary inference. Larger studies and the sequencing of full-length or near full-length genomes will needed to clarify the influence of host factors with the diversity on HEV-3 genotype variant.

During the last years, there was an uncontrolled proliferation of Calabrian wild boars that led to an increase in hunting activity and, consequently, in potential local consumption of wild boar products (meat and liver) by consumers ("Coldiretti - CALABRIA").

To date, studies regarding the detection of HEV in the Calabria region were performed only in swine (Costanzo et al., 2015; Pavia et al., 2021). Furthermore, as described by Montagnaro et al., (2015) and Wu et al., (2011), there is evidence of environmental interaction between wild boars and swine. Hence, considering this topic, our study focused on the prevalence and characterization of circulating HEV genotype in the Calabrian wild boars' population since wild boars are considered reservoirs for HEV and are widespread all over the country (Aprea et al., 2018).

Based on the georeferentiation of collected samples, our results highlighted that positive samples were detected in restricted areas of Catanzaro's province. This could suggest a higher exposure to the virus in these territories with a potential increment of the risks related both to the consumption of wild boars' meat and their professional handling by hunters (Montone et al., 2019). In fact, in the last decade, in Italy, it has been observed a trend in continuous increase of human HEV reported cases (Stroffolini et al., 2015) with a 100 % increment observed in 2019 compared to 2018 (SEIEVA, 2020). It seems mainly due to foodborne outbreaks that occurred in Marche, Lazio, and Abruzzo regions where wild boars' meat is mostly consumed (SEIEVA, 2020) even if its consumption in other Italian regions or other countries cannot be ruled out.

263 Although, no human HEV cases have been notified in the last year, in the Calabria region, our results
264 showed the presence of HEV RNA in 26.7% of wild boar livers which suggest a potential HEV
265 underdiagnosis and/or asymptomatic infections (Ricci et al., 2017). Furthermore, it is known that
266 patients infected with subtype 3c were at a lower risk of hospitalization than those infected with
267 subtypes 3f or 3e (Abravanel et al., 2020; Subissi et al., 2019).
268 Our results, apparently in contrast with the results previously mentioned, could be explained by the
269 existing gap between animal and food sanitary surveillance and the development of HEV outbreaks
270 in humans. In fact, In Italy, although the notification of hepatitis E virus is mandatory thus providing
271 data on acute cases of the disease, until now, none of the cases notified have been directly linked
272 to food-borne transmission (Montone et al., 2019) suggesting a potential underestimation of HEV
273 incidence (Alfonsi et al., 2018). Hence, based on the evidence that the HEV-3c subtype causes fewer
274 hospitalizations, more studies are now needed to clarify the influence of host factors and virus
275 diversity on HEV-3 pathogenesis (Nicot et al., 2021).
276 Moreover, biomolecular methods performed in our study detected only the HEV RNA positivity of
277 analyzed samples without information about the virus viability. Consequently, it is not possible to
278 fully estimate the risk related to the consumption of pigs or wild boar's liver sausages or meat
279 products.
280

281 **5. CONCLUSIONS**

282 The circulation of HEV-3 in Calabrian wild boars was detected in 23/86 (26.7%) liver samples by the
283 amplification of a short fragment. Most positive samples belonged to HEV-3c subtypes and different
284 strains can be found, confirming the high heterogeneity of HEV in wild boars. Although, no human
285 HEV cases have been notified in the last year, in the Calabria region, our results showed a high HEV
286 RNA prevalence in wild boar livers suggesting a potential HEV underdiagnosis and/or asymptomatic

287 infections. Furthermore, biomolecular methods performed in our study detected only the HEV RNA
288 positivity of analyzed samples without information about the virus viability.
289 to confirm the HEV genetic variability found in our study, future studies will carried out using whole
290 genomic sequences or at least the complete capsid. Our results highlight the need for further studies
291 in order to investigate the virus viability and to better evaluate the relationship between the wild
292 boar's meat consumption and HEV human seroprevalence in Italian regions (Abruzzo, Lazio,
293 Campania and Calabria) where typical wild boar's products are consumed. In this way, the
294 Competent Authorities could implement risk management in order to establish proper measures to
295 ensure the public health and prevent relative human disease. Therefore, currently, in the absence
296 of specific control measures for HEV, hunters should be informed about implementing good hygiene
297 practices during slaughtering procedures to minimize the potential zoonotic and foodborne risks of
298 exposure for humans.
299
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304 **REFERENCES:**
305 Abravanel, F., Dimeglio, C., Castanier, M., Péron, J.M., Kamar, N., Lhomme, S., Izopet, J., 2020.
306 Does HEV-3 subtype play a role in the severity of acute hepatitis E? Liver Int. 40, 333–337.
307 https://doi.org/10.1111/liv.14329
308 Adlhoch, C., Avellon, A., Baylis, S.A., Ciccaglione, A.R., Couturier, E., de Sousa, R., Epštein, J.,
309 Ethelberg, S., Faber, M., Fehér, Á., Ijaz, S., Lange, H., Manďáková, Z., Mellou, K., Mozalevskis,
310 A., Rimhanen-Finne, R., Rizzi, V., Said, B., Sundqvist, L., Thornton, L., Tosti, M.E., van Pelt, W.,

311 Aspinall, E., Domanovic, D., Severi, E., Takkinen, J., Dalton, H.R., 2016. Hepatitis E virus:
312 Assessment of the epidemiological situation in humans in Europe, 2014/15. J. Clin. Virol. 82,
313 9–16. https://doi.org/10.1016/j.jcv.2016.06.010
314 Adlhoch, C., Wolf, A., Meisel, H., Kaiser, M., Ellerbrok, H., Pauli, G., 2009. High HEV presence in
315 four different wild boar populations in East and West Germany. Vet. Microbiol. 139, 270–278.
316 https://doi.org/10.1016/J.VETMIC.2009.06.032
317 Alfonsi, V., Romanò, L., Ciccaglione, A.R., La Rosa, G., Bruni, R., Zanetti, A., Libera, S. Della,
318 Iaconelli, M., Bagnarelli, P., Capobianchi, M.R., Garbuglia, A.R., Riccardo, F., Tosti, M.E.,
319 Ferrigno, L., Crateri, S., Galli, C., Tagliacarne, C., Giordani, M.T., Morelli, E., Vit, A., Bertin, T.,
320 Marinello, S., De Checchi, G., Zotti, C., Amprino, V., Rossati, A., Di Vito, A., Guidoni, C.,
321 Fiacchini, D., Marinelli, K., Loffredo, M., De Angelis, G., Citarella, A., Megnia, A.S., 2018.
322 Hepatitis e in Italy: 5 years of national epidemiological, virological and environmental
323 surveillance, 2012 to 2016. Eurosurveillance 23. https://doi.org/10.2807/1560-
324 7917.ES.2018.23.41.1700517
325 Anheyer-Behmenburg, H.E., Szabo, K., Schotte, U., Binder, A., Klein, G., Johne, R., 2017. Hepatitis E
326 Virus in Wild Boars and Spillover Infection in Red and Roe Deer, Germany, 2013–2015 -
327 Volume 23, Number 1—January 2017 - Emerging Infectious Diseases journal - CDC. Emerg.
328 Infect. Dis. 23, 130–133. https://doi.org/10.3201/EID2301.161169
329 Aprea, G., Amoroso, M.G., Di Bartolo, I., D'Alessio, N., Di Sabatino, D., Boni, A., Cioffi, B.,
330 D'Angelantonio, D., Scattolini, S., De Sabato, L., Cotturone, G., Pomilio, F., Migliorati, G.,
331 Galiero, G., Fusco, G., 2018. Molecular detection and phylogenetic analysis of hepatitis E virus
332 strains circulating in wild boars in south-central Italy. Transbound. Emerg. Dis. 65, e25–e31.
333 https://doi.org/10.1111/tbed.12661
334 Aprea, G., Scattolini, S., D'Angelantonio, D., Chiaverini, A., Di Lollo, V., Olivieri, S., Marcacci, M.,

335 Mangone, I., Salucci, S., Antoci, S., Cammà, C., Di Pasquale, A., Migliorati, G., Pomilio, F., 2020.
336 Whole Genome Sequencing Characterization of HEV3-e and HEV3-f Subtypes among the Wild
337 Boar Population in the Abruzzo Region, Italy: First Report. Microorganisms 8, 1393.
338 https://doi.org/10.3390/microorganisms8091393
339 Bandelt, H.J., Forster, P., Röhl, A., 1999. Median-joining networks for inferring intraspecific
340 phylogenies. Mol. Biol. Evol. 16, 37–48.
341 https://doi.org/10.1093/oxfordjournals.molbev.a026036
342 Brayne, A.B., Dearlove, B.L., Lester, J.S., Kosakovsky Pond, S.L., Frost, S.D.W., 2017. Genotype-
343 Specific Evolution of Hepatitis E Virus. J. Virol. 91, 2241–2257.
344 https://doi.org/10.1128/JVI.02241-16/ASSET/6F9DC4B3-7537-41D2-B682-
345 2BB0BDB6099D/ASSETS/GRAPHIC/ZJV9991825400004.JPEG
346 Clement, M., Posada, D., Crandall, K.A., 2000. TCS: A computer program to estimate gene
347 genealogies. Mol. Ecol. 9, 1657–1659. https://doi.org/10.1046/j.1365-294X.2000.01020.x
348 Coldiretti, 2021. Coldiretti - CALABRIA [WWW Document]. URL
349 https://calabria.coldiretti.it/news/coldiretti-calabria-laltopiano-della-sila-ma-non-solo-invaso-
350 dai-cinghiali-occorre-moltiplicare-gli-sforzi/ (accessed 12.14.21).
351 Costanzo, N., Sarno, E., Peretti, V., Ciambrone, L., Casalinuovo, F., Santoro, A., 2015. Serological
352 and molecular investigation of swine hepatitis e virus in pigs raised in southern Italy. J. Food
353 Prot. 78, 2099–2102. https://doi.org/10.4315/0362-028X.JFP-15-159
354 De Sabato, L., Amoroso, M.G., Ianiro, G., Esposito, C., De Grossi, L., Fusco, G., Barone, A., Martini,
355 E., Ostanello, F., Di Bartolo, I., 2020. Detection of Hepatitis E Virus in Livers and Muscle
356 Tissues of Wild Boars in Italy. Food Environ. Virol. 12, 1–8. https://doi.org/10.1007/s12560-
357 019-09405-0
358 De Sabato, L., Lemey, P., Vrancken, B., Bonfanti, L., Ceglie, L., Vaccari, G., Di Bartolo, I., 2018a.

359 Proposal for a new subtype of the zoonotic genotype 3 Hepatitis E virus: HEV-3l. Virus Res.
360 248, 1–4. https://doi.org/10.1016/J.VIRUSRES.2018.02.010
361 De Sabato, L., Ostanello, F., De Grossi, L., Marcario, A., Franzetti, B., Monini, M., Di Bartolo, I.,
362 2018b. Molecular survey of HEV infection in wild boar population in Italy. Transbound. Emerg.
363 Dis. 65, 1749–1756. https://doi.org/10.1111/tbed.12948
364 Di Bartolo, I. Di, Diez-Valcarce, M., Vasickova, P., Kralik, P., Hernandez, M., Angeloni, G., Ostanello,
365 F., Bouwknegt, M., Rodríguez-Lázaro, D., Pavlik, I., Ruggeri, F.M., 2012. Hepatitis E Virus in
366 Pork Production Chain in Czech Republic, Italy, and Spain, 2010 - Volume 18, Number 8—
367 August 2012 - Emerging Infectious Diseases journal - CDC. Emerg. Infect. Dis. 18, 1282–1289.
368 https://doi.org/10.3201/EID1808.111783
369 Di Cola, G., Fantilli, A.C., Pisano, M.B., Ré, V.E., 2021. Foodborne transmission of hepatitis A and
370 hepatitis E viruses: A literature review. Int. J. Food Microbiol. 338, 108986.
371 https://doi.org/10.1016/j.ijfoodmicro.2020.108986
372 Di Pasquale, S., De Santis, P., La Rosa, G., Di Domenico, K., Iaconelli, M., Micarelli, G., Martini, E.,
373 Bilei, S., De Medici, D., Suffredini, E., 2019. Quantification and genetic diversity of Hepatitis E
374 virus in wild boar (Sus scrofa) hunted for domestic consumption in Central Italy. Food
375 Microbiol. 82, 194–201. https://doi.org/10.1016/j.fm.2019.02.005
376 Doceul, V., Bagdassarian, E., Demange, A., Pavio, N., 2016. Zoonotic hepatitis E virus: Classification,
377 animal reservoirs and transmission routes. Viruses 8, 1–24.
378 https://doi.org/10.3390/v8100270
379 Drexler, J.F., Seelen, A., Corman, V.M., Fumie Tateno, A., Cottontail, V., Melim Zerbinati, R., Gloza-
380 Rausch, F., Klose, S.M., Adu-Sarkodie, Y., Oppong, S.K., Kalko, E.K. V., Osterman, A., Rasche,
381 A., Adam, A., Muller, M.A., Ulrich, R.G., Leroy, E.M., Lukashev, A.N., Drosten, C., 2012. Bats
382 Worldwide Carry Hepatitis E Virus-Related Viruses That Form a Putative Novel Genus within

the Family Hepeviridae. J. Virol. 86, 9134–9147. https://doi.org/10.1128/jvi.00800-12

Forzan, M., Pacini, M.I., Periccioli, M., Mazzei, M., 2021. Hepatitis E Virus RNA Presence in Wild Boar Carcasses at Slaughterhouses in Italy. Anim. an open access J. from MDPI 11. https://doi.org/10.3390/ANI11061624

Gouy, M., Guindon, S., Gascuel, O., 2010. Sea view version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Mol. Biol. Evol. 27, 221–224. https://doi.org/10.1093/molbev/msp259

Hepatitis E Virus Genotyping Tool [WWW Document], n.d. URL https://www.rivm.nl/mpf/typingtool/hev/ (accessed 1.3.22).

Huang, F., Li, Y., Yu, W., Jing, S., Wang, J., Long, F., He, Z., Yang, C., Bi, Y., Cao, W., Liu, C., Hua, X., Pan, Q., 2016. Excretion of infectious hepatitis E virus into milk in cows imposes high risks of zoonosis. Hepatology 64, 350–359. https://doi.org/10.1002/HEP.28668/SUPPINFO

iTOL: Interactive Tree Of Life [WWW Document], n.d. URL https://itol.embl.de/ (accessed 2.23.22).

Johne, R., Dremsek, P., Reetz, J., Heckel, G., Hess, M., Ulrich, R.G., 2014. Hepeviridae: An expanding family of vertebrate viruses. Infect. Genet. Evol. 27, 212–229. https://doi.org/10.1016/j.meegid.2014.06.024

Kamar, N., Izopet, J., Pavio, N., Aggarwal, R., Labrique, A., Wedemeyer, H., Dalton, H.R., 2017. Hepatitis E virus infection. Nat. Rev. Dis. Prim. 3. https://doi.org/10.1038/nrdp.2017.86

Katoh, K., Rozewicki, J., Yamada, K.D., 2018. MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. Brief. Bioinform. 20, 1160–1166. https://doi.org/10.1093/bib/bbx108

King, N.J., Hewitt, J., Perchec-Merien, A.M., 2018. Hiding in Plain Sight? It's Time to Investigate Other Possible Transmission Routes for Hepatitis E Virus (HEV) in Developed Countries. Food Environ. Virol. 10, 225–252. https://doi.org/10.1007/s12560-018-9342-8

La Bella, G., Basanisi, M.G., Nobili, G., Terio, V., Suffredini, E., La Salandra, G., 2021. First Report of Hepatitis E Virus in Shellfish in Southeast Italy. Appl. Sci. 11, 1–6. https://doi.org/10.3390/app11010043

La Rosa, G., Proroga, Y.T.R., De Medici, D., Capuano, F., Iaconelli, M., Della Libera, S., Suffredini, E., 2018. First Detection of Hepatitis E Virus in Shellfish and in Seawater from Production Areas in Southern Italy. Food Environ. Virol. 10, 127–131. https://doi.org/10.1007/s12560-017-9319-z

leafletR: Interactive Web-Maps Based on the Leaflet JavaScript Library. R package version 0.4-0 [WWW Document], n.d. URL http://cran.r-project.org/package=leafletR (accessed 1.3.22).

Leigh, J.W., Bryant, D., 2015. POPART: Full-feature software for haplotype network construction. Methods Ecol. Evol. 6, 1110–1116. https://doi.org/10.1111/2041-210X.12410

Letunic, I., Bork, P., 2019. Interactive Tree Of Life (iTOL) v4: recent updates and new developments. Nucleic Acids Res. 47. https://doi.org/10.1093/NAR/GKZ239

Martelli, F., Caprioli, A., Zengarini, M., Marata, A., Fiegna, C., Di Bartolo, I., Ruggeri, F.M., Delogu, M., Ostanello, F., 2008. Detection of Hepatitis E virus (HEV) in a demographic managed wild boar (Sus scrofa scrofa) population in Italy. Vet. Microbiol. 126, 74–81. https://doi.org/10.1016/j.vetmic.2007.07.004

Montagnaro, S., De Martinis, C., Sasso, S., Ciarcia, R., Damiano, S., Auletta, L., Iovane, V., Zottola, T., Pagnini, U., 2015. Viral and Antibody Prevalence of Hepatitis E in European Wild Boars (Sus scrofa) and Hunters at Zoonotic Risk in the Latium Region. J. Comp. Pathol. 153, 1–8. https://doi.org/10.1016/J.JCPA.2015.04.006

Montone, A.M.I., De Sabato, L., Suffredini, E., Alise, M., Zaccherini, A., Volzone, P., Di Maro, O., Neola, B., Capuano, F., Di Bartolo, I., 2019. Occurrence of HEV-RNA in Italian Regional Pork and Wild Boar Food Products. Food Environ. Virol. 11, 420–426.

https://doi.org/10.1007/s12560-019-09403-2

Moor, D., Liniger, M., Baumgartner, A., Felleisen, R., 2018. Screening of Ready-to-Eat Meat Products for Hepatitis E Virus in Switzerland. Food Environ. Virol. 10, 263–271. https://doi.org/10.1007/S12560-018-9340-X

Mulder, A.C., Kroneman, A., Franz, E., Vennema, H., Tulen, A.D., Takkinen, J., Hofhuis, A., Adlhoch, C., Aberle, S., Subissi, L., Suin, V., Midgley, S., Kuznetsova, T., Izopet, J., Pavio, N., Baechlein, C., Baylisa, S.A., Corman, V.M., Fabera, M., Johne, R., Kamp, C., Wenzel, J.J., Coughlan, S., Di Bartolo, I., Bruni, R., Ciccaglionea, A.R., Garbuglia, A.R., Suffredini, E., Boxman, I., Hogema, B., van der Poel, W., Zaaijera, H., Sousaa, R. de, Velebit, B., Avellóna, A., Buti, M., Girones, R., Quer, J., Widén, F., Norder, H., Nyström, K., Bachofen, C., Sahli, R., Ijaza, S., Treagus, S., Kulka, M., Rizzi, V., 2019. HEVnet: A one health, collaborative, interdisciplinary network and sequence data repository for enhanced hepatitis e virus molecular typing, characterisation and epidemiological investigations. Eurosurveillance 24, 1–6. https://doi.org/10.2807/1560-7917.ES.2019.24.10.1800407

Nicot, F., Dimeglio, C., Migueres, M., Jeanne, N., Latour, J., Abravanel, F., Ranger, N., Harter, A., Dubois, M., Lameiras, S., Baulande, S., Chapuy-Regaud, S., Kamar, N., Lhomme, S., Izopet, J., 2021. Classification of the Zoonotic Hepatitis E Virus Genotype 3 Into Distinct Subgenotypes. Front. Microbiol. 11, 1–8. https://doi.org/10.3389/fmicb.2020.634430

Nicot, F., Jeanne, N., Roulet, A., Lefebvre, C., Carcenac, R., Manno, M., Dubois, M., Kamar, N., Lhomme, S., Abravanel, F., Izopet, J., 2018. Diversity of hepatitis E virus genotype 3. Rev. Med. Virol. 28, 1–9. https://doi.org/10.1002/rmv.1987

O'Hara, Z., Crossan, C., Craft, J., Scobie, L., 2018. First Report of the Presence of Hepatitis E Virus in Scottish-Harvested Shellfish Purchased at Retail Level. Food Environ. Virol. 10, 217–221. https://doi.org/10.1007/s12560-018-9337-5

455 Oeser, C., Vaughan, A., Said, B., Ijaz, S., Tedder, R., Haywood, B., Warburton, F., Charlett, A., Elson,
456 R., Morgan, D., 2019. Epidemiology of hepatitis e in england and wales: A 10-year
457 retrospective surveillance study, 2008-2017. J. Infect. Dis. 220, 802–810.
458 https://doi.org/10.1093/infdis/jiz207
459 Okamoto, H., 2007. Genetic variability and evolution of hepatitis E virus. Virus Res. 127, 216–228.
460 https://doi.org/10.1016/J.VIRUSRES.2007.02.002
461 Oliveira-Filho, E.F., Bank-Wolf, B.R., Thiel, H.J., König, M., 2014. Phylogenetic analysis of hepatitis E
462 virus in domestic swine and wild boar in Germany. Vet. Microbiol. 174, 233–238.
463 https://doi.org/10.1016/J.VETMIC.2014.09.011
464 Palombieri, A., Tsachev, I., Sarchese, V., Fruci, P., Profio, F. Di, Pepovich, R., Baymakova, M.,
465 Marsilio, F., Martella, V., Martino, B. Di, 2021. veterinary sciences A Molecular Study on
466 Hepatitis E Virus (HEV) in Pigs in Bulgaria.
467 Pavia, G., Gioffrè, A., Pirolo, M., Visaggio, D., Clausi, M.T., Gherardi, M., Samele, P., Ciambrone, L.,
468 Di Natale, R., Spatari, G., Visca, P., Casalinuovo, F., 2021. Seroprevalence and phylogenetic
469 characterization of hepatitis E virus in pig farms in Southern Italy. Prev. Vet. Med. 194.
470 https://doi.org/10.1016/J.PREVETMED.2021.105448
471 Pierini, I., Di Bartolo, I., Manuali, E., Pirani, S., Bazzucchi, M., Moscati, L., De Mia, G.M.,
472 Giammarioli, M., 2021. Hepatitis E virus (HEV) genotype 3 diversity: Identification of a novel
473 HEV subtype in wild boar in Central Italy. Transbound. Emerg. Dis. 68, 2121–2129.
474 https://doi.org/10.1111/tbed.13860
475 Price, M.N., Dehal, P.S., Arkin, A.P., 2010. FastTree 2--approximately maximum-likelihood trees for
476 large alignments. PLoS One 5. https://doi.org/10.1371/JOURNAL.PONE.0009490
477 Ricci, A., Allende, A., Bolton, D., Chemaly, M., Davies, R., Fernandez Escamez, P.S., Herman, L.,
478 Koutsoumanis, K., Lindqvist, R., Nørrung, B., Robertson, L., Ru, G., Sanaa, M., Simmons, M.,

Skandamis, P., Snary, E., Speybroeck, N., Ter Kuile, B., Threlfall, J., Wahlström, H., Di Bartolo, I., Johne, R., Pavio, N., Rutjes, S., van der Poel, W., Vasickova, P., Hempen, M., Messens, W., Rizzi, V., Latronico, F., Girones, R., 2017. Public health risks associated with hepatitis E virus (HEV) as a food-borne pathogen. EFSA J. 15. https://doi.org/10.2903/J.EFSA.2017.4886

Rivero-Juarez, A., Risalde, M.A., Frias, M., García-Bocanegra, I., Lopez-Lopez, P., Cano-Terriza, D., Camacho, A., Jimenez-Ruiz, S., Gomez-Villamandos, J.C., Rivero, A., 2018. Prevalence of hepatitis E virus infection in wild boars from Spain: a possible seasonal pattern? BMC Vet. Res. 2018 141 14, 1–6. https://doi.org/10.1186/S12917-018-1377-4

Rose, N., Lunazzi, A., Dorenlor, V., Merbah, T., Eono, F., Eloit, M., Madec, F., Pavio, N., 2011. High prevalence of Hepatitis E virus in French domestic pigs. Comp. Immunol. Microbiol. Infect. Dis. 34, 419–427. https://doi.org/10.1016/J.CIMID.2011.07.003

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537–7541. https://doi.org/10.1128/AEM.01541-09

SEIEVA, 2020. Bullet 6 - Update 2019, March 2020.

Smith, D.B., Izopet, J., Nicot, F., Simmonds, P., Jameel, S., Meng, X.-J., Norder, H., Okamoto, H., van der Poel, W.H.M., Reuter, G., Purdy, M.A., 2020. Update: proposed reference sequences for subtypes of hepatitis E virus (species Orthohepevirus A). J. Gen. Virol. 101, 692–698. https://doi.org/10.1099/jgv.0.001435

Stroffolini, T., Rapicetta, M., Chionne, P., Esvan, R., Madonna, E., Lombardo, F., Toccaceli, F., Pisani, G., Ciccaglione, A., Bortolotti, F., 2015. Evidence for the presence of autochthonous (locally acquired) cases of acute hepatitis E virus infections in Italy since the 80s. Eur. J. Intern.

Med. 26, 348–350. https://doi.org/10.1016/j.ejim.2015.03.012

Subissi, L., Peeters, M., Lamoral, S., Klamer, S., Suin, V., Van Gucht, S., 2019. Subtype-specific differences in the risk of hospitalisation among patients infected with hepatitis E virus genotype 3 in Belgium, 2010–2018. Epidemiol. Infect. 147, 0–3. https://doi.org/10.1017/S0950268819001122

Takahashi, M., Nishizawa, T., Sato, H., Sato, Y., Jirintai, Nagashima, S., Okamoto, H., 2011. Analysis of the full-length genome of a hepatitis E virus isolate obtained from a wild boar in Japan that is classifiable into a novel genotype. J. Gen. Virol. 92, 902–908. https://doi.org/10.1099/VIR.0.029470-0

Terio, V., Bottaro, M., Pavoni, E., Losio, M.N., Serraino, A., Giacometti, F., Martella, V., Mottola, A., Di Pinto, A., Tantillo, G., 2017. Occurrence of hepatitis A and E and norovirus GI and GII in ready-to-eat vegetables in Italy. Int. J. Food Microbiol. 249, 61–65. https://doi.org/10.1016/j.ijfoodmicro.2017.03.008

Treagus, S., Wright, C., Baker-Austin, C., Longdon, B., Lowther, J., 2021. The Foodborne Transmission of Hepatitis E Virus to Humans. Food Environ. Virol. 13, 127–145. https://doi.org/10.1007/S12560-021-09461-5

Wenzel, J.J., Preiß, J., Schemmerer, M., Huber, B., Plentz, A., Jilg, W., 2011. Detection of hepatitis E virus (HEV) from porcine livers in Southeastern Germany and high sequence homology to human HEV isolates. J. Clin. Virol. 52, 50–54. https://doi.org/10.1016/J.JCV.2011.06.006

World Health Organization (WHO), 2021. Hepatitis E [WWW Document]. URL https://www.who.int/news-room/fact-sheets/detail/hepatitis-e (accessed 12.14.21).

Wu, N., Abril, C., Hinic, V., Brodard, I., Thür, B., Fattebert, J., Hüssy, D., Ryser-Degiorgis, M.P., 2011. Free-ranging wild boar: A disease threat to domestic PIGS in Switzerland? J. Wildl. Dis. 47, 868–879. https://doi.org/10.7589/0090-3558-47.4.868

Zecchin, B., Schivo, A., Milani, A., Fusaro, A., Zamperin, G., Bellinati, L., Ceglie, L., Natale, A., Bonfanti, L., Cunial, G., Obber, F., Di Bartolo, I., Citterio, C., Monne, I., 2019. Identification of a zoonotic genotype 3 hepatitis E subtype in wildlife in north-eastern Italy. Infect. Genet. Evol. 71, 16–20. https://doi.org/10.1016/j.meegid.2019.03.005

551 **TABLES**

552 **TABLE 1. GEOREFERENTIATION**

553

Numbers of samples	Municipality	Latitude	Longitude
3	**Andali - CZ**	**39,0145**	**16,7693**
30	**Borgia - CZ**	**38,82627**	**16,5077**
3	**Germaneto- CZ**	**38,88579**	**16,5768**
9	**Montauro - CZ**	**38,7498**	**16,5142**
5	**Fossato Serralta - CZ**	**38,9968**	**16,5787**
4	**San Floro - CZ**	**38,839**	**16,5195**
8	**Satriano - Laganosa - CZ**	**38,6797**	**16,5272**
18	**Simeri Crichi - CZ**	**38,9548**	**16,6412**
6	**Squillace - CZ**	**38,7805**	**16,513**

554

555 **Table 1**. Sampling details of wild boars hunted in nine municipalities of Calabria region.

556

557 **TABLE 2. PHYLOGENETIC RESULTS**

558

Haplotype	Samples	Genotype	Subtype (bootstrap%)	Reference Description	Hits/ Identity (%)
Hap_1	ANDALI_1,ANDALI_2,SIMERICRICHI_1,SIMERICRICHI_2,SIMERICRICHI_3,SIMERICRICHI_8, SIMERICRICHI_9,SANFLORO_1,SANFLORO_2 SANFLORO_3	HEV3	HEV3c (72%)	Germany – Sus scrofa (FJ705359)	296/305 (97%)
Hap_2	SIMERICRICHI_4,SIMERICRICHI_5,SIMERICRICHI_6, SIMERICRICHI_7	HEV3	Not assigned	Germany – Sus scrofa (FJ705359)	293/302 (97%)
Hap_3	BORGIA_1,BORGIA_2, BORGIA_3, BORGIA_4	HEV3	HEV3c (74%)	Germany – Sus scrofa (FJ705359)	294/305 (96%)
Hap_4	SQUILLACE_1,SQUILLACE_2,SQUILLACE_3,SQUILLACE_4,SQUILLACE_5	HEV3	HEV3c (70%)	Germany – Sus scrofa (FJ705359)	295/305 (97%)

559

560 **Table 2. Summary of dataset**. the list of identical samples included and the assignments to genotype
561 and subtypes inferred by HEVnet was reported for each haplotype. Subtype column includes the
562 bootstrap support values from the phylogenetic trees generated in HEVnet. Inference at subtype

563 level wasn’t possible for Hap_2 (bootstrap < 70%). The column identity reports the match details
564 between the haplotype and the reference best hit.
565
566
567

FIGURES

Figure 1. GEOREFENTIATION OF HEV-POSITIVE WILD BOARS

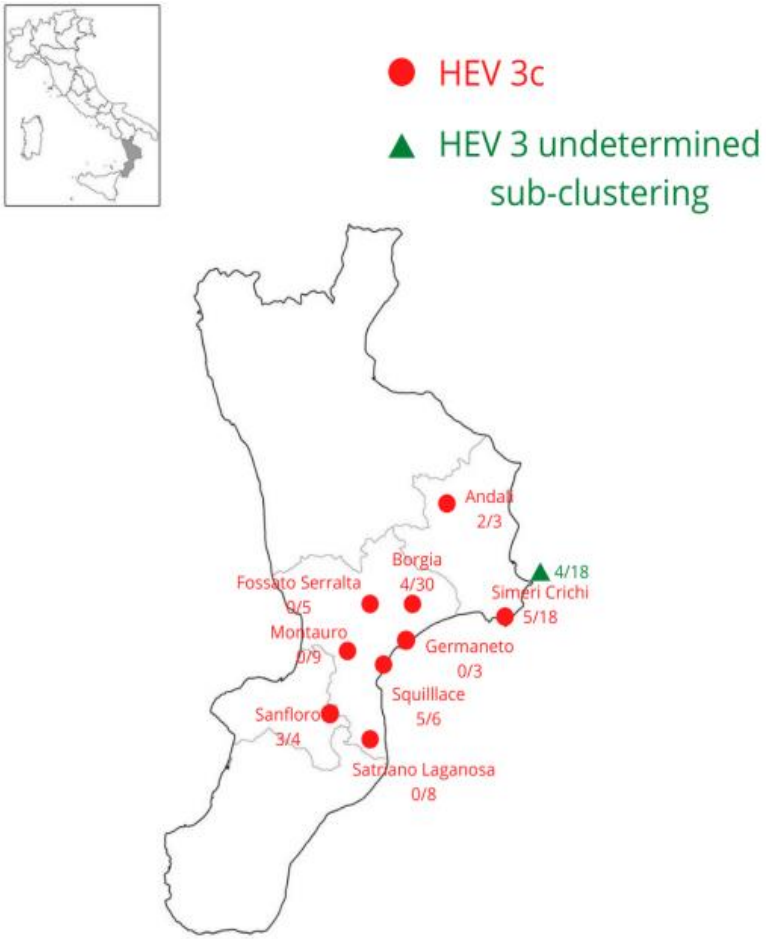


Figure 1: The map shows the municipalities of Catanzaro's province where samples were collected, and the number of HEV-positive samples. Symbols indicate different HEV sub clustering as specified in the legend.

Figure 2. SAMPLE MAP AND HAPLOTYPES

Figure 2: Map of sampling and corresponding haplotypes found in each municipality. Black circles indicate sites where HEV-positive wild boars were absents.

Figure 3. Maximum Likelihood tree of HEV 3 genotype using the RNA-dependent RNA polymerase.

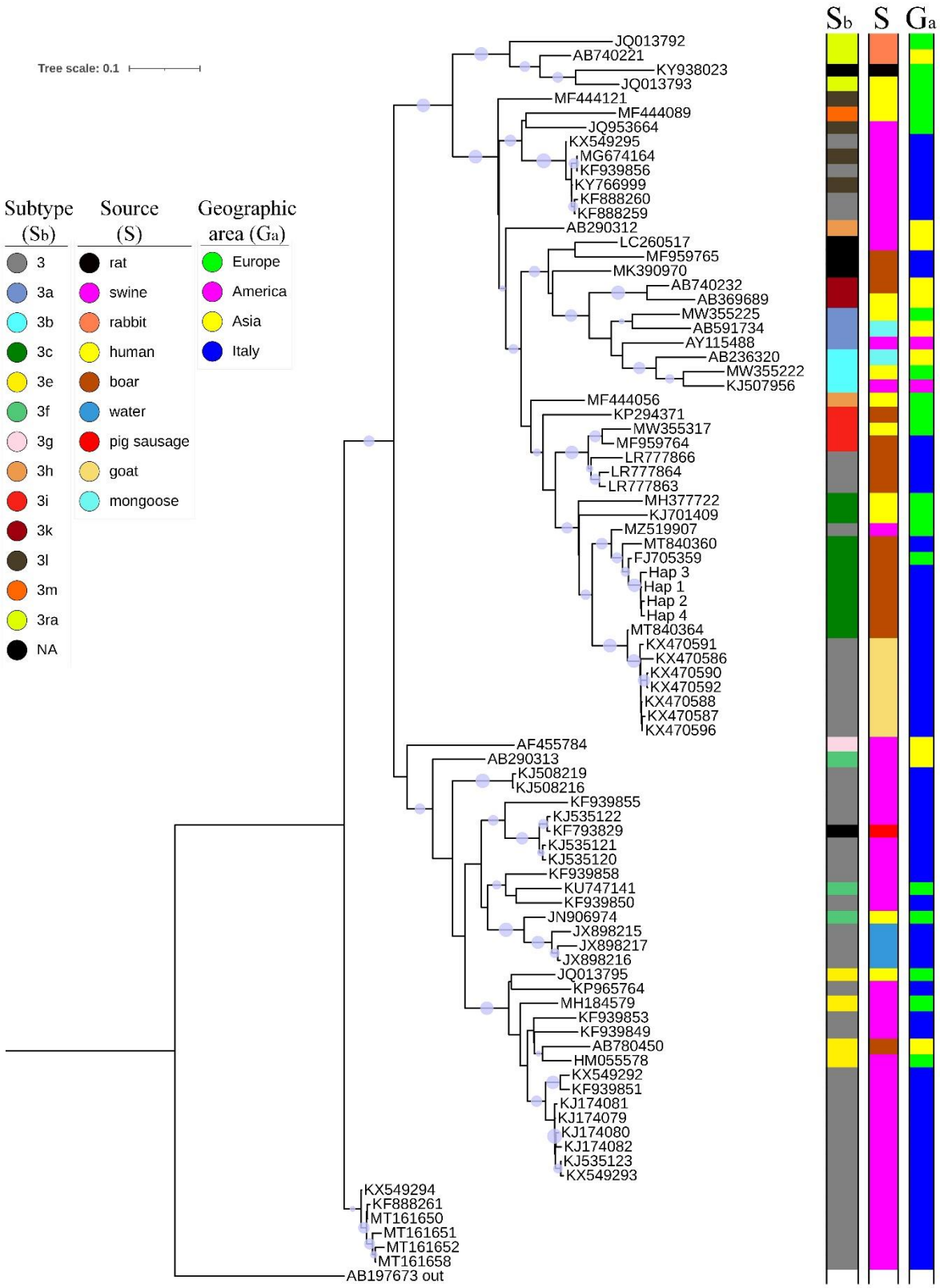
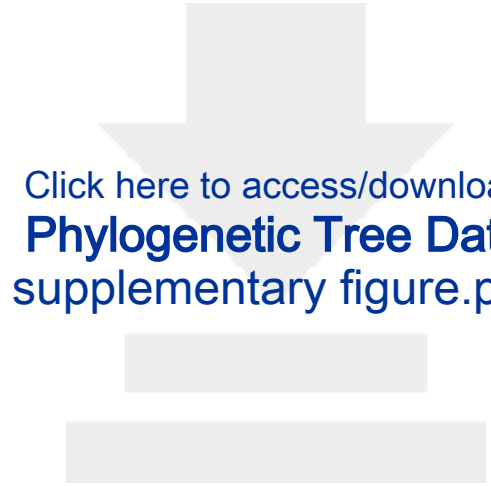


Figure 3. Maximum Likelihood tree of HEV 3 genotype using the RNA-dependent RNA polymerase. The tree included our four haplotypes and all samples available from other Italian regions and sources. Representatives' sequences of other geographic areas were also included and one HEV-4 strain was used as outgroup (AB197673). Bootstraps >70% were showed as symbols (dots) proportional to the values. Metadata associated to sequences were showed by three colour code panels: Subtype (Sb), Source (S) and Geographic area (Ga).

Supplementary figure



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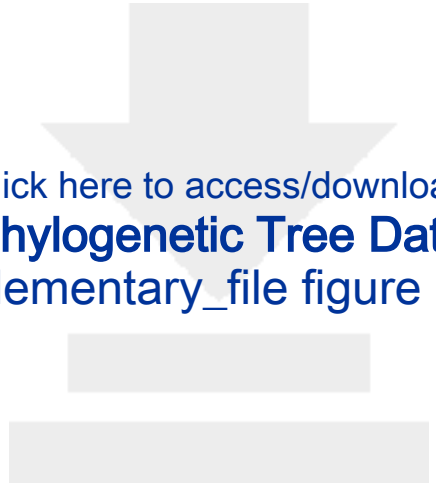
Supplementary file figure 3



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Phylogenetic Tree Data

Supplementary_file figure 3.xlsx

Conflict of Interest and Authorship Conformation Form

Please check the following as appropriate:

X All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.

X This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.

X The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript

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