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Hepatitis E virus strains infecting Swedish domestic pigs are unique for each pig farm and remain in the farm for at least 2 years

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Abstract

Hepatitis E virus (HEV) genotype 3 (HEV3) is distributed globally and infects both humans and animals, mainly domestic pigs and wild boars, which are the major reservoirs. In this study, the prevalence of HEV among Swedish pigs was investigated by HEV RNA analysis in 363 faecal samples from 3-month-old piglets sampled twice (2013 and 2014) in 30 Swedish pig farms. Four different types of farms were investigated; organic, conventional closed (keeping the sow), satellites in a sow pool (conventional farms sharing sows) and conventional non-closed farms (purchasing gilts). More than two-thirds (77%) of the farms had HEV-infected pigs. HEV RNA was found in faeces from 79 pigs (22%). Partial ORF1 could be sequenced in 46 strains. Phylogenetic analysis revealed a unique HEV3 strain for each farm. Strains sampled more than a year apart from the same farm were closely related, indicating that the same HEV strain is present for several years on the farm. Despite that only 4% of the Swedish pig farms were investigated, two farms had strains similar to those from humans, another had strains similar to wild boar HEV. The uniqueness of strains from each farm indicates a possibility to identify a source of infection down to farm level. This knowledge may be used by the farms to investigate the effectiveness of good hygiene routines to reduce the amount of HEV and thus the infection risk in the farm, and for Swedish public health authorities to identify cases of HEV transmissions from consumption of uncooked pork.

KEYWORDS
anti-HEV, HEV, HEV RNA, zoonosis

1 | INTRODUCTION

Hepatitis E virus (HEV) is a non-enveloped RNA virus in the Hepeviridae family. Strains infecting humans belong to the genus *Orthohepevirus A* (Purdy et al., 2017) and are classified into five genotypes HEV1-4, and 7. The infection mainly has faecal-oral spread, but blood-borne transmission also occurs (Kamar et al., 2017; Kumar, Subhadra, Singh, & Panda, 2013). HEV1 and HEV2 are human specific and associated with large waterborne outbreaks in developing countries with poor sanitation mainly in Asia and Africa. Pregnant women infected with HEV1 may develop a severe form of hepatitis with up to 25% mortality (Aggarwal, 2011; Kamar et al., 2017). Domestic pigs and wild boars are the major reservoirs for HEV3 and HEV4 and may transmit the virus to humans and between each other, whereas for HEV7 the reservoir is camel

(Doceul, Bagdassarian, Demange, & Pavio, 2016). HEV3 and 4 are associated with sporadic infections in humans, often with unclear transmission routes (Purdy & Khudyakov, 2011). However, zoonosis through ingestion of undercooked meat/liver from swine, deer and wild boar has been shown for HEV3 (Choi et al., 2013; Colson et al., 2010; Guillois et al., 2016; Li et al., 2005; Meng, 2011; Takahashi, Kitajima, Abe, & Mishiro, 2004). This genotype is found worldwide while HEV4 is commonly found in Asia, but has also been isolated in Europe (Bouamra et al., 2014; Colson et al., 2012; Midgley, Vestergaard, Dalgaard, Enggaard, & Fischer, 2014). Most human HEV infections are self-limiting, however, chronic infections may occur in immunosuppressed patients and are associated with progressive liver damage (Fujiwara et al., 2014). What determines the severity of the infection is still unclear.

In Sweden, the seroprevalence of immunoglobulin G (IgG) against HEV (anti-HEV IgG) is about 17% in the general population, which is comparable to neighbouring countries, like Germany and Norway, with a seroprevalence ranging from 13.5% to 16.8% (Faber et al., 2012; Lange et al., 2017), and at least one out of 7,986 blood donations is positive for HEV RNA (Baylis, Gartner, Nick, Ovemyr, & Blumel, 2012; Norder et al., 2016). The number of notified HEV cases is increasing annually in Sweden, from seven cases in 2008 to 42 in 2017, but they are still very few in relation to the high seroprevalence suggesting that many infections have a subclinical course. This has been shown during an outbreak in France where about 70% of the infected persons did not develop disease (Guillois et al., 2016). However, the few number of Swedish cases reported annually suggests that clinical HEV infections may also be underdiagnosed.

All endemic Swedish HEV infections are caused by HEV3 strains. This genotype is classified into nine subtypes, 3a-c, 3e-j. These nine subtypes genetically form two major clades in the phylogenetic analysis, clade 3I with subtypes 3abchij and clade 3II with 3efg (Norder et al., 2009; Widén et al., 2010). Swedish strains from most human cases with acute HEV and HEV from pigs and wild boar belong to subtype 3f in clade 3II (Norder et al., 2009; Roth et al., 2016). Zoonotic transmission of HEV by direct or indirect contact with infected animals, as pigs, may therefore be a further factor (Huang, Huang, Wagner, Chen, & Lu, 2018; Krumbholz et al., 2012, 2014; Sommerkorn, Schauer, Schreiner, Fickenscher, & Krumbholz, 2017) contributing to the surprisingly high seroprevalence in Sweden.

In Sweden there are about 900 pig farms, each with 2,000–3,000 pigs. Each sow can give birth to two litters annually, with each litter consisting of 12–14 piglets. The piglets stay with their mother for 4–6 weeks and are thereafter transferred to another section in the farm together with pigs of the same age, where they stay until they are 3 months old. Their maternal antibodies disappear at the age of 1–2 months (Feng et al., 2011; Meng et al., 1997). At the age of 3 months the pigs are transferred to the fattening stables where they stay for about another 3 months before slaughter. It is in these fattening stables most pigs get infected by HEV and they excrete the virus for 2–11 weeks (Kanai et al., 2010; Kasorndorkbua et al., 2004; Meng et al., 1998). The seroprevalence of HEV in pigs ranges from 42.7% up to 80% in European countries, and it is increasing with the age of the pig (Costanzo et al., 2015; Dremsek et al., 2013; Ivanova et al., 2015; Krumbholz et al., 2013; Lange et al., 2017). By the time of slaughter most pigs have cleared the HEV infection, but HEV RNA has been detected in between 0% and 42% faecal samples from slaughter-aged pigs (Kaba et al., 2009; Leblanc et al., 2007).

This study was conducted to examine the prevalence of HEV among Swedish pigs and if virus strains from pig farms also may be identified from infected humans. The purpose was also to investigate if each farm's unique HEV strain previously identified (Widén et al., 2010) remained in the farm for a longer period or if the HEV strain in the farms changes genetically over time by sampling pigs from the same farm twice, with each sampling occasion 1 year apart.

2 | MATERIALS AND METHODS

2.1 | Materials

Faecal samples from 363 pigs aged about 3 months were collected in the growing stables in 30 Swedish pig farms were investigated. The pig farms were distributed in central and southern Sweden (Figure 1). Six pigs were sampled in each farm, with 12–17 months between the samplings. The first sampling was carried out between April and early June 2013 and the second sampling was from late April to September 2014 in the same farms.

Four different types of pig farms were investigated, organic farms (nine farms), conventional closed farms with their own recruitment of gilts (seven farms), conventional satellites in a sow pool sharing sows (seven farms) and conventional non-closed farms purchasing gilts (seven farms; Table 1). The animals are kept together in groups of the same age and moved from department to department in age-sectioned systems in the farms. It is 'all in all out' breeding with emptying of pigs and careful washing of the stables before new groups are introduced. The animals go on concrete floors with a small part of the surface having draining gaps. Mostly straw but shavings and peat are also used for scattering on the floors. In the organic farming all pigs go out for at least 5 months during the summer time/grazing period.

Hepatitis E virus strains in serum samples from 36 Swedish human cases with endemic hepatitis E of unknown source of infection in 2011–2017 were sequenced for this study. The study is approved by the Regional Ethical Review Board in Gothenburg, with registration number 737-12.

2.2 | RNA detection

2.2.1 | Nucleic acid extraction

About 1 g of stool sample was homogenized in tubes containing glass beads in phosphate-buffered saline (pH 7.4). The suspensions were centrifuged at 2,700 *g* for 10 min.

Two hundred and fifty microlitre of the stool suspensions were added to 2 ml of lysis buffer and incubated for 10 min at room temperature. Nucleic acids were extracted by using NucliSens® easyMag® instrument and reagents (NucliSENS easyMag; bioMérieux, SA, France) and eluted in 110 µl extraction buffer according to the manufacturers' protocol.

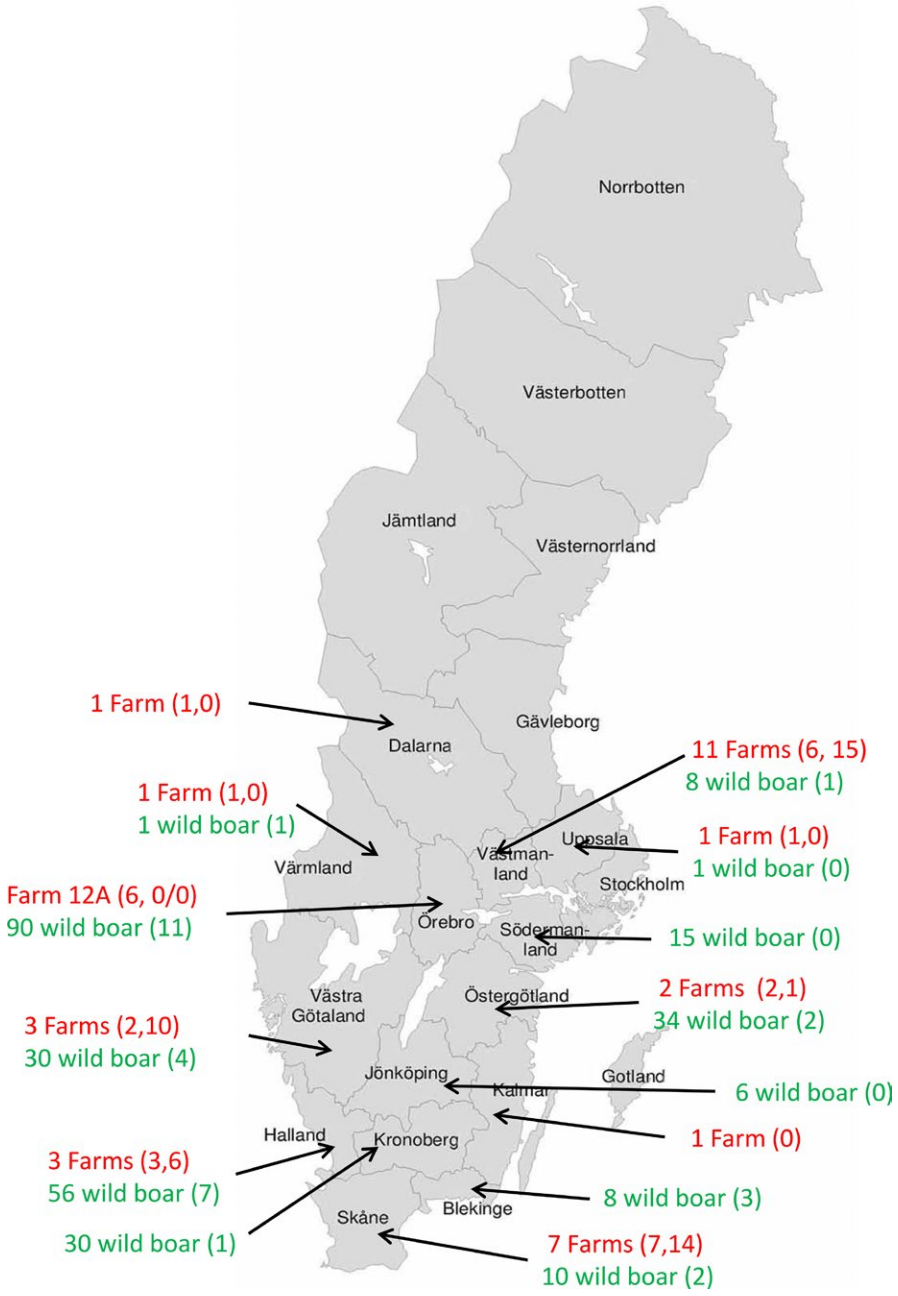


FIGURE 1 Map of Sweden showing the counties and number of farms per county (in red) where samples were collected for this study in 2013/2014 and from farm 12A in 2007. The figures in parentheses depict the number of farms with positive piglets, the number of farms with strains that could be sequenced. The number and region of hunting of 289 sampled wild boars shot in Sweden between 2007 and 2016 and previously published are shown in green (Roth et al., 2016; Widén et al., 2010). The figures in parenthesis depict the number of PCR positive wild boars

2.2.2 | RT-qPCR assay

The extracted nucleic acids from the stool samples were analysed for HEV RNA in a one-step TaqMan RT-qPCR assay targeting the ORF2/3 region of HEV1- HEV4 as previously described (Roth et al., 2016; Widén et al., 2010). The amplification and multicomponent plots were monitored and only those samples with delta Rn above the threshold and increased reporting dye were considered reactive.

2.2.3 | cDNA synthesis

cDNA synthesis was carried out in a 20 µl reaction mix containing 5 µl extracted RNA as previously described (Roth et al., 2016).

2.2.4 | PCR amplification for sequencing

To amplify HEV3 strains for sequencing, a semi-nested PCR in ORF1 was performed in a 50 µl reaction mix with 5 µl cDNA as template as previously described (Norder et al., 2009; Roth et al., 2016).

2.2.5 | Sequencing

The sequencing of the amplified PCR products was carried out with the same primers as in the nested PCR with the Big Dye Ready Reaction kit 3.1 (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions as previously described (Roth et al., 2016;

TABLE 1 Type and number of farms and number of piglets investigated, and number of farms with HEV excreting piglets

Type of farm	Number of farms	Number of faecal samples from piglets/year		Sub total	Number of farms with HEV RNA positive piglets per year		Total number of farms with positive piglets (%)
		2013	2014		2013 (%)	2014 (%)	
Non-closed purchased gilt	7	42	42	84	6 (86)	5 (71)	7 (100)
Closed	7	42	45	87	3 (43)	3 (43)	5 (71)
Pool sharing sows	7	42	42	84	3 (43)	3 (43)	4 (57)
Organic	9	54	54	108	5 (56)	3 (33)	7 (78)
Total	30	180	183	363	17 (57)	14 (47)	23 (77)

Widén et al., 2010), and the sequences were analysed with the 3130xl Genetic Analyzer (Applied Biosystems).

2.2.6 | Phylogenetic analysis

The sequences obtained were analysed in the program DNAStar SeqMan in the DNAStar program package version 10.1.2 (DNA Star Inc, Madison, WI). The sequences were aligned with the corresponding region of 347 sequences representing HEV3 obtained from GenBank, and one moose HEV strain (Lin, Norder, Uhlhorn, Belak, & Widen, 2014) to root the tree. Phylogenetic analysis was carried out with the PHYLIP package version 3.65 (Felsenstein, 1996). Evolutionary distances were calculated using the F84 algorithm in the DNADIST program with a transition/transversion ratio of 4.29. Boot strap analysis was performed on 1,000 replicas with the Seqboot program of the PHYLIP package. Phylogenetic trees were constructed using the unweight pair-group method using arithmetic averages (UPGMA) and the neighbour-joining method in the NEIGHBOR program of the PHYLIP package. The trees were visualized using the program TREEVIEW, version 1.6.6. All sequences obtained in the study are deposited in GenBank with accession numbers MK582523-MK582474.

2.2.7 | Statistical analysis

Statistical calculations were performed with Fisher's test, using GraphPad Prism 6 (http://www.graphpad.com/quickcalcs/). $p < 0.05$ was considered significant.

3 | RESULTS

Pigs excreting HEV were found in 77% of all farms regardless of type during the 2 years of sampling (Table 1; Figure 1). The number of infected pigs in each farm and year of sampling is given in Table 2. Eight of the farms had pigs excreting HEV both years, 15 farms had positive pigs at one occasion, while there was no positive faecal sample identified from seven farms. There was no difference in number of farms with HEV infected pigs and type of farm.

There were in total 79 pigs (22%) with HEV RNA-positive faecal samples from 23 different farms. Significantly more positive samples were identified in 2013 (51 samples from 17 farms) compared to 2014, when only 28 samples from 14 farms were positive ($p = 0.0033$; Table 2). The largest difference was seen in the nine farms with organic breeding, 21 positive pigs in four farms in 2013 versus 4 positive pigs in three farms in 2014. Only one of these farms had HEV-positive pigs at both sampling occasions.

Partial ORF1 could be amplified and sequenced in 46 (59%) of the 79 HEV RNA-positive samples from the pigs. These samples derived from 14 of the 23 farms with HEV RNA-positive pigs. Phylogenetic analysis of the sequences revealed that each farm had a unique HEV3 strain confirmed with significant boot strap values (Figure 2). Strains from farms from the same county of Sweden did not have genetic similarities and were intermixed with strains from other counties in the phylogenetic tree. There were only two farms, 16 and 18, from the same geographical region that had HEV strains forming a common branch on the phylogenetic tree, which may indicate that these strains have a common ancestor and may have diverged from each other rather recently (Figure 2).

The same unique HEV strain found at the first sampling was also isolated at the second sampling a year later in four of five farms with HEV RNA-positive pigs at both test occasions (Farms 1, 5, 12 and 16), as was found in the previous study with piglets in three farms being infected with the same HEV strain in 2007 and 2009 (Widen et al., 2011). All except one farm (Farm 12) had pigs infected with HEV3 strains belonging to subtype 3f (Figure 2). In Farm 12 a subtype 3e strain was isolated (Figure S1).

The HEV strains from the farms with positive pigs were compared to strains from humans and pigs in the Swedish farms investigated during 2007 and 2009 and from Swedish wild boars (Roth et al., 2016; Widén et al., 2010). The 3e strain from Farm 12 was similar with 85% bootstrap value to a strain from a human infected in Sweden in 2011 (Figure S1).

There was one clade formed by subtype 3f strains from pigs in Farm 20 in 2013 and Farm 12A in 2007 and humans infected in 2002, 2008 and 2016 (Figure 2). Another clade was formed by the strain from Farm 2 in 2014 and strains from wild boars hunted during 2007, 2008 and 2014 in the same geographical region as the farm (Figure 2). Strains from Farms 4 and 29 formed one clade found on the same branch as a strain from a human infected in 2009 and a wild boar strain isolated in 2007.

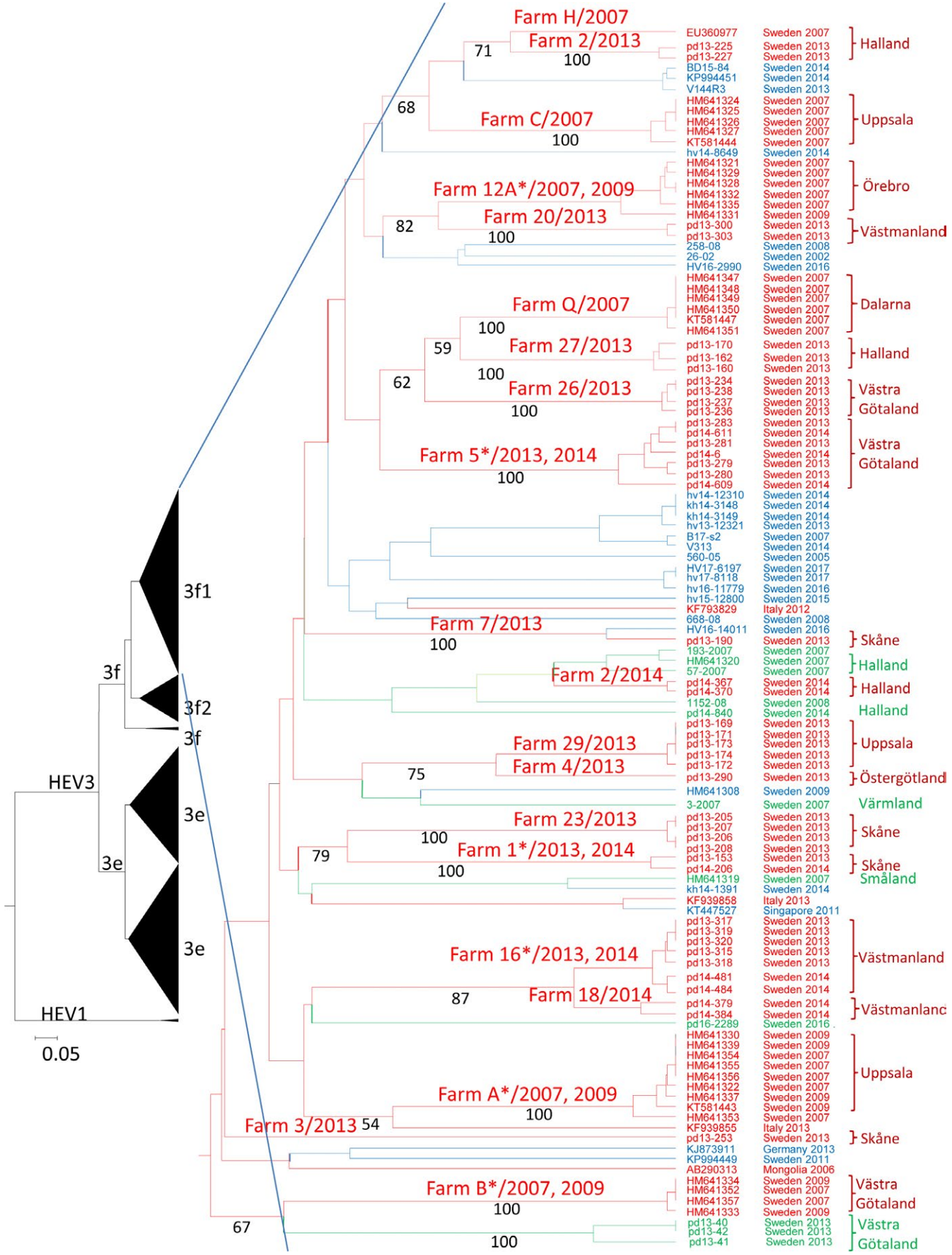
TABLE 2 Number of HEV excreting piglets in each farm at both test occasions, and number of strains sequenced

Farm number	Number HEV RNA-positive piglets/number of analysed		Months between samplings	Total number HEV RNA-positive piglets	Number sequenced strains
	2013	2014			
1	1/6	1/6	13	2/12	2
2	3/6	4/6	14	7/12	4
3	1/6	0/6	15	1/12	1
4	1/6	0/6	14	1/12	1
5	5/6	2/6	16	7/12	6
6	0/6	2/6	15	2/12	
7	1/6	1/6	17	2/12	1
8	0/6	1/6	13	1/12	
9	0/6	0/6	12	0/12	
10	0/6	1/6	13	1/12	
11	0/6	0/6	14	0/12	
12	1/6	3/12	13	4/18	4
13	3/6	0/6	13	3/12	
14	1/6	0/3	13	1/12	
15	0/6	0/6	12	0/12	
16	6/6	2/6	15	8/12	7
17	0/6	1/6	12	1/12	
18	5/6	6/6	14	11/12	2
19	0/6	0/6	15	0/12	
20	2/6	0/6	12	2/12	2
21	0/6	0/6	12	0/12	
22	2/6	0/6	12	2/12	
23	6/6	1/6	14	7/12	4
24	0/6	0/6	17	0/12	
25	0/6	2/6	13	2/12	
26	5/6	0/6	12	5/12	4
27	2/6	0/6	17	2/12	2
28	0/6	1/6	13	1/12	
29	6/6	0/6	17	6/12	6
30	0/6	0/6	14	0/12	
Total	51/180	28/183		79/363	46

4 | DISCUSSION

In this study, a persistent high prevalence of HEV infections in Swedish pigs was revealed through a 2-year investigation of 30 farms in central and southern Sweden. The previously assumed uniqueness of each farm having its own HEV strain was confirmed (Widén et al., 2010). The high incidence of farms with pigs excreting HEV was similar to that described from other European countries, as Italy, Estonia and Finland where 60%–90% of the farms had HEV-infected pigs (Ivanova et al., 2015; Kantala, Heinonen, Oristo, von Bonsdorff, & Maunula, 2015; Monini et al., 2015). These previous studies investigated only six to nine different farms

FIGURE 2 The enlarged branch of HEV3 subtype 3f strains in a UPGMA phylogenetic tree based on 325 nucleotides of partial ORF1 for genotype 3. Bootstrap values from 1,000 replicas are given below the branches. The strains from pigs are given in red, those from wild boars in green and those from humans in blue. The farm designations where the strains were collected are given on the branches and the region of origin of the farms are given at the nodes. The farms in which samples were collected in 2007 and 2009 are indicated with year after the name of the farm. Farms marked with an asterisk indicate farms with strains collected more than a year apart. Accession number and origin of the strains are given at the nodes


Farm H/2007
Farm 2/2013
Farm C/2007
Farm 12A*/2007, 2009
Farm 20/2013
Farm Q/2007
Farm 27/2013
Farm 26/2013
Farm 5*/2013, 2014
Farm 7/2013
Farm 2/2014
Farm 29/2013
Farm 4/2013
Farm 23/2013
Farm 1*/2013, 2014
Farm 16*/2013, 2014
Farm 18/2014
Farm A*/2007, 2009
Farm 3/2013
Farm B*/2007, 2009
HEV3
HEV1
3f1
3f
3f2
3e
3e
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0.05
EU360977 Sweden 2007
pd13-225 Sweden 2013
pd13-227 Sweden 2013
BD15-84 Sweden 2014
KP994451 Sweden 2014
V144R3 Sweden 2013
HM641324 Sweden 2007
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HM641326 Sweden 2007
HM641327 Sweden 2007
KT581444 Sweden 2007
hv14-8649 Sweden 2014
HM641321 Sweden 2007
HM641329 Sweden 2007
HM641328 Sweden 2007
HM641332 Sweden 2007
HM641335 Sweden 2007
HM641331 Sweden 2009
pd13-300 Sweden 2013
pd13-303 Sweden 2013
258-08 Sweden 2008
26-02 Sweden 2002
HV16-2990 Sweden 2016
HM641347 Sweden 2007
HM641348 Sweden 2007
HM641349 Sweden 2007
HM641350 Sweden 2007
KT581447 Sweden 2007
HM641351 Sweden 2007
pd13-170 Sweden 2013
pd13-162 Sweden 2013
pd13-160 Sweden 2013
pd13-234 Sweden 2013
pd13-238 Sweden 2013
pd13-237 Sweden 2013
pd13-236 Sweden 2013
pd13-283 Sweden 2013
pd14-611 Sweden 2014
pd13-281 Sweden 2013
pd14-6 Sweden 2014
pd13-279 Sweden 2013
pd13-280 Sweden 2013
pd14-609 Sweden 2014
hv14-12310 Sweden 2014
kh14-3148 Sweden 2014
kh14-3149 Sweden 2014
hv13-12321 Sweden 2013
B17-s2 Sweden 2007
V313 Sweden 2014
560-05 Sweden 2005
HV17-6197 Sweden 2017
hv17-8118 Sweden 2017
hv16-11779 Sweden 2016
hv15-12800 Sweden 2015
KF793829 Italy 2012
668-08 Sweden 2008
HV16-14011 Sweden 2016
pd13-190 Sweden 2013
193-2007 Sweden 2007
HM641320 Sweden 2007
57-2007 Sweden 2007
pd14-367 Sweden 2014
pd14-370 Sweden 2014
1152-08 Sweden 2008
pd14-840 Sweden 2014
pd13-169 Sweden 2013
pd13-171 Sweden 2013
pd13-173 Sweden 2013
pd13-174 Sweden 2013
pd13-172 Sweden 2013
pd13-290 Sweden 2013
HM641308 Sweden 2009
3-2007 Sweden 2007
pd13-205 Sweden 2013
pd13-207 Sweden 2013
pd13-206 Sweden 2013
pd13-208 Sweden 2013
pd13-153 Sweden 2013
pd14-206 Sweden 2014
HM641319 Sweden 2007
kh14-1391 Sweden 2014
KF939858 Italy 2013
KT447527 Singapore 2011
pd13-317 Sweden 2013
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pd13-315 Sweden 2013
pd13-318 Sweden 2013
pd14-481 Sweden 2014
pd14-484 Sweden 2014
pd14-379 Sweden 2014
pd14-384 Sweden 2014
pd16-2289 Sweden 2016
HM641330 Sweden 2009
HM641339 Sweden 2009
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HM641355 Sweden 2007
HM641356 Sweden 2007
HM641322 Sweden 2007
HM641337 Sweden 2009
KT581443 Sweden 2009
HM641353 Sweden 2007
KF939855 Italy 2013
pd13-253 Sweden 2013
KJ873911 Germany 2013
KP994449 Sweden 2011
AB290313 Mongolia 2006
HM641334 Sweden 2009
HM641352 Sweden 2007
HM641357 Sweden 2007
HM641333 Sweden 2009
pd13-40 Sweden 2013
pd13-42 Sweden 2013
pd13-41 Sweden 2013
Halland
Uppsala
Örebro
Västmanland
Dalarna
Halland
Västra Götaland
Västra Götaland
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Östergötland
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at once occasion and there were often several HEV strains present in the farms. In this study, the same farms were visited twice, and it was shown that the unique farm-specific strain remained in the farm and did not change with time. All HEV strains in this study belonged to HEV3. Most were of subtype 3f, and a few were 3e. This is in accordance with previous studies from Sweden and Italy (Caruso et al., 2015; Widén et al., 2010) whereas studies from other countries demonstrate a more widespread subtype distribution of HEV3 in the pig farms (Oliveira-Filho, Bank-Wolf, Thiel, & Konig, 2014; Rutjes, Lodder, Bouwknegt, & de Roda Husman, 2007; Steyer, Naglic, Mocilnik, Poljsak-Prijatelj, & Poljak, 2011; Vasickova et al., 2009). This difference may be due to differences in production or when the pigs were sampled. If the pigs are kept in the same farm until time of slaughter, they are exposed only to the HEV strain(s) in that farm. However, if the pigs are moved to other farms for fattening, there may be an intermixing of different strains and HEV-free pigs may become infected. In this study the pigs were sampled while still in the growing stage and had not been transferred to another farm. Some Swedish farms keep the pigs until slaughter, while others send them to fattening farms, where they are usually being held together until slaughter. Typing the HEV strains and thereby deploy their genetic uniqueness could be used to indicate if humans are infected with food items based on uncooked pork that have been imported or locally acquired. In addition, knowledge of the unique farm-specific strains could be used by public health authorities to monitor human HEV infections acquired by uncooked pork of Swedish origin, perhaps even down to farm level, since about 75% of all pork consumed in Sweden is produced in the country (Lannhard-Öberg, 2018).

Hepatitis E virus infection among pigs may not be a large problem for the production of pigs, since pigs appear clinically healthy and asymptomatic during the infection (Banks et al., 2004; Di Bartolo et al., 2008; Meng et al., 1997). However, it is estimated that an infected pig may infect eight others during the replicative phase of the infection (Bouwknegt et al., 2008), which may result in transmission of the virus between the pigs for several months in the farm. In Sweden, most of the pig production is indoors and almost all pigs are produced batch-wise, which may hinder the spread of infectious agents between batches or from elder to younger animals. However, HEV can survive for a long time in the environment, such as in faeces or urine from the pigs or in wastewater (Banks et al., 2004; Bouwknegt et al., 2008; Schielke, Filter, Appel, & Johne, 2011; Yugo & Meng, 2013). Factors involved in the transmission of the strain between the pigs in the farms may thus be by direct contact with contaminated material from the previous batch due to incorrect cleaning routines or through contaminated water or feed. The virus may also spread through manure ditches into the environment and water wells (Fernandez-Barredo et al., 2006). Another route may be through infected rats in the surrounding of the farms, since rats infected with HEV from pigs have been reported from the USA (Lack, Volk, & van den Bussche, 2012) and pig farms in Japan (Kanai et al., 2012). Even if transmission from the sow cannot be excluded, it is probably uncommon, since more than 85% of pigs older than 5 months have developed anti-HEV (Di Bartolo, Ponterio, Castellini, Ostanello, & Ruggeri, 2011; Jori et al., 2016; Lange et al., 2017), and only one study from Spain has shown HEV RNA in faecal samples from sows in breeding farms (Fernandez-Barredo et al., 2007). In addition, if the sow was the main source of HEV infection in the farms in this study, the virus strain would most probably change with the herds. In the pools of farms the sow could be sent for breeding to any of the farms in the pool, however, each farm in the pool had its own unique strain regardless of the sow present. This was also shown for the farms buying gilts for the breeding, since these farms had one unique HEV strains regardless of change in sow. The farm-specific HEV strains identified in this study may give indications of the importance to clean the section between each new breeding occasion to minimize the risk of the HEV strain spreading in the new herd. Typing the strains may also indicate if the viability of the viruses would be reduced with time, by investigating how long time that would be needed before introducing a new batch of pigs to reduce transmission of the viruses in the stables.

There was no difference between type of farm and number of farms with HEV-positive pigs in this study. However, the closed farms had fewer HEV-positive pigs per farm during the 2-year period of sampling. This type of production may lower the risks of introducing infectious agents into the herd (Alvasen, Hansson, Emanuelson, & Westin, 2017). The other types of farms had similar number of HEV-excreting pigs during the whole study period. Fewer infected pigs were found in the organic farms during the second year of sampling. This may be a coincidence and must be confirmed by further testing. The organic farms sampled in this study, offer access to natural outdoor behaviour for the pigs during at least 5 months of the year, which may lead to contact with wild animals (Hovi, Sundrum, & Thamsborg, 2003). In this study, we found several similar HEV strains from wild boars and pigs, indicating a zoonotic transmission between them, which is line with a study from Estonia (Ivanova et al., 2015). However, only one of these farms had organic production, and the route for introduction of the wild boar strains into the pig farms needs to be investigated. There may be a significant risk that direct or indirect contacts between wild boars and domestic pigs can introduce other diseases in the herds, such as porcine circovirus, *Yersinia* ssp., *Salmonella* ssp. and *Toxoplasma gondii*, which are all carried by Swedish wild boars (Quintern & Sundrum, 2006; Malmsten, Magnusson, Ruiz-Fons, Gonzalez-Barrio, & Dalin, 2018; Sanno, Rosendal, Aspan, Backhans, & Jacobson, 2018). Therefore, the dissemination of HEV and other diseases between wild boars and domestic pigs needs further investigation.

The increasing number of acute but also chronic human HEV infections underlines the necessity for accurate methods for analysis and genetic comparison of HEV strains to identify routes of transmission, which are almost always unknown for identified endemic cases, and if there are differences in disease progressions caused by certain HEV strains. Phylogenetic analysis of the HEV strains in this study revealed that Swedes both with clinical and subclinical HEV infection were infected with similar HEV strains as

those from Swedish pigs. Most persons have an unknown source of infection, but the similarity of the infecting strains to those from pigs, indicate transmission by contaminated food items. Direct contact with pigs may also be a route of transmission, since the seroprevalence of anti-HEV antibodies has been shown significantly higher in pig farm workers than in controls (Chaussade et al., 2013; Galiana, Fernandez-Barredo, Garcia, Gomez, & Perez-Gracia, 2008). In Sweden a high prevalence of anti-HEV antibodies was found both in 40- to 60-year-old pig farm workers and their controls living nearby (Olsen, Axelsson-Olsson, Thelin, & Weiland, 2006), which indicates that further studies on HEV transmissions are needed.

In summary, HEV strains identified in each pig farm were divergent from the strains in other farms. Some were similar to those from wild boars and others to those from clinical and subclinical HEV-infected individuals. These findings are important for the identification of zoonotic transmission of HEV from domestic pigs to humans as well as between wild boars and domestic pigs. This knowledge can also be used for tracing the source of infection to limit the spread of HEV. This work increases our knowledge of HEV prevalence among Swedish pigs and can be used to monitor, control and, preferably, also limit zoonotic spread of HEV from pork. Continued study of HEV in pigs can be developed into an effective public health tool to reduce the spread of HEV in the society.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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