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Isolation, detection and characterization of swine hepatitis E virus from herds in Costa Rica

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Although swine HEV isolates from North America, Europe, and Asia have been genetically characterized, little is known about the strains presumed to be circulating in Latin America. In this study, seven commercial swine production sites in Costa Rica were surveyed for HEV. Using RT-PCR, with primers located in ORF2, 19/52 fecal samples produced a product of the expected size following two rounds of amplification. Most positive samples were from swine between the ages of 1.5 and 4 months. This study provides documented evidence for the endemicity of HE infections in swine residing in Central America. Through nucleic acid sequencing, isolates were found to be genetically similar, if not identical, with no amino acid substitutions. By comparison of swine and human HEV strains representing all four genotypes and phylogenetic analysis, our isolates closely resembled the US swine and human and other Genotype III strains, with 85–93% nucleic acid identity.

Keywords: hepatitis E virus; zoonoses; public health; disease reservoirs; surveillance; molecular epidemiology

Introduction

Hepatitis E virus (HEV) is the major hepatotrophic agent responsible for sporadic cases of acute viral hepatitis in developing countries of Asia and Africa, and is the known etiological agent of waterborne hepatitis epidemics within these same areas (Emerson and Purcell 2003). Similar to hepatitis A, hepatitis E infections are acute and self-limited with no apparent chronicity or sequelae. Mortality is in the order of 1% except for pregnant women, where the case-fatality rate can reach 27% (Jaiswal et al. 2001; Kumar et al. 2004). In developed countries, including the US, hepatitis E occurs rarely and is typically attributed to travel to an endemic region. However, reports document the existence of autochthonous cases of hepatitis E infections and an unexplained relatively high seroprevalence level of HEV antibodies in otherwise healthy individuals (Mast et al. 1997; Thomas et al. 1997; Meng et al. 2002).

Sero-epidemiological studies indicate that HEV has a wide host range including swine residing in human hepatitis E endemic and non-endemic areas. The discovery of almost

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genetically indistinguishable swine and human strains, in multiple countries, suggest a potential role of swine in the transmission of HEV to humans (Meng 2003). Moreover, direct evidence of zoonotic spread involving the group consumption of undercooked wild boar meat or raw Sika deer meat has been established in two case studies (Tei et al. 2003; Tamada et al. 2004). Other routes of human exposure to swine HEV (sHEV) are uncertain but may include direct and indirect animal contact and exposure to fecal matter. There is only limited information regarding HEV seropositivity among those professionally in contact with swine. Results from one study conducted on swine workers from North Carolina found that nearly 11% (18/165) showed evidence of HEV antibodies (Withers et al. 2002).

Comparative studies of the nucleotide sequences of geographically diverse human HEV strains suggested that at least four genotypes exist (Emerson and Purcell 2003). Strains belonging to Genotype I and II have been well characterized for over a decade and, at the current time, consist solely of strains of human origin from developing countries (Arankalle et al. 2002). Strains of HEV belonging to Genotype III and IV provide a much different natural history, with the existence of human strains from industrialized countries along with swine strains representing both developed and lesser developed areas of the world. One hypothesis, with limited experimental support, is that only swine strains belonging to genotype III or IV can cross species barriers (Meng et al. 1998a, 1998b; Cooper et al. 2005).

Although sHEV prevalence has been studied in North America, Europe, Asia and elsewhere, little is known about HEV circulation in swine from Latin America. Recent published studies conducted with selected Brazilian and Mexican swine populations noted a high level of anti-HEV IgG antibodies ($>80\%$ tested) (Cooper et al. 2005; Vitral et al. 2005). Genetic analysis of the Mexican sHEV strain revealed it to be most similar to strains found in developed countries including the US and not similar to the outbreak strain detected previously in Mexico (Velazquez et al. 1990; Cooper et al. 2005). Importantly, a thorough investigation has not been conducted to examine the prevalence and genetic heterogeneity of HEV circulating in swine populations elsewhere in Central America.

The purpose of this study is two-fold: to document the extent of sHEV infection in Central America and to obtain data on the molecular genetic properties of any detected HEV strains by nucleic acid comparisons with other HEV strains. Accordingly, seven commercial swine production sites in Costa Rica were surveyed for fecal HEV in freshly passed swine feces collected from individual barns. Repeat sampling was done for two farms.

Materials and methods

Study design

Seven commercial farms served by the Veterinary School within the National University of Costa Rica agreed to participate and were surveyed in two visits (August 2001 and March 2002). Farms followed a traditional farrow-to-finish management system in which swine are bred and raised to market weight within the same site. All farms had an onsite liquid waste management system. Farm size varied greatly; Farms 1 and 2 had 20,000 and 10,000 pigs, respectively, while Farms 3–7 were smaller with herd sizes of 2500–5000. All farms are centrally located within a 50-mile radius of the city, Alajuela, but are geographically distinct as no two farms were closer than 20 miles. In Costa Rica, the Central Valley is an area of intensive agricultural production due to the fertile soil and temperate climate.

Sample collection

Fecal grab samples of freshly passed stool from the floor of different pens were collected. Specifically, a total of 10 individual samples of approximately 5 g each were collected from each barn and placed in a clean, sealable bag. Fecal samples were treated with chloroform for the inactivation of enveloped viruses (e.g. Classical Swine Fever virus) per USDA requirement prior to transportation into the US.

Virus extraction, concentration and recovery

A 10% fecal suspension in sterile phosphate buffered saline was prepared for each sample. Samples were vortexed at full speed for 2 min and then centrifuged at 5000 ***g*** for 15 min at 4°C. The solid fractions were resuspended in 3% Beef Extract (5 × volume of solids). The mixtures were then subjected to chloroform extraction to facilitate virus separation from organic solids. After centrifuging, the supernatant from the extracted fecal solids was recovered and added to the initial fecal supernatant. Viral RNA was recovered and purified using the QiaAmp Viral RNA kit (Qiagen Inc., Valencia, CA, USA) following recommended procedures and stored at −70°C. Assuming that as little as 1 genomic copy of purified target viral RNA could be successfully amplified, this method made it possible to detect 1 genomic copy of target viral RNA in as little as 14 ml of undiluted fecal matter or about 70 genomic copies of target viral RNA per gram of fecal matter.

Virus detection by RT-PCR

Primers used (Primer Set 1; Table 1) for initial screenings of all samples are specific for the US sHEV and are located in the Open Reading Frame 2 (ORF2) capsid protein region of the viral genome. They have been applied previously to the detection of the novel US sHEV Strain (Meng et al. 1998a). Since the nucleic acid sequence of any sHEV isolated in Costa Rica could potentially differ significantly from previously reported strains, samples were also screened with additional primer sets. The nucleotide sequences of 20 full-length HEV genomes encompassing all HEV genotypes were aligned. Regions for all three open reading frames showing the greatest homology were examined and primer sets were selected (Primer Set 2, Table 1).

Table 1. Primers used in PCR assays.

	Primer name	Orientation	Amplicon size[†]	Nucleotide sequence (5′ to 3′)
Primer Set 1	SHEVFEXT	Sense	429 bp	AGCTCCTGTACCTGATGTTGACTC
	sHEVREXT	Anti – sense	(5526 – 5955)	CTACAGAGCGCCAGCCTTGATTGC
	sHEVFINT	Sense	288 bp	GCTCACGTCATCTGTCGCTGCTGG
	sHEVRINT	Anti – sense	(5596 – 5884)	GGGCTGAACCAAAATCCTGACATC
Primer Set 2	JAKFExt	Sense	508 bp	ACAGAATTGATTTCGTCGGC
	JAKRExt	Anti – sense	(6323 – 6831)	TTAGTK(G/T)GTR(G/A)CCW(A/T)GC CTCCC
	JAKFInt	Sense	127 bp	GTY(C/T)GTCTCR(G/A)GCCAATGGC
	JAKRInt	Anti – sense	(6371 – 6498)	TAATCCTGR(G/A)ATAACY(C/T)ACA CG

[†]Sequence position relative to US swine HEV given in parentheses; ORF, Open Reading Frame; K – Guanosine/Thymidine; R – Adenosine/Guanosine; W – Adenosine/Thymidine; Y – Cytosine/Thymidine; S – Guanosine/Cytosine.

Two-Step RT-PCR

For the initial screening of all samples, Primer Set 1 (Table 1) was used along with the Two-Step RT-PCR protocol, as it was found to produce the greatest sensitivity in terms of lower level of detection (Kase, unpublished data). All two-step RT-PCR reagents were from the Gene Amp RNA PCR kit (Applied Biosystems; Foster City, CA, USA) with previously described PCR conditions and manufacturer recommendations used (Meng et al. 1998a). The subsequent nested PCR assay used 10 μl of the first round PCR product and 40 cycles of: 1 min (94°C), 1 min (52°C), and 2 min (72°C). Positive (e.g. US sHEV) and negative controls were included.

One-Step RT-PCR

For a second screening of selected samples, Primer Set 2 (Table 1) was used along with the One-Step RT-PCR kit (Qiagen Inc., Valencia, CA, USA) instructions. This method was found to produce the most sensitivity using this particular primer pair (Kase, unpublished data) with cycling conditions: 50°C for 30 min, 95°C for 15 min and 40 cycles consisting of 1 min (94°C), (52°C), and (72°C), with a 10-min final step (72°C). A second round of amplification followed with the internal primer pair. Negative and positive (e.g. US sHEV) controls were included.

Sequence analysis of PCR amplicons

Nucleic acid sequencing of probable HEV amplicons was done through the University of North Carolina (UNC-CH) Automated DNA sequencing Facility. In preparation for sequencing, PCR reactions were subjected to the QIAquick PCR purification kit (Qiagen Inc., Valencia, CA, USA). All resulting sequencing information was compared with other HEV genomic sequences available through the GenBank database (National Center for Biotechnology Information, National Institutes of Health, www.ncbi.nlm.gov) for confirmation of identity. The construction and drawing of evolutionary distance trees was accomplished using TREECON for Windows, version 1.3b (Van de Peer and De Wachter 1994). Distance estimation analysis was conducted by either the Jukes and Cantor or Saitou and Nei method based upon a 179 nucleotide region of ORF2 (Jukes and Cantor 1969; Saitou and Nei 1987). Trees were constructed using the Neighbor-joining method and any difference in resulting phylogenetic tree construction using the two methods of distance estimation was noted. Bootstrap values, providing validity for a particular constructed tree, were generated based upon 1000 resamplings of the data set. Both single-sequence rooted and unrooted trees were constructed for comparison.

Results

Screening of samples from Farms 1–4 using Primer Set 1 indicates that seven out of 21 samples analyzed contained HEV RNA (Table 2). Notably, viral material was detected in at least one sample from each of the farms. Five out of seven of positive samples were from pigs between the ages of approximately 1.5–3 months; sHEV was not detected in samples taken from younger pigs. In addition, samples from adult sows were positive for HEV RNA from both Farm 1 and 2 (Samples 1/C and 2/B).

To assess if viral RNA was not detected in some samples because of the specificity of Primer Set 1 for the US sHEV, a second PCR analysis was conducted using Primer Set 2

Table 2. Results from PCR analysis using both Primer Set 1 and 2 on Costa Rican swine fecal samples–Round 1 Survey: Farms 1–4.

Farm/code	Sample source description	PCR result with Primer Set 1	PCR result with Primer Set 2
1/A	Sows + piglets (few days old)	negative	negative
1/B	Sows + piglets	negative	negative
1/C	Sows + piglets (12 days old)	POSITIVE	POSITIVE
1/D	Sows + piglets (4 days old)	negative	negative
1/E	Pigs (~3 mths)	POSITIVE	POSITIVE
1/F	Pigs (~3 mths)	POSITIVE	POSITIVE
1/G	Pigs (~1 mth)	negative	negative
1/H	Adult swine (5 mths and up)	negative	negative
2/A	Adult sows (lactating)	negative	negative
2/B	Adult sows (maternity)	POSITIVE	negative
2/C	Pigs (~4 mths)	negative	negative
2/D	Pigs (~1–2 mths)	POSITIVE	POSITIVE
2/E	Pigs (ranging from 2–5 mths)	negative	negative
3/A	Adult sows (gestation)	negative	negative
3/B	Adult sows (maternity)	negative	negative
3/C	Pigs (<1.5 mths)	negative	negative
3/D	Pigs (1.5–2.5 mths)	POSITIVE	POSITIVE (?)
3/E	Pigs (ranging from 2.5–5 mths)	negative	negative
4/A	Adult sows (gestation)	negative	negative
4/B	Adult sows (maternity)	negative	negative
4/C	Pigs (~3 mths)	POSITIVE	POSITIVE

Negative – Detectable amounts of hepatitis E virus were not found; Positive – hepatitis E virus was detected.

(Table 1). As shown in Table 2, no new positive samples were revealed. Six out of the seven samples found to be positive for HEV RNA by analysis with Primer Set 1 were confirmed as positives with Primer Set 2. In previous work, a 10-fold less sensitivity in the detection of serial dilutions of US sHEV strain was noted compared to Primer Set 1 (Kase, unpublished data). Since degenerate nucleotide positions exist in Primer Set 2 primers, it is possible that the difference in robustness is related to the extent of sequence homology in the primer binding region.

Round 2 survey of Farms 1 and 2 also confirm the presence of sHEV. As seen previously, it was primarily swine near 3 months of age excreting sHEV (Table 3). In addition, positive samples included pregnant sows and swine over 1.5 months of age, both from Farm 2. None of the other samples from the adult swine contained detectable amounts of viral RNA.

The second round of sampling also gave evidence of detectable genomic HEV RNA in samples from the three farms surveyed for the first time (Farms 5–7; Table 3). Only one sample for each of Farms 5 and 7 contained detectable amounts of sHEV. In contrast, five out of seven samples from Farm 6 were positive. Unlike data collected from other farms, positive samples were identified in swine ranging in age from approximately 1–12 months.

The identity of all Round 1 PCR amplicons considered probable positives for HEV based upon amplicon size (molecular weight) was confirmed by nucleic acid sequencing. GenBank accession numbers for all Costa Rican swine sequences are DQ677372–DQ677383. Although, several nucleotide variations were observed between these previously recognized HEV strains and those identified in Costa Rican swine and between the samples obtained from the different Costa Rican farms, none of those differences represent amino acid changes. These results indicate considerable similarities among the

Table 3. Results from PCR Analysis using Primer Set 1 on Costa Rican swine fecal samples – Round 2 Survey: Farms 1, 2, 5–7.

Farm/code	Sample source description	PCR result with Primers Set 1
1/I	Pigs (~2.5–3.5 mths)	Positive
1/J	Pigs (~4 mths)	Positive
1/K	Sows + piglets (18–21 days old)	Negative
1/L	Sows (lactating)	Negative
2/F	Sows (maternity)	Positive
2/G	Pigs (~3.5 mths)	Positive
2/H	Pigs (1.6 mths)	Positive
2/I	Pigs (1.7 mths)	Negative
2/J	Sows + piglets (17 days old)	Negative
2/K	Pigs (5.5 mths)	Negative
2/L	Pigs (~5 mths)	Negative
5/A	Sows + piglets (18 days old)	Negative
5/B	Sows (gestation)	Negative
5/C	Sows + piglets (13 days old)	Negative
5/D	Pigs (~2 mths)	Negative
5/E	Pigs (~3 mths)	Positive
5/F	Pigs (>4 mths)	Negative
5/G	Pigs (>5 mths)	Negative
6/A	Pigs (~1 mths)	Positive
6/B	Pigs (~2 mths)	Positive
6/C	Pigs (~5 mths)	Negative
6/D	Pigs (~4 mths)	Positive
6/E	Pigs (~12 mths)	Positive
6/F	Pigs (~13 mths)	Negative
6/G	Pigs (~7 mths)	Positive
7/A	Sows (maternity)	Negative
7/B	Pigs (3–4 mths)	Positive
7/C	Sows + piglets (18 days old)	Negative
7/D	Pigs (~1 mths)	Negative
7/E	Pigs (2–3 mths)	Negative
7/F	Pigs (~5 mths)	Negative

Negative – Detectable amounts of hepatitis E virus were not found; Positive – hepatitis E virus was detected.

US, Canadian and Costa Rican HEV strains found in swine and humans HEV genotype III strains. On the other hand, substantially more variation exists between the sequences from the Costa Rica farms and Mex-14, a human Mexican genotype II HEV strain.

PCR amplicons from Round 2 sampling of Farms 1 and 2 underwent nucleic acid sequencing. Comparisons with Round 1 Costa Rican sequences indicate high homology and little diversity. Moreover, a number of unique nucleotide substitutions seen in Round 1 Farm 2 samples (2/B and 2/D) were also observed in the Round 2 Farm 2 sample (2/G).

To address the issue of possible contamination contributing to positive results, a portion of the PCR product generated from the HEV positive control material underwent nucleic acid sequencing using the same method as applied to other samples. The nucleotide similarity between the amplified material from the positive control HEV and the GenBank published US sHEV strain was 100% within the region examined (data not shown).

Percentage nucleotide identity between selected Costa Rican and representative HEV strains with overlapping sequences from all known genotypes is shown in Table 4. CR 1/E, 2/H, 3C and 4/C demonstrate the greatest nucleotide identity (84.9–93.3%) with the swine and human US isolates and other Genotype III strains. CR isolates appear to be divergent

Table 4. Percentage nucleotide identity between selected HEV isolates over a 179 base ORF 2 region.

CR isolates	Genotype I	Genotype II	Genotype III	Genotype IV
1/E	79.3–82.7	86.0	87.2–93.3	80.4–84.4
2/B	78.8–82.7	84.7	87.7–92.7	81.0–83.8
2/H	81.0–83.8	82.1	86.0–90.5	81.0–86.0
3/D	78.8–82.7	84.7	87.7–92.7	81.0–83.8
4/C	78.8–82.1	83.8	84.9–92.7	81.6–84.9

GenBank accession numbers: Genotype I AF051830 (Nepal), M73218 (Burma), AF185822 (Pakistan), M80581 (Pakistan), M94177 (China), D11093 (China), X98292 (India), and AY204877 (Africa); Genotype II M74506 (Mexico; Mex–14); Genotype III AF082843 (US swine), AF060668 (US; US1), AF060669 (US; US2), AF347692 (Canada swine), AB073912 (Japan swine); Genotype IV AJ272108 (China), AB108537 (China), AB074915 (Japan), AB099347 (Japan), AB097811 (Japan swine); CR Isolates DQ677373 (1/E), DQ677377 (2/B), DQ677381 (2/H), DQ677382 (3/D), DQ677383 (4/C).

but similar to Mex-14 (Genotype II) with the percentage of identical nucleotides ranging from 82.1–86.0% compared to slightly less similarity with selected Genotype I and IV strains (78.8–83.8% and 80.4–86.0%, respectively). The diversity among all 12 Costa Rican isolates was 0–7.8%. Within farms, a number of isolates were found to be genetically indistinguishable including 2/G and 2/H and 1/F, 1/I, and 1/J. In addition, isolates from Farm 1 (1/C), Farm 2 (2B) and Farm 3 (3C) are identical.

Nucleic acid comparisons among the various Costa Rican strains reveal some diversity, although the strains most resemble one another compared to other swine and human strains. However, phylogenetic analysis revealed several distinct clusters as seen in Figure 1. Farm 1 and 2 isolates from both Round 1 and 2 sampling show a high resemblance but also appear in different groupings. Interestingly, 2/F, originating from sows, has the greatest genetic similarity to strains coming from Farm 1 swine between 2.5 and 4 months of age (1/I, 1/F, and 1/J). The other sow isolates (1/C and 2/B) are located in a different grouping with strains recovered from Farm 1 and 3. Isolate 4/C is perhaps the most divergent with all Farm 2 isolates from pigs between 1 and 3.5 months of age (2/D, 2/H, and 2/G) also forming a distinct group. Repeated analysis was conducted with the same genetic information using several available models (e.g. Saitou and Nei) within the TREECON software package (Van de Peer and De Wachter 1994). None resulted in an appreciably different dendogram than that produced by the Jukes and Cantor method (Jukes and Cantor 1969). Moreover, the genotypic groupings for the previously characterized HEV strains follow earlier published data. Efforts are currently underway to sequence a larger region within ORF 2 to better genetically characterize these Costa Rican sHEV strains.

Discussion

Although little information has been available regarding the HEV status of swine herds from Central and South America, data from several geographic areas considered either endemic or non-endemic for human HEV suggest a global endemicity of sHEV infection (Meng 2003). In this study, viral RNA of sHEV was successfully detected in feces collected from each of the seven commercial farms surveyed. To our knowledge, this is the first evidence of endemic HEV in swine in Costa Rica with confirmation through isolation and genetic characterization of the virus.

Overall, the majority of samples identified as containing sHEV RNA were taken from swine between the ages of 1.5 and 4 months of age (Tables 2 and 3). On Farms 1, 4, and 6,

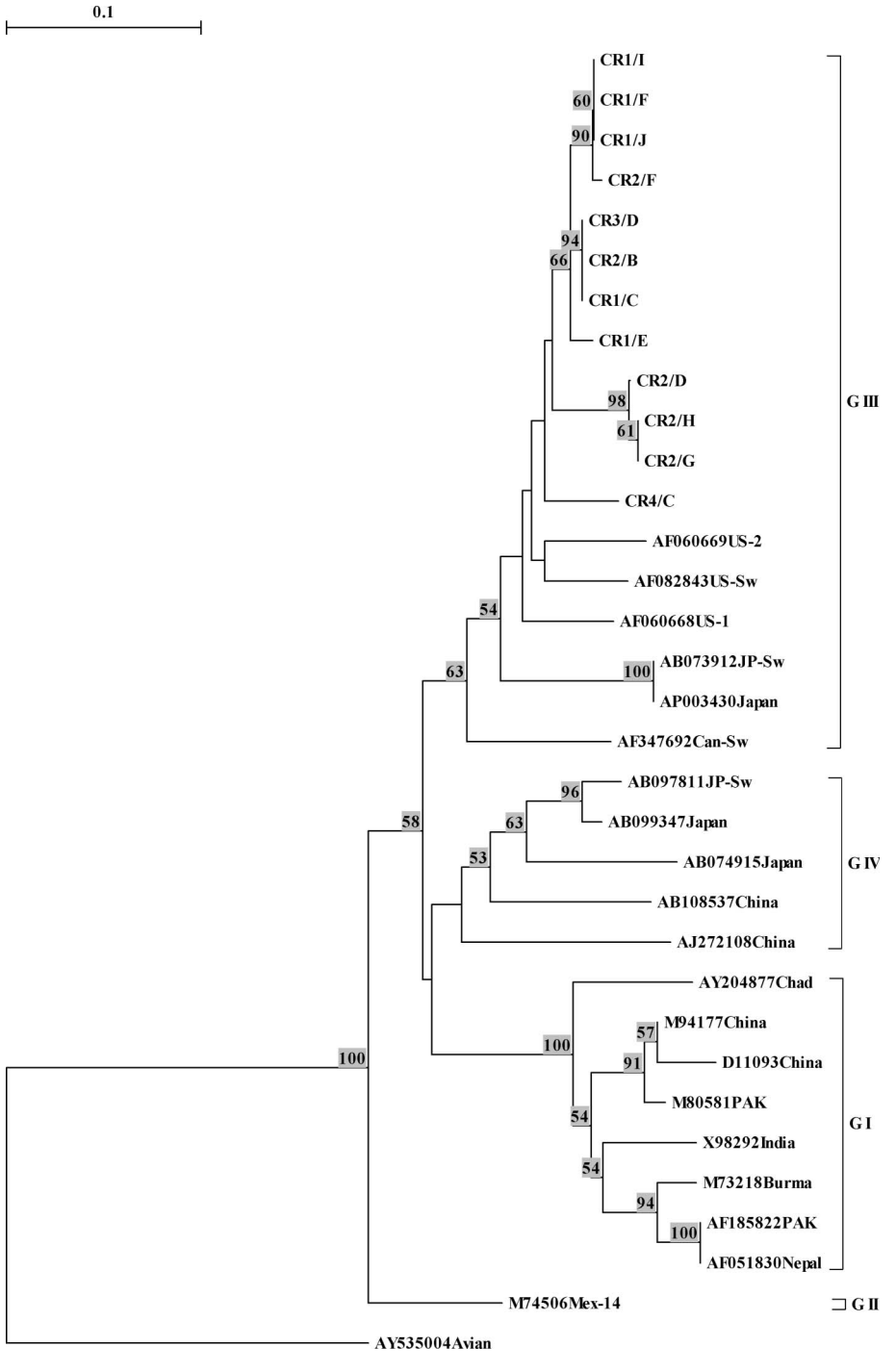

Figure 1. Dendogram showing the evolutionary relationship between the sequenced strain of Costa Rican swine and other published human and swine sequences. Phylogenetic analysis on 179 nucleotides was executed using TREECON for Windows computer software with Jukes and Cantor correction for evolutionary rate. Bootstrap analyses were conducted and percentages above 50% are shown at the respective nodes. Avian HEV was chosen as the out-group sequence.

all samples from swine belonging to this age group were found to contain the virus by detecting genomic HEV material in feces. However, this was not the situation for Farms 2, 3, 5, and 7. Possible explanations for the HEV occurrence differences include variability in the levels of HEV shed in swine feces, limitations in the sensitivity (lower detection limit) of sHEV detection in swine feces and inaccuracies in the reported swine ages. Ages were recorded from inventory sheets attached to the containment pens, although in some instances, we elicited the information from farm workers.

Swine reported to be 3 months of age were positive but those slightly younger or older were not consistently positive. Possible reasons for this observation are the presence of viral genomic material at undetectable levels; presence of pigs recently infected and not yet excreting virus; or, recovery from recent infection but no longer excreting the virus. Finally, there is little documentation of the clinical course of HEV infection in swine and the extent of virus shedding in feces based on virus concentrations in feces and the duration of shedding during natural infection in different swine herds and genetic clones. Regardless, the results of sHEV occurrence in swine observed in this study are generally consistent with and compliment those found in previous studies and support the belief that swine become susceptible to sHEV infection following weaning and subsequent waning of protective maternal HEV antibodies (Meng et al. 1997; Wu et al. 2002).

In a few cases, fecal samples coming from adult swine were found to contain genomic HEV RNA (Farms 1, 2, and 6) (Tables 2 and 3). Similar results for occasional sHEV occurrence in adult swine, specifically sows, were seen in a study conducted on North Carolina farms (Kase, unpublished data). However, sHEV fecal shedding in adult swine seems to be an exception rather than the norm. Such fecal shedding may be related to changes in immunological status with pregnancy, as all three positive samples from Farms 1 and 2 occurred in either pregnant sows (2/B and 2/F) or in a sow that had recently given birth (1/C). Pregnant sows have been found to be susceptible to HEV infection experimentally without vertical transmission to offspring (Kasorndorkbua et al. 2003). Furthermore, because other swine, especially juveniles, located on these farms were excreting the virus, physical transfer and horizontal spread of HEV from these young animals to additional barns cannot be ruled out.

All sHEV isolates were found to be genetically similar but distinct from other characterized HEV strains. By genetic sequence comparisons and phylogenetic analysis, our sHEV isolates most closely resembled the apparent indigenous human and swine US HEV strains (Table 4). This genetic similarity may be a reflection of the US origin of many swine breeding stocks in Costa Rica. In fact, one producer reported importing US swine as recently as 1999. Considering that all nucleotide changes in the sHEV swine isolates were in third codons of amino acids and therefore were silent mutations, such changes could be indicative of low levels of genetic drift from introduced US sHEV strains.

The existence of identical sequences from several farms suggests little genetic variability of HEV in Costa Rican swine. While all the farms were located within the agriculturally concentrated Central Valley, there was no indication of extensive sharing of animals between farms. Nucleic acid sequencing of a larger portion of the sHEV genome might be useful in resolving the extent of genetic diversity that exists within this area. More analyses, including determination of the lineages of swine herds and the extent of sHEV occurrence, are needed to better understand and document the origin, spread, persistence and evolution of sHEV. Only one human HEV strain from Latin America, Mex-14 (the prototype genotype II strain), has been fully characterized (Velazquez et al. 1990). Our

analysis has revealed that the Costa Rica farm isolates of sHEV most closely resemble Genotype III strains versus MEX-14. Recent work involving Mexican swine has shown the identified sHEV to group phylogenetically with Genotype III strains (Cooper et al. 2005). Unfortunately, a different area of ORF 2 gene was targeted in that study, thus preventing direct comparisons between the strains from Mexico and Costa Rica. Nonetheless, both the Costa Rican and the Mexican swine strains were found most similar to geographically local strains and share approximately 90% nucleotide sequence identity to other Genotype III strains.

Although it is difficult to identify a particular reason for the perpetuation of sHEV infection within the farm environment, the results from this study support the position that farm size and bio-security measures have little impact on sHEV prevalence. For example, Farms 5 and 7 are similar in size but varied greatly in terms of animal husbandry practices, yet fecal samples of similar swine age groups were found positive. Given the typical practice of feeding bovines solid swine excrement as a supplemental food source and evidence of HEV infection in a variety of animals including cattle, bovines asymptomatically infected with HEV could be reservoir animals excreting appreciable amounts of HEV genomic RNA and perhaps even contribute to the perpetuation of sHEV infection on farms (Tien et al. 1997; Arankalle et al. 2001).

Conclusion

In summary, this study provides the first documented evidence for the existence of HE infections in swine residing in Costa Rica. Although only a small sub-set of farms were examined, results suggest that the infection is widespread and is not necessarily affected by farm size or particular bio-security measures designed to curb the spread of swine-related infections. No epidemiological data exist to substantiate any claims that practices such as discharge of swine waste to surface waterways or feeding of swine fecal material to cattle has any influence on the spread of HE infections to either humans or other animals. Previous studies have indicated that the cross-species transmission of sHEV is possible and that infectious virus can persist in the environment for relatively long periods of time (Meng et al. 1998b; Pina et al. 1998). Moreover, HEV is known to circulate among humans in Costa Rica without causing outbreaks of clinically evident disease, as reported in an unpublished seroprevalence study conducted in San Ramon in 2000 (La Nacion 2000). It would be useful to have genetic information for isolates of human origin so that nucleic acid comparisons between human and swine could be made. Especially compelling would be evidence for a high degree of homology between swine and human isolates, as was found in previous studies conducted in the US, Japan, Taiwan, UK, and Korea (Meng et al. 1997; Hsieh et al. 1999; Choi et al. 2003; Takahashi et al. 2003; Banks et al. 2004). In those countries, co-circulation of genetically indistinguishable human and sHEV strains has been found. Alternatively, the human and swine strains of HEV in Costa Rica could belong to different genotypic groups as demonstrated in isolates from India, Thailand, and Mexico (Arankalle et al. 2002; Cooper et al. 2005).

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References

Arankalle VA, Joshi MV, Kulkami AM, Gandhe SS, Chobe LP, Rautmare SS, Mishra AC, Padbidri VS. 2001. Prevalence of anti-hepatitis E virus antibodies in different Indian animal species. J Viral Hepat. 8:223–227.

Arankalle VA, Chobe LP, Joshi MV, Chadha MS, Kundu B, Walimbe AM. 2002. Human and swine hepatitis E viruses from Western India belong to different genotypes. J Hepat. 36:417–425.

Banks M, Bendall R, Grierson S, Heath G, Mitchell J, Dalton H. 2004. Human and porcine hepatitis E virus strains, United Kingdom. Em Infect Dis. 10:953–955.

Choi IS, Kwon HJ, Shin NR, Yoo HS. 2003. Identification of swine hepatitis E virus (HEV) and prevalence of anti-HEV antibodies in swine and human populations in Korea. J Clin Microbiol. 41:3602–3608.

Cooper K, Huang FF, Batista L, Rayo CD, Bezanilla JC, Toth TE, Meng XJ. 2005. Identification of Genotype 3 hepatitis E virus (HEV) in serum and fecal samples from pigs in Thailand and Mexico, where Genotype 1 and 2 HEV strains are prevalent in the respective human populations. J Clin Microbiol. 43:1684–1688.

Emerson SU, Purcell RH. 2003. Hepatitis E virus. Rev Med Virol. 13:145–154.

Hsieh SY, Meng XJ, Wu YH, Liu ST, Tam AW, Lin DY, Liaw YF. 1999. Identity of a novel swine hepatitis E virus in Taiwan forming a monophyletic group with Taiwan isolates of human hepatitis E virus. J Clin Microbiol. 37:3828–3834.

Jaiswal SPB, Jain AK, Naik G, Soni N, Chitnis DS. 2001. Viral hepatitis during pregnancy. Int J Gynecol Obstet. 72:103–108.

Jukes TH, Cantor CR. 1969. Evolution of protein molecules. In: Munro HN, editor. Mammalian protein metabolism. New York: Academic Press. p. 21–32.

Kasorndorkbua C, Thacker BJ, Halbur PG, Guenette DK, Buitenwerf RM, Royer RL, Meng XJ. 2003. Experimental infection of pregnant gilts with swine hepatitis E virus. Can J Vet Res. 67:303–306.

Kumar A, Beniwal M, Kar P, Sharma JB, Murthy NS. 2004. Hepatitis E in pregnancy. Int J Gynecol Obstet. 85:240–244.

La Nacion. Costa Rica. 2000.

Mast EE, Kuramoto IK, Favorov MO, Schoening VR, Burkholder BT, Shapiro CN, Holland PV. 1997. Prevalence of and risk factors for antibody to hepatitis E seroreactivity among blood donors in Northern California. J Infect Dis. 176:34–40.

Meng XJ, Purcell RH, Halbur PG, Lehman JR, Webb DM, Tsareva TS, Haynes JS, Thacker BJ, Emerson SU. 1997. A novel virus in swine is closely related to the human hepatitis E virus. Proc Natl Acad Sci USA. 94:9860–9865.

Meng XJ, Halbur PG, Tsareva TS, Bruna JD, Royer RL, Purcell RH, Emerson SU. 1998a. Experimental infection of pigs with newly identified swine hepatitis E virus (swine HEV) but not with human strain of HEV. Arch Virol. 143:1405–1415.

Meng XJ, Purcell RH, Sharpiro MS, Govindarajan S, Bruna JD, Mushahwar IK, Emerson SU. 1998b. Genetic and experimental evidence for cross-species infection by swine hepatitis E virus. J Virol. 72:1143–1148.

Meng XJ, Wiseman B, Elvinger F, Guenette DK, Toth TE, Engle RE, Emerson SU, Purcell RH. 2002. Prevalence of antibodies to hepatitis E virus in veterinarians working with swine and in normal blood donors in the United States and other countries. J Clin Microbiol. 40:117–122.

Meng XJ. 2003. Swine Hepatitis E virus: Cross-species infection and risk in xenotransplantation. Curr Top Microbiol Immunol. 278:185–216.

Pina S, Jofre J, Emerson SU, Purcell RH, Girones R. 1998. Characterization of a strain of infectious hepatitis E virus isolated from sewage in an area where hepatitis E is not endemic. Appl Envr Micro. 64:4485–4488.

Saitou N, Nei M. 1987. The neighbor-joining method: A new method for reconstructing trees. Mol Biol Evol. 4:406–425.

Takahashi M, Nishizawa T, Miyajima H, Gotanda Y, Iita T, Tsuda F, Okamoto H. 2003. Swine hepatitis E virus strains in Japan form four phylogenetic clusters comparable with those of Japanese isolates of human hepatitis E virus. J Gen Virol. 84:851–862.

Tamada Y, Yano K, Yatsuhashi H, Inoue O, Mawatari F, Ishibashi H. 2004. Consumption of wild boar linked to cases of hepatitis E. J Hepat. 40:869–870.

Tei S, Kitajima N, Takahashi K, Mishiro S. 2003. Zoonotic transmission of hepatitis E virus from deer to human beings. Lancet. 362:371–373.

Thomas DL, Yarbough PO, Vlahov D, Tsarev SA, Nelson KE, Saah AJ, Purcell RH. 1997. Seroreactivity to hepatitis E virus in areas where the disease is not endemic. J Clin Microbiol. 35:1244–1247.

Tien NT, Clayson HT, Khiem HB, Sac PK, Corwin AL, Myint KS, Vaughn DW. 1997. Detection of immunoglobulin G to the hepatitis E virus among several animal species in Vietnam. Am J Trop Med Hyg. 57:211–213.

Van de Peer Y, De Wachter R. 1994. TREECON for Windows: A software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. Compu Applic Biosci. 10:569–570.

Velazquez O, Stetler HC, Avila C, Ornelas G, Alvarez C, Hadler SC, Bradley DW, Sepulveda J. 1990. Epidemic transmission of enterically transmitted non–A, non–B hepatitis in Mexico, 1986–1987. JAMA. 263:3281–3285.

Vitral CL, Pinto MA, Lewis-Ximenez LL, Khudyakov YE, dos Santos DR, Gaspar AMC. 2005. Serological evidence of hepatitis E virus infection in different animal species from the Southeast of Brazil. Mem Inst Oswaldo Cruz. 100:117–122.

Withers MR, Correa MT, Morrow M, Stebbins ME, Seriwatana J, Webster WD, Boak MB, Vaughn DW. 2002. Hepatitis E virus antibody levels in North Carolina swine workers, swine, and murids. Am J Trop Med Hyg. 66:384–388.

Wu JC, Chen CM, Chiang TY, Tsai WH, Jeng WJ, Sheen IJ, Lin CC, Meng XJ. 2002. Spread of hepatitis E virus among different-aged pigs: Two-year survey in Taiwan. J Med Virol. 66:488–492.