

*Brief Report*

**Enteric caliciviruses in domestic pigs in Hungary**

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**Summary**

Caliciviruses closely related to human norovirus and sapovirus were recently detected in domestic pigs, causing discussions about the animal reservoir and the potential for zoonotic transmission to humans. To detect porcine caliciviruses, 17 fecal samples collected on two swine farms in southwestern Hungary were tested by reverse transcription-polymerase chain reaction. Three (17.6%) samples were positive for caliciviruses. This study confirms the presence of caliciviruses, both porcine sapovirus (genus *Sapovirus*) and porcine norovirus (genus *Norovirus*), in domestic pigs in Hungary and provides additional information on the viral genetic diversity and relationship to viruses referred to as human caliciviruses.

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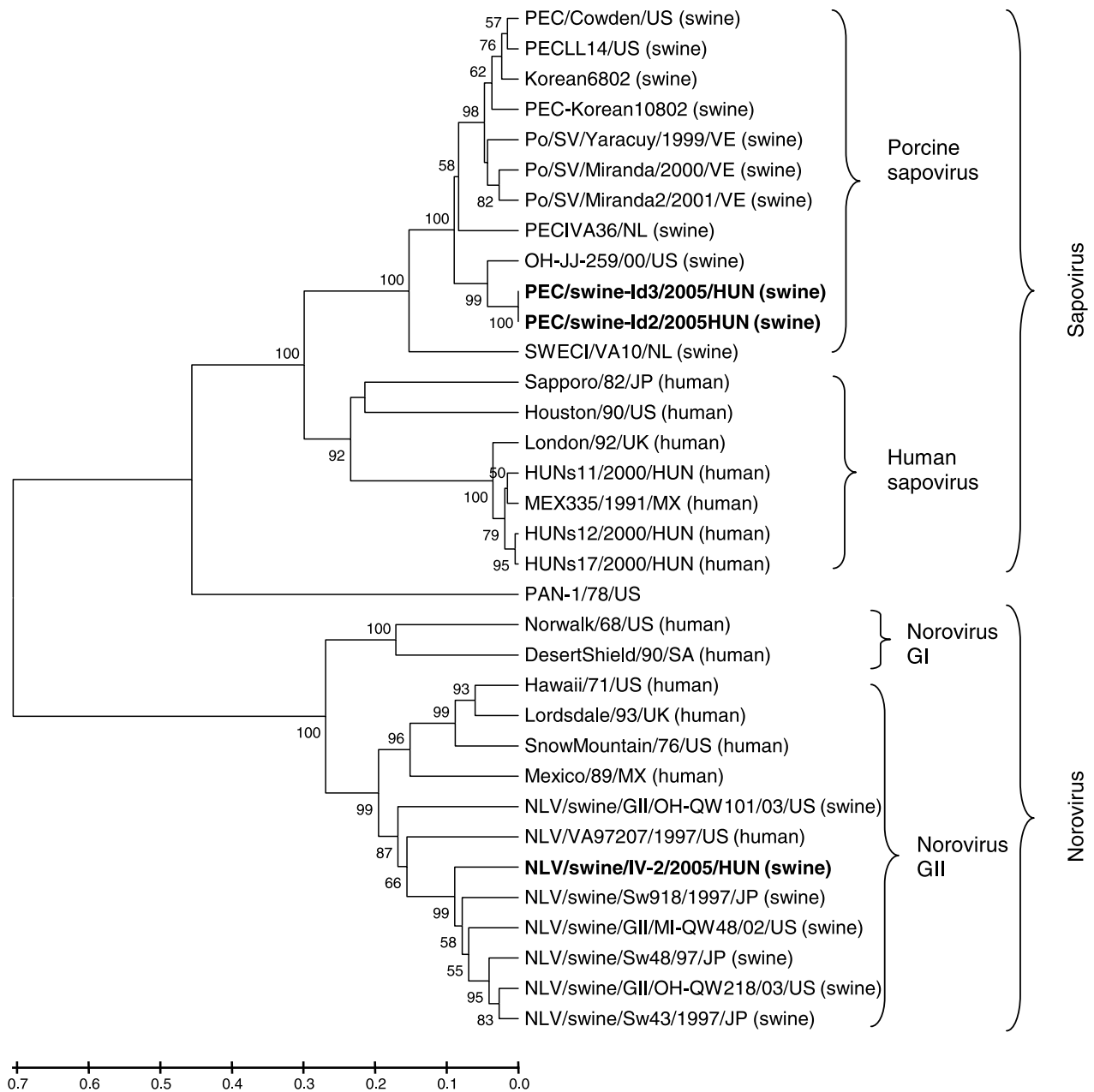
Caliciviruses (family *Caliciviridae*) are small, non-enveloped viruses with single-stranded, positive-

sense genomic RNA which are classified in four genera: *Vesivirus*, *Lagovirus*, *Norovirus* and *Sapovirus* [6]. Viruses within one genus are phylogenetically related, have common features in genomic organization, and have sequence similarities in the RNA polymerase and capsid regions [2]. The vesiviruses and noroviruses have three separate open reading frames (ORFs) in their genomes, whereas the lagoviruses and sapoviruses have similar genomic organizations composed of two ORFs. Norwalk and Sapporo viruses are prototype strains of the genera *Norovirus* and *Sapovirus*, respectively. Until now, it was generally thought that vesiviruses and lagoviruses were non-human pathogens and noroviruses and sapoviruses were human pathogens. Human caliciviruses, especially norovirus, are a common cause of gastroenteritis outbreaks in humans worldwide and the predominant etiological agent in epidemic gastroenteritis in Hungary [7]. Sapovirus is mainly associated with sporadic gastroenteritis in infants and children. Recently, it was shown that in some cases there was no sharp demarcation of host species for some calicivirus infections; some caliciviruses may have zoonotic potential, and animals such as domestic pig may act as a reservoir for caliciviruses [1, 9, 13]. Porcine sapovirus (historically called porcine enteric calicivirus – PEC) was first identified in the United

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Note: Nucleotide sequence data reported are available in the GenBank database under accession numbers DQ383274 and DQ864982.

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**Fig. 1.** Phylogenetic tree of porcine sapovirus (PEC/swine-Id2/2005/HUN and PEC/swine-Id3/2005/HUN, DQ383274) and porcine norovirus (NLV/swine/IV-2/2005/HUN, DQ864982) based on the 274-nt fragment of the RNA-dependent RNA polymerase gene (ORF1) of caliciviruses. The Hungarian strains detected in this study are in boldface. The phylogenetic tree was constructed using the UPGMA method by MEGA version 3.1 (available from <http://www.megasoftware.net>). Accession numbers in GenBank: PEC/Cowden/US (AF182760), PECLL14/US (AY425671), Korean6802 (AY289186), PEC-Korean10802 (AY289188), Po/SV/Yaracuy/1999/VE (AY633966), Po/SV/Miranda/2000/VE (AY633963), Po/SV/Miranda2/2001/VE (AY633965), PECIVA36/NL (AY615805), OH-JJ-259/00/US (AY826423), SWECI/VA10/NL (AY615807), Sapporo/82/JP (U77903), Houston/90/US (U95644), London/92/UK (U95645), HUNs11/2000/HUN (AF488717), MEX335/1991/MX (AY157869), HUNs12/2000/HUN (AF488718), HUNs17/2000/HUN (AF488720), Norwalk/68/US (M87661), Desert Shield/90/SA (U04469), Hawaii/71/US (U07611), Lordsdale/93/UK (X86557), Snow Mountain/76/US (U70059), Mexico/89/MX (U22498), NLV/VA97207/1997/US (AY038599), NLV/swine/GII/OH-QW101/03/US (AY823304), NLV/swine/Sw918/1997/JP (AB074893), NLV/swine/GII/MI-QW48/02/US (AY823303), NLV/swine/Sw48/97/JP (AB009413), NLV/swine/GII/OH-QW218/03/US (AY823307), NLV/swine/Sw43/1997/JP (AB074892) and primate vesivirus PAN-1/78/US (U52086)

States by electron microscopy in 1980 [8] and genetically characterized as a sapovirus in 1999 [2, 12]. Further porcine sapovirus strains have been detected in the Netherlands (van der Heide et al., unpublished data) and, recently, in South Korea [4] and Venezuela [5]. Porcine norovirus was first detected in Japan in 1998 [10] and, recently, in the Netherlands [11] and the United States and is related to genotype (G) II human noroviruses [13]. This is the second report of detection of porcine sapovirus as well as porcine norovirus in Europe.

Fecal samples were collected from 17 piglets from farms (6 and 11 samples) located in south-western Hungary in March 2005. Six pigs (three 1 day old and three 10–12 days old) on the first farm had a history of diarrhoea, while 11 pigs (two 4 days old, five 2–6 months old and four sows of 2 years old) at the second farm had no clinical history of diarrhoea. Reverse transcription-polymerase chain reaction (RT-PCR) was performed using primers designated p289/p290 that were specific for the viral RNA-dependent RNA polymerase region and produced products of 319 nt for norovirus and 331 nt for sapovirus as described previously [3, 7].

Three (17.6%) of 17 samples were found positive for caliciviruses. Two (both from 10–12-day-old pigs) from the first farm were positive for sapovirus and 1 (from a 4-month-old pig) from the second farm was positive for porcine norovirus (Fig. 1). Genetically identical porcine sapovirus strains (PEC/swine-Id2/2005/HUN and PEC/swine-Id3/2005/HUN) had 91, 82 and 57% nucleotide identity to the closest strain (OH-JJ259/00/US), to the prototype porcine sapovirus strain (PEC/Cowden/US), and to the prototype human sapovirus strain (Sapporo/82/JP), respectively. Nucleotide identity to the human sapovirus strain HUNs11/2000/HUN detected in the same region in Hungary was 61%. In addition, rotavirus gene segments were also identified in sapovirus-positive feces by PAGE (data not shown), indicating viral co-infection. The porcine norovirus strain (NLV/swine/IV-2/2005/HUN) had 86, 83, 75 and 62% nucleotide identity to the closest strain (Sw48/97/JP), to the prototype porcine norovirus strain (Sw43/1997/JP), to the closest human norovirus strain (VA97207/1997/US), and to the prototype norovirus strain (Norwalk/

68/US) strains, respectively. The NLV/swine/IV-2/2005/HUN strain showed 85 and 83% nucleotide identity, respectively, to the MI-QW48/02/US and OH-QW218/03/US strains described recently in the USA, the latter of which is a new, potentially recombinant genotype [13].

There are only a few enteric calicivirus sequences reported from domestic pigs [4, 6, 11, 13]. We report the molecular detection and phylogenetic analysis of porcine sapovirus and porcine norovirus in domestic pigs in Hungary. Detection and characterization of further porcine calicivirus strains from different geographic areas will be important for understanding the worldwide distribution and heterogeneity of calicivirus strains in swine and their association with zoonotic infection. This study confirmed the presence of caliciviruses in swine in Europe and provides additional information on their genetic diversity and relationship to viruses referred to as human caliciviruses.

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