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Short communication

Novel picornavirus in domestic rabbits (*Oryctolagus cuniculus var. domestica*)

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ABSTRACT

Picornaviruses (family *Picornaviridae*) are small, non-enveloped viruses with positive sense, single-stranded RNA genomes. The numbers of the novel picornavirus species and genera are continuously increasing. Picornaviruses infect numerous vertebrate species from fish to mammals, but have not been identified in a member of the Lagomorpha order (pikas, hares and rabbits). In this study, a novel picornavirus was identified in 16 (28.6%) out of 56 faecal samples collected from clinically healthy rabbits (*Oryctolagus cuniculus var. domestica*) in two (one commercial and one family farms) of four rabbit farms in Hungary. The 8364 nucleotide (2486 amino acid) long complete genome sequence of strain Rabbit01/2013/HUN (KT325852) has typical picornavirus genome organization with type-V IRES at the 5′UTR, encodes a leader (L) and a single $2A^{H\text{-}box/NC}$ proteins, contains a hepatitis-A-virus-like cis-acting replication element (*CRE*) in the 2A, but it does not contain the sequence forming a "barbell-like" secondary structure in the 3′UTR. Rabbit01/2013/HUN has 52.9%, 52% and 57.2% amino acid identity to corresponding proteins of species *Aichivirus A* (genus *Kobuvirus*): to murine *Kobuvirus* (JF755427) in P1, to canine *Kobuvirus* (JN387133) in P2 and to feline *Kobuvirus* (KF831027) in P3, respectively. The sequence and phylogenetic analysis indicated that Rabbit01/2013/HUN represents a novel picornavirus species possibly in genus *Kobuvirus*. This is the first report of detection of picornavirus in rabbit. Further study is needed to clarify whether this novel picornavirus plays a part in any diseases in domestic or wild rabbits.

1. Introduction

The family *Picornaviridae* consists of small, non-enveloped viruses with positive sense, single-stranded RNA genome. It contains 29 genera and 50 species and a number of unassigned picornaviruses (Knowles et al., 2012; Adams et al., 2014; http://www.picornaviridae.com).

Picornaviruses have a common genome organization with VPg-5′ UTR^{IRES}-L/P1-P2-P3-3′UTR-poly(A) (Knowles et al., 2012; Palmenberg et al., 2010; http://www.picornaviridae.com) except the members of the species *Cadicivirus A* in genus *Dicipivirus* (Woo et al., 2012). The genome begins with a 5'untranslated region (UTR) consisting of the internal ribosomal entry site (IRES). At present, five IRES types (type-I to type-V) are known in picornaviruses (Hellen and de Breyne, 2007; Sweeney et al., 2012). The viral genome encodes a single polyprotein transcribing from a single open reading frame (ORF). The polyprotein is cleavaged into smaller functionally active proteins: P1 encodes the viral capsid proteins; and P2 and P3 encode the non-structural proteins (Knowles et al., 2012; Palmenberg et al., 2010). In addition, most of the picornaviruses encode a leader (L) protein before the P1 region. At the 3′ end of the genome it has a 3′UTR and a poly(A)-tail. The picornavirus genomes consist of several additional secondary RNA structures (e.g. cis-acting replication element (*CRE*), "barbell"-like structures) (Boros et al., 2012, 2014; McKnight and Lemon, 1998; Steil and Barton, 2009; Yang et al., 2008). The genome location of *CREs* and their contribution to viral RNA replication have been determined and the "barbell"-like structure has been investigated in some picornaviruses (Boros et al., 2012; Pankovics et al., 2015; Steil and Barton, 2009).

Picornaviruses include a wide range of pathogens (Knowles et al., 2012; http://www.picornaviridae.com) and have been found in various vertebrate host species including humans and marine mammals (Kapoor et al., 2008), birds (Boros et al., 2014; Pankovics et al., 2015) and recently in reptiles (Heuser et al., 2014; Ng et al., 2015), amphibians (Reuter et al., 2015) and fish (Fichtner et al., 2013; Barbknecht et al., 2014; Phelps et al., 2014). The members of the genus *Kobuvirus* infect human and several domestic or wild animals such as canine, feline,

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murine, avian, ovine, bovine, ferret, caprine, porcine and wild boar (Li et al., 2009; Ng et al., 2014; Pankovics et al., 2015; Reuter et al., 2009, 2010).

Picornaviruses have not yet been reported in domestic rabbits (*Oryctolagus cuniculus var. domestica*); however, Equine rhinoviruses 1, and 2 and Seneca Valley virus can be cultured in rabbit derived cell lines (Knowles et al., 2012; Wutz et al., 1996). In rabbits, the most familiar viral diseases are the viral skin diseases myxomatosis (myxoma virus), shope fibromatosis (rabbit fibroma virus) and rabbitpox (rabbitpox virus), all pathogens belonging to the family *Poxviridae* (Krogstad et al., 2005; Meredith, 2013; Kerr and Donelly, 2013). The shope and oral papillomatosis are less frequent skin disease of rabbits caused by cottontail papillomavirus (*Papillomaviridae*) (Krogstad et al., 2005; Meredith, 2013; Kerr and Donelly, 2013). The enteric viruses in rabbits include rabbit haemorrhagic disease virus (genus *Lagovirus*, family *Caliciviridae*), lapine rotaviruses, enteric coronaviruses, astroviruses, bocaparvoviruses and hepatitis E virus (Martella et al., 2011; Meredith, 2013; Kerr and Donelly, 2013; Lanave et al., 2015; Xia et al., 2015).

In this study, we report the identification and complete genome characterization of a novel picornavirus from domestic rabbits.

2. Materials and methods

2.1. Sample collection

Between the years 2010 and 2014, a total of 56 faecal samples were collected from rabbits (*O. cuniculus var. domestica*), from four lapine farms in different geographical locations in Hungary. The animals have no clinical signs or symptoms at the sampling dates.

Lapine farm No1: Forty-four faecal samples (designated as NY1A–M, NY2A–N, NY3A–G and NY4A–J) were collected from a commercial rabbit farm from young (6-weeks to 3-months old) and adult rabbits in Somogysárd in March 2010. California female rabbits are graded up with Pannon Great white male rabbits and the animals are kept in deep litter method.

Lapine farm No2: Two faecal samples (Rabbit01–02) were collected from domestic rabbits in town Békéscsaba, in April 2013. The pooled faecal samples originated from two different beds of straw in separate cages. Six pieces of 2-week-old nestlings and a mother rabbit were kept in the first cage (Rabbit01) and five 3-months-old rabbits were kept in the second cage without parents (Rabbit02). The animals are kept for private consumption and all animals were vaccinated against myxomatosis virus.

Lapine farm No3: Two faecal samples (NYUL040423, NYUL050423) were collected from a 3-month-old male and a 3-year-old female domestic rabbit in the town of Pécs, during April 2013. Altogether 20–25 animals lived in a small stock-farm separated by a fence and they are kept for private consumption.

Lapine farm No4: Eight faecal samples (VR1–6, VR2013/I–VR2013/II) were collected from mature adult rabbits from a backyard animal farm in Városlőd, in February 2012 and 2013, respectively. The animals are kept in cages for private consumption.

2.2. Viral metagenomic analysis

Four specimens – two samples from each of *lapine farm No2* and *No3* – were randomly selected for viral metagenomics. Briefly, phosphate-buffered saline (PBS)-diluted specimens were passed through a 0.45-μm sterile filter (Millipore) and centrifuged at 6000 ×*g* for 5 min. The filtrate was then treated with a mixture of DNases and RNases to digest unprotected nucleic acids (Phan et al., 2013). Viral-particle protected nucleic acids were then extracted using QIAamp spin-column technique (Qiagen) and subjected to a viral metagenomic analysis using sequence independent random RT-PCR amplification (Victoria et al., 2009). Viral cDNA library was constructed by ScriptSeq™ v2 RNA-Seq Library Preparation Kit (Epicentre) and then the library was sequenced by the Miseq Illumina platform according to the manufacturer's instruction, and as described previously (Phan et al., 2013). The Illumina reads and assembled contigs greater than 100-bp were compared to the GenBank protein database (BLASTx) attending to significant cut-off value (E-value: 10^{-5}).

2.3. Genome acquisition of novel picornavirus

Specimens were diluted in PBS to 30% (*w*/*v*) and total RNA were extracted from 300 μl of faecal suspension using TRI Reagent (Molecular Research Centre, Cincinnati, OH, USA) according to the manufacturer's instruction. Specific primers were designed to the reads/contigs to obtain the complete viral genome by primer-walking, 5′/3′ RACE, dsRNA-RACE and TAIL-PCR methods (Boros et al., 2012; Liu and Chen, 2007; Pankovics et al., 2015). PCR-products were sequenced directly and then run on an automated sequencer (ABI Prism 310, Applied Biosystems, Stafford, USA). Screening primer-pairs Rabbit-picorna-3D-R (5′-AACGTGACGTCATAGATGG-3′ corresponding to nt positions 7916 to 7934 of rabbit picornavirus) and Rabbit-picorna-3D-F (5′-TGGAAGTGCTGTCGGCTGCAA-3′ corresponding to nt position 7428 to nt 7448 of rabbit picornavirus) were designed on the partial nucleotide sequences of the RNA-dependent RNA polymerase ($3D^{pol}$) region of rabbit picornavirus for RT-PCR amplification method.

2.4. Sequence and phylogenetic analyses

The nucleotide (nt) and amino acid (aa) sequence analysis was performed by NCBI BLASTn/BLASTp and GeneDoc. The amino acid (aa) identity analyses between representative picornaviruses and the study strain were estimated by SIAS web server (http://imed.med.ucm.es/Tools/sias) using the "gaps are taken into account" option. All evolutionary analysis was conducted in MEGA 6.06 (Tamura et al., 2013). The nucleotide sequences of representative picornavirus and the study strain were codon-based aligned and pre-tested with the best nt/aa model (ML) search. The dendrograms were constructed by the Maximum Likelihood method based on the General Time Reversible model with gamma distribution (+G) and invariable sites (+I). Possible polyprotein cleavage sites were predicted based upon the aa alignments of relevant picornavirus sequences.

2.5. RNA secondary structure analysis

The secondary RNA structures of the 5′UTR, including IRES and the CRE were predicted using Mfold (Zucker, 2003) with manual correction as described previously (Sweeney et al., 2012).

2.6. Nucleotide sequence accession numbers

The novel rabbit picornavirus sequences were submitted to GenBank under accession numbers KT325852 and KT325853.

3. Results

3.1. Viral metagenomic analysis of lapine samples

A total of 4 faecal samples from rabbits without any clinical symptoms from *lapine farm No2* and *No3* were used in this study. In one (Rabbit01, from lapine farm *No2*) out of four faecal samples, four picornavirus sequence reads (a: VP3, b: VP1, c: 2A and d: 2C) were identified by viral metagenomic analysis (Fig. 1). The VP3, VP1, 2A and 2C sequence reads showed 83%, 60%, 60% and 72% amino acid identity to that of *Kobuvirus* from sewage (JQ898342), canine *Kobuvirus* (JN387133) and the last two ones to Aichi virus (AB040749, NC_001918) using BLASTx, respectively.

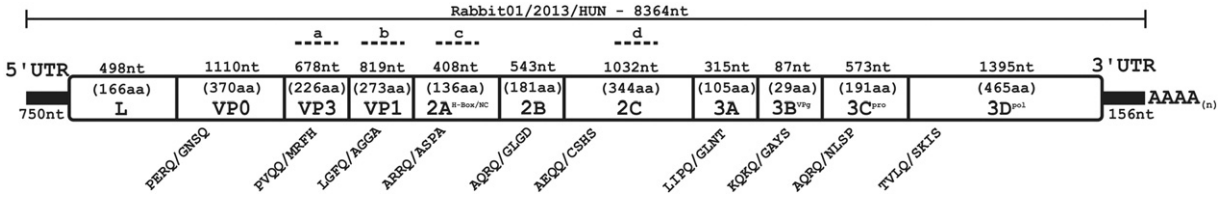

Fig. 1. Schematic genome organization of rabbit picornavirus strain Rabbit01/2013/HUN (KT325852). The length of the complete genome excluding the poly(A) tail is indicated above the scale bar; the nucleotide (nt) and amino acid (aa) lengths of the potential genes are indicated above and below the gene-boxes. The predicted aa cleavage sites are shown on the border of every regions. The viral metagenomic sequence contigs (a–d) are denoted with dashed lines above the corresponding gene boxes. The nt identity of contig (a) was 83% to *Kobuvirus* from sewage (JQ898342); contig (b) was 60% to canine *Kobuvirus* (JN387133); and contigs (c) and (d) were 60% and 72% to Aichi virus 1 (AB040749).

3.2. General genomic features of rabbit picornavirus

The complete RNA genome of strain Rabbit01/2013/HUN (KT325852) was 8364 nt-long, excluding the poly(A) tail with a typical picornaviral genome organization 5UTR$^{IRES\text{-}V}$/L/P1(VP0-VP3-VP1)/P2(2A$^{H\text{-}box/NC}$-2B-2C)/P3(3A-3B^{VPg}-3C^{Pro}-3D^{Pol})/3UTR-poly(A) (Fig. 1). Rabbit01/2013/HUN had a 55.2% G + C content with a 18.8% A, 26% U, 25.7% G and 29.5% C.

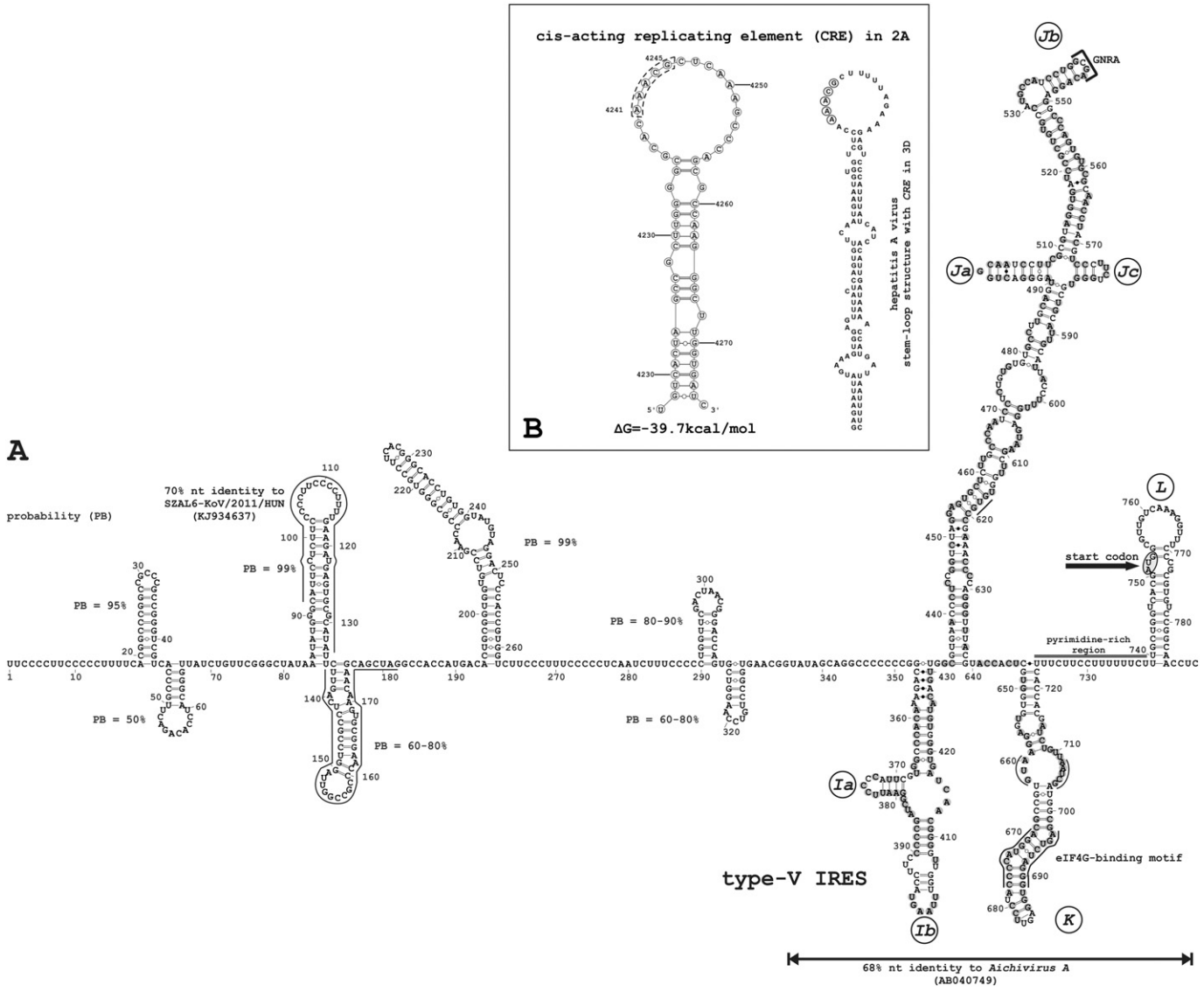

Fig. 2. A) Predicted RNA secondary structure of the 5′UTR and internal ribosomal entry site (IRES) of rabbit picornavirus strain Rabbit01/2013/HUN (KT325852). The type-V IRES have been annotated as proposed previously (Sweeney et al., 2012). Domains/hairpins are labelled from I–L except A–B, which are not found in rabbit picornavirus, maintaining the current nomenclature. The positions of conserved nt motifs and conserved nts between the rabbit picornavirus and the Aichi virus (AB040749) (*Aichivirus A*, genus *Kobuvirus*) and the polyprotein initiation **AUG** start codon are indicated by grey background and black lines, respectively. The seven stem-loop hairpin structures in the beginning of the 5′UTR were supported by the thermodynamic probability (PB) percentages are indicated next to the structures. The closest sequence identities to the closest viral sequences are marked by a bold line. B) Predicted RNA secondary structure of cis-acting replication element (*CRE*) in the 2A region. Bases contributing to the AAACG motif within the loop sequence are marked by dashed lines compared with hepatitis A virus *CRE* motif. Yang et al. (2008)

3.3. Description of 5′/3′ untranslated regions and cis-acting regulation element (CRE)

The 5′UTR of the study strain is 750 nt long (Fig. 1). The putative in-frame AUG initiation codon is at nt position 751–753 (CACG**A**$_{751}$**UG**G) in optimal Kozak context (Kozak, 1987). In the GenBank database, 68% sequence identity was found between Rabbit01/2013/HUN (from nt 340 to nt 750) and the Aichi virus (AB040749) 5′UTR (from nt 348 to nt 707). Based upon the nucleotide identity and the predicted secondary RNA structure of Rabbit01/2013/HUN 5′UTR-IRES it has a potential type-V IRES with conserved elements (Fig. 2A). The first 347 nt of the 5′UTR contained several Aichi virus-like stem–loop secondary structures with high 60.4% GC content and showed 70% partial sequence identity from nt 93 to nt 182 to European roller *Kobuvirus* (KJ934637) (from nt 118 to nt 207) (Fig. 2A), but the ori region (origin of replication) was not detected (Sweeney et al., 2012; Pankovics et al., 2015). The apex of Ia and Ib hairpins and Ja–Jc hairpins are highly similar to those of Aichi virus. The apex of the Jb contains the GNRA motif and the K hairpin also contains two highly conserved sequences, AGGUAC CCC (nt 669 to 677) and GGGAUCUGA (nt 689 to 697), as in *Kobuvirus* IRESs. This bipartite base-paired motif is functionally important, and interacts with initiation factor eIF4G in type-II IRESs (Yu et al., 2011). A significant polypyrimidine tract was found upstream of the initiation codon of Rabbit01/2013/HUN. The Y_n-X_m-AUG motif is Y_{18}-X_{10}-A_{751}UG where the 18 nt polypyrimidine (Y) tract was followed by a 10 nt spacer sequence (X) (Fig. 2A). The 3′UTR of Rabbit01/2013/HUN is 156 nt long and has no significant nt sequence identity to any viral sequences in GenBank and it does not contain the sequence forming the "barbell-like" secondary structure (Boros et al., 2014).

A total of 9 possible *CRE* motifs (6 of 5′-AAACA-3′ and 3 of 5′-AAACG-3′) were found in the genome of Rabbit01/2013/HUN. Based on the RNA secondary structure formation, thermodynamically the most favoured site (AAACG in 2A between nt 4241 and 4245; $\Delta G = -39.7$ kcal/mol) is located in the top loop of the structure of 2A (Fig. 2B), structurally similar to hepatitis A virus *CRE* in 3D (Yang et al., 2008).

3.4. Analysis of the polyprotein coding region

The Rabbit01/2013/HUN genome encodes a 2486 amino acid long polyprotein that is cleaved into proteins during the folding process (Fig. 1). The potential polyprotein cleavage sites are hypothesised based on the probable cleavage sites of the prototype strains of *Aichivirus A*, *Aichivirus B* and *Aichivirus C* (genus *Kobuvirus*), *Sakobuvirus A* (genus *Sakobuvirus*) and *Salivirus A* (genus *Salivirus*) (Fig. 1). The putative L protein is 498 nt (166 aa) long and neither putative conserved domains nor significant sequence identity was found using BLASTx. A potential N-terminal myristoylation motif (GxxxT/S, **G**$_{167}$NSQT) was reliably predicted in the N-terminal end of VP0 hypothetical protein. The conserved **H**-box/**NC** amino acid motifs (**H**$_{1064}$WAI and **N**$_{1126}$**C**STW) in 2A; the conserved nucleotide-binding domain of helicase (GRP**G**$_{1499}$**CGKS**) in 2C; the conserved picornavirus H–D–C catalytic triad (AS**H**$_{1873}$V, **S**$_{1913}$LVR instead of **D** and GL**C**$_{1973}$G where cysteine is the active-site of the cysteine protease) and the histidine (**H**$_{1991}$VAG) residue in the trypsin-like protease domain in 3C; and well-conserved aa motifs (**K**$_{2180}$DELR, **G**$_{2308}$NPSG, **Y**$_{2346}$GDD and **F**$_{2395}$LKR) in 3D are present in Rabbit01/2013/HUN. All P1, P2 and P3 protein products of Rabbit01/2013/HUN are related to kobuviruses with 52–59.3% amino acid identity except for the 2C protein where the *Salivirus A* were the closest relative (Fig. 3B). The rabbit picornavirus showed 53.2% and 52.9% amino acid identity to murine *Kobuvirus* (M-5/USA/2010, JF755427; *Aichivirus A*) in the complete polyprotein and in P1, 52–52% amino acid identity to

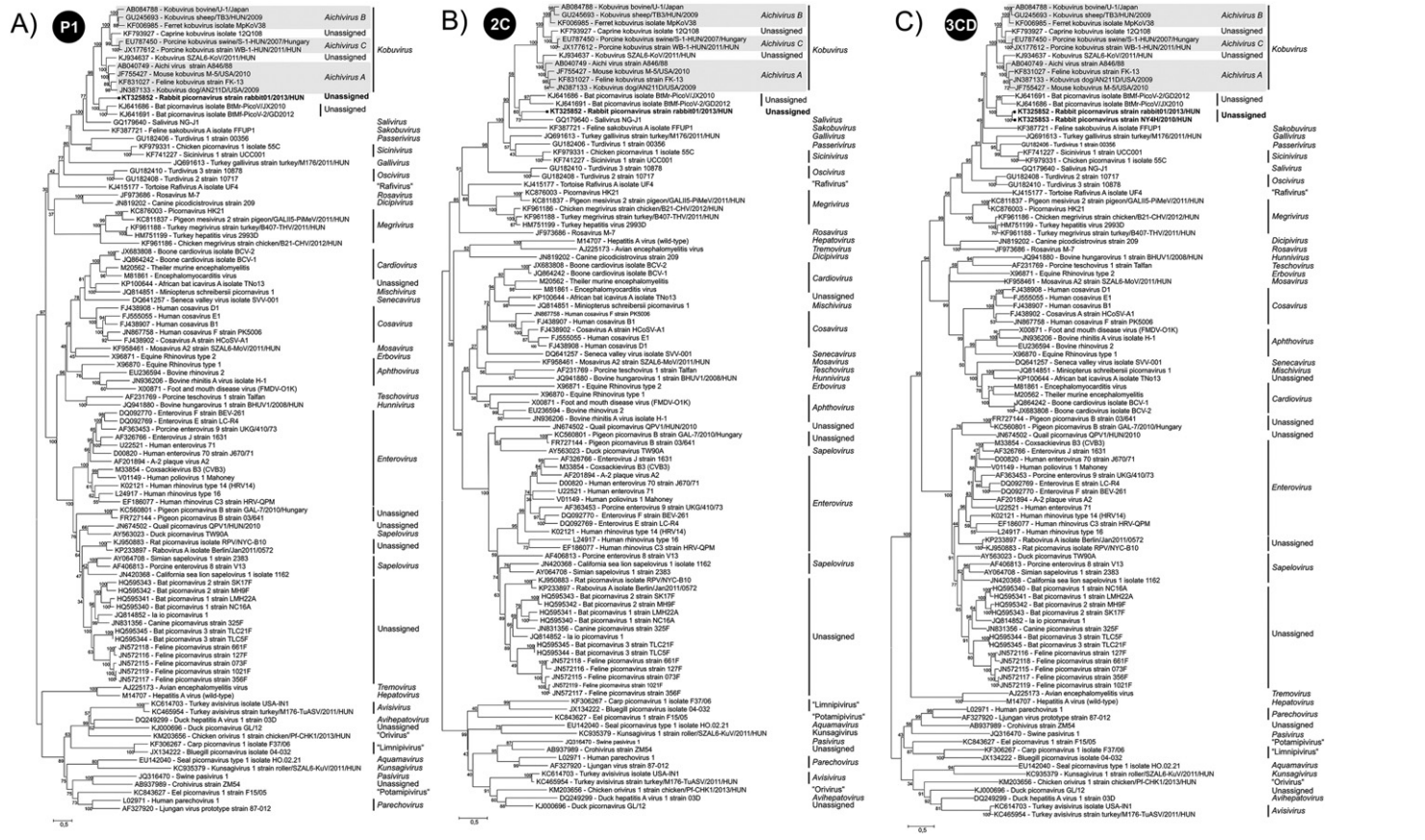

Fig. 3. Phylogenetic analysis of rabbit picornavirus strain Rabbit01/2013/HUN (KT325852 in bold) and representative picornaviruses based on the complete P1 (A), 2C (B) and 3CD (C) nucleotide sequences. The accepted *Kobuvirus* species are highlighted by light grey square. Bootstrap values were determined with 1000 replicates.

canine *Kobuvirus* (dog/AN211D/USA/2009, JN387133; *Aichivirus A*) and caprine *Kobuvirus* (black goat/12Q108/KOR/2012, KF793927; "*Aichivirus D*") in P2, 57.2% and 59.3% amino acid identity to feline *Kobuvirus* (FK-13/South Korea/2011, KF831027; *Aichivirus A*) in P3 and 3CD, and finally 52.4% amino acid identity to human *Salivirus* (hu/02394-01/USA/2002, GQ184145; *Salivirus*) in 2C.

The phylogenetic trees based on the P1, 2C and 3CD nucleotide sequence of Rabbit01/2013/HUN and representative members of picornaviruses show that Rabbit01/2013/HUN is related to kobuviruses in the complete viral capsid (P1) and protease–polymerase ($3C^{pro}D^{pol}$) regions and to saliviruses in the complete $2C^{hel}$ region, but it represents a novel phylogenetic lineage (Fig. 3A–C).

3.5. Detection of rabbit picornavirus in lapine faecal samples

Applying the screening primer-pairs Rabbit-picorna-3D-R/F, 16 (28.6%) out of 56 samples yielded a PCR-product of the expected amplicon size collected in 2 of the 4 farms. Fifteen (34%) and 1 (50%) of the faecal samples were positive for rabbit picornavirus in lapine farms *No1* and *No2* collected from young animals. By sequence analysis all sequences from lapine farm *No1* related to the sequence of Rabbit01/2013/HUN. One sample "Ny4H" was selected from lapine farm *No1* and the continuous 3CD-3′UTR part of Ny4H/2010/HUN (KT325853) genome was determined. Ny4H/2010/HUN showed 88% nucleotide and 98% amino acid identity to Rabbit01/2013/HUN in 3CD-3′UTR with seven amino acid changes in 3CD.

4. Discussion

This study reports the detection of a novel picornavirus in domestic rabbits. Based on the amino acid sequence identity and phylogenetic analyses, rabbit picornavirus is related to viruses in the lineage of the genera *Kobuvirus*, *Salivirus* and *Sakobuvirus*. According to the taxonomic genus and species demarcation criteria of International Committee on Taxonomy of Viruses (ICTV) (http://www.picornastudygroup.com/definition/genus_definition.htm), novel picornavirus genera are defined, if amino acid (aa) identity in P1, P2 and P3 regions are less than 40%, 40% and 50%, respectively. In addition, novel *Kobuvirus* species are classified, if the aa difference in the complete polyprotein, P1, 2C and 3CD proteins are less than 70%, 70%, 80% and 80%, respectively. In the complete polyprotein, P1, P2, P3 and 3CD regions of rabbit picornavirus showed 52–59% amino acid identity to the corresponding proteins of different types of kobuviruses (especially *Aichivirus A*) and 52% in 2C to *Salivirus*. Based on these data the rabbit picornavirus strain Rabbit01/2013/HUN is a novel picornavirus species and considered as a potential member of the genus *Kobuvirus*; however, the formal classification of rabbit picornavirus at the genus level is subject to further discussion by the ICTV. During the manuscript preparation, novel bat picornavirus sequences from respiratory and gastrointestinal samples of bats (*Miniopterus fuliginosus*) in China were deposited in GenBank as unpublished data. These two partial unassigned genome sequences (KJ641686 and KJ641691) from bats seems to be the closest relatives of rabbit picornavirus with 50.2/49.4%, 58.2/54.7% and 63.4/65% amino acid identities in P1, 2C and 3CD regions, respectively.

To our knowledge, kobuviruses (except *Aichivirus C*) and saliviruses have type-V internal ribosomal entry site (IRES) elements (Sweeney et al., 2012; Reuter et al., 2009). Considering the nucleotide identity and secondary RNA structural analysis rabbit picornavirus also has type-V IRES. But neither the SL-A, SL-B and SL-C pseudoknot structures described in Aichi virus nor the ori region (origin of replication) identified in canine and European roller kobuviruses were found in the first 120 nucleotides of the 5′UTR (Nagashima et al., 2008; Reuter et al., 2009; Sweeney et al., 2012; Pankovics et al., 2015). These structures have an essential role in picornaviral RNA replication and supposedly other secondary or tertiary structures provide these functions for the rabbit picornavirus (Sweeney et al., 2012; Pankovics et al., 2015). The 3′UTR of rabbit picornavirus does not contain a "barbell-like" structure, which is a common feature of species *Aichivirus A* and saliviruses (Boros et al., 2014; Pankovics et al., 2015).

Additionally, the secondary structures in the 5′/3′UTR picornaviruses internally located stem–loop structures also play a part in RNA replication and synthesis. After the first cis-acting replication element (*CRE*) was recognized in human rhinovirus 14 (McKnight and Lemon, 1998) this essential element was only investigated in a few picornavirus (Steil and Barton, 2009; Pankovics et al., 2015; Yang et al., 2008). The stem–loop structure of *CRE* in rabbit picornavirus is potentially located in 2A and showed structural similarity and sequence identity to that of human hepatitis A virus (Yang et al., 2008).

The leader (L) protein is present in 16 of the 29 picornavirus genera (http://www.picornaviridae.com). The L protein in Aichi virus (*Aichivirus A*, genus *Kobuvirus*) is involved in viral RNA replication and encapsidation without autocatalytic or polyprotein cleavage activity (Sasaki et al., 2003). Based on amino acid sequence similarities, L protein probably provides the same function to members of the species *Aichivirus B* and *Aichivirus C*. Hence the rabbit picornavirus L protein does not exhibit significant amino acid homology to *Kobuvirus* or other known picornavirus L proteins; however, it has 35% amino acid identity to bat picornavirus (KJ641686) L protein sequence recently deposited in GenBank database.

Kobuviruses have a wide range of host species identified from mammalian hosts, e.g. human (Yamashita et al., 1998), canine (Li et al., 2011), feline (Chung et al., 2013), murine (Phan et al., 2011), bovine (Yamashita et al., 2003), ovine (Reuter et al., 2010), ferret (Smits et al., 2013), porcine (Reuter et al., 2009), and caprine (Oem et al., 2014) and was also found in the faeces from a carnivorous bird, the European roller (Pankovics et al., 2015). In addition, kobuviruses are also detected in environmental samples (Ng et al., 2012). Rabbit can now be included as a potential host species of kobuviruses. Rabbits are common livestock animals and its breeding has a long tradition. Rabbits keep in large commercial farms, small house-keeping farms and as pets in close contact with humans. Picornaviruses were detected in rabbit faecal samples originated from two (commercial and family) different types of lapine farms in two geographical regions (270 km apart) in Hungary. Rabbits shedding picornavirus in the faeces did not show obvious clinical signs. These data indicate that rabbit is the potential host species of this novel picornavirus but further epidemiological and molecular studies will be required to investigate the biology, disease spectrum and pathogenesis of this picornavirus in domesticated and wild rabbits and other potential host species including humans.

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