Heliyon 9 (2023) e15702

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Characterization of a novel picornavirus prevalent in experimental rabbits (*Oryctolagus cuniculus*)

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ARTICLE INFO

Keywords:
Rabbit
Virome
Picornavirus
Metagenomics
Feces
Blood

ABSTRACT

Here, using viral metagenomic method a novel picornavirus (named UJS-2019picorna, GenBank accession number OP821762) was discovered in fecal and blood samples of experimental rabbits (*Oryctolagus cuniculus*). The complete genome size of UJS-2019picorna is 7832 bp excluding the poly(A)-tail, with GC content of 44.00% and a nucleotide composition of 28.0% A, 28.0% U, 21.5% G, and 22.5% C. The viral genome has a typical picornavirus organization pattern from the 5′-3′ direction: VPg-5′ UTR-(L)-P1, (VP4–VP2–VP3–VP1)–P2, (2 A-2B–2C)–P3, (3 A-3B–3C-3D)-3′ UTR-poly(A). The P1 region of UJS-2019picorna is related to *Erbovirus* with amino acid identity of 37.31%, while the P2 and P3 regions are the closest to *Bopivirus* with amino acid identity of 35.66%–39.53%. According to the *Picornaviridae* Study Group guidelines, UJS-2019picorna should be presumed to be a new genus belonging to the *Picornaviridae* family. Epidemiologic study revealed that this novel picornavirus was prevalent in a cohort of experimental rabbits, with prevalence rate of 23.68% (9/38) in feces and 18.4% (7/38) in blood samples. Further work is required to elucidate whether this virus is pathogenic to rabbits and whether it has influence on studies using rabbits as experimental animal.

1. Introduction

Thanks to the development and widespread application of sequencing technologies, the number of newly identified viruses has expanded rapidly in recent years, as is the number of known host species [1,2]. Members of the family *Picornaviridae* are spherical, non-enveloped, single-stranded positive-sense RNA viruses which infect vertebrates including fish, mammals, birds and even humans [3–5]. Numerous identified picornaviruses have been found in mammals and birds, but until now, novel picornaviruses have also been characterized in other animals such as amphibians and reptiles [2,6–8]. Some picornaviruses are important pathogens causing a wide range of diseases in humans as well as wild, domestic and laboratory animals including the common cold, polio, hand-foot-and-mouth disease, meningitis, diarrhea, hepatitis, etc [9–11]. However, little is known about whether these viruses can spread between humans

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https://doi.org/10.1016/j.heliyon.2023.e15702
Received 20 December 2022; Received in revised form 18 April 2023; Accepted 20 April 2023
Available online 23 April 2023

and animals and cause disease.

According to the classification principles of the International Committee for the Taxonomy of Viruses (ICTV), the family *Picornaviridae* currently consists of 158 species grouped into 68 genera. Viral genomes are approximately 7.1–8.9 kilobases (kb) in size and typically contain a single open reading frame (ORF) encoding a large polyprotein [12,13]. The polyprotein is cleaved by proteases into several functionally active proteins, such as P1 region encoding capsid proteins, P2 and P3 regions encoding non-structural proteins [14]. Typical picornaviruses encode a leader (L) protein between P1 and 5′ UTR and have a 3′UTR and a poly(A)-tail structure at the 3′ end of the genome. The characteristic organizational pattern of the viral genome from the 5′-3′ direction is as follows: VPg-5′ UTR-(L)-P1, (VP4–VP2–VP3–VP1)–P2, (2 A-2B–2C)–P3, (3 A-3B–3C-3D)-3′ UTR-poly(A) [4,14,15]. The P1 region contains four structural proteins, 1 A, 1 B, 1C, and 1D, which encode VP1-4, respectively, responsible for capsid formation and virus-receptor binding. The P2 and P3 proteins encode 2 A, 2 B, 2C and 3 A, 3 B, 3C, and 3D, respectively [13,16–18]. 2 A and 2 B mainly interfere with host cell function, while 2C is involved in vesicle formation. 3 A protein is involved in membrane protein presentation and inhibition of cellular protein transport. 3 B can be involved in viral replication and can serve as a primer for RNA synthesis. The 3C protein is the protease responsible for cleaving the P1 precursor protein, and the 3D protein is the RNA-dependent RNA polymerase required for viral replication [13].

In recent years, there have been some reports on the discovery of picornaviruses in rabbits [19]. So far, fewer complete picornaviruses have been identified in rabbits. Péter Pankovics et al. reported a novel complete picornavirus Rabbit 01/2013/HUN (KT325852) in domestic rabbits in 2015 [14]. Here, we report the identification and complete genome characterization of a novel picornavirus in experimental rabbits (*Oryctolagus cuniculus*).

2. Materials and methods

2.1. Sample collection and preparation

In May 2019, fresh fecal samples of 38 healthy adult experimental rabbits were collected using disposable absorbent cotton swabs at the Laboratory Animal Center of Jiangsu University. The weight range of rabbits is 3.5–5 kg. Whole blood samples were also collected from each rabbit. All samples were stored in sterile covered containers and shipped on dry ice. About 10 g of each fecal sample was resuspended using 5 mL DPBS and vortexed for 5 min vigorously, then incubated at 4 °C for 30 min. After centrifugation at 15,000 g for 10 min, 1 mL of each supernatant was transferred to new 1.5 mL centrifuge tubes and stored at −80 °C for later use [20]. Whole blood samples were centrifuged (10 min, 15,000 g) to collect plasma. Five samples of rabbit feces were randomly selected to construct the library and the remaining samples were used for PCR screening.

2.2. Viral metagenomic analysis

A total of 500 μL of supernatant was pipetted from 5 samples (100 μL per sample) and then collected into a new 1.5 mL tube. These samples were centrifuged at 12,000 g for 5 min at 4 °C and filtered through a 0.45 μm filter to remove non-viral particles. RNase and DNase were used to treat the filtrates followed by digestion of unprotected nucleic acids at 37 °C for 60 min [21]. Total nucleic acids were then extracted strictly using the manufacturer's protocol provided by QIAamp MinElute Virus Spin Kit. These nucleic acid samples containing DNA and RNA viral sequences were then subjected to reverse transcription reactions with SuperScript III reverse transcriptase and 100 pmol of a random hexamer primer, followed by a single round of DNA synthesis using Klenow fragment polymerase. Libraries were constructed using the Nextera XT DNA Sample Preparation Kit and sequenced on the NovaSeq Illumina platform with 250 bases paired ends with dual barcoding. Paired end reads of 250 bp generated by NovaSeq were debarcoded using vendor software from Illumina for bioinformatics analysis. Process sequencing data using an in-house analysis pipeline running on a 64-node Linux cluster. Potential host sequences were removed using Bowtie 2 software [22,23] and primer sequences of raw reads were trimmed using Trim Galore. The generated files were then quality controlled with options '–phred 33 –length 50 –stringency 3 –paired –fastqc'. QC reports were then integrated using MultiQC [24] with default parameters and confirmed manually. Duplicated reads were marked using PRINSEQ-lite v0.20.4 (-derep 1). The library was then assembled by MEGAHIT software [25] with the minimum length parameter of output contigs set to 1500 bp. All generated contigs and singlets reads were then matched against the NR database using blastx program built-in DIAMOND [26] with an E-value cutoff of $<10^{-5}$.

2.3. Acquisition and annotation of viral genomes

To obtain viral genomes of interest, those contigs which showed significant identity (E-value $<10^{-5}$) to vertebrate-related viruses were further analyzed. Putative viral open reading frames (ORFs) were also predicted by Geneious Prime v2022.0.1 with default parameters [27], and were further verified by comparison with related viruses. Annotations of these putative ORFs were based on comparisons with Conserved Domain Database (CDD). Finally, we obtained a relatively complete picornavirus genome with low amino acid sequence identity to other picornaviruses and incorporated it into subsequent analyses.

2.4. PCR amplification and screening

The SMARTer RACE 5'/3' Kit utilizing Rapid Amplification of cDNA Ends (RACE) technology was employed to amplify both the 3′ and 5′ ends of the target virus to obtain its full-length genome. Nested PCR screening was performed for the novel picornavirus in stool

and blood samples to facilitate investigation of prevalence. The nested PCR procedure involved the following conditions: initial pre-denaturation at 95 °C for 5 min, followed by 31 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C (first round) and 60 °C (second round) for 30 s, and elongation at 72 °C for 30–110 s, with a final elongation step at 72 °C for 5 min. The reaction system utilized the premixed enzyme rTaq (TaKaRa). The details of the sequences of the primers used in this research are listed in Table 1.

2.5. Phylogenetic analysis

To infer phylogenetic relationships, protein sequences of different reference viruses belonging to the family *Picornaviridae* were downloaded from the GenBank database. Protein sequences were aligned using the built-in alignment program in Geneious Prime and the results were further optimized using the MUSCLE program in MEGA v11 [28]. Phylogenetic trees were constructed by MrBayes v3.2 [29]. We specified "prset aamodelpr = mixed" for the phylogenetic analysis of amino acid sequences, enabling the program to employ its 10 pre-built amino acid models. The maximum number of generations was raised to 1 million until the standard deviation of split frequencies dropped below 0.01. Samples were taken every 50 generations, and the first 25% of Markov chain Monte Carlo (mcmc) samples were discarded as burn-in. Maximum Likelihood trees were also constructed using MEGA v11 [28] to validate all phylogenetic trees.

2.6. Prediction of tertiary structure

In this study, we utilized ColabFold [30] to forecast the three-dimensional arrangement of the viral capsid protein. Moreover, we employed SWISS-MODEL [31] to evaluate and filter out the models having comparable spatial structures from the PDB database. For visualization purposes, PyMOL v2.0 was employed.

2.7. Quality control

All experimental steps were strictly in accordance with relevant requirements to prevent sample cross-contamination and nucleic acid degradation during the process. Aerosol filter tips are used to reduce the probability of sample cross-contamination, and all other experimental materials, including microcentrifuge tubes and tips, that are in direct contact with nucleic acid samples are free of DNase and RNase. Samples were dissolved in DEPC-treated water containing RNase inhibitors.

To test blank controls, sterile ddH2O was prepared simultaneously and further processed under the same experimental conditions. During quality testing using agarose gel electrophoresis and Agilent bioanalyzer 2100, there was no detectable DNA in the control pool. During the sequencing process on the Illumina NovaSeq platform, the control pool generated a very small number of reads. No viral sequences were found in the control pool by BLASTx search.

2.8. Data availability

All raw reads are deposited in the Short Read Archive (SRA) of the GenBank database with accession no. SRR22164050.

The novel picornavirus sequence identified in this research has been uploaded to the GenBank database under accession number OP821762.

3. Results

3.1. Viral metagenomic overview

The rabbit fecal metagenomic library (rabbitFe01) generated a total of 13, 198, 586 raw reads on the Illumina NovaSeq platform, the number of clean reads obtained after quality control is 13, 194, 536. After queried of the clean reads against the nr database, a total of 3,572,430 reads had the best matches with viral proteins (Supplementary Table 1), accounting for 27.08% of the total clean reads. About 14 viral families were detected, the most abundant viral family was *Siphoviridae* (53.15% of the total clean viral reads), followed by *Microviridae* (26.72%), *Inoviridae* (7.91%), *Podoviridae* (6.53%), *Myoviridae* (3.73%), *Circoviridae* (1.43%), *Tombusviridae* (0.15%), and *Picornaviridae* (0.11%) (Supplementary Table 1). However, most of the detected viruses, particularly those related to vertebrates, exhibited a high degree of identity to the sequences in the GenBank database. Furthermore, there are 9694 reads that have not been clearly assigned to viruses at any taxonomic level (E value $> 10^{-5}$) and potentially novel viruses may be present in these reads.

Table 1
Primers used for specific PCR confirmation.

Primer	Targeted virus	Sequence (5′-3′)	Fragment size (bp)	Annealing temperature (°C)
Fw	UJS-2019picorna	ATTCCATTGCCTCCATGCCA	563	60
Rw		TCTGTCAAGCCCGGGAATTC		
Fn		GGCAACGGAACAGTGGGATA	294	60
Rn		CTCAGGATTGTCCAGCCGTT		

Fw, outer sense; Rw, outer antisense; Fn, inner sense; Rn, inner antisense.

3.2. Identification of a novel picornavirus

In this research, one viral contig (named UJS-2019picorna) related to *Picornaviridae* was obtained using the assemble sequences program in Geneious Prime, After queried against the nr database, this contig was initially considered to be a possible novel member of the family *Picornaviridae*. After RACE amplification, we obtained the whole genome of UJS-2019picorna. The complete genome size of UJS-2019picorna is 7832 bp excluding the poly(A)-tail, with GC content of 44.00% and a nucleotide composition of 28.0% A, 28.0% U, 21.5% G, and 22.5% C, with an average sequencing coverage of 20. The characteristic organizational pattern of the viral genome from the 5′-3′ direction is shown in Fig. 1A. The 5′ UTR and 3′ UTR are 590 and 141 nt long, respectively. There is a typical poly(A)-tail structure at the 3′ end of the genome. The search results of blastn showed that the 5′ UTR and 3′ UTR regions of UJS-2019picorna did not have any matches in GenBank. The putative in-frame AUG initiation codon is at nt position 591–593 in an optimal Kozak consensus (GUCGA$\mathbf{_{591}UGA}$). The UJS-2019picorna encodes a 7101 nt (2367 aa) long large polyprotein that is cleaved into serveal proteins during the folding process. The polyprotein cleavage sites were inferred from the annotated strains in the GenBank database. The putative L protein is 273 nt (91 aa) long, the P1 region encoding the capsid protein includes VP4, VP2, VP3 and VP1 with lengths of 189 (63 aa), 810 (270 aa), 678 (226 aa) and 921 (307 aa) nt, respectively. The lengths of 2 A, 2 B, 2C, 3 A, 3 B, 3C and 3D included in the P2 and P3 regions encoding non-structural proteins are 399 (133 aa), 216 (72 aa), 1002 (334 aa) nt, 537 (179 aa), 69 (23 aa), 615 (205 aa) and 1392 (464 aa) nt, respectively.

3.3. Prevalence of UJS-2019picorna

To investigate the prevalence of UJS-2019picorna in all collected samples, primers were designed based on 3C and 3D regions (Table 1). PCR screening results indicated that out of the 38 rabbit fecal samples, 9 (23.68%) tested positive for UJS-2019picorna. Of these 9 rabbits, 7 were found to be positive for blood as well. The sequences were obtained from the positive individuals and were identical.

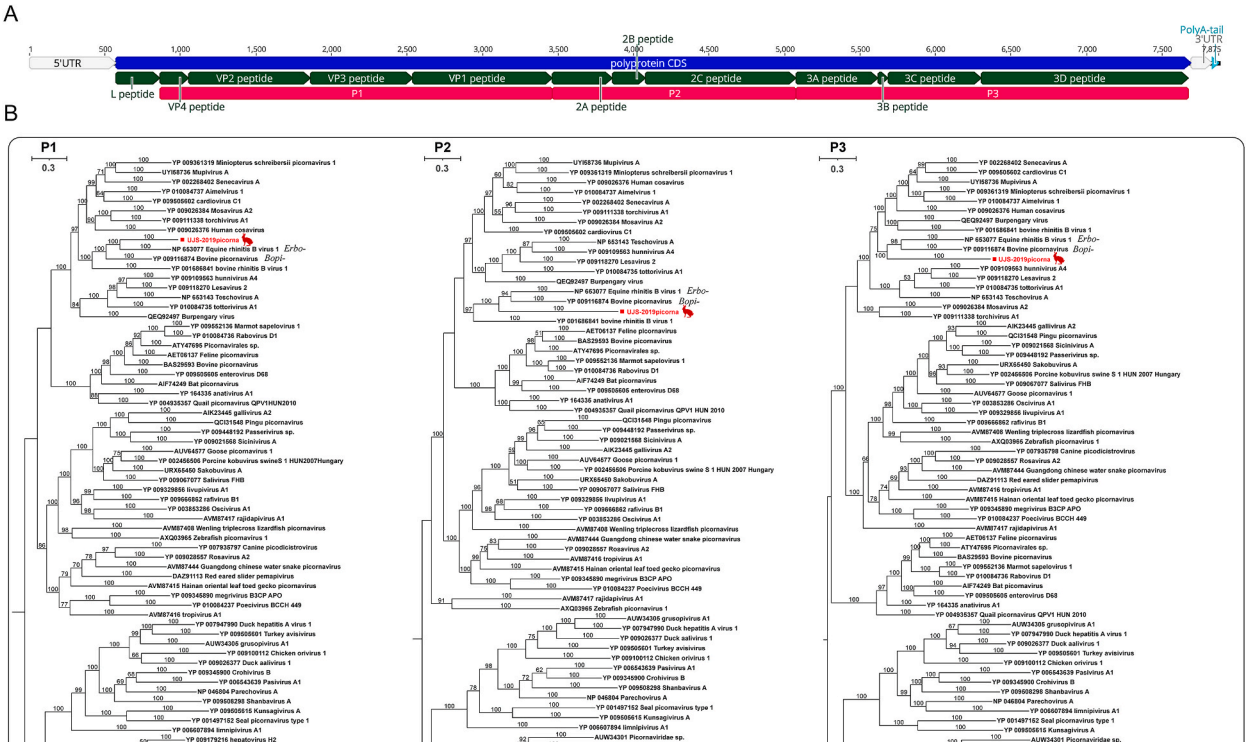

Fig. 1. (A) Genomic organization of UJS-2019picorna identified in rabbits. (B) Phylogenetic analysis of UJS-2019picorna identified in rabbits. Bayesian inference tree based on amino acid sequences of the P1, P2 and P3 regions of viruses belonging to *Picornaviridae* identified here. Bayesian posterior probabilities were displayed next to each node. Within each tree, the virus found in this study is marked with a red dot, and a silhouette of a rabbit is provided next to it. Each scale bar indicates 0.3 amino acid substitutions per site.

3.4. Phylogenetic analysis

Representative sequences of the current 68 genera of the family *Picornaviridae* were included in the subsequent analysis, phylogenetic analysis based on the amino acid sequences of the P1, P2 and P3 regions indicated that UJS-2019picorna clustered on a sister clade with Equine rhinitis B virus 1 (*Erbovirus*; NP_653,077) and Bovine picornavirus (*Bopivirus*; YP_009,116,874) isolated from horse and cattle, respectively (Fig. 1B). The amino acid sequence identity of the whole genome sequence with the best match in GenBank database is 37.20% (*Bopivirus*; YP_009,116,874). The P1 region of UJS-2019picorna is related to *Erbovirus* with amino acid identity of 37.31%, while the P2 and P3 regions are the closest to *Bopivirus* with amino acid identity of 35.66%–39.53%. The maximum likelihood trees constructed by MEGA v11 supports the above results.

Distance matrix analysis of the P1, P2 and P3 regions showed that UJS-2019picorna shared less than 40% identity with the amino acid sequences of any currently known virus belonging to the family *Picornaviridae* (Supplementary Figure 1 and Supplementary Table 2). Based on the *Picornaviridae* Study Group guidelines, members of different genera of the family *Picornaviridae* should share less than 40.0%, 40.0%, and 50.0% amino acid sequence identity in the P1, P2, and P3 region, respectively [4]. Therefore, UJS-2019picorna could be presumed to be a new genus of picornaviruses. In addition, the RDP4 software did not indicate any significant evidence of recombination signals.

3.5. Prediction of tertiary structure of UJS-2019picorna capsid protein (VP1-4)

To forecast and assess the likeness of spatial structure between the capsid protein of UJS-2019picorna and the structure encoded by presently available sequences, we obtained sequences from the GenBank database that correspond to the capsid protein region of UJS-2019picorna. Some of these sequences include *Erbovirus* (AAZ81659) and *Bopivirus* (UPG35835). We employed ColabFold to forecast the spatial structure of the sequences that encode capsid proteins. Furthermore, we used SWISS-MODEL to search and download the virus model (Seneca Valley Virus 3cji.1. B) that is most similar to UJS-2019picorna. We imported the PDB files generated above into PyMOL software for pairwise comparisons. Typically, an RMSD of less than approximately 2 Å would be regarded as very similar. In this study, UJS-2019picorna and *Erbovirus* (AAZ81659), *Bopivirus* (UPG35835) and Seneca Valley Virus (3cji.1. B) have good similarity in capsid protein spatial structure (Fig. 2A, B and C). This implies that UJS-2019picorna and these viruses might share similar characteristics in terms of virus-receptor binding.

4. Discussion

In recent decades, an increasing number of novel viruses have been discovered, largely due to innovations and advances in metagenomic sequencing, which allows researchers to detect viruses on the skin and *in vivo*. Genomic studies on human or animal samples have revealed dozens of unknown viruses in intestines, lungs, skin, and blood [32,33]. Some of these newly discovered viruses tend to accompany the emergence and progression of disease, but for the most part, some of them are apparently harmless to humans or animals. However, the presence of the virus in healthy animals does not preclude its pathogenic potential. Understanding how these newly discovered viruses affect humans or animals will allow us to determine whether their infections can be prevented and treated.

Rabbits, like other animals, often carry multiple pathogens such as viruses, mycoplasma, chlamydia, bacteria, fungi, and various parasites [34–39]. In some cases, rabbits encounter poultry raised by humans and living environments, the pathogens carried by them

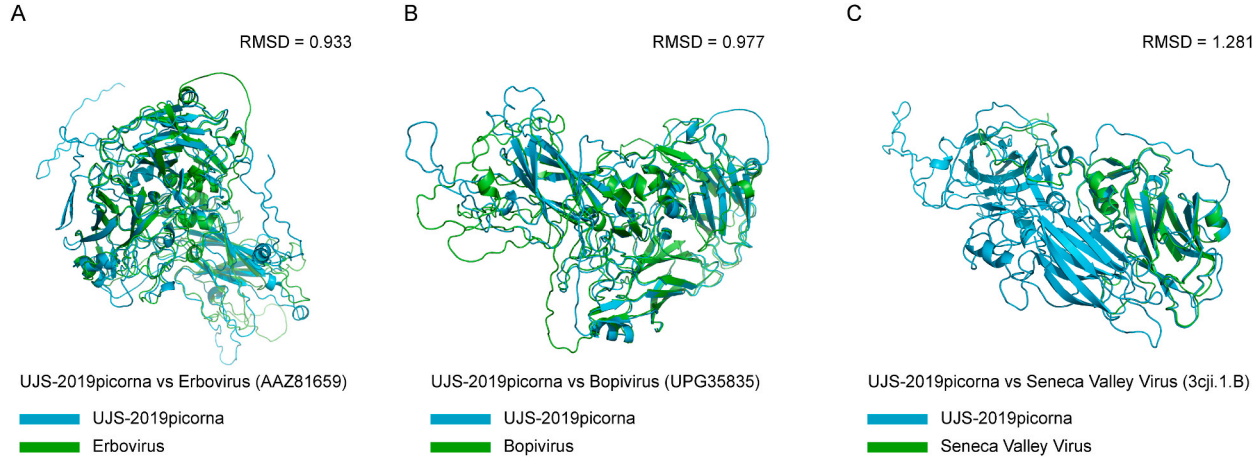

Fig. 2. Viral capsid protein structural model visualization. PDB files were visualized and pairwise aligned using PyMOL v2.0 software. (A) Prediction of the three-dimensional structural similarity between the capsid proteins of UJS-2019picorna and *Erbovirus* (AAZ81659). (B) Prediction of the three-dimensional structural similarity between the capsid proteins of UJS-2019picorna and *Bopivirus* (UPG35835). (C) Prediction of the three-dimensional structural similarity between the capsid proteins of UJS-2019picorna and Seneca Valley Virus (3cji.1. B).

may cause diseases in animals and even spread to humans. Tularemia [40] is an example, infections in humans can cause skin lesions, sepsis, and meningitis. However, in the past period, only a few complete picornaviruses (such as KT325852) have been identified in rabbits and uploaded to the GenBank database.

In this study, we assembled and obtained a complete virus belonging to the family *Picornaviridae* in a rabbit fecal library, named UJS-2019picorna. The whole genome size of UJS-2019picorna is 7832 bp excluding the poly(A)-tail, with GC content of 44.00%. The amino acid sequence identity of the best match in the blastx search of the UJS-2019picorna whole genome sequence is 37.20% (Bopivirus; YP_009,116,874), and there is no matching sequence in the blastn search. The P1, P2 and P3 regions of UJS-2019picorna share less than 40% amino acid identity with other known viruses. Phylogenetic analysis based on the amino acid sequence of RdRp showed that UJS-2019picorna has a distant relationship with 68 known genera of *Picornaviridae*. Based on the *Picornaviridae* Study Group guidelines, UJS-2019picorna could be presumed to be a new genus of picornaviruses. After a prevalence survey, UJS-2019picorna was positive in fecal samples of 9 rabbits, and 7 of them were also positive in blood samples. After three-dimensional structure prediction, UJS-2019picorna showed similarity to the capsid protein structure (VP1-4) of Seneca Valley Virus-001 (SVV-001) in the PDB database. There is a report that SVV-001 does not infect humans, but multiplies in tumor cells, displaying neuroendocrine features [41].

To some extent, this research has increased our understanding of the diversity of picornaviruses. In general, although there is a lot of evidence showing that picornaviruses are associated with various clinical diseases, there is little evidence that these viruses are directly related to specific pathological conditions, further dynamic monitoring of these viruses is necessary in the future.

Author contribution statement

Liye Zhou: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Xiang Lu: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Chunyan Zhao; Yu Zhang: Analyzed and interpreted the data.

Songyi Ning: Conceived and designed the experiments.

Wen Zhang: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Data availability statement

Data associated with this study has been deposited at All raw reads are deposited in the Short Read Archive (SRA) of the GenBank database with accession no. SRR22164050. The novel picornavirus sequence identified in this research has been uploaded to the GenBank database under accession number OP821762.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at [URL].

Animal and human rights statement

The study was approved by the Jiangsu university Ethics Committee on the use of animals and complied with Chinese ethics laws and regulations.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgments

This work was supported by National Key Research and Development Programs of China no. 2022YFC2603801 and the National Natural Science Foundation of China no. 82271264.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e15702.

References

[1] M. Shi, X.D. Lin, J.H. Tian, L.J. Chen, X. Chen, C.X. Li, et al., Redefining the invertebrate RNA virosphere, Nature 540 (7634) (2016) 539–543, https://doi.org/10.1038/nature20167. Epub 2016/11/24.

[2] R. Hargitai, P. Pankovics, A. Boros, R. Matics, E. Altan, E. Delwart, et al., Novel picornavirus (family Picornaviridae) from freshwater fishes (Perca fluviatilis, Sander lucioperca, and Ameiurus melas) in Hungary, Arch. Virol. 166 (9) (2021) 2627–2632, https://doi.org/10.1007/s00705-021-05167-y. Epub 2021/07/14.

[3] E. Altan, S.V. Kubiski, A. Boros, G. Reuter, M. Sadeghi, X. Deng, et al., A highly divergent picornavirus infecting the gut epithelia of zebrafish (Danio rerio) in research institutions worldwide, Zebrafish 16 (3) (2019) 291–299, https://doi.org/10.1089/zeb.2018.1710. Epub 2019/04/03.

[4] Y. Ao, Y. Zhou, D. Li, Z. Duan, A novel picornavirus identified in wild Macaca mulatta in China, Arch. Virol. 165 (2) (2020) 495–504, https://doi.org/10.1007/s00705-019-04442-3. Epub 2019/12/18.

[5] P. Pankovics, A. Boros, T. Kiss, G. Reuter, Identification and complete genome analysis of kobuvirus in faecal samples of European roller (Coracias garrulus): for the first time in a bird, Arch. Virol. 160 (1) (2015) 345–351, https://doi.org/10.1007/s00705-014-2228-7. Epub 2014/09/10.

[6] T.F. Ng, J.F. Wellehan, J.K. Coleman, N.O. Kondov, X. Deng, T.B. Waltzek, et al., A tortoise-infecting picornavirus expands the host range of the family Picornaviridae, Arch. Virol. 160 (5) (2015) 1319–1323, https://doi.org/10.1007/s00705-015-2366-6. Epub 2015/02/28.

[7] P. Pankovics, A. Boros, Z. Toth, T.G. Phan, E. Delwart, G. Reuter, Genetic characterization of a second novel picornavirus from an amphibian host, smooth newt (Lissotriton vulgaris), Arch. Virol. 162 (4) (2017) 1043–1050, https://doi.org/10.1007/s00705-016-3198-8. Epub 2016/12/23.

[8] Z. Liu, L. Li, Y. Guo, W. Xu, Y. Yuan, X. Liang, et al., Genome characterization and phylogenetic analysis of bovine hepacivirus in inner Mongolia, northeastern China, Zoon 2 (1) (2022), https://doi.org/10.15212/zoonoses-2022-0003.

[9] S.K. Lau, P.C. Woo, K.K. Lai, Y. Huang, C.C. Yip, C.T. Shek, et al., Complete genome analysis of three novel picornaviruses from diverse bat species, J. Virol. 85 (17) (2011) 8819–8828, https://doi.org/10.1128/JVI.02364-10. Epub 2011/06/24.

[10] S.E. Jacobs, D.M. Lamson, K. St George, T.J. Walsh, Human rhinoviruses, Clin. Microbiol. Rev. 26 (1) (2013) 135–162, https://doi.org/10.1128/CMR.00077-12. Epub 2013/01/09.

[11] H. Feng, A.L. Sander, A. Moreira-Soto, D. Yamane, J.F. Drexler, S.M. Lemon, Hepatovirus 3ABC proteases and evolution of mitochondrial antiviral signaling protein (MAVS), J. Hepatol. 71 (1) (2019) 25–34, https://doi.org/10.1016/j.jhep.2019.02.020. Epub 2019/03/17.

[12] P.C. Woo, S.K. Lau, G.K. Choi, Y. Huang, J.L. Teng, H.W. Tsoi, et al., Natural occurrence and characterization of two internal ribosome entry site elements in a novel virus, canine picodicistrovirus, in the picornavirus-like superfamily, J. Virol. 86 (5) (2012) 2797–2808, https://doi.org/10.1128/JVI.05481-11. Epub 2011/12/30.

[13] E.C. Scherbatskoy, K. Subramaniam, L. Al-Hussinee, K. Imnoi, P.M. Thompson, V.L. Popov, et al., Characterization of a novel picornavirus isolated from moribund aquacultured clownfish, J. Gen. Virol. 101 (7) (2020) 735–745, https://doi.org/10.1099/jgv.0.001421. Epub 2020/05/19.

[14] P. Pankovics, A. Boros, H. Biro, K.B. Horvath, T.G. Phan, E. Delwart, et al., Novel picornavirus in domestic rabbits (Oryctolagus cuniculus var. domestica), Infect. Genet. Evol. 37 (2016) 117–122, https://doi.org/10.1016/j.meegid.2015.11.012. Epub 2015/11/22.

[15] A. Boros, P. Pankovics, G. Reuter, Avian picornaviruses: molecular evolution, genome diversity and unusual genome features of a rapidly expanding group of viruses in birds, Infect. Genet. Evol. 28 (2014) 151–166, https://doi.org/10.1016/j.meegid.2014.09.027. Epub 2014/10/04.

[16] R. Zell, E. Delwart, A.E. Gorbalenya, T. Hovi, A.M.Q. King, N.J. Knowles, et al., ICTV virus Taxonomy profile: Picornaviridae, J. Gen. Virol. 98 (10) (2017) 2421–2422, https://doi.org/10.1099/jgv.0.000911. Epub 2017/09/09.

[17] P. Jiang, Y. Liu, H.C. Ma, A.V. Paul, E. Wimmer, Picornavirus morphogenesis, Microbiol. Mol. Biol. Rev. 78 (3) (2014) 418–437, https://doi.org/10.1128/MMBR.00012-14. Epub 2014/09/04.

[18] J.Y. Lin, T.C. Chen, K.F. Weng, S.C. Chang, L.L. Chen, S.R. Shih, Viral and host proteins involved in picornavirus life cycle, J. Biomed. Sci. 16 (2009) 103, https://doi.org/10.1186/1423-0127-16-103. Epub 2009/11/21.

[19] J.E. Mahar, M. Shi, R.N. Hall, T. Strive, E.C. Holmes, Comparative analysis of RNA virome composition in rabbits and associated ectoparasites, J. Virol. 94 (11) (2020), https://doi.org/10.1128/JVI.02119-19. Epub 2020/03/20.

[20] H. Wang, Y. Ling, T. Shan, S. Yang, H. Xu, X. Deng, et al., Gut virome of mammals and birds reveals high genetic diversity of the family Microviridae, Virus Evol 5 (1) (2019) vez013, https://doi.org/10.1093/ve/vez013. Epub 2019/06/14.

[21] W. Zhang, L. Li, X. Deng, B. Kapusinszky, P.A. Pesavento, E. Delwart, Faecal virome of cats in an animal shelter, J. Gen. Virol. 95 (Pt 11) (2014) 2553–2564, https://doi.org/10.1099/vir.0.069674-0. Epub 2014/08/01.

[22] B. Langmead, S.L. Salzberg, Fast gapped-read alignment with Bowtie 2, Nat. Methods 9 (4) (2012) 357–359, https://doi.org/10.1038/nmeth.1923. Epub 2012/03/06.

[23] B. Langmead, C. Wilks, V. Antonescu, R. Charles, Scaling read aligners to hundreds of threads on general-purpose processors, Bioinformatics 35 (3) (2019) 421–432, https://doi.org/10.1093/bioinformatics/bty648. Epub 2018/07/19.

[24] P. Ewels, M. Magnusson, S. Lundin, M. Kaller, MultiQC: summarize analysis results for multiple tools and samples in a single report, Bioinformatics 32 (19) (2016) 3047–3048, https://doi.org/10.1093/bioinformatics/btw354. Epub 2016/06/18.

[25] D. Li, R. Luo, C.M. Liu, C.M. Leung, H.F. Ting, K. Sadakane, et al., MEGAHIT v1.0: a fast and scalable metagenome assembler driven by advanced methodologies and community practices, Methods 102 (2016) 3–11, https://doi.org/10.1016/j.ymeth.2016.02.020. Epub 2016/03/26.

[26] B. Buchfink, K. Reuter, H.G. Drost, Sensitive protein alignments at tree-of-life scale using DIAMOND, Nat. Methods 18 (4) (2021) 366–368, https://doi.org/10.1038/s41592-021-01101-x. Epub 2021/04/09.

[27] M. Kearse, R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, et al., Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data, Bioinformatics 28 (12) (2012) 1647–1649, https://doi.org/10.1093/bioinformatics/bts199. Epub 2012/05/01.

[28] S. Kumar, G. Stecher, K. Tamura, MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets, Mol. Biol. Evol. 33 (7) (2016) 1870–1874, https://doi.org/10.1093/molbev/msw054. Epub 2016/03/24.

[29] F. Ronquist, M. Teslenko, P. van der Mark, D.L. Ayres, A. Darling, S. Hohna, et al., MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space, Syst. Biol. 61 (3) (2012) 539–542, https://doi.org/10.1093/sysbio/sys029. Epub 2012/02/24.

[30] M. Mirdita, K. Schutze, Y. Moriwaki, L. Heo, S. Ovchinnikov, M. Steinegger, ColabFold: making protein folding accessible to all, Nat. Methods 19 (6) (2022) 679–682, https://doi.org/10.1038/s41592-022-01488-1. Epub 2022/06/01.

[31] A. Waterhouse, M. Bertoni, S. Bienert, G. Studer, G. Tauriello, R. Gumienny, et al., SWISS-MODEL: homology modelling of protein structures and complexes, Nucleic Acids Res. 46 (W1) (2018) W296–W303, https://doi.org/10.1093/nar/gky427. Epub 2018/05/23.

[32] V. Aggarwala, G. Liang, F.D. Bushman, Viral communities of the human gut: metagenomic analysis of composition and dynamics, Mobile DNA 8 (2017) 12, https://doi.org/10.1186/s13100-017-0095-y. Epub 2017/10/14.

[33] G.D. Hannigan, Q. Zheng, J.S. Meisel, S.S. Minot, F.D. Bushman, E.A. Grice, Evolutionary and functional implications of hypervariable loci within the skin virome, PeerJ 5 (2017) e2959, https://doi.org/10.7717/peerj.2959. Epub 2017/02/15.

[34] J. Abrantes, W. van der Loo, J. Le Pendu, P.J. Esteves, Rabbit haemorrhagic disease (RHD) and rabbit haemorrhagic disease virus (RHDV): a review, Vet. Res. 43 (2012) 12, https://doi.org/10.1186/1297-9716-43-12. Epub 2012/02/14.

[35] C. Doyle, R. Nakamura, R. Bing, B. Rousseau, R.C. Branski, Mycoplasma affects baseline gene expression and the response to glucocorticoids in vocal fold fibroblasts, J. Med. Microbiol. 70 (5) (2021), https://doi.org/10.1099/jmm.0.001362. Epub 2021/05/27.

[36] S. Ciuria, M.S.M. Brouwer, M.M. de Gier, Y. van Zeeland, A. Bossers, B. Prahauser, et al., Chlamydia caviae in Swiss and Dutch Guinea pigs-occurrence and genetic diversity, Pathogens 10 (10) (2021), https://doi.org/10.3390/pathogens10101230. Epub 2021/10/24.

[37] K.L. Tyrrell, D.M. Citron, J.R. Jenkins, E.J. Goldstein, Periodontal bacteria in rabbit mandibular and maxillary abscesses, J. Clin. Microbiol. 40 (3) (2002) 1044–1047, https://doi.org/10.1128/JCM.40.3.1044-1047. Epub 2002/03/07.

[38] K. Hughes, A.M. Borman, Adiaspiromycosis in a wild European rabbit, and a review of the literature, J. Vet. Diagn. Invest. 30 (4) (2018) 614–618, https://doi.org/10.1177/1040638718772631. Epub 2018/05/03.

[39] C.M. Whipps, E.J. Gavard, J. Cohen, S.J. Ryan, Gastrointestinal parasites of the New England cottontail rabbit (Sylvilagus transitionalis) and eastern cottontail rabbit (Sylvilagus floridanus) in the Hudson Valley, New York, Parasitol. Res. 118 (7) (2019) 2257–2262, https://doi.org/10.1007/s00436-019-06351-5. Epub 2019/06/10.
[40] M. Faber, K. Heuner, D. Jacob, R. Grunow, Tularemia in Germany-A Re-emerging zoonosis, Front. Cell. Infect. Microbiol. 8 (2018) 40, https://doi.org/10.3389/fcimb.2018.00040. Epub 2018/03/06.
[41] S. Venkataraman, S.P. Reddy, J. Loo, N. Idamakanti, P.L. Hallenbeck, V.S. Reddy, Structure of Seneca Valley Virus-001: an oncolytic picornavirus representing a new genus, Structure 16 (10) (2008) 1555–1561, https://doi.org/10.1016/j.str.2008.07.013. Epub 2008/10/23.