Viromic Analysis of Feces From Laboratory Rabbits Reveals a Novel Circovirus

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Research Article

Keywords: laboratory rabbits, fecal virome, novel circovirus, complete genome

Posted Date: September 9th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-863446/v1

Abstract

Circoviruses are responsible for fatal diseases that can affect mammals and birds. Beak and feather disease virus (BFDV) is responsible for fatal diseases of birds, causing the psittacine beak and feather disease. The current study discovered a novel circovirus named RabCV from feces of laboratory rabbits, which shows close relationship to BFDVs. This novel circovirus was discovered from feces of rabbits, which showed low prevalence in the healthy laboratory rabbits. BFDV is responsible for fatal diseases that could affect birds, which suggested that the potential threat of the novel rabbit circovirus to the health of laboratory rabbits needs further study.

Main Text

Circoviruses belonging to the family *Circoviridae* are small, non-enveloped viruses with a circular, single-stranded DNA, which range from about 1.7 to 2.3 kb in size[1]. Circoviruses possess ambisense genomes with two major open reading frames encoding the replication-associated (Rep) and capsid (Cap) proteins. Rep is involved in the replication of the virus, while Cap is responsible for particle formation[2]. The first circovirus was described in a porcine cell culture and then a number of viruses with similar characteristics were discovered[3].

The family *Circoviridae* contains two genera: Circovirus and Cyclovirus. Members of the genus Circovirus are responsible for fatal diseases that can affect birds (Beak and feather disease virus, BFDV) and pigs (Porcine circovirus-2, PCV2) coming from veterinary science-related research[4, 5]. So far, many studies have identified the presence of circovirus genomes among some unconventional hosts such as freshwater fish and some mammals, including dogs, humans, bats, chimpanzees and so on. While those of the genus Cyclovirus have been identified not only in vertebrates but also in invertebrates such as insects[6].

In the current study, we identified a novel circovirus from the fecal samples of laboratory rabbits by using viral metagenomics and the complete genome sequence was assembled. Then the screening PCR was performed to count the positive rate of the rabbit circovirus (RabCV). The phylogenetic analysis based on the replication-associated protein was also performed.

Fecal samples were obtained using cotton swabs from 10 laboratory rabbits rased in the Animal Center of Jiangsu university in October 2018 and then stored at -80°C. When treating fecal samples, 500 μL DPBS was added into each 1.5 mL centrifuge tubes containing swabs and the tubes were vortexed for 5 min at room temperature. The fecal supernatants were then collected after centrifugation (5 min, 14,000 × g). The 10 fecal sample supernatants were then pooled into a sample pool. Sampling and all experiments in the present study were conducted with Ethical Approval given by Ethics Committee of Jiangsu University and the reference number is No. yzaujs2019121.

Removing eukaryotic and bacterial cell-sized particles were achieved by filtering the supernatant pool (500 μL) through a 0.45-μm filter (Millipore). The filtrates containing relative high concentration of viral

particles were then treated with a mixture of DNases and RNase where this step aimed to digest unprotected nucleic acid at 37°C for 90 min[7–9]. Remaining viral nucleic acid (including those from both DNA and RNA) was then extracted using QiaAmp Mini Viral RNA kit (Qiagen) according to manufacturer's protocol. A library was then constructed using the Nextera XT DNA sample preparation kit (Illumina) and nest generation sequencing (NGS) was performed on the NovaSeq Illumina platform with 250 bp paired ends with dual barcoding [10].

Paired-end reads of 250 bp produced by NovaSeq sequencing platform were debarcoded using vendor software of Illumina. An in-house analysis pipeline working on a 32-node Linux cluster was used to treat the NGS data. Clonal reads were abandoned, and low sequencing quality tails were removed using Phred quality score 30 as the threshold. Adaptors were removed using the default parameters of VecScreen that is NCBI BLASTn with specialized parameters designed for adapter removal. The remaining cleaned reads in the library were *de novo* assembled through the software of Ensemble assembler. The assembled contigs, along with the unassembled reads were compared to an in-house virus proteomic database using BLASTx with an E-value cutoff of $<10^{-5}$ [11, 12].

In order to assess the prevalence of the novel circovirus among the laboratory rabbits, nested-PCR was conducted uwing primers designed based on the rabbit circovirus genome. The PCR conditions were: 95℃ for 5 min, 31 cycles 95℃ for 30 s, 50℃ (for the first round) or 55℃ (for the second round) for 30 s and 72℃ for 40 s, a final extension at 72℃ for 5 min. The samples used in PCR screening included the 10 samples originally collected for library construction and 28 samples collected from 28 rabbits in the same animal center in May 2019. Primers used in PCR screening were designed based on the contig assembled from the sequence reads in the libraries. Primer sequences are shown in Table 1.

Phylogenetic analysis was established based on predicted virus amino acid sequences of this rabbit circovirus and the closest relatives on the best BLASTp hits in GenBank and representative members of related viral species or genera. Multiple sequence alignment was performed using CLUSTAL X (version 2.1) with the default settings. Phylogenetic trees were generated by using Bayesian Inference (BI) in MrBayes3.2. Settings were according to the specific sequence until average standard deviation of split frequencies was less than 0.01[13]. Putative ORFs in the virus genome were predicted in software Geneious10.0[14].

A library from fecal samples of laboratory rabbits was generated and sequenced using the Illumina NovaSeq platform. In this study, we detected a novel rabbit circovirus (named RabCV) in the fecal sample pool where the sequence reads could be assembled into a complete sequence genome with 1985 bp in length with a GC content of 47.9%, which showed a strong similarity to Beak and feather disease viruses based on the Blastx search, sharing the identify of 38.2%-42.5% with them based on the whole amino acid sequence of Rep protein.

PCR screening with primers designed according to this complete sequence was performed among all the 38 fecal samples, including the 10 samples for the libraries and the 28 samples collected in the second

time. PCR screening results indicated that 3 of the 38 samples were positive for this novel circovirus. The genomic structure was shown in Fig.1A. Genome characteristics include two putative ORFs encoding the Rep and Cap proteins, which are 229 aa and 130 aa long, respectively. A stem-loop was also observed between the ORFs of Cap and Rep (Fig 1B).

Phylogenetic analysis was performed based on the amino acid sequence of the Rep protein, including the best BLASTp searching matches and some available species of the genus Circovirus in the GenBank. The result indicated that the RabCV clustered more closely with Beak and feather disease viruses and other relative circoviruses from birds than with the circoviruses from mammals (Fig.2), which seems to be transitional between bird circoviruses and mammal circoviruses.

The development of viral metagenomics facilitates the identify of novel viruses among different animals and humans[8, 15–20]. In the present study, we used metagenomics to analysis the viral nucleic acids in the feces of laboratory rabbits from the school animal center.

A novel rabbit circovirus was identified which was named RabCV and the complete sequence of this virus was assembled with 1985 bp in length. The phylogenetic tree based on the amino acid sequence of the Rep protein was closely clustered with Beak and feather disease virus. Beak and feather disease virus (BFDV) is responsible for fatal diseases that could affect birds, causing the psittacine beak and feather disease (PBFD), which has emerged in recent years as an important threaten to Psittaciformes[4, 21], which suggested that the pathogenicity of the novel rabbit circovirus cannot be ruled out and the presence of possible pathogenicity needs to be confirmed.

Using viral metagenomic method, a novel circovirus was discovered from feces of laboratory rabbits, of which the complete genome was determined. Phylogenetic analysis indicated that this novel circovirus showed close relationship to BFDV. PCR screening showed this virus had low prevalence in a cohort of healthy laboratory rabbits. Whether this novel circovirus has potential threat to the health of laboratory rabbits needs further study.

Abbreviations

PCR: Polymerase Chain Reaction

BLAST: Basic Local Alignment Search Tool

BFDV: Beak and feather disease virus

PCV: Porcine circovirus

RabCV: Rabbit circovirus

Declarations

Availability of data and materials

The raw sequence reads from the metagenomic library were deposited in the Short Read Archive of GenBank database with accession no. SRX11616509. The genome sequence of the novel rabbit circovirus characterized in detail in this study was submitted to GenBank with the accession no. MZ695759.

Authors' contributions

SN and YX performed most of the experiments and data analysis, HW and YT designed this project, SN and YX wrote the manuscript, all authors read and approved the final version of this manuscript.

Funding

This work was supported by National Key Research and Development Programs of China No. 2017YFC1200201, Foundation for of Jiangsu University undergraduate student fund no. 202010299580X, and the key R & D projects of Jiangsu Province-industrial prospect and common key technology projects No. 2018040.

Acknowledgements

Not applicable

Ethics declarations

Ethics approval and consent to participate

Sample collection and all experiments in the present study were performed with Ethical Approval given by Ethics Committee of Jiangsu University the reference number is No. yzaujs2019121.

Consent for publication

All the authors have read the paper before submission and consent submission.

Conflict of interest

The authors declare that they have no conflict of interest.

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Table

Table 1

Primer sequences for circovirus screening

Specific virus	Primer ID	Application	Primer sequence (5'-3')
Circovirus	rabbitcirwF	First round	TCGCACGAATCTGTTCACCA
	rabbitcirwR		CCAACCTCCTCATCCACCAC
	rabbitcirnF	Second round	TGTGGTGAGAACTGCGTGAA
	rabbitcirnR		GCAGGCGTTGTGTGTCAAAG

Figures

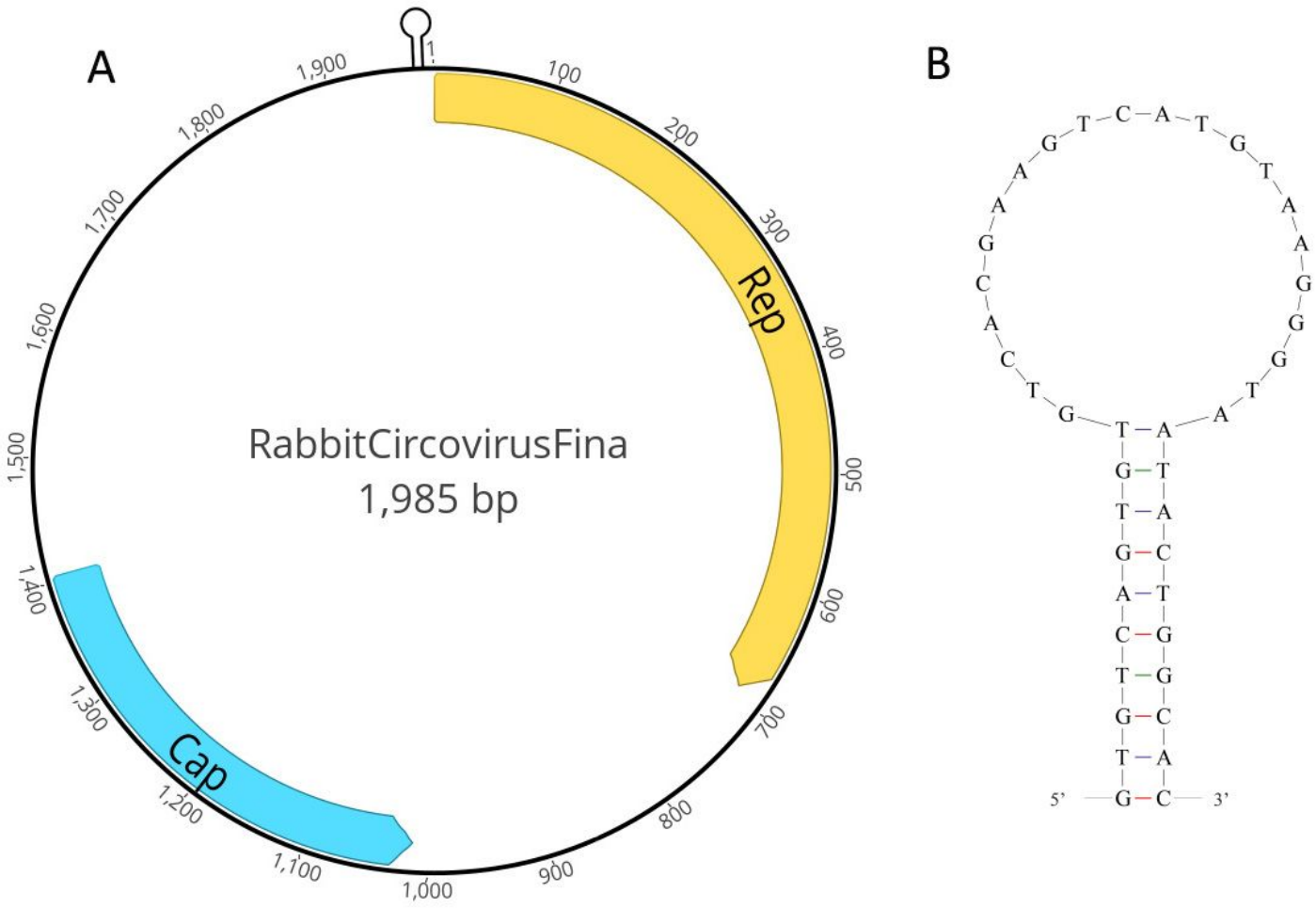

Figure 1

Genomic structure and the stem-loop of the novel rabbit circovirus (RabCV).

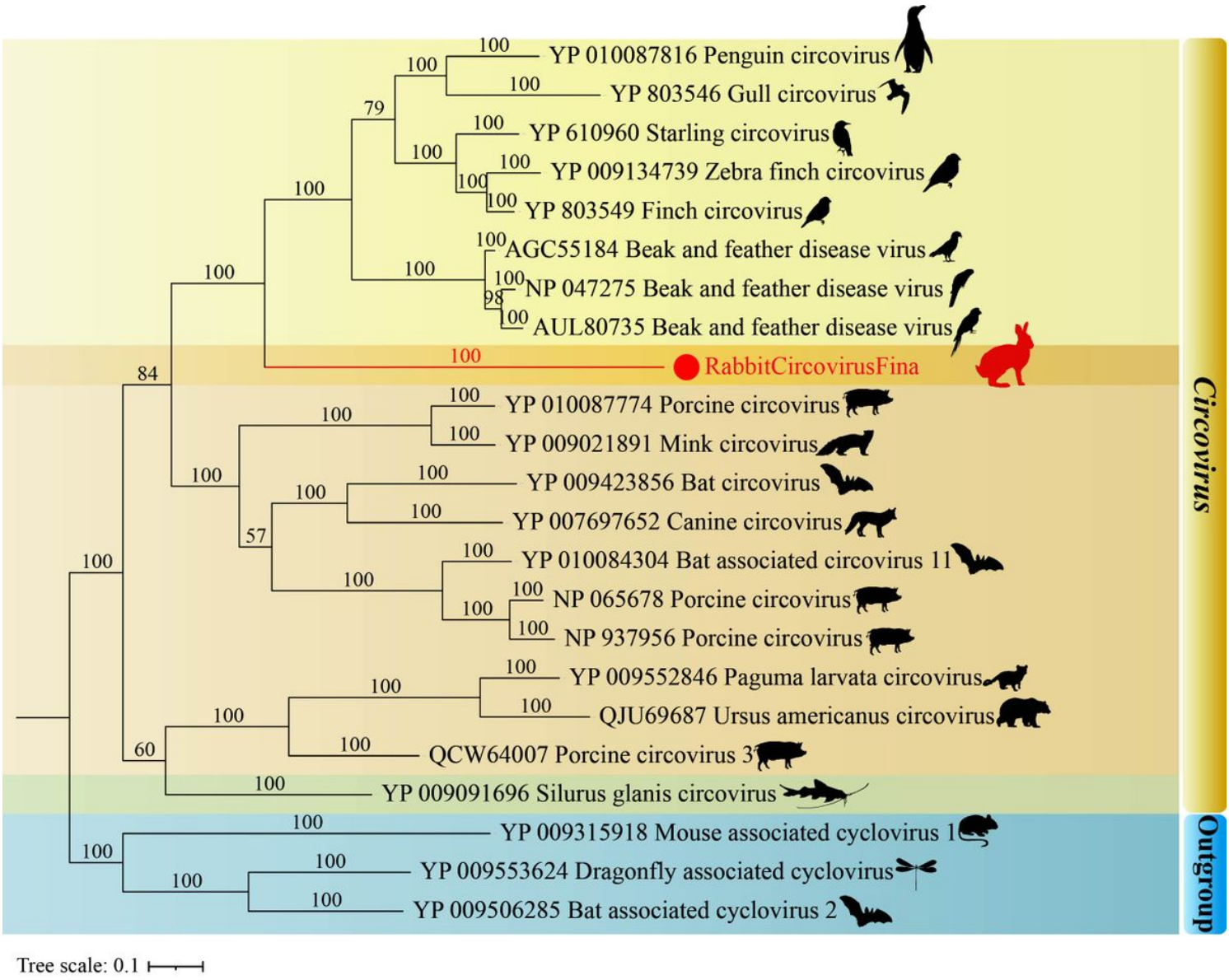

Figure 2

Phylogenetic analysis of the rabbit circovirus identified in the fecal samples of laboratory rabbits based on the amino acid sequence of Rep protein. Circovirus identified in this study was labeled in red.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- RabCV.fasta