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Research paper

Identification and genomic characterization of a novel rat bocavirus from brown rats in China

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ABSTRACT

Despite recent discoveries of novel animal bocaparvoviruses, current understandings on the diversity and evolution of bocaparvoviruses are still limited. We report the identification and genome characterization of a novel bocaparvovirus, rat bocaparvovirus (RBoV), in brown rats (*Rattus norvegicus*) in China. RBoV was detected in 11.5%, 2.4%, 16.2% and 0.3% of alimentary, respiratory, spleen and kidney samples respectively, of 636 brown rats by PCR, but not in samples of other rodent species, suggesting that brown rats are the primary reservoir of RBoV. Six RBoV genomes sequenced from three brown rats revealed the presence of three ORFs, characteristic of bocaparvoviruses. Phylogenetic analysis showed that RBoV was distantly related to other bocaparvoviruses, forming a distinct cluster within the genus, with ≤55.5% nucleotide identities to the genome of ungulate bocaparvovirus 3, supporting its classification as a novel bocaparvovirus species. RBoV possessed a putative second exon encoding the C-terminal region of NS1 and conserved RNA splicing signals, similar to human bocaparvoviruses and canine bocaparvovirus. In contrast to human, feline and canine bocaparvoviruses which demonstrates inter/intra-host viral diversity, partial VP1/VP2 sequences of 49 RBoV strains demonstrated little inter-host genetic diversity, suggesting a single genetic group. Although the pathogenicity of RBoV remains to be determined, its presence in different host tissues suggests wide tissue tropism. RBoV represents the first bocaparvovirus in rodents with genome sequenced, which extends our knowledge on the host range of bocaparvoviruses. Further studies are required to better understand the epidemiology, genetic diversity and pathogenicity of bocaparvoviruses in different rodent populations.

1. Introduction

Parvoviruses are small, non-enveloped, single-stranded DNA (ssDNA) viruses that can cause various diseases in animals and human. The family *Parvoviridae* is classified into two subfamilies based on their host range: *Parvovirinae* which infect vertebrates, and *Densovirinae* which infect arthropods such as insects. According to the International Committee on Taxonomy of Viruses, the subfamily *Parvovirinae* currently comprises eight different genera, including *Amdoparvovirus*, *Aveparvovirus*, *Bocaparvovirus*, *Copiparvovirus*, *Dependoparvovirus*, *Erythroparvovirus*, *Protoparvovirus* and *Tetraparvovirus* (Cotmore et al., 2014). In the past decade, a number of novel parvoviruses were discovered in animals and humans, which has led to various changes in the taxonomy and classification of parvoviruses. These include *Primate tetraparvovirus 1* (also known as human parvovirus 4 or PARV4) (Jones et al., 2005; Fryer et al., 2006, 2007) and related viruses in different animals (Lau et al., 2008; Tse et al., 2011; Adlhoch et al., 2010; Cadar et al., 2011; Sharp et al., 2010) which belong to the new genus *Tetraparvovirus*. Previously unclassified parvoviruses were also re-classified under *Aveparvovirus* and *Copiparvovirus* (Allander et al., 2001; Cheung et al., 2010; Day and Zsak, 2010). The discovery of novel parvoviruses is important for better understandings of their diversity and evolution.

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The genus *Bocaparvovirus* was named according to its initial two members, bovine parvovirus (BPV) and minute virus of canine (MVC) which were discovered in the 1960s to 1970s (Binn et al., 1970; Mochizuki et al., 2002; Spahn et al., 1966; Storz et al., 1978). The third bocaparvovirus, human bocavirus (HBoV), was discovered in 2005 from respiratory samples and later in stool samples of children (Allander et al., 2005; Lau et al., 2007; Sloots et al., 2006; Söderlund-Venermo et al., 2009). Four different HBoVs, HBoV1 to 4, now reclassified under *Primate bocaparvoviruses 1* and *2*, are known to be circulating worldwide, although their pathogenicity is still controversial (Allander et al., 2005; Arthur et al., 2009; Kapoor et al., 2009, 2010b). Subsequent to the discovery of HBoV, a number of novel bocaparvoviruses were discovered in different animals, including porcine bocaviruses (PBoVs) from swine (Blomström et al., 2009; Cheng et al., 2010; Lau et al., 2011; McKillen et al., 2011; Zeng et al., 2011; Zhai et al., 2010; Li et al., 2012; Shan et al., 2011a, 2011b), feline bocavirus (FBoV) (Lau et al., 2012), canine bocaviruses (CBoVs) (Kapoor et al., 2012; Li et al., 2013; Lau et al., 2012), gorilla bocavirus 1 (GBoV1) from Western gorillas (Kapoor et al., 2010a), California sea lion bocaviruses (Li et al., 2011), and bat bocaviruses (He et al., 2013; Wu et al., 2012). Some of these bocaparvoviruses also displayed interesting characteristics, such as inter- and intra-host genetic diversity, and/or recombination among human, porcine, feline and canine bocaviruses (Lau et al., 2011, 2012; Fu et al., 2011; Khamrin et al., 2013; Tyumentsev et al., 2014). Despite these recent findings, there are still many unknowns regarding the genetic and host diversity of bocaparvoviruses, as well as the evolutionary origin of HBoVs.

Similar to bats, rodents are also mammals with large species diversity and are reservoir for many viruses. We hypothesized that there are previously unrecognized bocaparvoviruses in rodents that may be closely related to human or other animal bocaparvoviruses. In this study, we examine the presence of bocaparvoviruses in wild and street rodents in Hong Kong and southern China. A novel bocaparvovirus, rat bocavirus (RBoV), was identified from brown rats, which represents the first bocaparvovirus in rodents with genome sequenced and characterized.

2. Materials and methods

2.1. Collection of rodent samples

All rodent samples were collected from August 2008 to August 2014 using procedures described previously (Lau et al., 2005, 2015). Samples from southern China were collected from rats in animal markets or restaurants. Samples from Hong Kong were collected from wild and street rodents with assistance of Agriculture, Fisheries and Conservation Department, and Food and Environmental Hygiene Department of the Hong Kong Special Administrative Region (HKSAR) respectively. Alimentary samples were placed in viral transport medium containing Earle's balanced salt solution (Invitrogen, New York, United States), 20% glucose, 4.4% $NaHCO_3$, 5% bovine albumin, 50,000 μg/ml vancomycin, 50,000 μg/ml amikacin, 10,000 units/ml nystatin, before transportation to the laboratory for DNA extraction. To prevent cross contamination, dissections were performed using disposable scalpels and tissues were collected from the center of each tissue after surface decontamination. Protective gloves were also changed after each tissue collection. The study was approved by the Committee on the Use of Live Animals for Teaching and Research, The University of Hong Kong.

2.2. Detection of bocaparvoviruses

DNA was extracted from all samples using QIAamp DNA Mini Kit (QIAgen, Hilden, Germany). DNA was subject to PCR for bocaparvoviruses, using forward primer 5′-GCCAGCACNGGNAARACMAA-3′ and reverse primer 5′-CATNAGNCAYTCYTCCCACCA-3′ targeted to 141-bp fragment of the non-structural protein 1 (NS1) gene, designed by multiple alignments of the nucleotide sequences of NS1 regions of known bocaparvoviruses including HBoV, GBoV, BPV, MVC and PBoV. Since potentially novel bocaparvoviruses were detected, subsequent bocaparvovirus detection was performed using specific primers (forward primer 5′-CAAGTAGGACATTGGATA-3′ and reverse primer 5′-TCCAACTGCATTTACAAT-3′ for RBoV, which targeted to 126-bp fragment of NS1) designed based on the obtained genome sequences. The PCR mixture (25 μl) contained DNA, PCR buffer (10 mM Tris–HCl pH 8.3, 50 mM KCl, 2 mM $MgCl_2$ and 0.01% gelatin), 200 μM of each dNTPs and 0.625 U *Taq* polymerase (Applied Biosystem, Foster City, CA, USA). PCR was performed in 40 cycles of 94 °C for 1 min, 50 °C for 1 min and 72 °C for 1 min and a final extension at 72 °C for 10 min in an automated thermal cycler (Applied Biosystem, Foster City, CA, USA). Standard precautions were taken to avoid PCR contamination and no false-positive was observed in negative controls. PCR products were gel-purified using the QIAquick gel extraction kit (QIAgen, Hilden, Germany). Both strands of the PCR products were sequenced twice with an ABI Prism 3700 DNA Analyzer (Applied Biosystems, Foster City, CA, USA), using the PCR primers. The sequences of the PCR products were compared with known sequences of NS1 regions of bocaparvoviruses in the GenBank database.

2.3. Genome sequencing and analysis

Nearly-complete genome sequences were determined for the potentially novel rat bocavirus, RBoV, from six samples of three rats, using the strategy described in our previous publications (Lau et al., 2008, 2011, 2012). DNA directly extracted from the specimens was used as templates and amplified by degenerate primers designed by multiple alignment of the genomes of HBoV, GBoV, BPV, MVC and PBoV, and additional primers covering the original degenerate primer sites were designed from the results of the first and subsequent rounds of sequencing. Primer sequences are available on request. The terminal sequences were confirmed by a modified protocol for RACE (Allander et al., 2005; Lau et al., 2011). Sequences were assembled and manually edited to produce final sequences of the viral genome. All assembled sequences were confirmed by independent PCR using specific primers across overlapping regions to ensure accuracy of the assembled sequences. The nucleotide sequences of the genomes and the predicted ORFs were compared to those of known bocaparvoviruses. Maximum likelihood (ML) phylogenetic tree was constructed using PhyML version 3.0 (Guindon and Gascuel, 2003) under the best evolutionary model (GTR + I + G) determined by Modelgenerator (Keane et al., 2006). Bootstrap values were estimated by using 100 replicates on the ML substitution model. Protein domain and family analysis was performed using InterProScan (Apweiler et al., 2001) and/or multiple sequence alignment.

2.4. Sequencing of partial VP1/VP2 sequences

The genetic diversity of RBoV strains were studied by amplifying and sequencing of their partial capsid protein sequences, using primers (forward primer 5′-GGCATCTATACTTTGCTAGATT-3′ and reverse primer 5′-GAATTTAGGATATGGAGCATA-3′) targeted to a 941-bp region of their VP1/VP2 genes respectively, designed by multiple alignments of the obtained genome sequences, and the above PCR conditions.

2.5. Nucleotide sequence accession numbers

The nucleotide sequences of RBoV have been lodged within the GenBank sequence database under accession no. KT454512 - KT454517 and KX901809 - KX901843.