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Research paper

Genetic and phylogenetic characterization of novel bocaparvovirus infecting chimpanzee☆

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ABSTRACT

Primate bocaparvoviruses were first described in 2005, since then further human and gorilla bocaparvoviruses have been identified. To uncover diversity of non-human primates' bocaparvoviruses, their phylogenetic relationship and potential to cross the host species barrier, we tested 153 fecal samples from 17 captive primate species. The only one captive female of central chimpanzee (coded CPZh2) has been identified as bocaparvovirus positive. Based on the full genome phylogenetic analyses, CPZh2 strain shows close relationship to HBoV3 and GBoV. Further recombination analysis confirmed expected mosaic origin of CPZh2 strain. According the phylogenetic position, following the ICTV recommendations, we propose a novel genotype within the *Primate bocaparvovirus* 1 species infecting chimpanzee.

1. Introduction

Members of the genus *Bocaparvovirus* (*Parvoviridae*, *Parvovirinae*) are small, non-enveloped viruses infecting broad spectrum of mammals including humans and non-human primates (NHP). Based on the International Committee on Taxonomy of Viruses 2013 release (http://ictvonline.org/virusTaxonomy.asp), primate bocaparvoviruses are divided into two species. *Primate bocaparvovirus* 1 includes *Human bocaparvoviruses* 1 and 3 (HBoV1, HBoV3) and *Gorilla bocaparvovirus* (GBoV) genotypes. *Human bocaparvoviruses* 2a-c and 4 (HBoV2a-c, HBoV4) are members of the *Primate bocaparvovirus* 2 species.

HBoV was first identified by molecular screening of nasopharyngeal secretions of children suffering from respiratory disease in 2005 (Allander et al., 2005). HBoV1 has been detected worldwide causing mild-to-severe lower and upper respiratory tract infections especially in young infants, either alone or in co-infection with other pathogens (Chieochansin et al., 2009; Rotzén-Östlund et al., 2014; Do Amaral De Leon et al., 2013; Jula et al., 2013). HBoV2-HBoV4 have been mostly detected in stool samples, but their role in gastroenteritis is still being discussed (Cashman and O'Shea, 2012; Kapoor et al., 2009). The detection and identification of bocaparvoviruses infecting western lowland gorillas (*Gorilla gorilla*) suffering from diarrhea (Kapoor et al., 2010a) indicate their possible involvement in gastrointestinal tract disorders.

Bocaparvovirus genome is formed by a single stranded DNA of approximately 5000 nucleotides (nt) in length. Beside other parvoviruses, members of this genus have 3 open reading frames (ORF). ORFs NS1 and NP1 encode non-structural proteins, ORF VP encodes two structural proteins VP1 and VP2 translated by the leaky scanning mechanism and forming the icosahedral capsid (Fédière et al., 2004; Allander et al., 2005). Bocaparvoviruses are characterized by a rapid evolution and frequent recombination events that were described as a source of new bocaparvovirus variants. HBoV3 is a chimeric form of HBoV1 and HBoV2, with recombination between NP1 derived from HBoV1 and VP derived from HBoV2 (Arthur et al., 2009; Kapoor et al., 2010b; Khamrin et al., 2013). HBoV2 was also proved to originate from a recombination between HBoV1 and HBoV4 (Fu et al., 2011). The rate of mutation events can be compared to those in RNA viruses (Babkin et al., 2013; Zehender et al., 2010).

☆ The GenBank accession number for the CPZh2 bocaparvovirus sequence is KT223502.

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Beside numerous studies on the presence of bocaparvoviruses in humans, there are two reports describing these viruses in chimpanzees and gorillas. Kapoor et al. (2010a) described whole genome of a new *Gorilla bocaparvovirus* (GBoV) in fecal samples from captive gorillas suffering from diarrhea. Sharp et al. (2010) examined fecal samples from free ranging chimpanzees and gorillas in Cameroon and reported bocaparvovirus DNA in two chimpanzees and two gorillas. In the same study, plasma samples of African great apes from Cameroonian wildlife sanctuaries were tested as seropositive for HBoV1/2 in 73% and 36% of chimpanzees and gorillas, respectively, suggesting a frequent exposure to the virus.

Despite high rates of seropositivity and detection of viral DNA in great apes, only few partial sequences of NS1 and VP2 ORFs are available, in addition to the full GBoV genome (Sharp et al., 2010; Kapoor et al., 2010a). Here we describe nearly full genome sequence of bocaparvovirus detected in a chimpanzee, clustering with HBoV3 and GBoV.

2. Materials and methods

2.1. Samples

Fecal samples from captive African primates were collected in 12 Czech and one Slovak zoos in 2012; all individuals were considered as healthy in time of sampling. Samples were obtained from chimpanzees (*Pan troglodytes*; n = 25), lowland gorillas (*Gorilla gorilla*; n = 10), *Thercopithecus gelada* (n = 5), *Mandrillus sphinx* (n = 12), *Papio anubis* (n = 4), *Papio hamadryas* (n = 18), *Chlorocebus sabaeus* (n = 13), *Colobus guereza* (n = 12), *Erythrocebus patas* (n = 10), *Lophocebus aterrimus* (n = 4), *Cercopithecus campbelli* (n = 4), *Cercopithecus diana* (n = 9), *Colobus angolensis palliatus* (n = 6), *Cercopithecus neglectus* (n = 8), *Miopithecus ogouensis* (n = 5), *Macaca sylvanus* (n = 6) and *Cercopithecus mitis* (n = 2). Fecal material was preserved in RNA*later*™ (Sigma, Germany) and stored at −20 °C until use.

2.2. DNA extraction

1.2 ml of a fecal material resuspended in RNA later was pelleted by centrifugation at 7000 rcf for 15 mins. Total DNA was isolated from a pellet using PSP® Spin Stool DNA Kit (Stratec, Germany) according to manufacturer's instructions, DNA was eluted in the final volume of 100 μl. Concentration and purity of isolated DNA was determined by spectrophotometric analysis at 260/280 nm (Infinite® 200 PRO; Tecan, Switzerland).

2.3. PCR screening

Samples were screened for the presence of bocaparvovirus DNA by nested PCR using degenerated primers located in NS1 gene derived from Sharp et al. (2010). The first round of amplification was performed in a total volume of 14 μl, using "PPP master mix" (Top-Bio, Czech Republic), 0.35 μM primers and 50 ng DNA as a template. One microliter of this reaction was carried over to the second round and amplified in a total volume of 14 μl under similar conditions as the first round. Cycling conditions were the same for both rounds: 5 min at 94 °C, followed by 30 cycles of 18 s at 94 °C, 30 s at 50 °C, 90 s at 72 °C, and a final extension of 10 min at 72 °C (Sharp et al., 2010). PCR products were visualized in 1% agarose gel and stained with Midori Green Advanced DNA stain (Nippon Genetics Europe GmbH, Germany).

PCR products of expected length of 533 nt were gel purified using QIAquick Gel Extraction Kit (Qiagen, Germany) according to manufacturer's instructions, DNA was eluted by sterile nuclease free water in a total volume of 30 μl. Purified PCR products were cloned into pGEM®-T Easy Vector System (Promega, USA) and sequenced by Macrogen capillary sequencing services (MacrogenEurope, The Netherlands).

Additional sets of primers located in conserved parts of genome were designed to overhang each neighbor pair (Table 1) based on alignments of newly obtained and previously published *Primate Bocaparvovirus* 1 and 2 sequences (HBoV1 NC_007455; HBoV2 NC_012042 and GU301644; HBoV3 EU918736; HBoV4 KC461233; and GBoV HM145750). Nested and semi-nested PCRs were performed; expected length of PCR products is shown in the Table 1. The first round of amplification was performed in total volume of 25 μl using 1.25 U LA Taq DNA polymerases mix (Top-Bio, Czech Republic), 10× LA PCR buffer, 2% DMSO, 500 μM dNTPs mix and 0.5 μM outer primers. One microliter of this reaction was carried over to the second round and amplified. The second round of amplification was performed in a total volume of 25 μl, using PPP master mix (Top-Bio, Czech Republic) and 0.5 μM inner primers. Cycling conditions were the same for all the first rounds of PCR: 5 min at 94 °C, 30 cycles of 15 s at 94 °C, 30 s at 50 °C, 150 s at 68 °C, and 10 min at 68 °C as final extension. Cycling conditions for the second round were the same except annealing temperatures (Table 1): 5 min at 94 °C, followed by 30 cycles of 15 s at 94 °C, 30 s at 50/52 °C, 2 min at 72 °C, and 10 min at 72 °C as a final extension. PCR products were purified, cloned and both strands sequenced by Macrogen Europe as described above. Acquired sequences were compared with sequences obtained from GenBank and analyzed using BioEdit software (Hall, 1999).

2.4. Phylogenetic analysis

Alignments were generated using MAFFT algorithm (Katoh et al., 2002); appropriate evolution models were determined by likelihood ratio test using R software (R Core Team, 2013). Phylogenetic analyses were performed using maximum likelihood method in PhyML 3.0 software (Guindon and Gascuel, 2003). Phylogenetic trees were visualized and edited in FigTree v1.4.1(http://tree.bio.ed.ac.uk/software/figtree/). Sequences used for the comparison involved the following genotypes: HBoV1 (GenBank accession number NC_007455, DQ000496, DQ000495, KC823115), HBoV2 (NC_012042, GU301644, EU082214, FJ170278, GU048664), HBoV3 (EU918736, GU048665, FJ948861, FJ973562, HM132056, FJ973563, GQ867667), HBoV4 (KC461233, FJ973561), GBoV (HM145750), wild chimpanzee (HQ113148, HQ113146, HQ113151, HQ113150) and gorilla (HQ113149, HQ113147, HQ113145) isolates and canine minute virus as an outgroup (FJ899734). Detection of possible recombination breakpoints was performed using Simplot (Lole et al., 1999) and GARD (Kosakovsky Pond et al., 2006) software, only breakpoints supported by 70% of permutated trees were followed. P-distances were computed using PAUP software version 4b.10 (Swofford, 2002)

3. Results

Bocaparvovirus detection in a set of 153 captive African primate fecal samples was performed using PCR with degenerated primers located in a conserved part of NS1 gene. Amplicons of expected length were detected in three samples, but sequencing confirmed the presence of bocaparvovirus DNA in a single captive (but wild born) female chimpanzee only (sample further referred as CPZh2). Paired fecal samples were collected one year later to obtain information about possible persistence of bocaparvovirus, but PCR results showed no evidence of bocaparvovirus DNA in these samples.

Nearly whole genome sequence of CPZh2 was amplified using additional sets of primers targeting all known GBoV and HBoV genotypes (Table 1). We described three ORFs spanning 4477 bp of CPZh2 genome: (i) ORF NS1 located at the 5'end of genome, consisting of 1938 nt, predicted to code for 646 amino acids (aa); (ii) ORF NP1 spanning 654 nt, predicted to code 218 aa; and (iii) VP1 and VP2 genes located within ORF VP at the 3'end of the genome, amplified only partially and spanning1616 and 1229 nt only.

Whole genome phylogenetic analysis revealed CPZh2 sequence clustering most closely to the clade formed by GBoV1 and HBoV1