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New species of Torque Teno miniviruses infecting gorillas and chimpanzees ☆

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ABSTRACT

Anelloviridae family is comprised of small, non-enveloped viruses of various genome lengths, high sequence diversity, sharing the same genome organization. Infections and co-infections by different genotypes in humans are ubiquitous. Related viruses were described in number of mammalian hosts, but very limited data are available from the closest human relatives – great apes and non-human primates.

Here we report the 100% prevalence determined by semi-nested PCR from fecal samples of 16 captive primate species. Only the *Mandrillus sphinx*, showed the prevalence only 8%. We describe three new species of gorillas' and four new species of chimpanzees' *Betatorqueviruses* and their co-infections in one individual. This study is also first report and analysis of nearly full length TTMV genomes infecting gorillas. Our attempts to sequence the complete genomes of anelloviruses from host feces invariably failed. Broader usage of blood /tissue material is necessary to understand the diversity and interspecies transmission of anelloviruses.

Introduction

Despite the 18 years from the first description of anellovirus in primate species – humans, very little is known about their origin and limited data are available from the closest human relatives – great apes and non-human primates. First member of new, currently unassigned family *Anelloviridae* was described in 1997 in Japanese patient with acute post transfusion hepatitis and was named human TTV (following the TT initials of index case patient, lately termed Torque Teno virus) (Nishizawa et al., 1997). TTV is a small, non-enveloped virus carrying negative, single-stranded DNA genome of approximate length of 3.8 kb (Mushahwar et al., 1999). Subsequently shorter analogs sharing the same genome organization were described in humans - TTV-like mini virus (TTMV) (Takahashi et al., 2000) with the genome length of about 2.9 kB and lately torque teno midi virus (TTMDV) with genome length of about 3.2 kb (Jones et al., 2005).

All anelloviruses share the genomic organization with three overlapping open reading frames (ORF1 to 3) and short GC rich sequence in the most conserved untranslated region. The expression profile of anelloviruses is not fully characterized, but the work of Qiu et al. shows that following transfection of human 293 cells at least 6 TTV proteins were produced by alternative splicing of three different mRNAs (Qiu et al., 2005). Further molecular characterization of anelloviruses as gene expression and protein functions remains poorly understood.

In humans, prevalence up to 100% and dual or triple infection by different species was published (Hussain et al., 2012; Ninomiya et al., 2008; Pinho-Nascimento and Leite, 2011), however so far without apparent association with any disease.

TTV analogues were first detected in farm animals, New World monkeys, macaques and chimpanzees (Cong et al., 2000; Leary et al., 1999; Okamoto et al., 2000a; Verschoor et al., 1999). Later authors

☆The GenBank accession number for the Cpz1cl1 sequence is KT027935, for the Cpz1cl2 sequence is KT027936, for the Cpz2cl1 sequence is KT027937, for the Cpz2cl2 sequence is KT027938, for the GorF sequence is KT027939, for the GorMcl3 sequence is KT027940, and for the GorMcl4 sequence is KT027941.

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(Kapusinszky et al., 2015; Thom et al., 2003) confirmed the presence of anelloviruses in great apes other than chimpanzees (gorillas and orangutans) and Old World monkeys (drills, mandrills, mangabeys and African green monkeys). Beside complex work on *Chlorocebus sabaeus* (Kapusinszky et al., 2015), only short parts of genome (mostly from conserved untranslated region) from sera samples were detected and analyzed. The reported genome sizes in non-primate animals are in general smaller than for the human variants ranging from 2019 nt in cats to 2878 nt in pigs (Biagini et al., 2007; Inami et al., 2000; Ng et al., 2009b; Okamoto et al., 2002, 2001, 2000a, 2000b; Van den Brand et al., 2012). Besides humans, chimpanzees are so far the only reported hosts infected by anelloviruses of various genome lengths (assigned to genera *Alphatorquevirus, Betatorquevirus* and *Gammatorquevirus*).

In this study, we examined presence and determined prevalence of TTV-like viruses in fecal samples of 17different African primate species in Czech and Slovak zoos. We also described almost full length genomes of two different species of TTMV co-infecting wild-born, captive living gorilla male and one captive born gorilla female and also coding sequences from two captive born chimpanzees. We reported TTV-like virus infection and prevalence in all 17 studied African primate species and first characterized gorilla's analogue of TTMV. The elucidation of host range and characterization of genome sequences of TTV-like viruses contribute to understanding of origin, evolution, and interspecies transmission potential of anelloviruses.

Results

To establish the prevalence of anelloviruses of captive African non-human primate species in 12 Czech and one Slovak zoos, fecal samples were collected. In total 153 samples from 17 different species were screened using semi-nested PCR for anellovirus detection from which 136 samples were positive. The prevalence rates range from 75 to 100% in all tested species (Table 1) with the only exception of *Mandrillus sphinx* where only single sample from 12 was positive (8.3%). Specificity of PCR reaction was confirmed by sequencing of 35 randomly chosen amplification products from representative primate species. Nucleotide sequence analysis showed high variability out of the primer binding sites and confirmed common co-infection by several annellovirus species in one individual (Supplementary file 1).

Table 1
Anellovirus prevalence in NHP species based on semi-nested PCR targeting the conserved UTR; species in which anelloviruses were described for the first time are in bold.

	+/all	%	Prevalence at the genus level
Gorilla gorilla	10/10	100.0	
Pan troglodytes	25/25	100.0	
Thercopithecus gelada	5/5	100.0	
Mandrillus sphinx	1/12	**8.3**	
Papio anubis	4/4	100.0	100.0
Papio hamadryas	18/18	100.0	
Chlorocebus sabaeus	13/13	100.0	
Colobus guereza	10/12	83.3	88.9
Colobus angolensis	6/6	100.0	
Erythrocebus patas	9/10	90.0	
Lophocebus aterrimus	4/4	100.0	
Cercopithecus campbelli	4/4	100.0	87.0
Cercopithecus diana	8/9	88.9	
Cercopithecus neglectus	6/8	75.0	
Cercopithecus mitis	2/2	100.0	
Miopithecus ogouensis	5/5	100.0	
Macaca sylvanus	6/6	100.0	

The DNA extracted from the whole blood/serum samples of fourteen NHPs was also used for the detection of anelloviruses by nested PCR. Except one guereza sample, all other tested DNAs were anellovirus positive. Using this DNA as template, the entire genomic sequence of anelloviruses from only 4 samples could be amplified by PCR with the further described inverted primers. The strongest PCR signal of anellovirus genome was found to be approx. 2.8–2.9 kb in size (in two gorillas' and two chimpanzees' samples), weaker signal was also detected in approx. size of 4 kb in some samples. Remaining nine blood/serum samples did not provide any PCR product in whole genome amplification. The 2.8–2.9 kb PCR product for each sample was molecularly cloned and seven TTV clones (GorF; GorM-cl3 and cl4; CPZ1-cl1 and cl2; and CPZ2-cl1 and cl2) were sequenced over the entire coding part of the genome. Missing UTR part between primers was amplified and sequenced for both clones of GorM and one clone of GorF samples resulting in first almost complete gorilla infecting anellovirus genomes. According to the genome length and phylogenetic analysis (Fig. 2, Supplementary file 2) all newly described anelloviruses infecting gorillas and chimpanzees were classified to the genus *Betatorquevirus*.

Gorilla TTMV

Anelloviruses infecting gorillas share the common genome length and structure including UTR as most conserved region, GC rich region and major ORF (so called ORF1 coding putative capsid protein) spanning 1992 nt, 1995 nt and 2025 nt respectively. The nearly full length gorillas' TTMV genomes were obtained due to difficulties to sequence the GC rich part of anellovirus' genomes. The length of obtained sequences was 2851 nt for GorM cl3, 2856 nt for GorM cl4 and 2568 nt for GorF, which corresponds to the length of anellovirus mini genomes wihout GC rich domain in humans and other primates. The three genomes displayed overall organization of anelloviruses, with main ORFs in the same orientation; nevertheless they differed in the number of these major ORFs.

Transcribed region of the genomes encompassed TATA-box (TATAA) which was recognized 100–160 bp before the start codon of the first ORF and ended by polyadenylation signal (ATAAA) detected 110–120 nt following stop codon of ORF3 protein. The complete sequence of GC rich region was not obtained for technical reasons. Anyway 40 nt long fragment of GC rich stretch identified in GorM cl3 genome had the GC content of 84%.

The largest ORF was 1992 nt, 2025 nt and 1995 nt long in GorM cl3, GorM cl4 and GorF respectively. Based on the similarity with other known anelloviruses, ORF1 is most likely coding putative nucleocapsid protein. The similarity of ORF1s is ranging from 53 to 65% at nucleotide and from 38 to 54% at amino acid level (Table 2). All of them also contained nuclear localization signals detected by both cNLS Mapper (Kosugi et al., 2009) and NLStradamus (Nguyen et al., 2009). Arginine rich region at the beginning of ORF1 gene was also found ranging from 22 to 25 arginine residues in first 50 amino acids.

ORF2 partially overlapped the ORF1 and resulted in 348 nt (GorM cl3 and GorF), and 342 nt (GorM cl4) long reading frames. TTV/CAV-common motif WX7HX3CXCX5H described previously (Jazaeri Farsani et al., 2013; Takahashi et al., 2000), was found in the ORF2 of the new gorilla TTMV described here (CX7HX3CXCX5H) at the amino acid positions 25–45 of GorM cl4, 19–39 of GorF and degenerated in one position to the sequence CX7HX3**R**XCX5H in positions 22–42 of GorM cl3.

Finally, ORF3 overlapping with 3' terminus of ORF1 was found in GorM cl3 (495 nt) and GorF (504 nt) genomes only. In agreement with previously described sequences, in both cases the ORF3