PARV4 found in wild chimpanzee faeces - alternate route of transmission?

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Abstract

Human parvovirus 4 (PARV4, family *Parvoviridae*, genus *Tetraparvovirus*) displays puzzling features, such as uncertain clinical importance/significance, unclear routes of transmission and discontinuous geographical distribution. The origin, or the general reservoir, of human PARV4 infection is unknown. We aimed to detect and characterize PARV4 virus in faecal samples collected from two wild chimpanzee populations and 19 species of captive non-human primates. We aimed to investigate these species as a potential reservoir and alternate route of transmission on the African continent. From almost 500 samples screened, a single

wild *Pan troglodytes schweinfurthii* sample tested positive. Full genome analysis, as well as single ORF phylogenies, confirmed species-specific PARV4 infection.

Keywords: PARV4, non-human primates, Africa, phylogeny

Human parvovirus 4 (PARV4, genus *Tetraparvovirus*, family *Parvoviridae*) displays puzzling features, such as uncertain clinical importance/significance, unclear routes of transmission and discontinuous geographical distribution. The first primate tetraparvovirus probably emerged in the 1980´s together with the appearance of the HIV virus infection [1] and, so far, three phylogenetically distinct genotypes have been described in humans. Since first human PARV-4 description in 2005, the epidemiology data are growing and were recently reviewed by Matthews *et al.* [2]. Human PARV4 G1 and G2 are predominant in Europe, North America and Middle East [3–10], and are most often described in patients with a history of parenteral drugs application suffering from HIV, HCV or HBV infections [10, 11]. Genotype 2 is also reported from Asia [6, 12]. Human PARV4 G3 is distributed across Africa [13, 14] and exhibits unique features of transmissibility. It has been detected in diverse cohorts in the absence of other blood-borne viruses and/or intravenous drug/therapy application history [13–16], suggesting transmission routes other than parenteral infection. High frequencies of seropositivity in wild chimpanzee and gorilla serum samples also support the existence of another route of transmission of tetraparvoviruses in Africa [17].

The origin, or the general reservoir, of the human PARV4 is unknown. Three independent transmission events, perhaps from chimpanzees or other primate species, could be the source of the three genotypes [1, 17] in humans. A limited number of sequenced non-human primate tetraparvoviruses have been found to be species-specific for chimpanzees and colobus monkeys [17, 18]. Despite evidence of frequent exposure of African hunters to non-human primate bush meat, no direct evidence of cross-species parvovirus transmission has been found [18].

All previously described tetraparvovirus sequences from NHPs were obtained from either blood or bush meat samples [17, 18]. Our aim was to detect and characterize tetraparvoviruses in fecal samples collected from chimpanzees and other NHP with different levels of contact with humans, based on samples from (i) a wild, non-habituated eastern chimpanzee population in Issa Valley (Tanzania), known to be SIV positive [19], (ii) a wild, habituated eastern chimpanzee population from Kalinzu Forest Reserve (Uganda), and (iii) captive African non-human primates from Czech and Slovak zoos.

In total, 202 unidentified faecal samples were collected from eastern chimpanzees, with very limited contact with humans, in the Issa Valley (Figure 1) in Tanzania (between March 2012 and November 2013). The study site comprises approximately ~100 km^2 and is one of the driest and most open chimpanzee habitats, situated east of Lake Tanganyika, in western Tanzania [20]. The population density of Issa chimpanzees is estimated to be ~0.25 individuals/km^2 [21]. Further faecal samples (in total 123, 1-10 per individual) were collected from 42 identified individuals of habituated eastern chimpanzees (20 males, 22 females), with daily distant contact with researchers, in the Kalinzu Forest Reserve (Figure 1) [22] during
April–July 2014. The forest reserve (~137 km^2) is one of the three largest forest blocks in Uganda, being located on the eastern ridge of the western Rift Valley. The chimpanzee population density is estimated to be ~1.67 individuals/km^2 [23]. Captive primates, with daily intensive contact with keepers, were sampled in 13 Czech and Slovak zoological gardens; 25 *Pan troglodytes*, 10 *Gorilla gorilla* and 118 faecal samples from 17 different species other African primates were obtained [24] (see Supplementary material).

All faecal samples were preserved in RNAlater™ (Ambion Inc., Austin, TX) and stored at -80 °C until further use. DNA was isolated using PSP® Spin Stool DNA Kit (Stratec, Germany) according to manufacturer's instructions. DNA was screened by nested PCR based on a protocol published by Sharp *et al.* [17]. PCR products of the expected length of 295 nt were gel purified using QIAquick Gel Extraction Kit (Qiagen, Germany) according to manufacturer's instructions, 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 primate tetraparvovirus and other tetraparvovirus genomes were designed to overlap each other. PCR products were purified, cloned and both strands sequenced as described above.

All obtained sequences were carefully edited using Geneious 11.0.2 [25] and compared with those available in GenBank by the BLASTn algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequence alignments were generated using the ClustalW algorithm. Phylogenetic trees were inferred by maximum likelihood method using IQ-TREE v. 1.6.beta4 [26]. A best-fit evolution model was then chosen based on the Bayesian information criterion (BIC) computed by ModelFinder [27]. Branch supports were assessed by the ultrafast bootstrap (UFBoot) approximation [28] and by SH-like approximate

likelihood ratio test (SH-aLRT) [29]. For detailed Material and Method see Supplementary material.

A total of 478 faecal samples was screened by nested PCR. Only a single sample from *P. t. schweinfurthii* (labelled U55) originating from the Issa Valley was found to be tetraparvovirus positive. The nearly whole U55 parvovirus genome of 4955 nt in length was sequenced (acc.no. MH215556). U55 chimpanzee PARV4 genome contains two main open reading frames (ORFs) and two additional small ORFs (ARF1 and 2) of unknown function, not observed in other members of the family *Parvoviridae* [14, 30]. The first main ORF of 1992 nt in length (U55 position of 59-2050) is located at the 5´ end of the genome and encodes non-structural protein NS1. The second main ORF is located at the 3´ end of the genome and consists of 2745 nt (positions 2147-4891), encoding two structural VP proteins (914 and 552 amino acids). Following current International Committee on Taxonomy of Viruses criteria, the U55 isolate belongs to the species *Primate tetraparvovirus 1* (with an amino acid sequence of NS1 protein identity 91.3-98.8% to human and chimpanzee genotypes within this species, Table).

In our phylogenetic analysis, the U55 sequence clusters together with other chimpanzee PARV4 sequences obtained from wild chimpanzees; *P. t. troglodytes* in Cameroon [17] and *P. t. verus* in Côte d'Ivoire [18] in both NS1 (Figure 2) and VP genes as well as in whole genome analysis (Supplementary material). This chimpanzee PARV4 clade is situated as a close outgroup to human genotypes with strong bootstrap support in all analyses (Figure 2, Supplementary material). Sequences published from colobus monkeys [18] formed separate clusters distinct from human as well as from chimpanzee PARV4 viruses. To further analyze the chimpanzee PARV4 genome, we performed a recombination analysis in Simplot software with reference strains of all human PARV4 genotypes and non-human primate PARV4 isolates other than chimpanzees. The analysis revealed no potential recombination sites that could have given rise to the novel chimpanzee PARV4 tetraparvovirus (data not shown).

Our study was inspired by growing evidence for alternate routes of transmission for PARV4 in sub-Saharan Africa [30–32]. Here we prove virus shedding is detectable in faeces through the detection of chimpanzee tetraparvovirus DNA in a single faecal sample collected from wild chimpanzees (*P. t. schweinfurthii*) from an SIV positive community in the Issa Valley (Tanzania). The low positivity rate observed among the Issa chimpanzee community can be explained by combined influence of low amount of excreted virus (detected only by nested, not simple PCR protocols) and shedding of the virus in limited time. Human PARV4 has been reported to be detected from faeces in a few cases, however, its shedding is very probably

intermittent or limited in time [13]. Detection of tetraparvoviruses in fecal samples and swabs was previously described in humans and NHP exclusively in sub-Saharan Africa [17, 18], suggesting this is either a unique characteristic of virus genotypes limited to Africa, or the impact of other factors unique to Africa, e.g. co-infection with other viruses/parasites (e.g. GI helminths), affecting tetraparvovirus shedding into the intestinal lumen. Repeated finding of PARV-4 virus in faeces is the first prerequisite to the faecal-oral route of transmission which deserves more attention as it implies a significant role in PARV4 infection spread in a period of acute infection in communities sharing territory and habits.

Phylogenetic and p-distance analyses of chimpanzee tetraparvovirus U55 described here place it along with sequences previously derived from *P. t. troglodytes* [17] and *P. t. verus* [18]. This fact, together with the lack of recombination sites detected in the genome proves the co-evolution of chimpanzee PARV-4 with their hosts, supporting opinion about host specificity of tetraparvoviruses [18]. Importantly, existence of chimpanzee-specific clade of PARV-4 suggests limited or nil possibility of zoonotic transfer of PARV-4 from chimpanzees to humans.

Complementary sets of blood/ faecal/nasal/other samples both from humans and non-human primates would be necessary to definitively address whether alternate routes of transmission of African tetraparvovirus occur and whether there is an influence of geographically restricted factors on the epidemiology of resulting infections.

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Compliance with Ethical Standards

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Ethical approval: This article does not contain any studies with human participants or animals performed by any

of the authors.

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Conflicts of interest: none

Table: Distance pairwise comparison of nucleotide and amino acid (boldface) distances of NS1 gene among strains belonging to the species *Primate tetraparvovirus 1*; cpzPARV4 – strains originating from chimpanzee, colPARV4 – strain originating from *Colobus polykomos*

		PARV4 G1	PARV4 G2	PARV4 G3	cpzPARV4			colPARV4
		EU546204	EU546205	EU874248	JN798203	U55	HQ113143	JN798211
PARV4 G1	EU546204		8,99	7,99	18,02	17,82	18,62	35,59
PARV4 G2	EU546205	**2,56**		8,4	18,17	17,77	18	36,41
PARV4 G3	EU874248	**2,85**	**2,7**		17,83	18,08	18,06	35,35
	JN798203	**8,3**	**8,6**	**9,17**		11,7	10,49	34,88
cpzPARV4	U55	**7,99**	**7,99**	**8,73**	**3,62**		5,5	35,49
	HQ113143	**8,43**	**8,43**	**9,17**	**1,34**	**3,15**		35,36
colPARV4	JN798211	**32,43**	**32,43**	**33,01**	**32,43**	**32,28**	**32,41**	

Figure 1: Map of study sites: Issa Valley (Tanzania) and Kalinzu (Uganda)

Figure 2: Phylogenetic analysis of full-length coding sequences of NS1 of strains belonging to the species *Primate tetraparvovirus 1* by maximum likelihood method using IQTREE software. Herein described strain is highlighted in red. Branch supports are displayed as % from 1000 replicates from SH-aLRT/UFBoot tests. Three different hokoviruses (EU200677, EU200669, JF504699) used as an outgroup are not displayed. Scale bar indicates a number of nucleotide substitutions per site.

Figure

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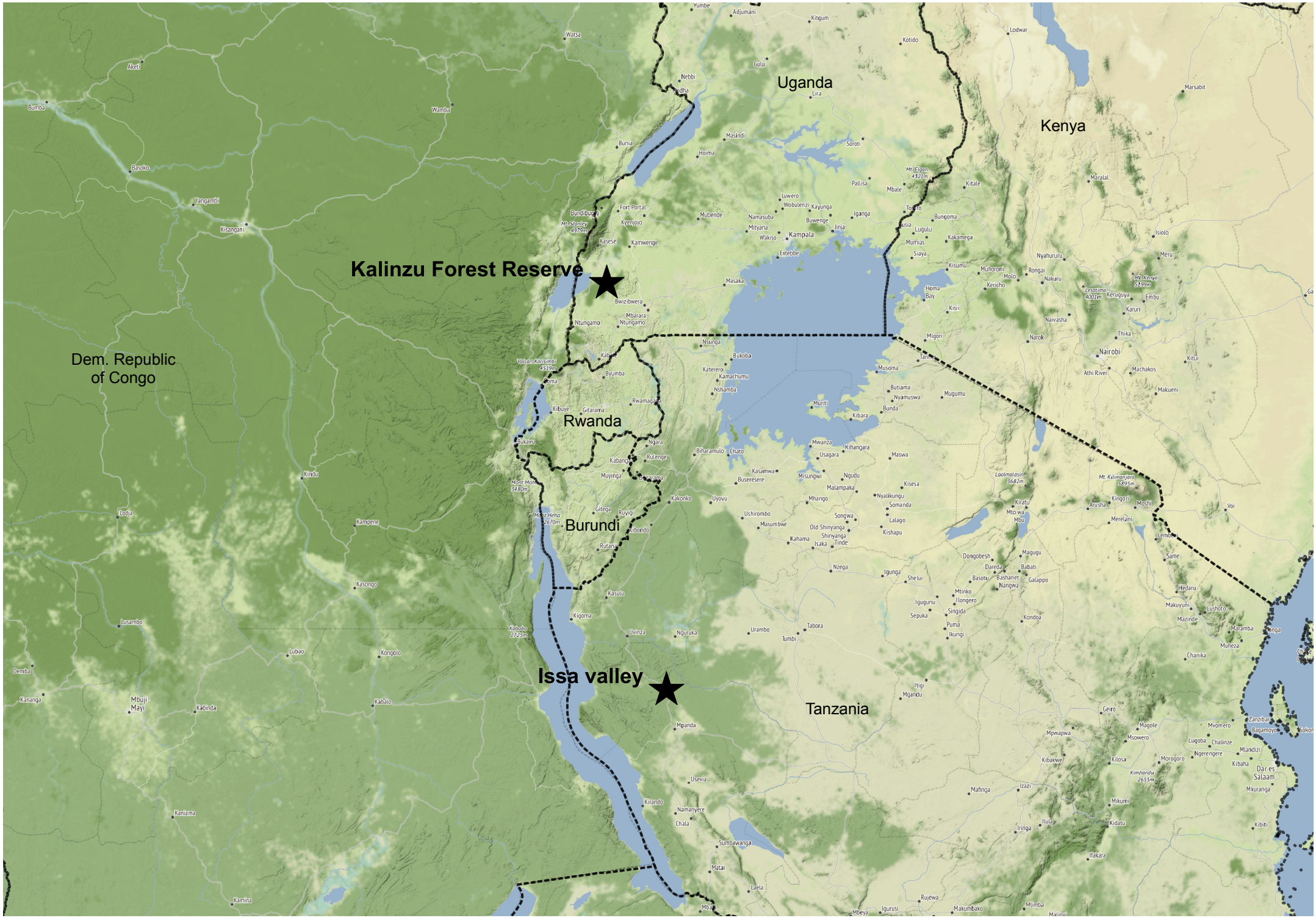

Figure2