JOURNAL OF VIROLOGY, Oct. 2011, p. 10774–10784 Vol. 85, No. 20
0022-538X/11/$12.00 doi:10.1128/JVI.00810-11

Novel Adenoviruses in Wild Primates: a High Level of Genetic Diversity and Evidence of Zoonotic Transmissions▽†

Diana Wevers,[1] Sonja Metzger,[2,3] Fred Babweteera,[4] Marc Bieberbach,[1] Christophe Boesch,[3] Kenneth Cameron,[5] Emmanuel Couacy-Hymann,[6] Mike Cranfield,[7] Maryke Gray,[8] Laurie A. Harris,[9] Josephine Head,[3] Kathryn Jeffery,[10,17,18] Sascha Knauf,[11,19] Felix Lankester,[12] Siv Aina J. Leendertz,[2] Elizabeth Lonsdorf,[12] Lawrence Mugisha,[13] Andreas Nitsche,[14] Patricia Reed,[5] Martha Robbins,[3] Dominic A. Travis,[15] Zinta Zommers,[16] Fabian H. Leendertz,[2] and Bernhard Ehlers[1]*

Division of Viral Infections, Robert Koch Institute, Berlin, Germany[1]; Research Group on Emerging Zoonoses, Robert Koch Institute, Berlin, Germany[2]; Department of Primatology, Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany[3]; Budongo Conservation Field Station, Budongo Forest, Uganda[4]; Wildlife Conservation Society, Global Health Program, Brazzaville, Republic of Congo[5]; LANADA/Laboratoire Central de la Pathologie Animale, Bingerville, Côte d'Ivoire[6]; Mountain Gorilla Veterinary Project, Davis, California[7]; The International Gorilla Conservation Programme, B.P. 931, Kigali, Rwanda[8]; Wildlife Health Center, School of Veterinary Medicine, University of California, Davis, California[9]; Agence Nationale des Parcs Nationaux, Libreville, Gabon[10]; Institut de Recherche en Écologie Tropicale, Libreville, Gabon[17]; University of Stirling, Stirling, Scotland, United Kingdom[18]; Department of Infection Pathology, German Primate Centre, Gottingen, Germany[11]; Department of Airway Immunology, Fraunhofer ITEM, Hanover, Germany[19]; Conservation and Science Department, Lincoln Park Zoo, Chicago, Illinois[12]; Makarere University, WARM Department, Kampala, Uganda[13]; Centre for Biological Security, Robert Koch Institute, Berlin, Germany[14]; Ecosystem Health Initiative, College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota[15]; and Wildlife Conservation Research Unit, Department of Zoology, University of Oxford, Tubney, United Kingdom[16]

Received 21 April 2011/Accepted 29 July 2011

Adenoviruses (AdVs) broadly infect vertebrate hosts, including a variety of nonhuman primates (NHPs). In the present study, we identified AdVs in NHPs living in their natural habitats, and through the combination of phylogenetic analyses and information on the habitats and epidemiological settings, we detected possible horizontal transmission events between NHPs and humans. Wild NHPs were analyzed with a pan-primate AdV-specific PCR using a degenerate nested primer set that targets the highly conserved adenovirus DNA polymerase gene. A plethora of novel AdV sequences were identified, representing at least 45 distinct AdVs. From the AdV-positive individuals, 29 nearly complete hexon genes were amplified and, based on phylogenetic analysis, tentatively allocated to all known human AdV species (*Human adenovirus A* to *Human adenovirus G* [HAdV-A to -G]) as well as to the only simian AdV species (*Simian adenovirus A* [SAdV-A]). Interestingly, five of the AdVs detected in great apes grouped into the HAdV-A, HAdV-D, HAdV-F, or SAdV-A clade. Furthermore, we report the first detection of AdVs in New World monkeys, clustering at the base of the primate AdV evolutionary tree. Most notably, six chimpanzee AdVs of species HAdV-A to HAdV-F revealed a remarkably close relationship to human AdVs, possibly indicating recent interspecies transmission events.

Primates are increasingly implicated as potential sources of emerging zoonotic diseases in humans (18, 59). In particular, interspecies transmission of various pathogens between humans and nonhuman primates (NHPs) is favored by their phylogenetic proximity (25, 35). For example, the worldwide pandemic of human immunodeficiency virus (HIV) resulted from various transmission events of the simian counterpart of the virus, simian immunodeficiency virus (SIV), from different primate species (19, 28). In addition, the emergence of certain acute diseases in humans, such as Ebola hemorrhagic fever, has been linked to contact with primates (44, 57). However, little is known about the potential of adenoviruses (AdVs), which are widespread in both humans and NHPs, to be transmitted between different primate species.

AdVs are nonenveloped icosahedral double-stranded DNA viruses of vertebrates. They belong to the family *Adenoviridae*, which is divided into five genera (*Mastadenovirus*, *Atadenovirus*, *Aviadenovirus*, *Siadenovirus*, and *Ichtadenovirus*) (8). As of 2009, 38 AdV species have been defined by the International Committee on Taxonomy of Viruses (ICTV) (http://talk.ictvonline.org). These species are populated by more than 100 AdV types, and many AdVs have been reported that potentially represent additional distinct serotypes (8, 46). Most

* Corresponding author. Mailing address: Robert Koch Institut, Nordufer 20, 13353 Berlin, Germany. Phone: 49187542347. Fax: 49187542598. E-mail: ehlersb@rki.de.

† Supplemental material for this article may be found at http://jvi.asm.org/.

▽ Published ahead of print on 10 August 2011.

mammalian and all human AdVs (>50 serotypes) belong to the genus *Mastadenovirus.* This genus includes eight species whose members have primate hosts: *Human adenovirus A*, *Human adenovirus B*, *Human adenovirus C*, *Human adenovirus D*, *Human adenovirus E*, *Human adenovirus F*, *Human adenovirus G*, and *Simian adenovirus A* (8). Abbreviations (HAdV-A, etc.) are adopted below to describe the viruses that group into the cognate genera (*Human adenovirus A*, etc.).

Typically, AdVs have a narrow host range restricted to one animal species or, as predicted by phylogenetic data in some cases, to closely related hosts (8, 58). Infections are predominantly subclinical, and overt disease develops upon the occurrence of immunosuppression or other cofactors (21, 32, 43, 58). Exceptions are canine AdVs, which cause epizootic respiratory disease (CAdV-1) or hepatitis (CAdV-2) in various carnivore species (13, 60). Certain HAdVs are associated predominantly with a specific pathology. Most prominent are conjunctivitis (HAdV-B and HAdV-E), keratoconjunctivitis (HAdV-D), acute respiratory disease (HAdV-B, HAdV-C, and HAdV-E), and (especially in children) gastroenteritis (HAdV-F) (47, 49).

A number of simian adenoviruses (SAdVs) have been described. They originate from macaques (SAdV-1 to SAdV-15), African green monkeys (SAdV-16 to SAdV-18 and SAdV-20), baboons (SAdV-19), and chimpanzees (SAdV-21 to SAdV-25) (1). More recently, 30 novel great ape AdVs, from chimpanzees, bonobos, and gorillas, as well as three macaque AdVs, were detected in captive NHPs held in facilities and zoological gardens in North America. Viruses were isolated from fecal samples, propagated in cell culture, completely sequenced, and tentatively named SAdV-25.2 to SAdV-50 (46). Most SAdVs were found to be very similar to HAdVs and grouped correspondingly into the HAdV-B, -C, -E, and -G groups (see Table S1 in the supplemental material) (4, 6, 33, 34, 36, 45, 46, 56). More distantly related simian AdVs were assigned to SAdV-A (8, 33). AdVs infecting New World monkeys (NWMs) have been reported by Shroyer et al. They identified adenovirus infections in owl monkeys, but no sequence data are available (51). AdVs infecting Asian apes (orangutans and gibbons) have not been reported so far. Simian members of the HAdV-B, -C, and -E groups were found only in great apes, and those of the HAdV-G group were found only in Old World monkeys (OWMs). Simian members of the HAdV-A, -D, and -F groups are presently not known. All human and simian AdVs for which complete genomes are available in GenBank are listed in Table S1 in the supplemental material.

Most of the SAdVs known to date were detected in or isolated from captive individuals and propagated in cell culture (3, 4, 6, 31, 33, 34, 36, 45, 46, 56). Unfortunately, little is known about SAdVs in wild animals. AdVs were detected in wild chimpanzees of Western Tanzania (53), and a number of AdVs attributable to the HAdV-B group were identified in a group of gorillas living in a remote area with little human presence in Loango National Park, Gabon (55).

In the present study, we set out to investigate adenoviruses in wild primates as part of a long-term project on the elucidation of the etiology of primate diseases (35). Our goal was to gain insight into the diversity of AdVs infecting individuals living in their natural environment, to compare their AdVs with those published previously for captive animals, and to unravel possible horizontal transmission events between humans and NHPs.

MATERIALS AND METHODS

Sample collection and processing. A total of 1,285 blood, fecal, and tissue samples were collected over a period of more than 10 years from live or deceased individuals of 51 primate species (great apes, OWMs, NWMs, and prosimians). Of these, 543 samples originated from wild primates in Africa ($n = 527$), Asia ($n = 7$), and South America ($n = 9$), 95 samples were from wild-born great apes housed in wildlife sanctuaries in West and East Africa, and 647 samples were derived from captive primates held in several zoological gardens and primate facilities in Europe and Africa. A complete list of species and samples analyzed is available on request. Necropsies of wild animals were performed under the highest possible safety standards, which included at least full body protection, a mask, face protection, and double gloves, and were performed in a defined area to which only protected personnel had access (35). Fecal samples were collected using single-use gloves and preserved in RNAlater (Qiagen, Hilden, Germany), in liquid nitrogen, or by drying over silica (2). DNAs from blood and tissue samples were prepared with a QiaAmp tissue kit (Qiagen) and DNAs from fecal samples were extracted with a GeneMATRIX stool DNA purification kit (Roboklon, Berlin, Germany), according to the instructions of the manufacturers.

Olive baboons at Lake Manyara National Park in the United Republic of Tanzania were sampled in accordance with the Tanzania Wildlife Research Institute's guidelines for conducting wildlife research and with permission from Tanzania National Parks (TNP/HQ/E.20/08B). Additional research permission was granted by the Commission for Science and Technology in Tanzania (2007-56-NA-2006-176).

PCR methods and sequence analysis. (i) Generic primate AdV PCR based on the DPOL gene. For universal detection of AdVs, a pan-primate PCR based on the DNA polymerase (DPOL) gene was used as described previously (55). Briefly, two pairs of degenerate and deoxyinosine-substituted (deg/dI) primers were applied in a nested setup, resulting in the amplification of a 610-bp (excluding primer-binding sites) DPOL sequence. The generic suitability was confirmed by amplification of several mastadenoviruses, including human members of all HAdV species, a bat AdV (GenBank accession no. FJ983127), and a bovine AdV (GenBank accession no. NC006324) (data not shown). If the amount of amplification product was too low to permit direct sequencing, a third-round PCR amplifying 325 bp (excluding primer-binding sites) of the DPOL gene was performed [sense primer, 5′-AACGCCCTCTA(y/i)GG(n/i)TC(n/i)TT; and antisense primer, 5′-CGTCCACATCCAG(r/i)AA(n/i)GT(r/i)AT]. PCR was run as in the second round, except that the elongation step was 1 min at 72°C.

(ii) Generic and LD-PCR based on the hexon gene. For amplification of partial hexon gene sequences, two pan-primate nested PCRs, targeting conserved regions at the 5′ and 3′ ends of the hexon gene, were performed as described previously, except that the elongation time was 1 min (55). Based on virus-specific primers (not shown) derived from the sequences of the hexon gene fragments, long-distance PCR (LD-PCR) was performed with the TaKaRa-EX PCR system according to the instructions of the manufacturer (Takara Bio Inc., Otsu, Japan).

(iii) PCR for amplification of PtroAdV-3. To amplify 454 bp of the DPOL gene of *Pan troglodytes* adenovirus 3 (PtroAdV-3), a nested PCR was performed using the sense primer 5′-GCTGGCATGTGCGGTTGCTT and the antisense primer 5′-TCCACCAGGGGGTCGACCTTT in the 1st round and the sense primer 5′-GGCGCTGCGTGGCAAAAGAG and the antisense primer 5′-AGGGTGT GAAGGCAAAGGTCATCT in the 2nd round. Conditions were as described for the second round of the generic DPOL PCR, except that the annealing temperature was 60°C and the elongation step was 1 min at 72°C.

(iv) Generic real-time PCR targeting AdVs related to HAdV-F and HAdV-G. For selective amplification of unknown AdVs related to HAdV-F and -G, primers (sense primer, GCATACTCACCCGAACAGC; and antisense primer, GG TCATCTTCCTCYGCRTCCA) and a TaqMan probe (FAM-CCACAGCGAT GCGGAAGAAAGTGA-BBQ [FAM, 6-carboxyfluorescein]) were selected which bind to sites conserved in the DPOL genes of HAdV-F and HAdV-G members only. The PCR was performed in a total volume of 25 μl with 0.2 μl Platinum *Taq* polymerase (Applied Biosystems, Darmstadt, Germany), 10 μM (each) primers, 10 nM probe, a 200 μM concentration of each deoxynucleoside triphosphate (dNTP), and 50 mM $MgCl_2$. An MX 3000P thermal cycler (Strata gene, Waldbronn, Germany) was used with the following cycling conditions: 95°C for 5 min and 45 cycles of 95°C for 30 s and 60°C for 30 s. Analysis was performed with MXPro V 4.0 software from Stratagene.

(v) Sequencing. All PCR products were purified by using a PCR purification kit (Qiagen) and were sequenced directly with a BigDye Terminator cycle sequencing kit (Applied Biosystems, Warrington, Great Britain) in a model 377 automated DNA sequencer (Applied Biosystems).

Phylogenetic analysis of DPOL sequences. Amino acid sequence alignments of DPOL gene sequences were generated using the MAFFT multiple-aligner plug-in of Geneious Pro 5.1.4 software (Biomatters Ltd., Auckland, New Zealand) (27). Regions that were considered too variable to be aligned with confidence, in addition to sites containing a gapping character in any sequence, were eliminated before using the alignment for phylogenetic inference. Phylogenetic trees were inferred from alignments of amino acid sequences (all alignments are available on request). Phylogenetic relationships were investigated with trees built by the maximum likelihood (ML) method, using the PhyML 2.0.1 plug-in (22). The nucleotide substitution model to which the data best fit was estimated by jModeltest v0.1.1 (23, 42), and the HKY85+I+G model was applied. Reliability of the trees was analyzed by bootstrap analysis (100-fold resampling).

Phylogenetic analysis of hexon sequences. Sequences were added to a data set consisting of all hexon gene sequences available from completely sequenced AdV genomes (see Table S1 in the supplemental material). The AdVs CAdV-1 and bovine adenovirus type 3 (BAdV-3) served as outgroups. Hexon protein sequences were first aligned in SeaView v4 by using the program Muscle (15, 16, 20). To identify and remove blocks of the alignment where the hypothesis of homology was likely to be overoptimistic, the Gblocks server (http://molevol.cmima.csic.es/castresana/Gblocks_server.html) was employed, a strategy confirmed to lead to better inference of phylogenetic trees (52). Corresponding blocks from the nucleotide alignment were eliminated, and the overall process resulted in a data set composed of 114 sequences of 1,403 nucleotides. The nucleotide substitution model to which the data were best fit was determined using jModeltest v0.1.1 (22, 42). Substitution schemes were examined along with rate variation (+I, +G, and +I+G) and base frequency (+F) modeling. Base trees for calculations were optimized by the ML method (23). According to the Akaike information criterion (AIC), comparisons of model likelihoods indicated that the global timer reversible GTR+I+G model was more favorable. Phylogenetic analyses were performed in both ML and Bayesian frameworks. ML analysis was performed with Sea View v4 (20), using the PhyML option under predefined conditions. Equilibrium frequencies, topology, and branch lengths were optimized, the starting tree was determined using BioNJ, and both nearest-neighbor interchange (NNI) and subtree pruning and regrafting (SPR) algorithms for tree searching were used. Branch robustness was analyzed by bootstrap analysis (100-fold resampling).

Bayesian analyses were performed using BEAST v1.6.1 (14). Calculations were run under the assumption of a relaxed, uncorrelated log-normal clock and using the Yule process speciation model. A run of 4,000,000 generations was performed. Trees and numerical values taken for all parameters were sampled every 1,000 generations. Tracer v1.5 was used to verify that the run converged on the same zones of parameter spaces and that chain mixing was satisfactory (global effective sample size value of >100) (14). Tree information was summarized on the maximum clade credibility tree by using TreeAnnotator v1.6.1 (distributed with BEAST). Posterior probabilities were taken as a measure of branch robustness.

Nucleotide sequence accession numbers. For the purposes of this paper, preliminary names and abbreviations for the candidate novel AdVs were derived from the species name of the host in which the virus was detected (for example, the abbreviation of *Pan troglodytes* adenovirus is PtroAdV). The GenBank accession numbers for their hexon sequences are as follows: *Pan troglodytes schweinfurthii* adenovirus 1, JN163971; *Pan troglodytes schweinfurthii* adenovirus 2, JN163972; *Pan troglodytes verus* adenovirus 3, JN163973; *Pan troglodytes verus* adenovirus 4, JN163974; *Pan troglodytes verus* adenovirus 5, JN163975; *Pan troglodytes schweinfurthii* adenovirus 6, JN163976; *Pan troglodytes schweinfurthii* adenovirus 7, JN163977; *Pan troglodytes verus* adenovirus 8, JN163978; *Pan troglodytes schweinfurthii* adenovirus 9, JN163979; *Pan troglodytes schweinfurthii* adenovirus 10, JN163980; *Pan troglodytes schweinfurthii* adenovirus 11, JN163981; *Pan troglodytes schweinfurthii* adenovirus 12, JN163982; *Pan troglodytes schweinfurthii* adenovirus 13, JN163983; *Pan troglodytes schweinfurthii* adenovirus 14, JN163984; *Gorilla gorilla gorilla* adenovirus 1, JN163985; *Gorilla gorilla gorilla* adenovirus 2, JN163986; *Gorilla gorilla beringei* adenovirus 3, JN163987; *Gorilla gorilla beringei* adenovirus 4, JN163988; *Gorilla gorilla beringei* adenovirus 5, JN163989; *Gorilla gorilla beringei* adenovirus 6, JN163990; *Papio hamadryas* adenovirus 1, JN163991; *Macaca fascicularis* adenovirus 1, JN163992; *Macaca mulatta* adenovirus 1, JN163993; *Colobus guereza* adenovirus 1, JN163994; *Colobus guereza* adenovirus 2, JN163995; *Colobus guereza* adenovirus 3, JN163996; *Piliocolobus badius* adenovirus 1, JN163997; *Piliocolobus badius* adenovirus 2, JN163998; and *Piliocolobus badius* adenovirus 3, JN163999.

TABLE 1. Sample material and adenovirus content

Sample material	No. of tested samples[a]	No. (%) of AdV-positive samples
Blood	295	1 (0.3)
Feces	291	172 (59)
Liver	123	7 (6)
Spleen	116	8 (7)
Lung	103	1 (1)
Lymph node	63	3 (5)
Kidney	58	1 (2)
Intestine	44	12 (27)
Muscle	40	4 (10)
Heart	34	0 (0)
Skin	30	3 (10)
Brain	18	0 (0)
Oral swab	11	1 (9)
Thymus	9	0 (0)
Bone marrow	7	2 (29)
Adrenal gland	6	0 (0)
Pancreas	6	0 (0)
Tonsil	5	1 (20)
Parotid gland	4	0 (0)
Urine	4	0 (0)
Eye eluate	3	0 (0)
Lip tissue	3	1 (33)
Tongue	3	1 (33)
Ascites	2	0 (0)
Testicle	2	0 (0)
Tumor	2	0 (0)
Abscess	1	0 (0)
Mouth mucosa	1	0 (0)
Esophagus	1	0 (0)
Total	1,285	218 (17)

[a] Samples were analyzed by DPOL PCR for generic detection of AdVs, and the products were sequenced.

RESULTS AND DISCUSSION

***De novo* detection of novel AdVs.** The aims of this study were to comprehensively investigate the diversity of AdVs in wild NHPs, to compare their AdVs with those published previously for captive animals, and to unravel possible horizontal transmission events between humans and NHPs. For these purposes, blood, tissue, and fecal samples (n = 1,285) which had been collected from live or deceased individuals of 52 primate species (great apes, OWMs, NWMs, and prosimians) were analyzed with a generic DPOL PCR. From 218/1,285 samples (17%), PCR products of the expected size were obtained, sequenced, and proven by BLAST analysis to originate from as yet unknown AdVs. Fecal samples were the predominant source of AdV sequences, since 59% were PCR positive. Far fewer AdV sequences were detected in organ specimens: of all organ types with >40 samples tested, the intestine, spleen, lymph nodes, and liver were the best sources of AdV sequences (27%, 7%, 5%, and 6% of samples, respectively). In contrast, AdVs were detected only once in kidney, lung, and blood samples (2%, 1%, and 0.3% of samples, respectively) (Table 1). In total, the sequences indicated the presence of 45 potentially novel AdVs. For six AdVs, only the third PCR round yielded enough product for direct sequence analysis (325 bp).

TABLE 2. DPOL-based detection of novel adenoviruses in nonhuman primate individuals

Host species	No. of AdV-positive individuals (living conditions)[a]							
	HAdV species							Unassigned AdVs
	A	B	C	D	E	F	G	
Great apes								
Chimpanzee (*Pan troglodytes*)	4 (w)	6 (w)	21 (w)	1 (w)	72 (w/s)	1 (w)		2 (w)
Bonobo (*Pan paniscus*)					7 (w)			
Gorilla (*Gorilla gorilla*)		53 (w/c)	4 (w)			1 (w)		
Old World monkeys								
Mantled guereza (*Colobus guereza*)								9 (c)
King colobus (*Colobus polykomos*)								1 (w)
Western red colobus (*Piliocolobus badius*)								17 (w)
Cynomolgus macaque (*Macaca fascicularis*)								2 (c)
Rhesus macaque (*Macaca mulatta*)							1 (c)	
Hamadryas baboon (*Papio hamadryas*)								2 (w)
New World Monkeys								
Common marmoset (*Callithrix jacchus*)								3 (c)
White-lipped tamarin (*Saguinus labiatus*)								1 (c)

[a] No individuals were positive for SAdV-A. w, wild; c, captive; s, born in the wild but kept in a sanctuary.

The AdV-positive primate hosts originated from eight countries in Africa, namely, Cameroon, Democratic Republic of Congo, Gambia, Côte d'Ivoire, Republic of Congo, Rwanda, Tanzania, and Uganda, as well as from a primate facility and several zoos in Germany (see Table 3). Primate hosts were members of eight different genera, six of the Catarrhini (*Colobus*, *Gorilla*, *Macaca*, *Pan*, *Papio*, and *Piliocolobus*) and two of the Platyrrhini (*Callithrix* and *Saguinus*). Prosimian AdVs were not detected. The AdV sequences identified in wild primates originated from chimpanzees, bonobos, gorillas, baboons, and two colobus species. Captive primate species revealing AdV sequences were mantled guerezas, rhesus macaques, cynomolgus macaques, marmosets, and tamarins. Taken together, 11/52 primate species yielded AdV sequences, with the moderate detection rate (17%) most likely being due to limitations in sample materials (e.g., the lack of fecal and intestinal samples). The primate species that yielded AdV sequence data and the tentative assignments of AdVs to HAdV species are listed in Table 2, and the countries of origin, sample types, and numbers of PCR-positive individuals are given in Table 3. The authenticity of the PCR-positive host individuals was controlled by amplification of *cytB* sequences from the specimens by PCR (data not shown), as performed previously (17).

BLAST analysis and preliminary phylogenetic tree construction using ML calculations revealed a high level of genetic diversity of the novel AdVs. They exhibited a broad evolutionary spectrum and were tentatively allocated to all HAdV species known (*Human adenovirus A* to *Human adenovirus G*) (Tables 1 and 3; Fig. 1). AdVs of great apes clustered predominantly in the HAdV-B, HAdV-C, and HAdV-E groups, i.e., most AdVs detected in gorillas clustered in HAdV-B (53/58 individuals), with AdVs from chimpanzees clustering mainly within HAdV-C (21/107 individuals) and HAdV-E (72/107 individuals). AdVs discovered in OWMs clustered in HAdV-G or could not be assigned to an AdV species, since they branched in the deeper part of the DPOL tree, in the vicinity of HAdV-G and SAdV-A, with weak statistical support (Fig. 1). Earlier reports already indicated that AdVs of captive NHPs can be allocated to all HAdV species (except for *Human adenovirus A* and *Human adenovirus D*) (8, 34, 46). However, a concern was raised about the true origin of AdVs from captive primates because humans and NHPs are frequently in close contact in zoos and primate facilities (46). With this in mind, our findings for wild primates are of significance because they confirm that AdVs of groups HAdV-B, -C, -E, -F, and -G populate NHPs. In addition, two observations were novel: (i) AdVs that grouped into HAdV-A, HAdV-D, HAdV-F, and SAdV-A were detected in great apes; and (ii) AdVs were identified in NWMs that clustered in a well-separated clade at the base of the primate AdV tree, possibly reflecting the split between NWMs and OWMs (Tables 2 and 3; Fig. 1).

The high level of conservation of the adenovirus DPOL gene ensured the broad detection of AdVs, but the limited length of the amplified fragment (610 bp) impeded the proper differentiation of AdVs allocated to the same species. Therefore, our inferences on the phylogeny of the novel AdVs required backup data for a more suitable gene. The hexon gene was chosen, since serotype specificity of AdV capsids correlates well with variations in hexon sequences (48). Furthermore, it is part of the species demarcation criteria applied by the ICTV (8). To determine nearly complete hexon gene sequences, we initially amplified short regions at the 5′ and 3′ ends of the hexon gene by use of degenerate primers. The products were then connected by LD-PCR, resulting in a contiguous hexon sequence of approximately 2.5 kb. This was successful for 29 of the 45 AdVs. For the remaining 16 AdVs, either one or both of the two generic hexon PCRs failed (data not shown). For most novel AdVs, both DPOL and hexon sequences were available from the same sample but could not be connected by LD-PCR amplification of the interspersed sequence. This was probably due to copy numbers being too low for amplification of >10 kb, to poor sample quality, or to the presence of more than one AdV. Therefore, it was not possible to unequivocally prove that both sequences derived from the same virus. In addition, five chimpanzee AdVs allocated to HAdV-E (PtroAdV-1, PtroAdV-11, PtroAdV-12, PtroAdV-13, and

TABLE 3. Origins of adenovirus DPOL sequences identified in this study

AdV species and PCR-positive primate host[a]	Origin of host (living conditions)[b]	PCR-positive tissue(s)	No. of PCR-positive individuals
Human adenovirus A			
Chimpanzee (*Pan troglodytes*)	Uganda (w), Côte d'Ivoire (w)	Feces, intestine, spleen	4
Human adenovirus B			
Chimpanzee (*Pan troglodytes*)	Gabon (w), Uganda (w)	Feces	6
Gorilla (*Gorilla gorilla*)	Rwanda (w), Democratic Republic of Congo (w), Germany (c)	Muscle, feces	53
Human adenovirus C			
Chimpanzee (*Pan troglodytes*)	Uganda (w), Côte d'Ivoire (w), Germany (c)	Feces	21
Gorilla (*Gorilla gorilla*)	Cameroon (s), Democratic Republic of Congo (w), Rwanda (w)	Lymph node, Feces	4
Human adenovirus D			
Chimpanzee (*Pan troglodytes*)	Uganda (w)	Feces	1
Human adenovirus E			
Bonobo (*Pan paniscus*)	Democratic Republic of Congo (w)	Feces	7
Chimpanzee (*Pan troglodytes*)	Gabon (w), Tanzania (w), Uganda (w/s), Côte d'Ivoire (w),	Feces	72
Human adenovirus F			
Chimpanzee (*Pan troglodytes*)	Gambia (w)	Liver, spleen, lung	1
Gorilla (*Gorilla gorilla*)	Gabon (w)	Skin	1
Human adenovirus G			
Rhesus macaque (*Macaca mulatta*)	Germany (c)	Skin	1
Unassigned AdVs			
Chimpanzee (*Pan troglodytes*)	Uganda (w)	Intestine	2
Cynomolgus macaque (*Macaca fascicularis*)	Germany (c)	Tongue, lip	2
Hamadryas baboon (*Papio hamadryas*)	Tanzania (w)	Oral swab, skin	2
King colobus (*Colobus polykomos*)	Côte d'Ivoire (w)	Buffy coat	1
Mantled guereza (*Colobus guereza*)	Germany (c)	Lymph node	9
Western red colobus (*Piliocolobus badius*)	Republic of Congo (w), Côte d'Ivoire (w)	Muscle, liver, intestine, spleen, lymph node, bone	17
Common marmoset (*Callithrix jacchus*)	Germany (c)	Liver	3
White-lipped tamarin (*Saguinus labiatus*)	Germany (c)	Intestine	1

[a] The allocation of the AdV DPOL sequences to adenovirus species is tentative.
[b] w, wild; c, captive; s, born in the wild but kept in a sanctuary.

PtroAdV-14) revealed distinct hexon sequences (pairwise hexon nucleic acid identities of 86.1 to 93.8%) but indistinguishable DPOL sequences in the respective samples. This indicated that identical DPOL sequences do not always originate from identical AdV genomes. We concluded that the 45 distinct AdV DPOL sequences detected in this study represent a subset of the AdVs present in the samples analyzed and that the allocation to certain AdV species, as well as provisional naming of the novel AdVs, had to be based on the hexon gene sequences. Since the occurrence of interspecies recombination involving the hexon gene had been suspected (11, 37–39, 54), this species allocation was tentative.

A hexon-based phylogenetic analysis was carried out using an ML calculation. The resulting tree (Fig. 2) was in overall accordance with the DPOL-derived tree (Fig. 1), and its general topology was the same as that revealed in previous reports (12, 46). In contrast to the DPOL tree, in which several deeper nodes were statistically poorly supported, thus inhibiting the determination of the relationships between the AdV clades, the hexon-based tree yielded high bootstrap support all over the tree (Fig. 2). In addition, a tree constructed by Bayesian analysis revealed the same tree topology (data not shown).

Since one of our goals was to comprehensively describe the diversity in AdVs in free-ranging individuals and to compare these viruses with AdVs infecting captive animals, several findings deserve attention. In light of current knowledge, AdVs of wild great apes would be expected to populate only the HAdV-B, -C, and -E clades, since AdVs were detected previously in captive individuals and allocated to HAdV-B, -C, and-E (45, 46). In addition, HAdV-B viruses were detected recently in wild Western lowland gorillas (*G. g. gorilla*) (55). However, in this study, we also detected AdVs in wild great apes that were attributable to groups HAdV-A, HAdV-D, HAdV-F, and SAdV-A. Three of these (PtroAdV-8 [HAdV-A], PtroAdV-10 [HAdV-D], and PtroAdV-3 [HAdV-F]), as well as two additional viruses (PtroAdV-7 [HAdV-B] and PtroAdV-14 [HAdV-E]), revealed a remarkably close relationship to human AdVs over the entire hexon gene sequence (Fig. 2).

Current knowledge about AdVs in wild NHPs is scarce (53,

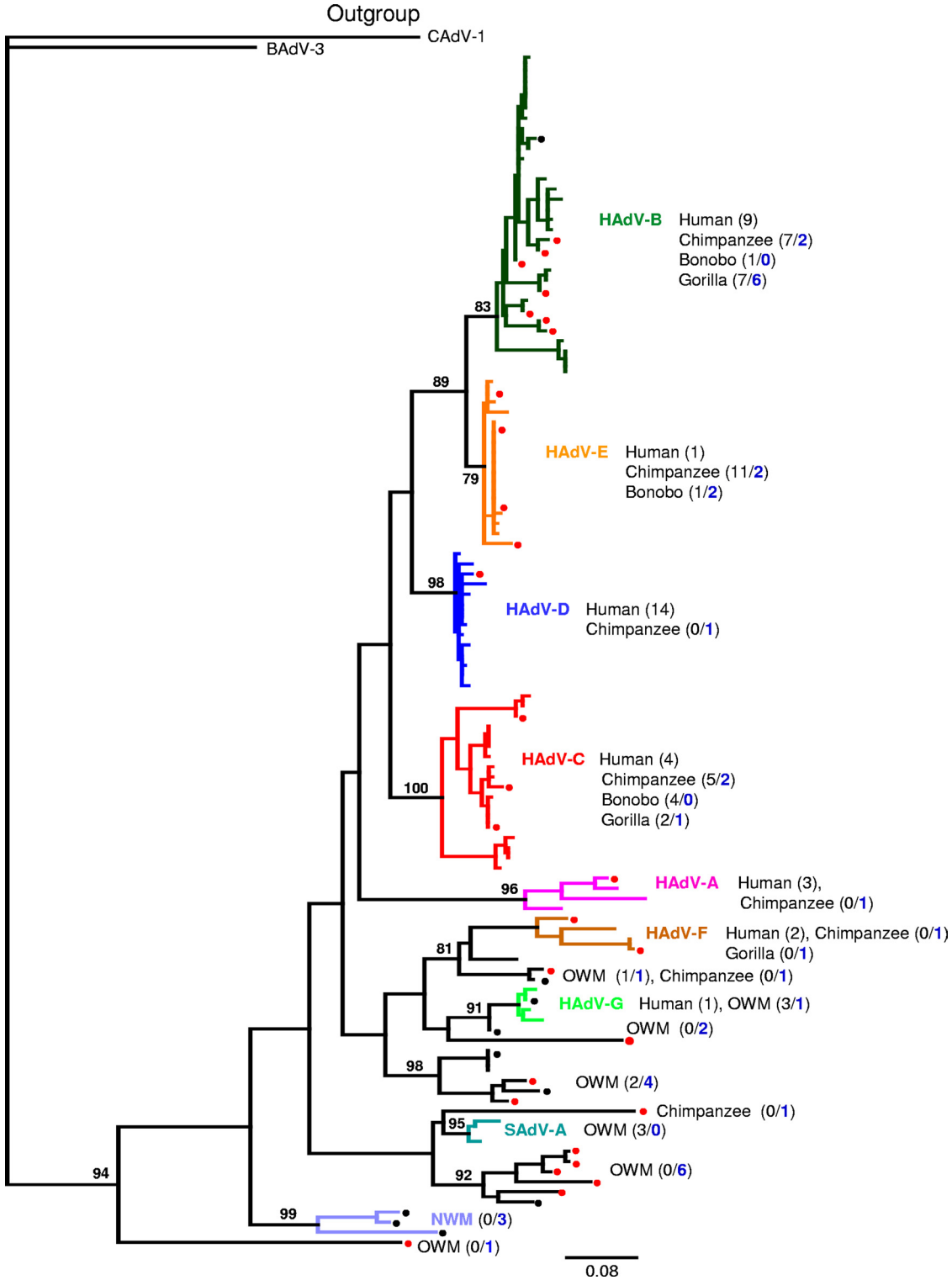
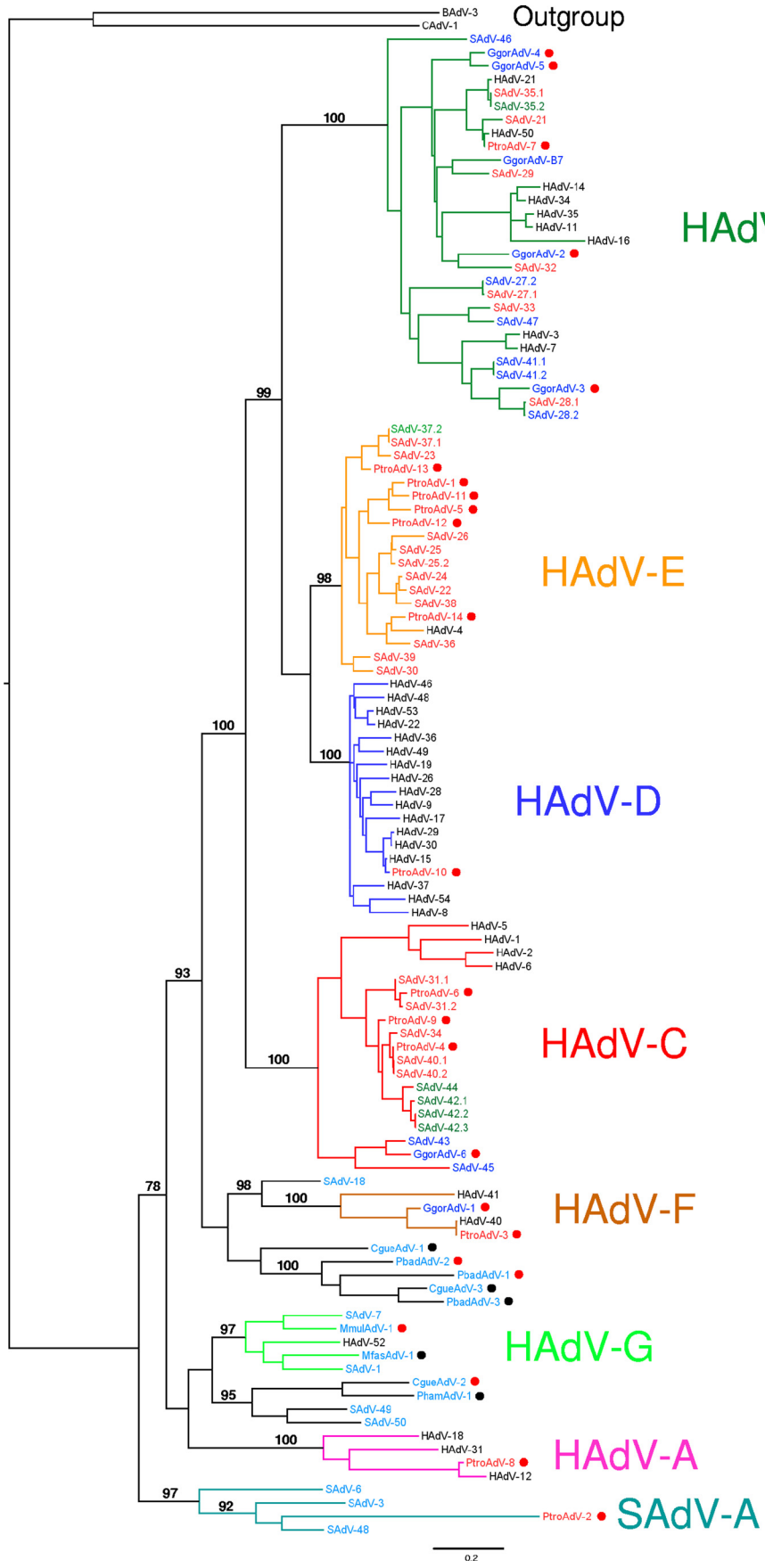


FIG. 1. DPOL sequence-based phylogenetic analysis. Partial DPOL gene sequences from the novel AdVs and from published NHP AdVs and human AdVs were aligned and subjected to phylogenetic analysis. The tree was reconstructed using maximum likelihood with the HKY85 model of substitutions, as described in Materials and Methods. AdVs from wild and captive NHPs discovered in this study are marked with red and black dots, respectively. All other NHP AdVs were reported previously and originated from captive animals. The branches of all AdVs that could tentatively be allocated to one of the species *Human adenovirus A* to *G* or to the species *Simian adenovirus A* are colored separately. Branches left without extra coloring belong to AdVs for which allocation to an AdV species was not possible. Bootstrap values are depicted at the nodes of the tree; nonsignificant values or those close to branch tips were omitted. AdV species and host compositions are indicated on the right. Numbers of AdVs found in each host species are given in parentheses: numbers of published AdVs are given before the slash, in black, and numbers of novel AdVs from this study are given behind the slash, in blue.



55), and most studies on NHP AdVs involve captive animals from zoos and animal facilities (3, 4, 6, 10, 24, 30, 31, 33, 34, 36, 40, 45, 46, 56, 61). Therefore, our study on wild primates is of importance because it provides insight into natural AdV diversity and evolution. A general outcome of our phylogenetic data set is that all seven HAdV species contain both human- and simian-derived AdVs (Fig. 1). However, we confirmed that two HAdV species contain AdVs from predominantly one host species or one host family only: AdVs classified into the HAdV-D species are found almost exclusively in humans, and AdVs of the HAdV-E species appear to originate almost entirely from chimpanzees (Fig. 1). The exceptions are as follows.

(i) HAdV-D. The chimpanzee AdV clustering in HAdV-D (PtroAdV-10) showed 99% pairwise hexon nucleic acid identity to HAdV-15 and 98% identity to HAdV-29 and -30. As expected, the PtroAdV-10 position was closest to HAdV-15 in the hexon-based phylogenetic tree (Fig. 2). This finding added a new quality to HAdV-D, i.e., a great ape AdV was added to a hitherto strictly human AdV cluster of 32 serotypes. Among human AdVs, HAdV-D is the largest and fastest growing species. Based on the variability of the genome regions responsible for serotype specificity, the ultimate number of serotypes may be very large (11). In contrast, PtroAdV-10 was found in only one individual, from the Budongo Forest area in Uganda. Although we did not search for PtroAdV-10 in chimpanzees by using a specific PCR, the lack of HAdV-D sequences in other primate individuals revealed by the generic DPOL PCR led us to hypothesize that PtroAdV-10 is of human origin, i.e., it is a putative variant of HAdV-15 circulating in the human population in Central Africa.

(ii) HAdV-E. One human AdV (HAdV-4), 11 chimpanzee AdVs, and 1 bonobo AdV (46) are presently allocated to HAdV-E. In the present study, the majority (79%) of the fecal samples from 94 chimpanzees and 7 bonobos that were positive in the generic DPOL PCR belonged to HAdV-E, and from the chimpanzee samples, six distinct hexon sequences could be amplified and tentatively named (PtroAdV-1, -5, -11, -12, -13, and -14). Notably, PtroAdV-14, identified in a wild animal in Uganda, was determined to be the closest known counterpart of HAdV-4, since it revealed a pairwise hexon nucleic acid identity of 93.6% and clustered most closely with HAdV-4 in the hexon-based tree (Fig. 2). This reinforces the previous hypothesis (43) that HAdV-4 is the result of a zoonotic transmission event from chimpanzees to humans.

(iii) HAdV-B and HAdV-C. The occurrence of a predominant host species, as in HAdV-D and HAdV-E, does not seem to be a hallmark for AdVs of HAdV-B and HAdV-C. Rather, these groups are populated with human AdVs and with those of different NHPs. HAdV-B encompasses AdVs of human, chimpanzee, gorilla, and bonobo origin. We discovered AdVs belonging to HAdV-B in 53 gorillas and 6 chimpanzees, thus confirming for wild individuals that members of HAdV-B widely infect great apes. However, viruses of the same host species stray throughout the clade, and several subclades comprising AdVs of different hosts are visible (Fig. 2). This was observed even for viruses (GgorAdV-3 to GgorAdV-5) that were identified in a geographically isolated population of mountain gorillas (*G. g. beringei*) (Fig. 2; Table 2). PtroAdV-7 deserves special attention, since it is the first AdV in a wild chimpanzee population with remarkable similarity to a human AdV of HAdV-B (pairwise hexon nucleic acid identity to HAdV-50, 99.2%). Taken together, the evidence shows that interspecies transmissions are a general driving force in the evolutionary history of human and NHP adenoviruses of the HAdV-B species. The completely mixed architecture of the HAdV-B clade does not allow speculations about the directionality of transmissions, although several viruses were detected in chimpanzee and gorilla individuals from remote areas without contact with humans. This may indicate that human HAdV-B originated in great apes, but further studies are clearly required to settle this issue. HAdV-C also encompasses AdVs of human, chimpanzee, gorilla, and bonobo origin. Of 101 fecal samples from chimpanzees, 21 tested positive for AdVs of the HAdV-C species. Also, AdVs of the HAdV-C species were detected in four fecal samples from wild gorillas. Interestingly, the viruses of each host species clustered in distinct subclades. This most likely reflects their origin by coevolutionary processes, with no evidence of horizontal transmission events (Fig. 2).

(iv) HAdV-A, HAdV-F, HAdV-G, and SAdV-A. Four primate AdV species (HAdV-A, HAdV-F, HAdV-G, and SAdV-A) presently comprise 10 AdVs or fewer (including our data) and are discussed separately below.

HAdV-A is presently populated with three human AdVs only. From a chimpanzee from Côte d'Ivoire, a hexon sequence was amplified and tentatively named PtroAdV-8. In the phylogenetic analysis, it was closely related to HAdV-12 (Fig. 2) (pairwise hexon nucleic acid identity of 95.7%), implying that PtroAdV-8 or its ancestor may have originated from a transmission event between humans and chimpanzees. However, we detected nearly identical HAdV-A DPOL sequences in chimpanzees from different subspecies and located at large geographic distances, i.e., two *P. t. verus* chimpanzees from Côte d'Ivoire and one *P. t. schweinfurthii* chimpanzee from Uganda, indicating that HAdV-A members are established in chimpanzees. More data from human and chimpanzee populations are needed to substantiate this hypothesis (Fig. 1 and 2).

HAdV-F is comprised of two human AdVs (HAdV-40 and -41), and an OWM-AdV (SAdV-18) may also belong to HAdV-F (Fig. 2). We discovered one chimpanzee and one

FIG. 2. Phylogenetic analysis of the hexon gene. The hexon gene sequences of the novel AdVs and of published NHP AdVs and human HAdVs were aligned and subjected to phylogenetic analysis. The tree was reconstructed using maximum likelihood with the GTR+I+G model of substitutions, as described in Materials and Methods. Human, chimpanzee, bonobo, and gorilla AdVs are depicted in black, red, green, and blue, respectively. AdVs from wild and captive NHPs discovered in this study are marked with red and black dots, respectively. All other NHP AdVs were reported previously and originated from captive animals. The branches of all AdVs that could be allocated to one of the species *Human adenovirus A* to *G* or to the species *Simian adenovirus A* are colored separately. Branches left without extra coloring belong to AdVs for which tentative allocation to an AdV species was not possible. Bootstrap values are depicted at the nodes of the tree; nonsignificant values or those close to branch tips were omitted.

TABLE 4. Novel adenoviruses and their primate hosts

Primate host species[a]	Origin of host (living conditions)[b]	PCR-positive tissue	Novel virus (abbreviation)	Human adenovirus species[c]
Family: Hominidae				
Chimpanzee (*P. t. schweinfurthii*)	Tanzania (w)	Feces	*Pan troglodytes* adenovirus 1 (PtroAdV1)	E
	Uganda (w)	Intestine	*Pan troglodytes* adenovirus 2 (PtroAdV2)	NA
Chimpanzee (*P.t. verus*)	Gambia (w)	Lung	*Pan troglodytes* adenovirus 3 (PtroAdV3)	F
	Côte d'Ivoire (w)	Feces	*Pan troglodytes* adenovirus 4 (PtroAdV4)	C
	Côte d'Ivoire (w)	Feces	*Pan troglodytes* adenovirus 5 (PtroAdV5)	E
Chimpanzee (*P. t. schweinfurthii*)	Uganda (w)	Feces	*Pan troglodytes* adenovirus 6 (PtroAdV6)	C
	Uganda (w)	Feces	*Pan troglodytes* adenovirus 7 (PtroAdV7)	B
Chimpanzee (*P. t. verus*)	Côte d'Ivoire (w)	Intestine	*Pan troglodytes* adenovirus 8 (PtroAdV8)	A
Chimpanzee (*P. t. schweinfurthii*)	Uganda (s)	Feces	*Pan troglodytes* adenovirus 9 (PtroAdV9)	C
	Uganda (w)	Feces	*Pan troglodytes* adenovirus 10 (PtroAdV10)	D
	Uganda (w)	Feces	*Pan troglodytes* adenovirus 11 (PtroAdV11)	E
	Uganda (w)	Feces	*Pan troglodytes* adenovirus 12 (PtroAdV12)	E
	Uganda (w)	Feces	*Pan troglodytes* adenovirus 13 (PtroAdV13)	E
	Uganda (s)	Feces	*Pan troglodytes* adenovirus 14 (PtroAdV14)	E
Gorilla (*G. g. gorilla*)	Gabon (w)	Skin	*Gorilla gorilla* adenovirus 1 (GgorAdV1)	F
	Gabon (w)	Feces	*Gorilla gorilla* adenovirus 2 (GgorAdV2)	B
Gorilla (*G. g. beringei*)	Democratic Republic of Congo (w)	Feces	*Gorilla gorilla* adenovirus 3 (GgorAdV3)	B
	Rwanda (w)	Feces	*Gorilla gorilla* adenovirus 4 (GgorAdV4)	B
	Rwanda (w)	Feces	*Gorilla gorilla* adenovirus 5 (GgorAdV5)	B
	Democratic Republic of Congo (w)	Feces	*Gorilla gorilla* adenovirus 6 (GgorAdV6)	C
Family: Cercopithecidae (Old World monkeys)				
Subfamily: Cercopithecinae				
Hamadryas baboon (*Papio hamadryas*)	Tanzania (w)	Skin	*Papio hamadryas* adenovirus 1 (PhamAdV-1)	NA
Cynomolgus macaque (*Macaca fascicularis*)	Germany (c)	Tongue	*Macaca fascicularis* adenovirus 1 (MfasAdV-1)	G
Rhesus macaque (*Macaca mulatta*)	Germany (c)	Skin	*Macaca mulatta* adenovirus 1 (MmulAdV-1)	G
Subfamily: Colobinae				
Mantled guereza (*Colobus guereza*)	Germany (c)	Liver	*Colobus guereza* adenovirus 1 (CgueAdV-1)	NA
	Germany (c)	Lymph node	*Colobus guereza* adenovirus 2 (CgueAdV-2)	NA
	Germany (c)	Spleen	*Colobus guereza* adenovirus 3 (CgueAdV-3)	NA
Western red colobus (*Piliocolobus badius*)	Côte d'Ivoire (w)	Intestine	*Piliocolobus badius* adenovirus 1 (PbadAdV-1)	NA
	Côte d'Ivoire (w)	Intestine	*Piliocolobus badius* adenovirus 2 (PbadAdV-2)	NA
	Côte d'Ivoire (w)	Feces	*Piliocolobus badius* adenovirus 3 (PbadAdV-3)	NA

[a] All primates listed belong to the Catarrhini.
[b] w, wild; c, captive; s, born in the wild but kept in a sanctuary.
[c] AdVs were tentatively allocated to an AdV species on the basis of hexon gene sequences. NA, no allocation to a certain AdV species.

gorilla AdV (PtroAdV-3 and GgorAdV-1) attributable to HAdV-F (Fig. 2). In addition, five novel OWM-AdVs were detected to cluster more closely with HAdV-F than with other HAdVs (Fig. 2 and Table 4; see Table S1 in the supplemental material). PtroAdV-3 was detected in necropsy samples from a wild chimpanzee from the River Gambia National Park, Gambia. The lungs, liver, and spleen were PCR positive (the intestine and feces were not available). PtroAdV-3 revealed a pairwise hexon nucleic acid identity of 99.8% to HAdV-40 (Fig. 1). Despite attempts to identify PtroAdV-3 in other chimpanzees by use of a specific PCR (data not shown), the virus was detected only in the index individual. Since the chimpanzee from Gambia had been fed by humans over a long period, it is possible that the virus was transmitted from humans to this chimpanzee. Nothing is known about possible contacts of the gorilla from Gabon hosting GgorAdV-1.

For HAdV-G, four novel monkey AdVs were detected to have a close relationship to HAdV-52 (Fig. 1 and 2). This HAdV type was discovered recently in the United States in samples from human patients with gastroenteritis of unknown etiology, and its close relatedness to some SAdVs was discussed as evidence of a recent common ancestor (26). However, concern was raised that HAdV-52 was in fact a simian AdV contaminating the primary monkey cell culture used for the isolation of this virus, and evi-

dence of its circulation in other parts of the world is lacking (5). The four novel monkey AdVs (MfasAdV-1, MmulAdV-1, PhamAdV-1, and CgueAdV-2) detected in this study and those identified very recently in captive rhesus monkeys in China (36), all branching closely around HAdV-52, underscore the above hypothesis that HAdV-52 may be a simian AdV originating from cultivated monkey cells.

The novel AdV species SAdV-A presently comprises only one AdV (of rhesus macaques), and two additional monkey AdVs (of cynomolgus and rhesus macaques) have been allocated tentatively (8, 46). We discovered a chimpanzee AdV (PtroAdV-2) as potentially belonging to SAdV-A, as revealed by hexon-based phylogenetic analysis (Fig. 2). PtroAdV-2 was discovered in a chimpanzee of the Budongo Forest area, Uganda. Since PtroAdV-2 has no closely related monkey counterpart, it remains unclear if it is a genuine great ape AdV or an AdV of monkey origin. Nevertheless, it adds a new qualifier to the species SAdV-A, and it is of great interest whether additional members of SAdV-A are circulating in great ape or human populations.

AdVs of clades HAdV-A, -B, -D, and -F and SAdV-A were identified only rarely in wild chimpanzees, and HAdV-G was not detected at all (Table 1; Fig. 1). Instead, 87% of the AdVs detected were HAdV-E and -C members, possibly inhibiting the detection of other AdV types by generic PCR. Therefore, we set up a real-time PCR with nondegenerate primers for the selective amplification of HAdV-F and -G members in order to exclude the possible detection of any other HAdV species. Fecal samples of chimpanzees (n = 113) were tested, but sequences attributable to HAdV-F or -G were not detected (data not shown). We concluded from these findings that HAdV-F and -G do not genuinely occur in chimpanzees, and since HAdV-F viruses are endemic in most countries (29, 50), the single chimpanzee HAdV-F virus (PtroAdV-3) with 99.8% identity to HAdV-40 is most likely the result of a very recent human-to-chimpanzee interspecies transmission event. In addition, the obvious lack of HAdV-G in chimpanzees further supports the above notion that HAdV-52 may have originated from an OWM.

Although extensive molecular evidence has accumulated in the literature to indicate that AdVs coevolved with their respective hosts, showing remarkable restrictions in their host range (7, 12), previous reports also corroborate the possibility of interspecies transmission of primate AdVs (26, 43). The most striking evidence of the zoonotic potential of primate AdVs emerged from the very recent respiratory illness of a scientist who appeared to have been infected with an unknown AdV while investigating a fatal outbreak of pneumonia and hepatitis in a colony of Titi monkeys (NWMs) at the California National Primate Research Centre in Davis, CA (10). In the present study, several lines of genetic evidence of recent interspecies transmissions of AdVs were obtained. Taken together, these data underscore the concept of AdVs having the potential to cross the borders between closely related host species, in particular those between NHPs and humans.

It is estimated that 75% of the infectious diseases affecting humans are of zoonotic origin, with severe consequences for public health (41). Since primates, particularly great apes, and humans are very similar in their physiological and genetic properties, studying the primate "virome" may provide information on viral candidates with the potential to infect humans (18). Transmission of such viruses is most likely to occur at places with close physical contact between NHPs and humans, such as zoos and other animal facilities (10). Sub-Saharan Africa deserves special attention, since hunting and preparation of primate meat create high exposure rates. In addition, the susceptibility to viral infections may be enhanced due to high HIV infection rates in local populations (9). Given the need to protect both public health and endangered NHPs and the fact that a number of AdVs have been linked firmly to human diseases, the large variety of known and novel AdVs in great apes and monkeys calls for larger studies to understand the diversity of AdVs currently circulating in African primates as well as in local human populations (18, 35). This may answer the intriguing question of whether NHPs and humans have an intersected "adeno-virosphere" and may provide the basis for elucidating the potential pathological consequences of interspecies AdV transmissions.

ACKNOWLEDGMENTS

The excellent technical assistance of Sonja Liebmann, Nezlisah Yasmum, and Cornelia Walter is gratefully acknowledged. We thank Balazs Harrach, Barbara Biere, and Andreas Kurth for the provision of human, bat, and bovine AdV DNAs, respectively; Kerstin Mätz-Rensing for the provision of tissue samples from captive NHPs; and Anja Blasse, Adeelia Goffe, Sophie Köndgen, Ulla Thiesen, Alexander Hübner, Tobias Hasenberg, and Kevin Merkel for DNA extraction. We thank the Agence Nationale des Parcs Nationaux (ANPN) and the Centre National de la Recherche Scientifique et Technique (CENAR EST) of Gabon for permission to conduct research in Loango National Park. We thank L. Rabanal, L. Makaga, E. R. Guizard, E. Fairet, M. Gregoire, L. Rankin, E. Wright, and the other field assistants of the Loango Ape Project for their help in collecting the samples. We also thank the Centre International de Recherches Médicales de Franceville (CIRMF), especially E. Leroy, and the Ministère des Eaux et Forets du Gabon. For samples from Côte d'Ivoire, we thank the Ivorian authorities for their long-term support, especially the Ministry of Environment and Forests as well as the Ministry of Research, the directorship of the Taï National Park, the Office Ivoirien des Parcs et Réserves, and the Centre Suisse de Recherches Scientifiques in Abidjan. We thank E. Batamuzi, J. Keyyu, Lake Manyara National Park Headquarters Staff, D. M. Kambarage, F. J. Kaup, and K. Mätz-Rensing for their altruistic support and scientific as well as personal advice. We also thank the Chimpanzee Sanctuary & Wildlife Conservation Trust, Uganda Wildlife Authority, and Uganda National Council of Science and Technology for permission and permits to collect chimpanzee samples from the Ngamba Island Chimpanzee Sanctuary. Permission and support to carry out research at Gombe were granted by the Government of Tanzania, Tanzania National Parks, the Tanzania Commission for Science and Technology, the Tanzania Wildlife Research Institute, and the Jane Goodall Institute. We especially thank Richard Ssuna, Michael Wilson, and Anthony Collins. Samples were also provided for analysis from the 2010 Virunga gorilla census, which was a close collaboration between the Congolese Institute for the Conservation of Nature, the Rwanda Development Board, and the Uganda Wildlife Authority, with the support of the International Gorilla Conservation Programme, the Max Planck Institute for Evolutionary Anthropology, the Dian Fossey Gorilla Fund International, and the Mountain Gorilla Veterinary Project. For samples from bonobos, we thank the Lui Kotale Bonobo Project, specifically B. Fruth and G. Hohmann, as well as the authorities from the Democratic Republic of Congo.

We thank the Société pour la Conservation et le Développement (SCD) and the Wildlife Conservation Society (WCS) for financial and logistical support. This work was supported by the German Academic Exchange Program (D/06/43974), the Christian Vogel Fond (2006), WAZA (07002), the University of Leipzig, the Justus Liebig University of Giessen, the Scil Animal Care Company, Telinject Inc., and Wuppertal Zoo. This work was also supported by grants from the U.S. Fish and Wildlife Great Ape Conservation Fund and the Arcus Founda-

tion. The analyses were financed by the Robert Koch Institute and the Deutsche Forschungsgemeinschaft (grant LE1818/4-1).

REFERENCES

1. **Andrewes, C. A.** 1989. Adenoviridae, p. 249–282. *In* J. S. Porterfield (ed.), Andrewes' viruses of vertebrates, 5th ed. Bailliere Tindall, London, United Kingdom.
2. **Arandjelovic, M., et al.** 2010. Effective non-invasive genetic monitoring of multiple wild western gorilla groups. Biol. Conserv. **143:**1780–1791.
3. **Asher, D. M., et al.** 1978. Persistent shedding of adenovirus in urine of chimpanzees. Infect. Immun. **21:**129–134.
4. **Bányai, K., et al.** 2010. Molecular detection of novel adenoviruses in fecal specimens of captive monkeys with diarrhea in China. Vet. Microbiol. **142:**416–419.
5. **Banyai, K., et al.** 2009. Searching for HAdV-52, the putative gastroenteritis-associated human adenovirus serotype in Southern Hungary. New Microbiol. **32:**185–188.
6. **Basnight, M., Jr., N. G. Rogers, C. J. Gibbs, Jr., and D. C. Gajdusek.** 1971. Characterization of four new adenovirus serotypes isolated from chimpanzee tissue explants. Am. J. Epidemiol. **94:**166–171.
7. **Benkö, M., and B. Harrach.** 2003. Molecular evolution of adenoviruses. Curr. Top. Microbiol. Immunol. **272:**3–35.
8. **Benkö, M., et al.** 2005. Family Adenoviridae, p. 213–228. *In* C. M. Fauquet, M. A. Mayo, J. Maniloff, U. Desselberger, and L. A. Ball (ed.), Virus taxonomy. VIIIth report of the International Committee on Taxonomy of Viruses. Elsevier, New York, NY.
9. **Bennett, E. L., et al.** 2007. Hunting for consensus: reconciling bushmeat harvest, conservation, and development policy in West and Central Africa. Conserv. Biol. **21:**884–887.
10. **Chen, E., et al.** 2011. Cross-species transmission of a novel adenovirus associated with a fulminant pneumonia outbreak in a New World monkey colony. PLoS Pathog. **7:**e1002155.
11. **Crawford-Miksza, L. K., and D. P. Schnurr.** 1996. Adenovirus serotype evolution is driven by illegitimate recombination in the hypervariable regions of the hexon protein. Virology **224:**357–367.
12. **Davison, A. J., M. Benko, and B. Harrach.** 2003. Genetic content and evolution of adenoviruses. J. Gen. Virol. **84:**2895–2908.
13. **Decaro, N., V. Martella, and C. Buonavoglia.** 2008. Canine adenoviruses and herpesvirus. Vet. Clin. North Am. Small Anim. Pract. **38:**799–814.
14. **Drummond, A. J., and A. Rambaut.** 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. **7:**214.
15. **Edgar, R. C.** 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics **5:**113.
16. **Edgar, R. C.** 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. **32:**1792–1797.
17. **Ehlers, B., et al.** 2007. Identification of novel rodent herpesviruses, including the first gammaherpesvirus of *Mus musculus*. J. Virol. **81:**8091–8100.
18. **Gillespie, T. R., C. L. Nunn, and F. H. Leendertz.** 2008. Integrative approaches to the study of primate infectious disease: implications for biodiversity conservation and global health. Am. J. Phys. Anthropol. Suppl. **47:**53–69.
19. **Gnanadurai, C. W., et al.** 2010. Genetic identity and biological phenotype of a transmitted/founder virus representative of nonpathogenic simian immunodeficiency virus infection in African green monkeys. J. Virol. **84:**12245–12254.
20. **Gouy, M., S. Guindon, and O. Gascuel.** 2010. SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Mol. Biol. Evol. **27:**221–224.
21. **Gray, G. C., J. D. Callahan, A. W. Hawksworth, C. A. Fisher, and J. C. Gaydos.** 1999. Respiratory diseases among U.S. military personnel: countering emerging threats. Emerg. Infect. Dis. **5:**379–387.
22. **Guindon, S., et al.** 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst. Biol. **59:**307–321.
23. **Guindon, S., and O. Gascuel.** 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. **52:**696–704.
24. **Hillis, W. D., and R. Goodman.** 1969. Serologic classification of chimpanzee adenoviruses by hemagglutination and hemagglutination inhibition. J. Immunol. **103:**1089–1095.
25. **Jones, K. E., et al.** 2008. Global trends in emerging infectious diseases. Nature **451:**990–993.
26. **Jones, M. S., II, et al.** 2007. New adenovirus species found in a patient presenting with gastroenteritis. J. Virol. **81:**5978–5984.
27. **Katoh, K., K. Misawa, K. Kuma, and T. Miyata.** 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. **30:**3059–3066.
28. **Keele, B. F., et al.** 2006. Chimpanzee reservoirs of pandemic and nonpandemic HIV-1. Science **313:**523–526.
29. **Kidd, A. H., J. E. Banatvala, and J. C. De Jong.** 1983. Antibodies to fastidious faecal adenoviruses (species 40 and 41) in sera from children. J. Med. Virol. **11:**333–341.
30. **Kidd, A. H., D. Garwicz, and M. Oberg.** 1995. Human and simian adenoviruses: phylogenetic inferences from analysis of VA RNA genes. Virology **207:**32–45.
31. **Kim, C. S., E. S. Sueltenfuss, and S. S. Kalter.** 1967. Isolation and characterization of simian adenoviruses isolated in association with an outbreak of pneumoenteritis in vervet monkeys (Cercopithecus aethiops). J. Infect. Dis. **117:**292–300.
32. **Kojaoghlanian, T., P. Flomenberg, and M. S. Horwitz.** 2003. The impact of adenovirus infection on the immunocompromised host. Rev. Med. Virol. **13:**155–171.
33. **Kovacs, G. M., A. J. Davison, A. N. Zakhartchouk, and B. Harrach.** 2004. Analysis of the first complete genome sequence of an Old World monkey adenovirus reveals a lineage distinct from the six human adenovirus species. J. Gen. Virol. **85:**2799–2807.
34. **Kovacs, G. M., B. Harrach, A. N. Zakhartchouk, and A. J. Davison.** 2005. Complete genome sequence of simian adenovirus 1: an Old World monkey adenovirus with two fiber genes. J. Gen. Virol. **86:**1681–1686.
35. **Leendertz, F. H., et al.** 2006. Pathogens as drivers of population declines: the importance of systematic monitoring in great apes and other threatened mammals. Biol. Conserv. **131:**325–337.
36. **Lu, J., Q. Wang, H. Wang, G. Li, and G. Gao.** 2011. Molecular characterization of adenoviruses in fecal samples of captively bred rhesus macaques in China. Vet. Microbiol. **149:**461–466.
37. **Lukashev, A. N., O. E. Ivanova, T. P. Eremeeva, and R. D. Iggo.** 2008. Evidence of frequent recombination among human adenoviruses. J. Gen. Virol. **89:**380–388.
38. **Mautner, V., and M. E. G. Boursnell.** 1983. Recombination in adenovirus: DNA sequence analysis of crossover sites in intertypic recombinants. Virology **131:**1–10.
39. **Mautner, V., and N. Mackay.** 1984. Recombination in adenovirus: analysis of crossover sites in intertypic overlap recombinants. Virology **139:**43–52.
40. **Mwenda, J. M., A. Nyachieo, D. K. Langat, and D. A. Steele.** 2005. Serological detection of adenoviruses in non-human primates maintained in a colony in Kenya. East Afr. Med. J. **82:**371–375.
41. **Palmarini, M.** 2007. A veterinary twist on pathogen biology. PLoS Pathog. **3:**e12.
42. **Posada, D.** 2008. jModelTest: phylogenetic model averaging. Mol. Biol. Evol. **25:**1253–1256.
43. **Purkayastha, A., et al.** 2005. Genomic and bioinformatics analysis of HAdV-4, a human adenovirus causing acute respiratory disease: implications for gene therapy and vaccine vector development. J. Virol. **79:**2559–2572.
44. **Rouquet, P., et al.** 2005. Wild animal mortality monitoring and human Ebola outbreaks, Gabon and Republic of Congo, 2001–2003. Emerg. Infect. Dis. **11:**283–290.
45. **Roy, S., et al.** 2004. Complete nucleotide sequences and genome organization of four chimpanzee adenoviruses. Virology **324:**361–372.
46. **Roy, S., et al.** 2009. Isolation and characterization of adenoviruses persistently shed from the gastrointestinal tract of non-human primates. PLoS Pathog. **5:**e1000503.
47. **Russel, W.** 2005. Adenoviruses, p. 439–447. *In* B. W. J. Mahy and V. ter Meulen (ed.), Topley and Wilson's microbiology and microbial infections, 9th ed. Hodder Arnold, London, United Kingdom.
48. **Rux, J. J., and R. M. Burnett.** 2000. Type-specific epitope locations revealed by X-ray crystallographic study of adenovirus type 5 hexon. Mol. Ther. **1:**18–30.
49. **Schmitz, H., R. Wigand, and W. Heinrich.** 1983. Worldwide epidemiology of human adenovirus infections. Am. J. Epidemiol. **117:**455–466.
50. **Shinozaki, T., K. Araki, H. Ushijima, and R. Fujii.** 1987. Antibody response to enteric adenovirus types 40 and 41 in sera from people in various age groups. J. Clin. Microbiol. **25:**1679–1682.
51. **Shroyer, E. L., S. T. Kelley, P. C. Taylor, P. Vanderloo, and T. L. Lester.** 1979. Three serologic types of adenovirus infections of owl monkeys. Am. J. Vet. Res. **40:**532–536.
52. **Talavera, G., and J. Castresana.** 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst. Biol. **56:**564–577.
53. **Tong, S., et al.** 2010. Short report: identification of adenoviruses in fecal specimens from wild chimpanzees (*Pan trogylodytes schweinfurthii*) in Western Tanzania. Am. J. Trop. Med. Hyg. **82:**967–970.
54. **Walsh, M. P., et al.** 2009. Evidence of molecular evolution driven by recombination events influencing tropism in a novel human adenovirus that causes epidemic keratoconjunctivitis. PLoS One **4:**e5635.
55. **Wevers, D., et al.** 2010. A novel adenovirus of Western lowland gorillas (*Gorilla gorilla gorilla*). Virol. J. **7:**303.
56. **Wigand, R., M. Mauss, and T. Adrian.** 1989. Chimpanzee adenoviruses are related to four subgenera of human adenoviruses. Intervirology **30:**1–9.
57. **Wittmann, T. J., et al.** 2007. Isolates of Zaire ebolavirus from wild apes reveal genetic lineage and recombinants. Proc. Natl. Acad. Sci. U. S. A. **104:**17123–17127.
58. **Wold, W. S. M., and M. S. Horwitz.** 2007. Adenoviruses, p. 2395–2436. *In* D. M. Knipe et al. (ed.), Fields virology, 5th ed., vol. 2. Lippincott Williams & Wilkins, Philadelphia, PA.
59. **Wolfe, N. D., C. P. Dunavan, and J. Diamond.** 2007. Origins of major human infectious diseases. Nature **447:**279–283.
60. **Wright, N. G.** 1976. Canine adenovirus: its role in renal and ocular disease: a review. J. Small Anim. Pract. **17:**25–33.
61. **Xiang, Z., et al.** 2006. Chimpanzee adenovirus antibodies in humans, sub-Saharan Africa. Emerg. Infect. Dis. **12:**1596–1599.