Short Report: Identification of Adenoviruses in Fecal Specimens from Wild Chimpanzees (*Pan troglodytes schweinfurthii*) in Western Tanzania

Suxiang Tong,* Jatinder Singh, Susan Ruone, Charles Humphrey, Cyril C. Y. Yip, Susanna K. P. Lau, Larry J. Anderson, and Taranjit Kaur

Gastroenteritis and Respiratory Viruses Laboratory Branch, Division of Viral Diseases, and Infectious Disease Pathology Branch, Division of Viral and Rickettsial Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia; Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Technology, Blacksburg, Virginia; Department of Microbiology, Research Centre of Infection and Immunology, and State Key Laboratory of Emerging Infectious Diseases, The University of Hong Kong, Hong Kong, People's Republic of China

Abstract. DNA of two distinctive adenoviruses was detected in wild chimpanzees in western Tanzania that showed clinical signs of acute, upper respiratory disease, notably coughing. The amplified sequences from part of the capsid hexon gene suggests that one virus is a novel adenovirus serotype candidate and the other virus is a species C adenovirus most closely related to recent isolates from captive chimpanzees in the United States, Simian AdV 37 with 86% nucleic acid identity and Simian AdV 40 with 95% nucleic acid identity, respectively. The species C adenovirus sequences suggest possible recombination with a human adenovirus. The source of these viruses and disease association is not known.

The family *Adenoviridae* includes a group of icosahedral nonenveloped viruses containing a double-stranded DNA genome. Members of this family are classified into two previously established genera, *Mastadenovirus* and *Aviadenovirus*, comprising mammalian and avian isolates, respectively, and three recently accepted genera, *Atadenovirus*, *Siadenovirus*, and *Ichtadenovirus*, comprising a broad range of hosts including mammalian, amphibian, reptile, and fish isolates. Human adenoviruses (HAdVs) are located in the genus *Mastadenovirus* and include 54 serotypes that can be divided into seven species subgroups (A, B, C, D, E, F, and G). The genomes of serotypes within a species are highly related and show modest divergence between species.^{1,2}

Adenoviruses in humans cause a broad spectrum of diseases including respiratory tract infections, acute conjunctivitis, cystitis, gastroenteritis and systemic infections in immunocompromised patients.¹ Polymerase chain reaction (PCR)-based diagnostic assays have made it possible to detect adenoviruses by family, serotype, or species, with good sensitivity. Pan adenovirus family PCR primers are specific for the highly conserved regions in hexon gene of known members of the family *Adenoviridae* and enable detection of known and previously unrecognized adenoviruses. We show the utility of the pan adenovirus family PCR approach in identification of two distinctive adenoviruses from wild chimpanzees in western Tanzania.

M group chimpanzees (*Pan troglodytes schweinfurthii*) at Mahale Mountains National Park in Tanzania are accustomed to humans and tolerate observation from close proximity for extended periods. We previously demonstrated that a human metapneumovirus was the likely causative agent associated with an acute, fatal respiratory disease outbreak in this group.³ With the aim of reducing the morbidity and mortality within this chimpanzee group, we established an intensive health surveillance program, which includes daily health observations and fecal collections.⁴ On October 16, 2007, an adult female chimpanzee was observed coughing repeatedly and had a loose stool. Approximately one week later, on October 22, severe

cough and dry stool developed in an adolescent female chimpanzee. Fecal samples from both animals were collected from the forest floor immediately after defecation and placed in 4% paraformaldehyde and RNAlater (Qiagen, Valencia, CA). Specimens were collected by personnel using gloved hands and wearing facemasks who had no symptoms or clinical signs of respiratory, ocular, gastrointestinal or other diseases. Samples from the adult female (MM07-1016) and the adolescent female (MM07-027) chimpanzees were shipped at ambient temperature to the Centers for Disease Control and Prevention (Atlanta, GA) for analysis.

Stool specimens were examined by negative stain electron microscopy (EM) according to a previously described protocol.⁵ Particles resembling adenovirus were seen in the stool specimen from chimpanzee MM07-1016 but not in the specimen from MM07-027 (Figure 1). The particles had a low titer. Despite the poor definition of the particles, the size, shape, and capsid surface structures were suggestive of an adenovirus.

Total nucleic acids from stool samples were extracted from a suspension in phosphate-buffered saline. Total nucleic acids from each stool sample was screened for selective viral respiratory pathogens (coronaviruses, adenoviruses, influenza viruses, and paramyxoviruses) by each of the four universal pan viral nested or semi-nested reverse transcription-PCRs (RT-PCRs) and nested or semi-nested PCRs. To avoid PCR contamination, sample extraction, PCR reagent preparation, PCR, and sequencing were carried out in separate rooms. Negative controls (water) were included in each step. The pan primers for each tested viral family were designed from a specific viral gene or open reading frame as follows: adenovirus (hexon gene),⁶ coronavirus (polymerase 1b open reading frame),⁷ paramyxovirus (polymerase L gene),⁸ and influenza virus (polymerase PB1 gene) (Tong S and others, unpublished data). All RT-PCRs and PCRs were performed and analyzed according to previously described protocols.^{6–8}

The pan paramyxovirus, pan influenza virus, and pan coronavirus RT-PCRs were nonreactive but the pan adenovirus PCR was reactive for both samples; water extracted in parallel to stool samples and non-template, water controls generated no amplicons. The PCR products from the adenovirus assays were purified and both strands of the amplicons were sequenced by using a BigDye Terminator version 3.1 ready reaction cycle sequencing kit on an ABI Prism 3130 automated

*Address correspondence to Suxiang Tong, Centers for Disease Control and Prevention, 1600 Clifton Road, Atlanta, GA 30333. E-mail: sot1@cdc.gov

FIGURE 1. Negative stain electron micrograph of virus-like structures images (**A** and **B**) isolated from fecal samples of from wild chimpanzees MM07-1016 (*Pan troglodytes schweinfurthii*) in western Tanzania. The structure of the particles was varied and generally poor, likely because of the condition in which they were collected and stored. Size and general structure is consistent with that of adenovirus. The specimens were stained in a 5% ammonium molybdate–1% trehalose. The pH was adjusted to 6.9 with 0.5 N ammonium hydroxide. Scale bars indicate 100 nm.

sequencer (Applied Biosystems, Foster City, CA) and corresponding PCR primers. Phylogenetic trees were reconstructed by using the neighbor-joining method (MEGA package, version3) (<http://www.megasoftware.net/>) by 1,000 bootstrap resamplings.

Phylogenetic analysis (Figure 2) of the two isolates was conducted by using nucleotide sequences of partial hexon gene amplicons (approximately 700 basepairs) and compared with the other known representative adenovirus sequences of human and non-human primate species. The sequence from chimpanzee MM07-027 (clone_327) was novel by BLAST search and most closely related to the recently identified simian adenovirus 37 (FJ025928) from captive chimpanzees in the United States and showed 86% nucleotide sequence identity. The sequence from chimpanzee MM07-1016 (clone_326) was closely related to the recently identified simian adenovirus-40 (SAdV-40) (FJ025926) from captive chimpanzees in the United States and showed 95% nucleotide sequence identity.

Because adenoviruses are known to recombine,^{1,9,10} we also performed bootscan analysis to look for evidence of an origin of recombination in both of these two newly identified adenoviruses from wild chimpanzee in western Tanzania. Nucleotide alignments of the sequences of the two viruses and the corresponding sequences of other closely related adenoviruses were generated by using ClustalX version 1.83 (<http://bips.u-strasbg.fr/fr/Documentation/ClustalX/>) and edited manually. Bootscan analysis was performed as described by using Simplot version 3.5.1 (F84 model; window size = 300 basepairs; step = 10 basepairs (<http://sray.med.som.jhmi.edu/SCSoftware/simplot/>)) with both viruses independently as a query.¹¹ This analysis showed a possible recombination site

FIGURE 2. Phylogenetic trees for adenoviruses generated in MEGA version 4.0 (<http://www.megasoftware.net/>) by neighbor-joining method. Two adenovirus nucleotide sequences of partial hexon gene amplicons (approximately 700 basepairs) from this study are shaded and marked by an arrow. The other known 60 corresponding adenovirus sequences of human and non-human primate species are designated by HAd and SAd, respectively, and their serotype number. The adenovirus species subgroups are labeled according to the most recent update by the International Committee on Taxonomy of Viruses. Bootstrap values shown for the major branches were based on 1,000 replicates. Scale bars indicate nucleotide substitutions per site.

between positions 400 and 480 of clone_326 (MM07-1016) (Figure 3). As shown in the Supplemental Figure (available at www.ajtmh.org), supplfig sequences from 5' end to position 400 clustered most closely with HAdV-2, species C (GenBank accession no. NC_001405), and sequences from position 480 to the 3' end clustered most closely with HAdV-6, as noted for the overall full 700-basepair comparison with higher bootstrap support. Because the percentage of permuted trees for these sequences were substantially below 100%, the existing adenovirus sequences do not identify likely parent viruses for recombination. No evidence of recombination was found for the clone_327 sequence from chimpanzee MM07-027.

We have identified two adenoviruses, one species C-like (clone_326) and one species D-like (clone_327) from stool samples of wild chimpanzees with acute, upper respiratory signs in western Tanzania. This finding is the first report of detection of adenoviruses in wild chimpanzees. Previously, more than 15 strains representing different serotypes of adenoviruses have been identified in captive chimpanzees and have not been linked to disease.^{9,12-14} Characterization of these isolates from captive chimpanzees by neutralization and hemagglutination inhibition studies have shown that these viruses are related to different HAdVs of species B, C and E subgroups.^{12,14}

In this report, clone_326 is most similar to SAdV-40, which is a human species C virus, and clone_327 is most similar to SAdV-37, which is a human species E virus.¹⁴ The parital hexon gene sequences of clone_327 and the corresponding region in SAdV-37 cluster with human species D subgroup viruses, and other regions of the SAdV-37 viruses cluster with species E adenoviruses.¹²⁻¹⁴ Obviously, more sequences from clone_327

are required to confirm the species identity of clone_327. Typically, in humans, respiratory tract diseases are associated with adenovirus species B1, C, and E, and gastrointestinal diseases are associated with species A and F.^{2,15} Clone_326 and clone_327 were isolated from stool samples of chimpanzees with acute upper respiratory signs.

Our pan viral family PCRs showed negative results for coronaviruses, influenzaviruses, and paramyxoviruses, a finding that is consistent with these adenoviruses being associated with the respiratory illnesses. However, there are a number of other respiratory pathogens for which we did not test, e.g., rhinoviruses, which could have caused the observed symptoms. Another alternative could be that the pan viral family PCRs failed to detect the virus because it was not present or was below the limit of detection of the assay. Consequently, the role of the detected adenoviruses in these illnesses is not clear. Because adenoviruses can be detected in asymptomatic humans, they may also be present without disease in chimpanzees. Persistence of adenovirus after an acute infection is illustrated by the study by Garnett and others, in which they detected species C adenoviruses in human tonsillar T lymphocytes in non-ill humans.¹⁶ More recently, persistently shedding of adenovirus from the gastrointestinal tract have been reported in non-human primates.¹⁴

The sequence data suggests the possibility of human-to-chimpanzee transmission with a recombinant virus or possibly infection followed by a recombination event resulting in clone_326, the HAdV 6-like sequence. Clone_327 sequence is distinct from previously reported human adenovirus sequences; thus, it is difficult to infer a source for this virus. Because we have limited information on adenoviruses in wild chimpanzees, the virus could be relatively new to wild

FIGURE 3. Bootscan analysis of sequence of adenovirus clone 326. Boot scanning was conducted with Simplot version 3.5.1 (<http://sray.med.som.jhmi.edu/SCSoftware/simplot/>) on a gapless nucleotide alignment generated with ClustalX (<http://bips.u-strasbg.fr/fr/Documentation/ClustalX/>) with clone_326 sequence as the query sequence. The brown indicates human adenovirus 6 (DQ149613), the gray line indicates human adenovirus 2 (NC_001405), the green line indicates human adenovirus 5 (AC_000008), the navy blue ----- indicates human adenovirus 1 (AC_000017), the light blue ---- indicates simian adenovirus 1 (NC_006879), and the purple .-.-. indicates simian adenovirus 7 (DQ792570). This figure appears in color at www.ajtmh.org.

chimpanzees and introduced from another species or had been circulating for a long time but only recently detected. As we acquire more information on adenoviruses in chimpanzees, it may become clearer how long a given virus may have been circulating and how often humans have been a likely source or *vice versa*.

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Authors' addresses: Suxiang Tong, Susan Ruone, and Larry J. Anderson, Division of Viral Diseases, Centers for Disease Control and Prevention, Atlanta, GA. Jatinder Singh and Taranjit Kaur, Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Technology, Blacksburg, VA. Charles Humphrey, Division of Viral and Rickettsial Diseases, Centers for Disease Control and Prevention, Atlanta, GA. Cyril C. Y. Yip and Susanna K. P. Lau, Department of Microbiology, Research Centre of Infection and Immunology, and State Key Laboratory of Emerging Infectious Diseases, The University of Hong Kong, Hong Kong, People's Republic of China.

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