Naturally Circulating Hepatitis A Virus in Olive Baboons, Uganda

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Hepatitis A (HA V; family Picornaviridae; genus Hepatovirus) is an ≈7.5-kb single-stranded positive-sense RNA virus that causes acute inflammation of the liver in humans and nonhuman primates. Although HA V has been detected in recently imported captive primates after spontaneous outbreaks of acute hepatitis in animal facilities, but the definitive hosts of this virus have remained obscure (2,3). We identified by next-generation sequencing HA V in the blood of a free-living olive baboon (Papio anubis) from Kibale National Park, Uganda, sampled in September 2010. Subsequent testing of a separate Kibale olive baboon troop in 2014 indicated the virus was prevalent and shed in feces.

As part of a long-term study of nonhuman primate health and ecology, 23 animals were immobilized and sampled in 2010 as previously described (4). All animal protocols received prior approval from the Uganda National Council for Science and Technology (Kampala, Uganda), the Uganda Wildlife Authority (Kampala, Uganda), and the University of Wisconsin–Madison Animal Care and Use Committee (Madison, WI, USA). All samples were shipped in accordance with international laws under Convention on International Trade in Endangered Species of Wild Fauna and Flora Ugandan permit no. 002290.

During May 2012, we subjected total RNA from 1 mL of blood plasma of each animal to next-generation sequencing as previously described (4); results showed HA V-like sequences in 1 of 23 baboons. De novo assembly of these reads yielded a nearly complete HA V genome, which we term KibOB-1. KibOB-1 is most similar (94.2% nt identity; Figure) to AGM-27, an HA V originally detected in an African green monkey (Chlorocebus aethiops) imported to a Russian primate facility from Kenya (3).

For 11 baboons, we also collected a paired fecal sample, which we analyzed for evidence of viral shedding. Samples were preserved in RNAlater (Ambion Inc., Austin, TX, USA) at –20°C, and viral RNA was isolated by using the ZR Soil/Fecal RNA Microprep kit (Zymo Research, Irvine, CA, USA) following manufacturer’s protocols. Reverse transcription PCR (RT-PCR) of RNA was primed with random hexamers by using the RNA to cDNA Ecodry Premix (Random Hexamers) (Clontech Laboratories, Inc., Mountain View, CA, USA), and diagnostic PCR was conducted with primers flanking the C-terminal extension of the HA V viral protein (VP) 1 gene (pX) by using the High Fidelity PCR Master Mix–Ecodry Premix (Clontech Laboratories, Inc.). Five of 11 paired fecal samples tested positive for HA V by RT-PCR, indicating a higher prevalence of the virus in feces than in blood.

We then surveyed a second troop of habituated olive baboons at the same field site during February–April 2014 (5). From these baboons, 7 of 19 fecal samples tested positive by RT-PCR. Confirmatory Sanger sequencing of RT-PCR amplicons was successful for 3 of these 7 animals (GenBank accession nos. KT819576–KT819578). Phylogenetic analyses of these sequences demonstrate monophyly and a low degree of interhost variability (≥94% nt identity).
The risk to humans posed by KibOb-1 remains unknown. Although human infection with HAV genotype V has not been reported, evidence suggests that HAV variants might be capable of infecting a diversity of primate hosts (6). Although it is not known whether the closely related AGM-27 strain was discovered infecting its natural host, the similarity of KibOb-1 and AGM-27 raises the possibility of a recent host transfer. Major host shifts characterize the evolutionary histories of recently discovered bat and rodent hepatoviruses (7). Host fidelity of KibOb-1 is similarly unknown, but experimental infection of several nonhuman primate species with the similar AGM-27 virus found varying pathogenicity in different species (6). In particular, the AGM-27 caused productive infection in chimpanzees, with stimulation of a broadly reactive HAV immunoglobulin response (6).

Human and simian HAVs are considered a single serotype (6); thus, serosurveillance for HAV in humans might be unable to distinguish between human and zoonotic simian HAV infection, enabling the possibility of cryptic zoonotic transmission. Similarly, detection of HAV antibodies in wild primates, such as in a recent study of baboons in South Africa living in close proximity to humans (8), might not indicate anthropogenic transmission of human viruses but rather infection with an endemic HAV.

Prior studies have documented cross-species transmission between the primates of Kibale National Park and neighboring human populations, especially of gastrointestinal pathogens (9). A study tracking food-crop-raiding events on 97 farms within 0.5 km of Kibale’s forest edge found that 72% of households faced baboon raids over a 23-month period, including 228 discrete baboon raids (10). This finding suggests that a major portion of the local community remains at risk for exposure to potentially infectious baboon excreta. Such exposure, in addition to the evidence presented here that HAV is prevalent in wild baboons of Uganda and is shed into the environment, merits increased attention to the zoonotic risk for simian hepatoviruses.

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Preliminary results from this study were presented at the 3rd International One Health Congress, March 15–18, 2015, Amsterdam, the Netherlands; and at the Conference of the German Veterinary Medical Association, March 8–10, 2015, Fulda, Germany.

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To the Editor: In 2013, a 6-week-old female piglet kept in a flatdeck cage had coughing, growth retardation, and diarrhea and was taken to a local veterinarian in Hannover, Germany; the piglet was euthanized. After necropsy at the University of Veterinary Medicine in Hannover, histologic investigation found interstitial pneumonia; a mild, multifocal, lymphohistiocytic panencephalitis that affected the cerebrum and cerebellum, including brain stem and medulla oblongata; and a mild, multifocal, lymphohistiocytic panencephalitis. Results from screening for typical neurotropic viruses (classical swine fever virus, suid herpesvirus 1, rabies virus, teschovirus, porcine enterovirus 8, 9, and 10) were negative; *Mycoplasma hyorhinis* was detected by multiplex PCR (Institute of Virology, University of Veterinary Medicine Hannover) within the lung and pulmonary lymph nodes. Cerebral tissue from the pig was processed for viral metagenomics by random RNA and DNA virus screening and next-generation sequencing (NGS) with the 454 sequencing platform (GS Junior; Roche, Basel, Switzerland), as described (7), and 21,359 reads were obtained. Analysis by using blastn and blastx (2) showed 10 reads had ≥97% nt identity with porcine bocavirus (PBoV) KU14. No other viral sequences were detected.

By using primers based on sequence data of the PBoV, partially overlapping PCR amplicons were obtained to confirm and extend the NGS data of the isolate, which was named PBoV S1142/13 (1; GenBank accession no. KU311698). A total of 2,176 nt of PBoV S1142/13 were obtained, consisting of the partial nucleoprotein (NP) 1 and the nearly complete viral protein (VP) 1 gene. By using MAFFT version 7 (http://mafft.cbrc.jp/alignment/server/), we aligned the nearly complete VP1 gene of PBoV S1142/13 with various closely related members of the genus *Bocaparvovirus* and built a maximum-likelihood tree by using the general time reversible plus invariable sites plus gamma distribution method, as determined by jModelTest 2.0 (3) and default parameters in MEGA6.06 (4). Results confirmed that PBoV S1142/13 was most closely related to PBoV KU14 (Figure, panel A). The partial genome of PBoV S1142/13 differed at 8 nt positions from PBoV KU14, resulting in 99.6% nt identity. Of these nucleotide differences, 4 resulted in an amino acid difference, including position 2733 (T→C on the basis of PBoV KU14 as a reference genome), which is part of the NP1 stopcodon of PBoV KU14. These results indicate that the stopcodon was located 39 nt farther downstream than for PBoV KU14. The other 3 aa differences were present in the VP1 protein; each of these differences was within the same group of amino acids as those detected in PBoV KU14.

For further substantiation of a potential cause-effect relationship of histologic (Figure, panel B) and NGS results, we performed fluorescent in situ hybridization (FISH) on formalin-fixed, paraffin-embedded central nervous system (CNS) sections of the diseased animal and of a control pig with no CNS lesions. We used an RNA probe specific for the obtained NP1 and VP1 sequences covering 1,153 nt (Affymetrix, Santa Clara, CA, USA) according to the manufacturer’s protocol, with minor variations (ViewRNA ISH Tissue 1-Plex Assay Kit and ViewRNA Chromogenic Signal Amplification Kit, Affymetrix). A probe specific for porcine ubiquitin (*Sus scrofa* ubiquitin