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Terrestrial Animal-Derived Rabies Virus in a Juvenile Indian Flying Fox in Sri Lanka

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Unlike the bats of the New World, Old World bats are not considered to transmit rabies virus (RABV). Two RABV variants are known to circulate in Sri Lanka; one variant is known to circulate among dogs and other animals. The other variant has been identified in a wild civet species. There is a possibility that other RABV variants also circulate among wild animals in Sri Lanka. Therefore, we performed molecular characterization of the RABVs present in Sri Lankan wild animals. Samples from wild animals, dogs, cats, and humans with suspected rabies infection, which were deposited at the Department of Rabies Diagnosis and Research, Medical Research Institute (MRI), Colombo, Sri Lanka, were used in this study. We identified a RABV in a Sri Lankan bat species, which is commonly known as the Indian flying fox (*Pteropus medius*). Phylogenetic analysis of the N gene showed that the RABV from the bat formed a separate phylogenetic lineage within the Sri Lankan RABV group. The RABVs of this lineage originated in wild animals only, and contained 3 nucleotide substitutions in their genomes compared with others. This observation indicated that Old World bats may be infected with RABV, and therefore, further studies are required.

In Sri Lanka, the most common canine RABV variant has been identified in dogs and other animals, whereas the other RABV variant was reported in a wild civet species (1). In Sri Lanka, RABV infects large numbers of wild animals (2), and thus, other RABV variants are possibly enzootic to wild animals. Mongooses are the main wild animals that are affected by rabies in Sri Lanka (2), and therefore, these animals are potential carriers of RABV variants. Other potential carriers are bats, although in Asia, RABV infection in bats remains unconfirmed (3). In this study, we identified a RABV in a bat in Sri Lanka.

From 2009 through 2012, samples from 44 wild animals (Table 1) with suspected rabies were deposited at the Medical Research Institute (MRI), Colombo for the diagnosis of rabies, which was based on a fluorescent antibody test (FAT) using brain tissue, and subsequently, confirmed by reverse transcription (RT)-PCR (1, 4). Overall, 19 samples (43.2%) were determined to be RABV-positive by RT-PCR, and full-length sequences of the N gene were determined for 13 samples, including one bat and 4 mongoose samples. For comparison, the full sequence of the N gene was determined in 2 of 6, 4 of 8, and 13 of 31 arbitrarily selected, RABV-positive dog, cat, and human samples, respectively, all of which were deposited at the MRI during the period of study. The study was approved by the ethics committee of the MRI.

The bat sample was obtained from the village of Makola in the Gampaha district, and there are no jungles or caves in the vicinity of this village. In May 2011, a large bat was found dead on the side of a road with a live juvenile bat nearby, which died the following day. The carcass of the juvenile bat was packed in ice, and brought to the MRI by a villager on the same day. The bat had no visible injury, and was designated as H-528-11. This term was also used to designate the brain

Table 1. Results obtained for wild animals tested and those positive for rabies virus by reverse transcription-PCR, including the number of samples with full-length N gene sequences

Animal	Submitted number	Rabies positive number	Number of full-length N gene sequence
Mongoose	9	5	4
Civet	11	5	2
Wild cat	3	2	2
Bat	3	1	1
Squirrel	2	2	2
Monkey	2	1	1
Fishing cat	1	1	0
Deer	2	1	0
Rock squirrel	7	0	0
Ruddy mongoose	2	0	0
Grey mongoose	1	1	1
Bandicoot	1	0	0
Total	44	19 (43.2%)	13 (29.5%)

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sample of the bat and the virus that was detected in the sample. The sample tested negative by FAT; however, it tested positive for RABV by RT-PCR. The whole-genome sequencing of the viral DNA was attempted, as described previously (4).

We determined the sequences of the 5′ untranslated region (70 nucleotides, nt), N gene (1,353 nt), and N-P noncoding region (90 nt), and the partial sequence of the phosphoprotein gene (106 nt) of strain H-528-11. The N gene of H-528-11 shared 98.4–99.9% (99.3–100%) nucleotide (amino acid) identity with Sri Lankan canine RABVs and 96.9% (99.3%) with a civet RABV. The N genes were subjected to phylogenetic analysis, and phylogenetic trees were constructed by the neighbor-joining method with MEGA ver. 5. The branching patterns of the trees were evaluated by bootstrap analysis using 1,000 replicates. The results showed that H-528-11 formed a separate cluster with a significant bootstrap value within the canine RABV group, which consisted of RABVs that were detected in a wild cat, monkey, civet, and mongoose from the districts of Kalutara and Gampaha (Fig. 1). Compared with the other RABVs, the

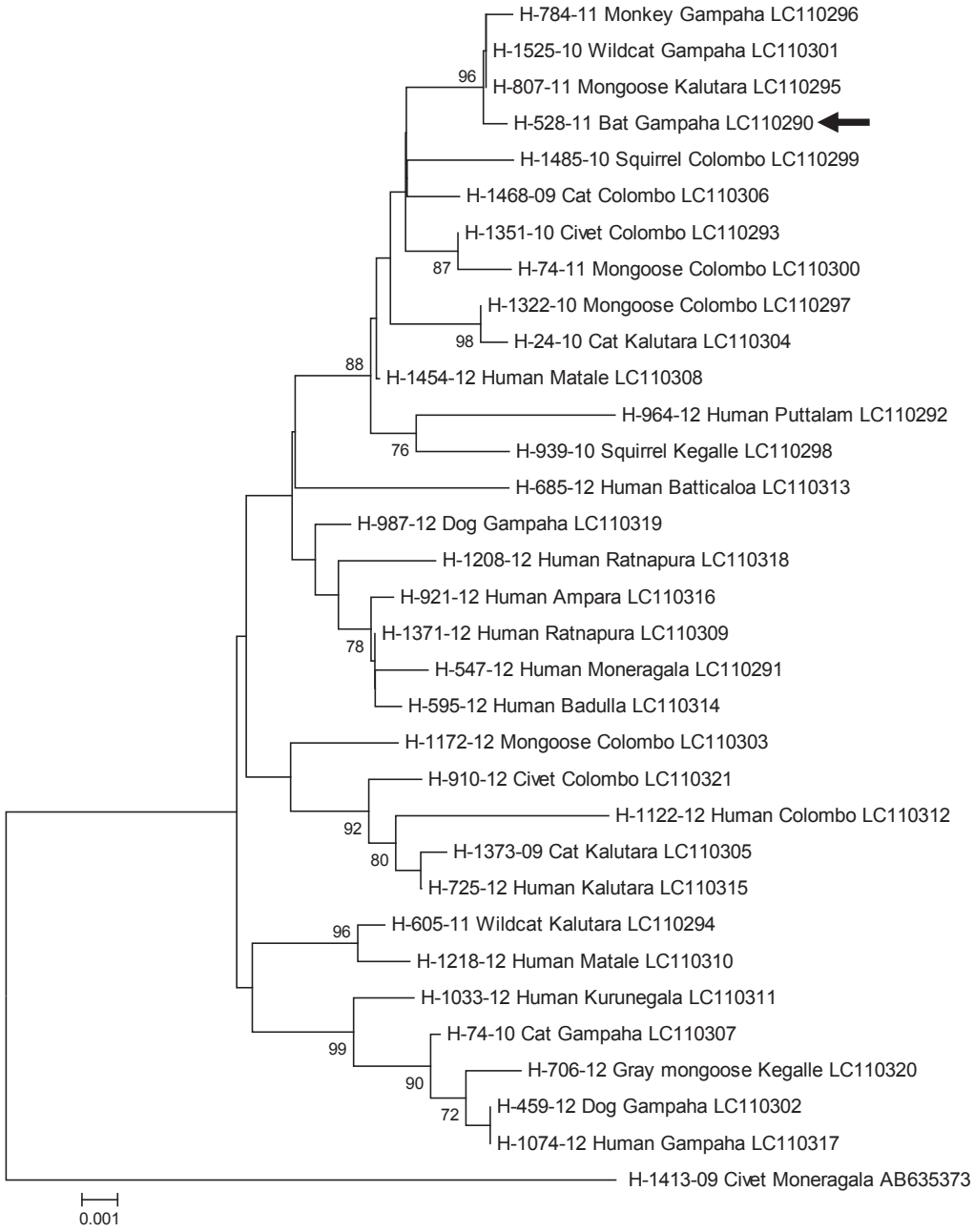

Fig. 1. Phylogenetic tree constructed using the nucleotide sequences of the complete nucleoprotein gene of rabies viruses (RABVs) from wild animals in Sri Lanka. RABV variant from golden palm civet was used as an out-group. According to the phylogenetic analysis, bat RABV H-528-11 belonged to the canine RABV group and formed a cluster with a significant bootstrap value, which included RABVs from a wild cat, monkey, civet, and mongoose from the Gampaha, and Kalutara districts. The numbers adjacent to the nodes represent the bootstrap values, where values < 70% are not included. The scale bar shows the genetic distance as nucleotide substitutions per site. The arrow indicates the bat strain. Strain number is followed by species of origin, district name, and the DNA Data Bank of Japan/European Molecular Biology Laboratory/GenBank accession numbers of the strains described in this study.

members of this cluster had 3 nucleotide substitutions in residues 879 (G→A), 951(T→C), and 1,236 (T→C) of their genomes.

The cytochrome b (*CYTB*) gene of mtDNA was amplified and sequenced from the bat sample (accession number LC121525) (5). BLAST (www.ncbi.nlm.nih.gov/blast) analysis of the partial nucleotide sequence (1,120 nt) of *CYTB* (1,140 nt) showed that it shared 99% identity with that of the Indian flying fox (*Pteropus medius*).

Infections in New World bats by bat variants of RABV have been documented; however, the presence of RABVs in Old World bats is unclear. A case of rabies in a human following a bat bite was reported in India in 1954 (6); however, the presence of RABV was not confirmed in the laboratory. In 1978, an RABV was isolated from the brain of a flying fox in Chandigarh, India (6), and in approximately 1967, from dog-faced fruit bats (*Cynopterus brachyotis*) in Thailand (7). However, genetic analyses were not performed, and therefore, the presence of these bat variants of RABV was not confirmed. There have been no previous reports of bat-to-human transmission of RABV or of bats carrying RABVs, in Sri Lanka. However, recently, a novel lyssavirus named Gannoruwa bat lyssavirus, which is closely related to RABV, has been reported in *P. medius* in Sri Lanka (3). In the present study, we confirmed the presence of an RABV in a juvenile *P. medius* in Sri Lanka.

The sequence identity and phylogenetic analysis indicated that H-528-11 belongs to a separate cluster within the group of Sri Lankan canine RABVs. The strains in this cluster were detected in a group of wild animals in the same location, but not in dogs, and these strains have characteristic nucleotide substitutions compared with other canine RABVs.

The cause of the death of these bats was not known. The juvenile bat exhibited no abnormal behavior that was suggestive of rabies while still alive. The incubation period of rabies in bats is highly variable — less than 14 days to more than 290 days— which may be related to site of infection , the specific RABV variant, and the immunocompetence of the animal (8).

The duration for which the carcass of the juvenile bat remained at ambient temperature before packing with ice and transportation to the MRI is not known. The bat sample had no apparent signs of brain decomposition, and was processed separately in order to avoid cross contamination from other samples. We considered that a small quantity of virus was possibly present in the sample, which may explain why the sample tested negative by FAT, whereas it tested were positive for RABV by RT-PCR. The extent of production of the mRNA of the RABV genes varies according to the following order: N > P > M > G > L (9), which may explain our success in the complete sequencing of the N gene and the partial sequencing of the P gene, while we failed to sequence the entire genome of the RABV present in this sample.

Excluding the abovementioned report of the incidence in India (6) and the present study, there have been no reports of RABV infection in the bats of genus *Pteropus* (10). In conclusion, a RABV strain, which shares similar genetic characteristics with strains that circulate among terrestrial animals, was detected in an Indian flying fox in Sri Lanka. Possibly, this strain was transmitted from a terrestrial animal to the bat. Alternatively, this strain could be maintained among bats after a strain was transmitted from a terrestrial animal to an Indian flying fox. It is necessary to elucidate whether bats in Asia are potential vehicles of RABV transmission to humans.

Conflict of interest None to declare.

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