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Short Communication

Novel adenoviruses and herpesviruses detected in bats

Máté Jánoska [a,1], Márton Vidovszky [a], Viktor Molnár [b], Mátyás Liptovszky [b], Balázs Harrach [a], Mária Benkő [a,*]

[a] Veterinary Medical Research Institute, Hungarian Academy of Sciences, 1581 Budapest, P.O. Box 18, Hungary
[b] Budapest Zoological and Botanical Garden, 1371 Budapest, P.O. Box 469, Hungary

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ABSTRACT

Samples from native Hungarian or captive bats were tested by PCR for the presence of adenoviruses and herpesviruses. Two novel adenoviruses from a common noctule (*Nyctalus noctula*) and a greater horseshoe (*Rhinolophus ferrum-equinum*) bat were detected. In captive Egyptian fruit bats (*Rousettus aegyptiacus*), DNA from two novel herpesviruses was demonstrated. Phylogenetic analysis facilitated provisional taxonomic placement of the newly detected viruses. Such analysis and the existence of unique, shared early proteins (E3 and E4) suggest that canine adenoviruses may have originated in vespertilinoid bats.

Relative to the very many bat species and our increasing awareness of their role as virus reservoirs (Calisher et al., 2006) or as 'donors' of novel adapting viruses, the number of adenovirus (AdV) and herpesvirus (HV) types identified in bats is small. The isolation of the first recognised bat AdV was published recently by Maeda et al. (2008) and over the past 3 years a dozen or so HV types have been identified in bats of different megachiropteran and microchiropteran species (Wibbelt et al., 2007; Molnár et al., 2008; Razafindratsimandresey et al., 2009). However, this probably represents only the 'tip of the iceberg' in terms of the actual number of AdVs and HVs that infect bats. In this study we carried out PCR-based assessment of organ and faecal samples collected from bats and identified two novel AdVs and two novel HVs, respectively.

A total of 57 samples originated from native species of bat, including three common noctules (*Nyctalus noctula*), two serotine bats (*Eptesicus serotinus*) and one parti-coloured bat (*Vespertilio murinus*). The animals had been found moribund or dead in the vicinity of Budapest during the previous 3 years and were submitted to Budapest Zoo which provides a nature conservation service for such threatened species. The moribund animals were euthanased by a veterinarian with expertise in handling bats and were processed *lege artis*. In addition, 43 Egyptian fruit bats (*Rousettus aegyptiacus*) and five Lyle's flying foxes (*Pteropus lylei*) captive-bred within the zoo, were examined. These bats had died from a range of different causes, but a viral aetiology was not suspected and pathognomonic lesions had not been evident on post-mortem examination. DNA extraction was carried out on an organ pool of liver, lungs and small intestines. Three faecal samples from greater horseshoe bats (*Rhinolophus ferrum-equinum*) randomly collected at the Aggtelek National Park in North-eastern Hungary in November 2009 were also examined.

Nested PCR was used in the viral detection process with degenerate, consensus primers targeting the DNA-dependent DNA polymerase gene of AdVs (Wellehan et al., 2004) or HVs (VanDevanter et al., 1996). The PCR products of 318 and 321 bp from the AdVs and of 219 and 228 bp from the HVs, respectively, were purified and sequenced directly using the corresponding inner PCR primers.

Of the 57 samples, three were found to contain adenoviral DNA. In two of the faecal samples from the greater horseshoe bats, identical sequences were demonstrated implying the presence of a hitherto unknown AdV (GenBank Accession number GU289918). A further novel AdV sequence was detected in the pooled organs of a common noctule (Accession number GU198877). Phylogenetic tree reconstruction indicated that these novel viruses were mastadenoviruses (Fig. 1).

Two novel HV sequences were detected in two samples from Egyptian fruit bats (Accession numbers. FJ797654 and FJ597655). On phylogenetic analysis, these HVs were found to belong to the subfamilies *Betaherpesvirinae* and *Gammaherpesvirinae*, respectively (Fig. 2).

The first AdV (designated FBV1) identified in a bat was isolated during attempts to establish a specific cell line from the megachiropteran Ryukyu flying fox (*Pteropus dasymallus yaeyamae*) (Maeda et al., 2008). Isolation of the first AdV (designated PPV1) from a microchiropteran bat, the common pipistrelle (*Pipistrellus pipistrellus*) was reported in Germany (Sonntag et al., 2009). More recently, the isolation and genomic analysis of an additional microchiropteran AdV (BtAdV-TJM) originating from a Rickett's big-footed bat

* Corresponding author. Tel.: +36 1 4674081; fax: +36 1 4674076.
E-mail address: benko@vmri.hu (M. Benkő).
[1] This article is dedicated to the memory of the late Máté Jánoska.

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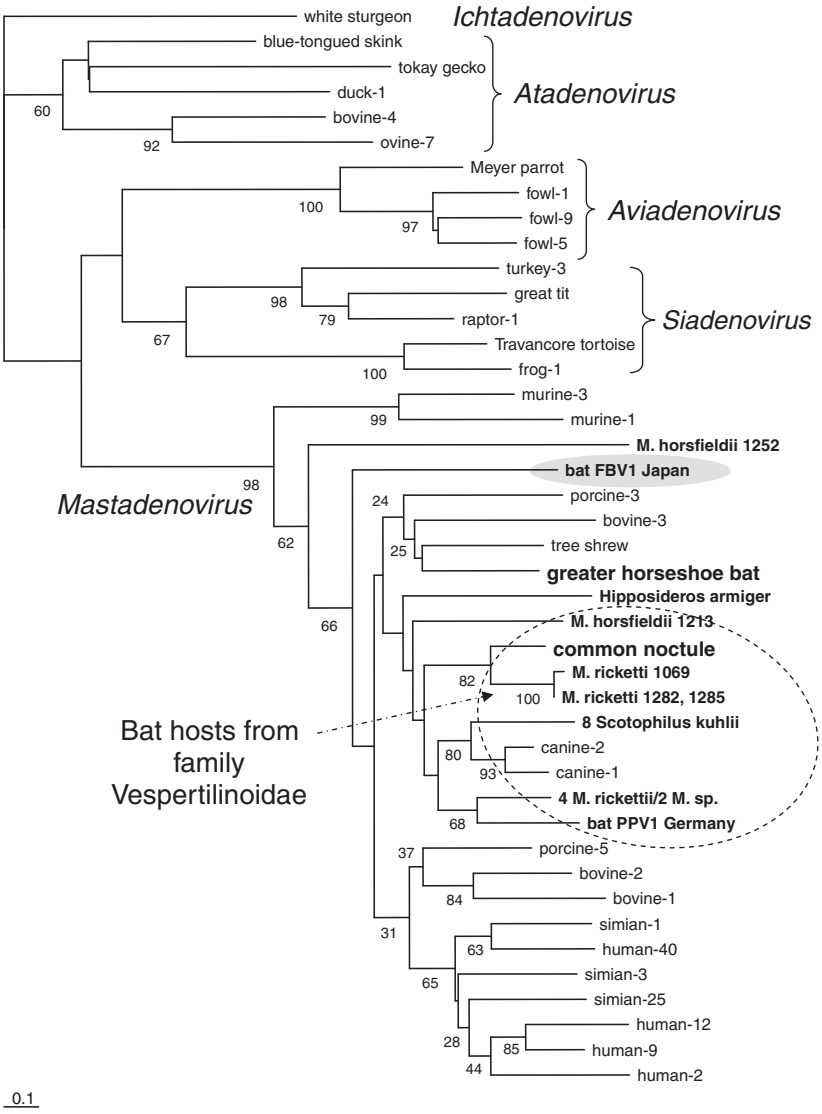

Fig. 1. Phylogenetic tree reconstruction of adenoviruses (AdVs) based on distance matrix analysis of a 91 amino acid-long alignment from partial sequences of the viral DNA-dependent DNA polymerase (ProtDist categories model, Fitch global rearrangements). AdVs associated with bats are printed in bold. Unrooted tree: the white sturgeon AdV was selected as an 'outgroup'. Bootstrap values are shown at branch nodes when they confirm the calculated topology. Latin names designate AdVs detected in bats in China. The branch marked '4 M. rickettii/2 M. Sp.' includes the sequence from the BtAdV-TJM strain (Li et al., 2010). The virus demonstrated from a megachiropteran fruit bat (*Pteropus dasymallus yaeyamae*) is highlighted by a grey background. The virus cluster that contains the canine AdVs and the AdVs from bats of the family Vespertilinoidae, is circled with a discontinuous line. *M. ricketti*, *Myotis ricketti*; bat FBV1, fruit bat AdV (Maeda et al., 2008); bat PPV1, AdV from *Pipistrellus pipistrellus* (Sonntag et al., 2009). Numbers in front of the Latin name of the host indicate the number times identical AdV sequences were detected.

(*Myotis ricketti*) has been reported in China (Li et al., 2010). These authors, using the same PCR (Wellehan et al., 2004) found 19 positives in >300 samples from bats. These positive samples represented six putative novel AdVs (Fig. 1) and were from Rickett's big-footed bats (*Myotis ricketti*), lesser Asiatic yellow house bats (*Scotophilus kuhlii*), Horsfield's bats (*Myotis horsfieldii*), and a great roundleaf bat (*Hipposideros armiger*). It is noteworthy that 102 faecal samples from *Rhinolophus* species in China were found to be negative, whereas two out of three similar samples from bats of a different species of the Rhinolophus genus found in Hungary were positive.

It is possible to make direct comparisons between the putative AdVs found in the bats in Hungary with those detected in Japan, Germany, and China (Fig. 1). In general, the divergence among the different bat AdVs was very large, considerably exceeding that observed among the AdVs of primates. The order Chiroptera is divided into suborders Megachiroptera and Microchiroptera. The Ryukyu flying fox is currently the only megachiropteran bat known to be infected with an AdV (strain FBV1), and the AdVs detected in microchiropterans are quite distinct from the strain FBV1. With one exception, the AdVs found in microchiropteran bats from the family Vespertilinoidae form a monophyletic clade (Fig. 1). This lineage is close to branches of the AdVs found in the two bats of the Rhinolophidae family, *Hipposideros armiger* and *Rhinolophus ferrum-equinum*.

Phylogenetic tree topology suggests there has been a long co-evolution of most AdVs found in bats with their hosts. A striking exception to this is the AdV detected in a sample (No. 1252) of Horsfield's bats (Li et al., 2010), which occupies a branch distant from the cluster of AdVs found in the vespertilinoid bats. The fact that the latter clade also includes the two canine AdV types (CAdV-1 and 2), leads us to speculate on the origin of CAdVs that have an unusually broad host range. The early regions of CAdVs show striking similarity with those of BtAdV-TJM (Li et al., 2010), including