

LETTER TO THE EDITOR

Novel European lineages of bat astroviruses identified in Hungary

G. KEMENESI^{1,2}, B. DALLOS^{1,2}, T. GÖRFÖL^{3,6}, S. BOLDOGH⁴, P. ESTÓK⁵, K. KURUCZ², M. OLDAL^{1,2},
V. NÉMETH^{1,2}, M. MADAI^{1,2}, K. BÁNYAI⁶, F. JAKAB^{1,2*}

¹Virological Research Group, János Szentágotthai Research Center, University of Pécs, Ifjúság útja 20., H-7624, Pécs, Hungary;

²Institute of Biology, Faculty of Sciences, University of Pécs, Pécs, Hungary; ³Department of Zoology, Hungarian Natural History Museum, Budapest, Hungary; ⁴Aggtelek National Park Directorate, Jósavató, Hungary; ⁵Department of Zoology, Eszterházy Károly College, Eger, Hungary; ⁶Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary

Received October 3, 2013; accepted February 24, 2014

Keywords: bat, astroviruses, coronaviruses, snRT-PCR, Hungary

Bats (the order *Chiroptera*) as a distinct group of mammals are remarkable for their high diversity and broad geographic distribution, representing over 20% of all living species of mammals, and are known from all continents except Antarctica (1). The decreasing area of natural habitats, the forest clearances, and the growing urbanization jointly cause bats to move closer to human-inhabited territories. This phenomenon increases the number of direct contacts between bats and domestic animals as well as humans, and given that bats have been recognized as important reservoir hosts for many viruses that can cross species barriers (2), it raises serious veterinary and public health issues. The presence of numerous virus groups in *Microchiroptera* bats has been evidenced all over the world; data have been published to report both DNA viruses (i.e. herpesviruses and adenoviruses) and RNA viruses (i.e. lyssaviruses, coronaviruses, astroviruses and orthoreoviruses) in these animals. Many of the bat origin RNA viruses are readily transmissible to humans, therefore collecting data about their occurrence, frequency and diversity in any location seems to be of interest. In the

present pilot study, we conducted a viral screening from bat fecal samples to determine the presence of different bat-borne viruses in Hungary.

Samples were collected from netted bats at swarming caves at two different locations (Bakony and Mecsek mountains) in Hungary in 2012. All bat species captured for sampling were healthy and identified for species by a chiropterologist. Bats were placed in paper bags individually and were left hanging for a maximum of 30 min, in order to let them defecate, than fecal samples were collected from the bottom of the bags. After sample collection, bats were released at the netting site. Stool samples were homogenized in 500 µl of PBS, then viral RNA was extracted from 200 µl of supernatants using DiaExtract Viral NA Isolation Kit (DIAGON Ltd., Hungary) following the manufacturer's recommendations. Samples were tested for the presence of viral RNA of coronaviruses, orthoreoviruses and astroviruses, using RT-PCR (RT-PCR) or RT-seminested PCR (RT-snPCR) assays. Amplification was performed with OneStep RT-PCR Kit and Dia Taq Kit (DIAGON Ltd., Hungary), respectively (3–5). PCR products were analyzed by electrophoresis in 2% agarose gel in TBE buffer stained with GelGreen. Amplified DNA products were purified by the QIAquick Gel Extraction Kit (Qiagen) and sequenced using BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems). The dye-labeled products were analyzed on an ABI Prism 310 DNA Sequencer (Applied

*Corresponding author. E-mail: jakabf@gamma.ttk.pte.hu; phone: +36-72-503-600 (ext.: 29044).

Abbreviations: AstV = astrovirus; BatAstV = bat-associated astrovirus; RT-snPCR = RT-seminested PCR

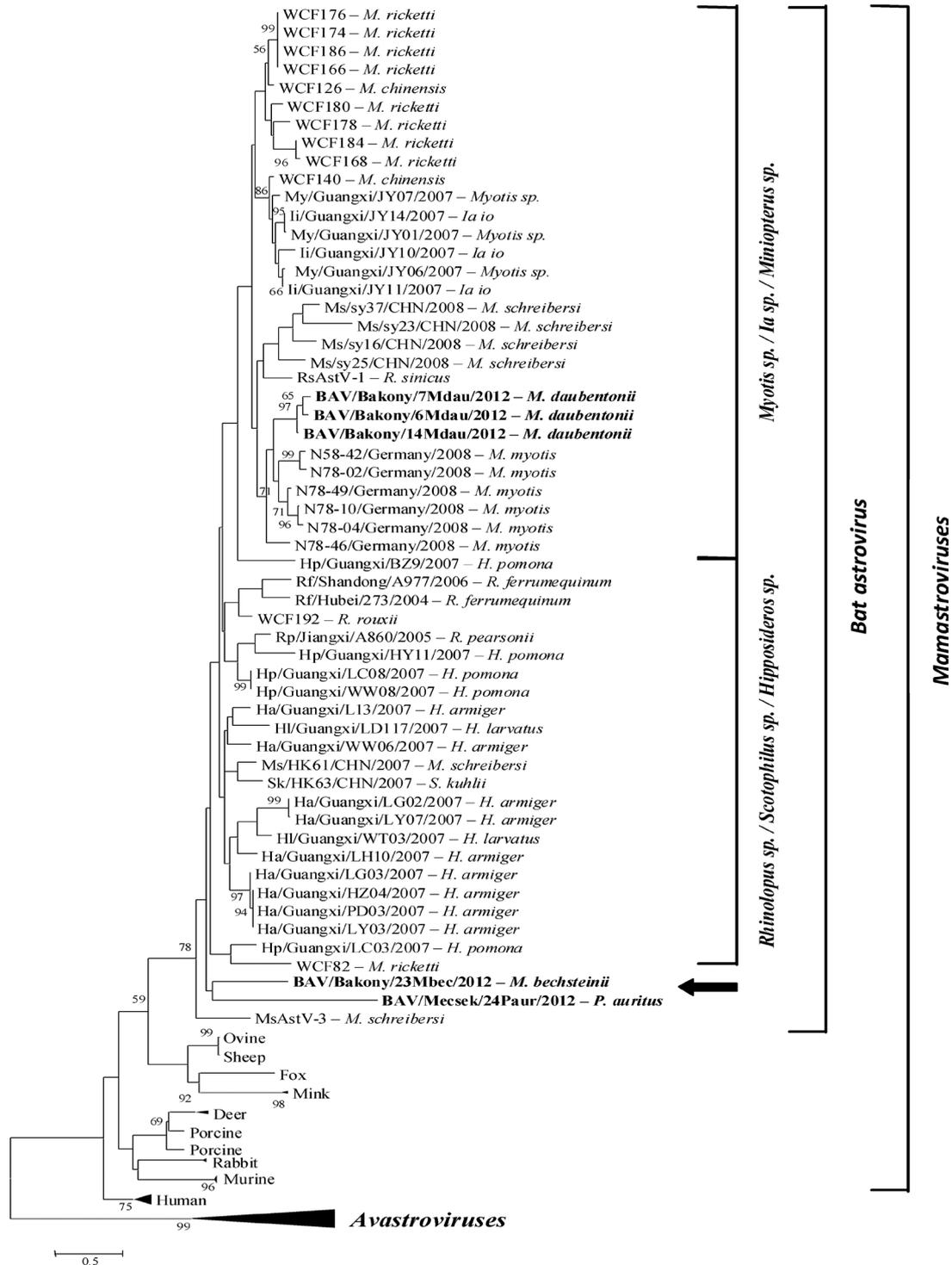


Fig. 1

Phylogenetic tree of BatAstV strains labeled with the corresponding host species

The tree was constructed based on a 265 nt-long consensus region of the RdRp gene with MEGA v5.0 software using the Maximum-Likelihood method, based on the General Time Reversible model. Number of bootstrap replications was 1000. Hungarian BatAstV strains identified in this study are marked in bold face. The black arrow indicates the new Hungarian BatAstV lineages. Abbreviations: *M. ricketti* (*Myotis ricketti*), *M. chinensis* (*Myotis chinensis*), *M. schreibersi* (*Miniopterus schreibersi*), *R. sinicus* (*Rhinolophus sinicus*), *M. daubentonii* (*Myotis daubentonii*), *M. myotis* (*Myotis myotis*), *H. pomona* (*Hipposideros pomona*), *R. ferrumequinum* (*Rhinolophus ferrumequinum*), *R. rouxii* (*Rhinolophus rouxii*), *R. pearsonii* (*Rhinolophus pearsonii*), *H. armiger* (*Hipposideros armiger*), *H. larvatus* (*Hipposideros larvatus*), *S. kuhlii* (*Scotophilus kuhlii*), *M. bechsteinii* (*Myotis bechsteinii*), *P. auritus* (*Plecotus auritus*).

Biosystems). Basic sequence manipulation and verification was performed using GeneDoc 2.7 software. Nucleic acid sequences of the new bat AstV isolates were compared to reference strains deposited in GenBank and identification was established based upon the greatest identity. Nucleotide sequences were aligned by ClustalX v2.0 software, then phylogenetic tree was constructed from the nucleic acid sequence alignments using Maximum-Likelihood method based on the General Time Reversible model of the program MEGA v5.0 software. The number of bootstrap replications was 1000.

Fecal samples were collected from 60 bats, represented nine different species (22 *Myotis bechsteinii*, 11 *Plecotus auritus*, 7 *Myotis daubentonii*, 6 *Myotis myotis*, 4 *Myotis nattereri*, 4 *Myotis dasycheme*, 3 *Eptesicus serotinus*, 2 *Myotis blythii*, and 1 *Pipistrellus pipistrellus*). Among the viruses we screened for, only bat-associated astroviruses (BatAstV) were detected. BatAstVs were found in 5 of 60 (8.3%) bats by RT-snPCR. The detection rate of BatAstV was the greatest in *Myotis daubentonii* (42.8%), followed by *Plecotus auritus* (9.1%) and *Myotis bechsteinii* (4.5%). No BatAstVs were detected in other host species. Phylogenetic analysis (Figure 1) showed that the Hungarian BatAstV isolates clustered together with BatAstVs from China and Europe and were unambiguously separated from other mammalian AstVs. In correspondence with other related studies, we also observed a notable genetic variability within BatAstV isolates. With the exception of a few sequences, viruses from the genus of *Myotis*, *Ia* and *Miniopterus* created a separate group, while AstVs identified in *Hipposideros*, *Rhinolophus* and *Scotophilus* genus created a distinct phylogenetic branch. Although viruses BAV/Bakony/6Mdau/2012, BAV/Bakony/7Mdau/2012 and BAV/Bakony/14Mdau/2012, which were identified from *Myotis daubentonii* species, created a separate monophyletic group, they were most closely related to the strains detected in Germany. Furthermore, BAV/Bakony/23Mbec/2012 and BAV/Mecsek/24Paur/2012 isolates derived from *Myotis bechsteinii* and *Plecotus auritus*, respectively, created two distinct lineages. The amplified RNA polymerase gene is the most conservative region of the AstV genome, yet the short fragments we analyzed indicated that multiple lineages of BatAstVs may be co-circulating in Hungarian bat populations. To our best knowledge, only the species *Myotis myotis* was tested in Europe so far and was found to be positive for BatAstV in Germany (6). However, it is of note that the number of research papers describing BatAstVs is limited to a few reports from China (5, 7, 8, 9), Germany and the United States (6, 10).

Mammalian orthoreoviruses had been detected in bats in Italy; these viruses have a wide geographic distribution, and are capable of infecting humans as well (11). Coronaviruses are predominantly associated with *Microchiroptera*, which are the most ecologically diverse suborder. Due to their wide

geographic range coronaviruses might be more prevalent than other bat transmitted viruses (12). Coronaviruses were identified in bats in some European countries such as Germany, United Kingdom, and SARS-like coronaviruses in Slovenia and Bulgaria (12–15). In this study, no evidence for circulation of other viruses was obtained; however, because data from Europe indicate that both orthoreoviruses and coronaviruses are present in bats, further efforts are needed to identify the carrier role of Hungarian bats for these other zoonotic RNA viruses of bat origin.

In the present study, intriguing new findings were obtained about BatAstVs. We clearly demonstrated that a wider range of European bat species carry AstVs. Also our data imply a remarkable genetic variability of BatAstV in Hungary. These findings suggest that an extension of the present pilot study is needed to gain a more penetrating insight into the ecology, epidemiology, evolution and taxonomy of the newly described BatAstVs.

Acknowledgement. Research activity of Ferenc Jakab was supported by the TÁMOP 4.2.4. A/2-11-1-2012 0001 – National Excellence Program Elaborating and operating an inland student and researcher personal support system. The project was subsidized by the European Union and co-financed by the European Social Fund. Krisztián Bányai was supported by the “Momentum program”.

References

- Gunnell GF, Simmons NB, *J. Mammalian Evol.* 12, 201. Gunnell GF, Simmons NB, *J. Mammalian Evol.* 12, 209–246, 2005. <http://dx.doi.org/10.1007/s10914-005-6945-2>
- Calisher CH, Childs JE, Field HE, Holmes KV, Schountz T, *Clin. Microbiol. Rev.* 19, 531–545, 2006. <http://dx.doi.org/10.1128/CMR.00017-06>
- Stephensen CB, Casebolt DB, Gangopadhyay NN, *Virus Res.* 60, 181–189, 1999. [http://dx.doi.org/10.1016/S0168-1702\(99\)00017-9](http://dx.doi.org/10.1016/S0168-1702(99)00017-9)
- Leary PL, Erker JC, Chalmers ML, Cruz AT, Wetzel JD, Desai SM, Mushahwar IK, Dermody TS, *Clin. Microbiol.* 40, 1368–1375, 2002. <http://dx.doi.org/10.1128/JCM.40.4.1368-1375.2002>
- Chu DK, Poon LL, Guan Y, Peiris JS, *J. Virol.* 82, 9107–9114, 2008. <http://dx.doi.org/10.1128/JVI.00857-08>
- Drexler JF, Corman VM, Wegner T, Tateno AF, Zerbinati RM, Gloza-Rausch F, Seebens A, Müller MA, Drosten C, *Emerg. Infect. Dis.* 17, 449–456, 2011. <http://dx.doi.org/10.3201/eid1703.100526>
- Wu Z, Ren X, Yang L, Hu Y, Yang J, He G, Zhang J, Dong J, Sun L, Du J, Liu L, Xue Y, Wang J, Yang F, Zhang S, Jin Q, *J. Virol.* 86, 10999–11012, 2012. <http://dx.doi.org/10.1128/JVI.01394-12>
- Xiao J, Li J, Hu G, Chen Z, Wu Y, Chen Y, Chen Z, Liao Y, Zhou J, Ke X, Ma L, Liu S, Zhou J, Dai Y, Chen H, Yu S, Chen Q, *Arch. Virol.* 156, 1415–1423, 2011. <http://dx.doi.org/10.1007/s00705-011-1011-2>

9. Zhu HC, Chu DK, Liu W, Dong BQ, Zhang SY, Zhang JX, Li LF, Vijaykrishna D, Smith GJ, Chen HL, Poon LL, Peiris JS, Guan Y, J. *Gen. Virol.* 90, 883–887, 2009. <http://dx.doi.org/10.1099/vir.0.007732-0>
10. Li L, Victoria JG, Wang C, Jones M, Fellers GM, Kunz TH, Delwart, J. *Virol.* 84, 6955–6965, 2010. <http://dx.doi.org/10.1128/JVI.00501-10>
11. Lelli D, Moreno A, Lavazza A, Bresaola M, Canelli E, Boniotti MB, Cordioli, *Zoonoses and Public Health* 60, 84–92, 2013. <http://dx.doi.org/10.1111/zph.12001>
12. Gloza-Rausch F, Ipsen A, Seebens A, Göttsche M, Panning M, Drexler JF, Petersen N, Annan A, Grywna K, Müller M, Pfefferle S, Drosten C, *Emerg. Infect. Dis.* 14, 626–631, 2008. <http://dx.doi.org/10.3201/eid1404.071439>
13. August TA, Mathews F, Nunn MA, *Vector-Borne and Zoonotic Diseases.* 12, 530–533, 2012. <http://dx.doi.org/10.1089/vbz.2011.0829>
14. Rihtarič D, Hostnik P, Steyer Jože Grom A, Toplak I, *Arch. Virol.* 155, 507–514, 2010 <http://dx.doi.org/10.1007/s00705-010-0612-5>
15. Drexler JF, Gloza-Rausch F, Glende J, Corman VM, Muth D, Goettsche M, Seebens A, Niedrig M, Pfefferle S, Yordanov S, Zhelyazkov L, Hermanns U, Vallo P, Lukashev A, Müller MA, Deng H, Herrler G, Drosten C, *J. Virol.* 84, 11336–11349, 2010. <http://dx.doi.org/10.1128/JVI.00650-10>