



HAL
open science

Bat Astrovirus in Mozambique

Flora Hoarau, Gildas Le Minter, Léa Joffrin, M. Corrie Schoeman, Erwan Lagadec, Beza Ramasindrazana, Andréa dos Santos, Steven Goodman, Eduardo S Gudo, Patrick Mavingui, et al.

► **To cite this version:**

Flora Hoarau, Gildas Le Minter, Léa Joffrin, M. Corrie Schoeman, Erwan Lagadec, et al.. Bat Astrovirus in Mozambique. *Virology Journal*, 2018, 15 (1), pp.104. 10.1186/s12985-018-1011-x . pasteur-01909356

HAL Id: pasteur-01909356

<https://riip.hal.science/pasteur-01909356v1>

Submitted on 31 Oct 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.




Distributed under a Creative Commons Attribution 4.0 International License

SHORT REPORT

Open Access



Bat Astrovirus in Mozambique

Flora Hoarau¹, Gildas Le Minter¹, Léa Joffrin¹, M. Corrie Schoeman², Erwan Lagadec¹, Beza Ramasindrazana^{1,3,7}, Andréa Dos Santos⁴, Steven M. Goodman^{3,5}, Eduardo S. Gudo⁶, Patrick Mavingui¹ and Camille Lebarbenchon^{1*} 

Abstract

Astroviruses (AstVs) are responsible for infection of a large diversity of mammalian and avian species, including bats, aquatic birds, livestock and humans. We investigated AstVs circulation in bats in Mozambique and Mayotte, a small island in the Comoros Archipelago located between east Africa and Madagascar. Biological material was collected from 338 bats and tested for the presence of the AstV RNA-dependent RNA-polymerase gene with a pan-AstV semi-nested polymerase chain reaction assay. None of the 79 samples obtained from Mayotte bats (*Pteropus seychellensis comorensis* and *Chaerephon pusillus*) tested positive; however, 20.1% of bats sampled in Mozambique shed AstVs at the time of sampling and significant interspecific variation in the proportion of positive bats was detected. Many AstVs sequences obtained from a given bat species clustered in different phylogenetic lineages, while others seem to reflect some level of host-virus association, but also with AstVs previously reported from Malagasy bats. Our findings support active circulation of a large diversity of AstVs in bats in the western Indian Ocean islands, including the southeastern African coast, and highlight the need for more detailed assessment of its risk of zoonotic transmission to human populations.

Keywords: Mammastrovirus, Mayotte, Mozambique, Madagascar, *Triaenops afer*

Astroviruses (AstVs) are small non-enveloped RNA viruses, transmitted via the fecal-oral route. They have been detected from over 80 vertebrate host species [1], and represent a significant source of morbidity and economic losses. Worldwide, AstVs account for 2 to 9% of all acute non-bacterial gastroenteritis in children [2]; they are also responsible for diseases in livestock, poultry and domestic pets [3]. In wild animals, AstVs have been mostly detected in bats [4] and in aquatic birds [5], although detection in other host types have been reported, such as in marine mammals [6] and non-human primates [7].

Current knowledge on the epidemiology of AstVs in African bats is limited [8, 9]. In a previous study, we detected high genetic diversity of AstVs in Malagasy bats [10]. Detection of AstVs on other islands of the western Indian Ocean has not been reported. The goal of this study was to investigate AstV circulation in bats in Mozambique and on Mayotte, a small island in the Comoros archipelago located between east Africa and Madagascar.

Biological material was collected on Mayotte at several locations (Bandrele, Chiconi, Coconi, Kwale, Mangajou, Passamainty, Sohoabe, Tsoundzou), in November–December 2014, and in Mozambique (Inhassoro district) in February and May 2015. Bats were captured using mist nets and harp traps. On Mayotte, rectal swabs were obtained with sterile rayon-tipped applicators (Puritan, Guilford, ME, USA) from 21 *Pteropus seychellensis comorensis*, and droppings were collected from 58 *Chaerephon pusillus*. Swabs and droppings were placed in 1.5 mL of Virus Transport Media (VTM; [10]), and were immediately frozen in liquid nitrogen. In Mozambique, one rectal and one buccal swab were collected for each sampled bat. The two swabs were then placed in the same tube, containing 1.5 mL of VTM, and were immediately frozen in liquid nitrogen. Sampled bat species and number of tested samples are presented in Table 1.

RNA extraction was performed with the QIAamp Viral RNA Mini Kit (QIAGEN, Valencia, CA, USA). Reverse transcription was performed on 10 µL of RNA using the ProtoScript II Reverse Transcriptase and Random Primer 6 (New England BioLabs, Ipswich, MA, USA) using a previously published protocol [10]. cDNAs were

* Correspondence: camille.lebarbenchon@univ-reunion.fr

¹Université de La Réunion, UMR Processus Infectieux en Milieu Insulaire Tropical (PIMIT), INSERM 1187, CNRS 9192, IRD 249, 2 rue Maxime Rivière (GIP CYROI), 97490 Sainte-Clotilde, La Réunion, France

Full list of author information is available at the end of the article



Table 1 Family, species day roosts, and number of bats sampled and tested for the presence of Astroviruses, in Mozambique

Family	Species	Day roosts	N tested	N positive
Hipposideridae	<i>Hipposideros caffer</i>	Caves	57	10
Miniopteridae	<i>Miniopterus mossambicus</i>	Caves	21	2
Molossidae	<i>Mops condylurus</i>	Houses	52	1
Nycteridae	<i>Nycteris thebaica</i>	Caves	14	4
Rhinolophidae	<i>Rhinolophus lobatus</i>	Caves	9	0
	<i>Rhinolophus mossambicus</i>	Caves	20	0
	<i>Rhinolophus rhodesiae</i>	Caves	31	0
Rhinycteridae	<i>Triaenops afer</i>	Caves	51	35
Vespertilionidae	<i>Neoromicia nana</i>	Rolled-up banana leaves	2	0
	<i>Scotophilus viridis</i>	Free-flying	2	0

tested for the presence of the AstV RNA-dependent RNA-polymerase (RdRp) gene using a pan-AstV semi-nested polymerase chain reaction (PCR) assay [10, 11]. PCRs were performed with the GoTaq G2 Hot Start Green Master Mix (Promega, Madison, WI, USA) in an Applied Biosystems 2720 Thermal Cycler (Thermo Fisher Scientific, Waltham, MA, USA). Electrophoresis were performed on 1.5% agarose gels stained with 2% GelRed (Biotium, Hayward, CA, USA). Chi square tests were conducted to investigate the effect of the host species, sampling period (month), and sex, on the probability of successful detection of AstV RdRp genes. Statistical analyses were conducted with R, version 3.2.3 [12].

PCR products of the expected size were submitted for direct Sanger sequencing (Genoscreen, Lille, France). The 31 sequences obtained in this study were aligned with 112 reference AstV RdRp partial nucleotide sequences, with CLC Sequence Viewer version 7.7.1 (CLC Bio, Aarhus, Denmark). A maximum-likelihood analysis was performed. Phylogenetic trees were constructed by maximum likelihood with the software PhyML 3.1 [13]. The evolutionary model was selected by Model Generator 0.85 (GTR + I + Γ , $I = 0.10$, $\alpha = 0.71$; [14]), and nodal supports were assessed with 1000 bootstrap replicates. A Bayesian Markov Chain Monte Carlo coalescent analysis was also performed, with the program BEAST, version 1.8.4 [15], and the Shapiro-Rambaut-Drummond-2006 (SRD06) nucleotide substitution model [16]. A strict molecular clock and a constant population size were selected. The analysis was performed with a chain length of 60 million generations sampled every 1000 iterations, with first 10% trees discarded as burn-in. The maximum clade credibility tree was visualized with FigTree, version 1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree>).

None of the 79 samples collected on Mayotte tested positive for the presence of AstV. Although this negative result may be affected by the relatively small sample size and differences in sampling protocols (swabs vs

droppings), it may also suggest temporal variation in AstVs shedding and circulation in bat populations, as previously documented [17]. Additional studies are thus needed before concluding that AstVs do not circulate in Mayotte bats.

In Mozambique, 52 of the 259 bats tested positive for the presence of AstV RdRp (mean detection rate \pm 95% confidence interval: 20.1% \pm 4.9%). This detection rate was similar to other studies using the same PCR assay, including the one we reported on Malagasy bats (22.5% \pm 6.1%; [10]). For the Mozambique samples, five of the ten bat species tested positive (Table 1 and Additional file 1 for details), with significant variation between species ($\chi^2 = 104$, $P < 0.001$). A high detection rate was found in *Triaenops afer* (68.6% \pm 12.7%), as compared to other species (Table 1). Significant variation was also found between the two sampling sessions ($\chi^2 = 9$, $P < 0.005$) with a higher detection rate in May (25.3% \pm 6.5%) than in February (10.1% \pm 6.3%), in particular for *T. afer* ($\chi^2 = 13$, $P < 0.001$; 20% \pm 24.7% in February, and 80.5% \pm 12.1% in May). This variation may be associated with factors related with bat population dynamics facilitating or limiting virus transmission (e.g. population size, density, age structure, body condition [18, 19]). No significant difference was found in AstV detection rate between males and females ($\chi^2 = 0.4$, $P < 0.5$).

High genetic diversity was detected among AstVs sequences obtained from Mozambican bats (pairwise distance up to 45%), without strong support for host family or species restriction (Fig. 1 and Additional file 2 for details), as commonly described for bat AstVs [8, 10, 11, 20, 21]. Most of the detected viruses clustered in large phylogenetic lineages, in particular for *Triaenops afer* and *Hipposideros caffer*, although statistical support was limited. Sequences of AstVs detected in *Nycteris thebaica* and *Mops condylurus* were mostly highly divergent and not included in larger genetic lineages comprising viruses of same bat family or the same geographic area (Fig. 1).

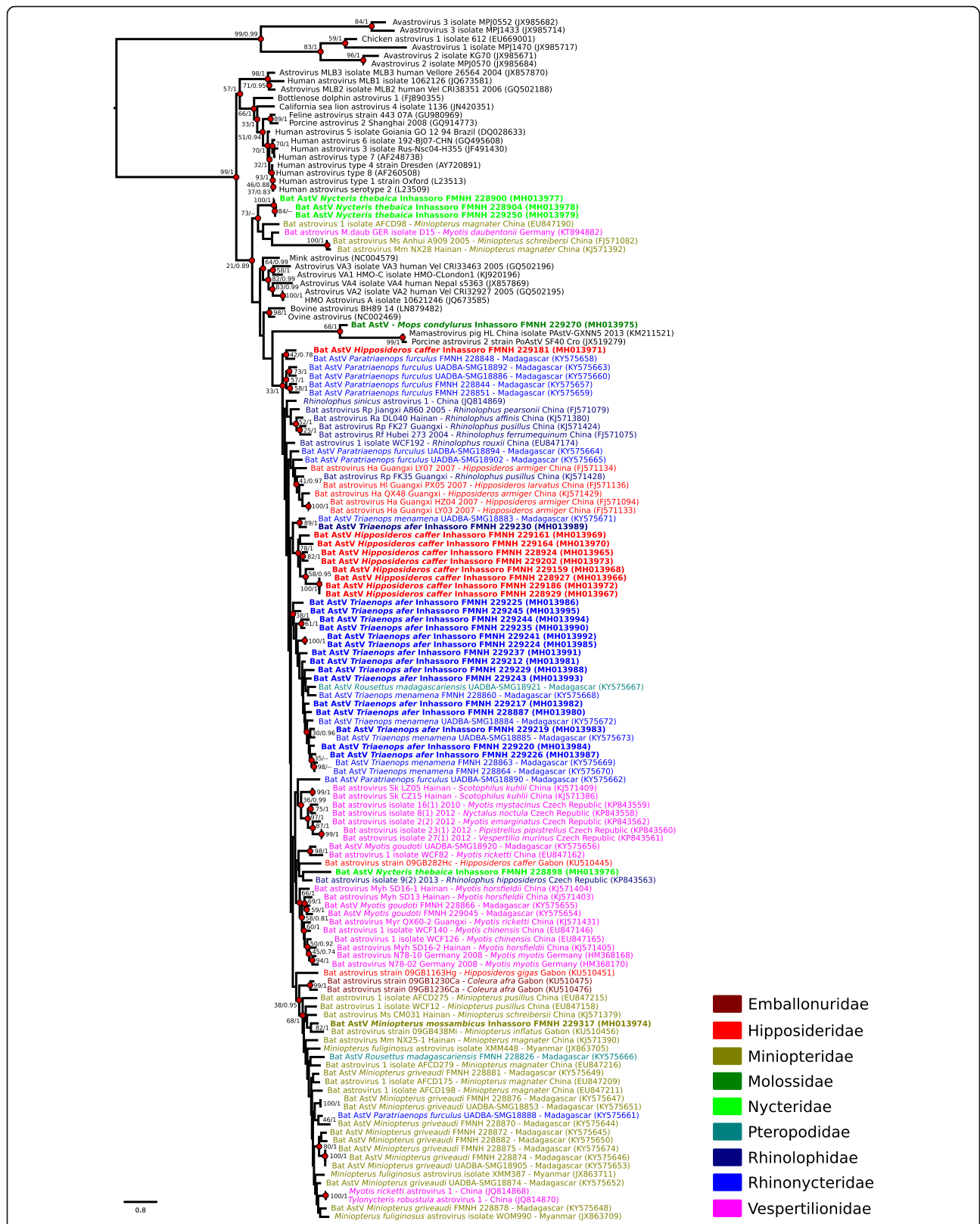


Fig. 1 (See legend on next page.)

(See figure on previous page.)

Fig. 1 Maximum Likelihood (ML) consensus tree derived from 143 Astrovirus (AstV) RNA-dependent RNA-polymerase partial nucleotide sequences (380 bp). Colored circles indicate nodes with bootstrap values > 70 in the ML tree, or posterior probabilities higher than 0.7 in the maximum clade credibility tree. Sequence names in bold indicate bat AstVs detected in this study, and were colored according to the bat family. Scale bar indicates mean number of nucleotide substitutions per site

The limited genetic information available for AstVs in public databases [1], as well as the high saturation of their genome [22], considerably affects the resolution of phylogenetic trees. Current understanding of the long-time evolutionary history of *Astroviridae* therefore remains limited. In addition, ecological factors involved in AstV infection in bats need to be better assessed. High temporal dynamics of viral infection has been documented before [17], and the risk of spillover to other hosts, including humans, has also been demonstrated to coincide with changes in bat behavior and population structure [23]. The high propensity of AstVs for host shifts highlight the need for a better assessment of zoonotic transmission risk to human populations, particularly in relationship to some unique aspects of bat immunology and ecology.

Additional files

Additional file 1: Detailed results of Astrovirus detection in samples from Mozambique. (ODS 30 kb)

Additional file 2: List of the bat families and species included in the phylogenetic tree. (PDF 44 kb)

Abbreviations

AstV: Astrovirus; PCR: Polymerase chain reaction; RdRp: RNA-dependent RNA-polymerase; VTM: Virus Transport Media

Acknowledgements

We thank Koussay Dellagi and Hervé Pascalis for the development and the management of the 'partenariat Mozambique-Réunion dans la recherche en santé: pour une approche intégrée d'étude des maladies infectieuses à risque épidémique (MoZaR)' research program.

Funding

Field research was funded by the 'Partenariat Mozambique-Réunion dans la recherche en santé: pour une approche intégrée d'étude des maladies infectieuses à risque épidémique (MoZaR)' (Fond Européen de Développement Régional, Programme Opérationnel de Coopération Territoriale) and by the 'Centre National de la Recherche Scientifique' (Projets Exploratoires Premier Soutien BATMAN). Molecular analyses were funded by tutorship institutions of the UMR PIMIT. LJ is supported by a 'Région Réunion, European Regional Development Funds (FEDER 2014-2020)' PhD fellowship. BR's post-doctoral fellowship was supported by the 'Run Emerge' European Union's Seventh Framework Program (FP7/2007-2013; Grant agreement NO 263958), the 'Fonds de Coopération Régionale, Prefecture de La Réunion' and The Field Museum of Natural History, Chicago, through the Ralph and Marian Falk Medical Research Trust. CL is supported by a 'Chaire mixte: Université de La Réunion - INSERM'. The funding agencies did not have any involvement in the study design, implementation or publishing of this study, and the research presented herein represents the opinions of the authors but not necessarily the opinions of the funding agencies.

Availability of data and materials

Sequences generated in this study have been deposited in GenBank under the accession numbers MH013965 to MH013995.

Authors' contributions

CL conceived and designed the study. EL, GLM, and BR collected biological material on Mayotte. SMG, GLM, ADS, and MCS collected biological material in Mozambique. FH and LJ performed the molecular analyses. FH and CL analyzed the data and wrote the paper. ESG and PM contributed to the project management between French and Mozambican institutions and to the first draft of the paper. All authors edited, read, and approved the final manuscript.

Ethics approval

All procedures have been evaluated and approved by an ethic committee (Agreement number A974 001; Comité d'éthique du CYROI number 114; Cyclotron Reunion Océan Indien, Sainte Clotilde, Reunion Island), and authorized by the French Ministry of Education and Research (Reference numbers 03584.01 and APAFIS#2638-2015110616208322v1). On Mayotte, research permits were issued by the 'Direction de l'Environnement, de l'Aménagement et du Logement' (Arrêté n°158/DEAL/SEPR/2014). In Mozambique, research permits were issued by the Museum of Natural History (Ref. 01/MHN/E.27/2015) and the Ministry of Health (N°S/N/SDI/0233/15).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details

¹Université de La Réunion, UMR Processus Infectieux en Milieu Insulaire Tropical (PIMIT), INSERM 1187, CNRS 9192, IRD 249, 2 rue Maxime Rivière (GIP CYROI), 97490 Sainte-Clotilde, La Réunion, France. ²School of Life Sciences, Biological Sciences Building, University of Kwa-Zulu Natal, South Ring Road, Westville Campus, Kwa-Zulu Natal 3630, South Africa. ³Association Vahatra, BP 3972, 101 Antananarivo, Madagascar. ⁴Veterinary Faculty, Eduardo Mondlane University, Maputo, Mozambique. ⁵Field Museum of Natural History, 1400 South Lake Shore Drive, Chicago 60605, USA. ⁶Instituto Nacional de Saúde, Maputo, Mozambique. ⁷Present Address: Institut Pasteur de Madagascar, Ambatofotsikely, 101 Antananarivo, Madagascar.

Received: 8 March 2018 Accepted: 3 June 2018

Published online: 20 June 2018

References

- Mendenhall IH, Smith GJD, Dhanasekaran V. Ecological drivers of virus evolution: Astrovirus as a case study. *J Virol*. 2015;89:6978–81.
- Bosch A, Pinto RM, Guix S. Human Astroviruses. *Clin Microbiol Rev*. 2014;27:1048–74.
- De Benedictis P, Schultz-Cherry S, Burnham A, Cattoli G. Astrovirus infections in humans and animals - molecular biology, genetic diversity, and interspecies transmissions. *Infect. Genet. Evol*. 2011;11:1529–44.
- Fischer K, dos Reis VP, Balkema-buschmann A. Bat Astroviruses: towards understanding the transmission dynamics of a neglected virus family. *Viruses*. 2017;9:34.
- Chu DKW, Leung CYH, Perera HKK, Ng EM, Gilbert M, Joyner PH, et al. A novel group of avian astroviruses in wild aquatic birds. *J Virol*. 2012;86:13772–8.
- Rivera R, Nollens HH, Venn-Watson S, Gulland FMD, Wellehan JFX. Characterization of phylogenetically diverse astroviruses of marine mammals. *J Gen Virol*. 2010;91:166–73.

7. Karlsson EA, Small CT, Freiden P, Feeroz MM, Matsen FA, San S, et al. Non-human primates harbor diverse mammalian and avian Astroviruses including those associated with human infections. *PLoS Pathog.* 2015;11: e1005225.
8. Rougeron V, Suquet E, Maganda GD, Jiolle D, Mombo IM, Bourgarel M, et al. Characterization and phylogenetic analysis of new bat astroviruses detected in Gabon, Central Africa. *Acta Virol.* 2016;60:386–92.
9. Waruhiu C, Ommeh S, Obanda V, Agwanda B, Gakuya F, Ge XY, et al. Molecular detection of viruses in Kenyan bats and discovery of novel astroviruses, caliciviruses and rotaviruses. *Virol Sin.* 2017;32:101–14.
10. Lebarbenchon C, Ramasindrazana B, Joffrin L, Bos S, Lagadec E, Le MG, et al. Astroviruses in bats, Madagascar. *Emerg Microbes Infect.* 2017;6:e58.
11. Chu DKW, Poon LLM, Guan Y, Peiris JSM. Novel astroviruses in insectivorous bats. *J Virol.* 2008;82:9107–14.
12. R Core Team R. A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2015.
13. Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst Biol.* 2010;59:307–21.
14. Keane TM, Creevey CJ, Pentony MM, Naughton TJ, McInerney JO. Assessment of methods for amino acid matrix selection and their use on empirical data shows that ad hoc assumptions for choice of matrix are not justified. *BMC Evol Biol.* 2006;6:29.
15. Drummond AJ, Rambaut A. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol Biol.* 2007;7:214.
16. Shapiro B, Rambaut A, Drummond AJ. Choosing appropriate substitution models for the phylogenetic analysis of protein-coding sequences. *Mol. Biol. Evol.* 2006;23:7–9.
17. Drexler JF, Corman VM, Wegner T, Tateno AF, Zerbinati RM, Gloza-Rausch F, et al. Amplification of emerging viruses in a bat colony. *Emerg Infect Dis.* 2011;17:449–56.
18. Seltmann A, Corman V, Rasche A, Drosten C, Czihak G, Bernard H, et al. Seasonal fluctuations of Astrovirus, but not coronavirus shedding in bats inhabiting human-modified tropical forests. *EcoHealth.* 2017;14:272–84.
19. Mendenhall IH, Skiles MM, Neves ES, Borthwick SA, Low DHW, Liang B, et al. Influence of age and body condition on astrovirus infection of bats in Singapore: an evolutionary and epidemiological analysis. *One Heal.* 2017;4: 27–33.
20. Kemenesi G, Dallos B, Gorfal T, Boldogh S, Estok P, Kurucz K, et al. Molecular survey of RNA viruses in Hungarian bats: discovering novel astroviruses, coronaviruses, and caliciviruses. *Vector Borne Zoonotic Dis.* 2014;14:846–55.
21. Lacroix A, Duong V, Hul V, San S, Davun H, Omaliss K, et al. Diversity of bat astroviruses in Lao PDR and Cambodia. *Infect. Genet. Evol.* 2017;47:41–50.
22. Lukashov VV, Goudsmit J. Evolutionary relationships among Astroviridae. *J. Gen. Virol.* 2002;83:1397–405.
23. Plowright RK, Foley P, Field HE, Dobson AP, Foley JE, Eby P, et al. Urban habituation, ecological connectivity and epidemic dampening: the emergence of Hendra virus from flying foxes (*Pteropus* spp.). *Proc Biol Sci.* 2011;278:3703–12.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

