VETERINARIA ITALIANA
RIVISTA DI SANITÀ PUBBLICA VETERINARIA

Paper

Activity of BatIFIT5 in different species of healthy and naturally infected with rabies virus bats

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Veterinaria Italiana, Vol. 60 No. 2 (2024) DOI: 10.12834/VetIt.3427.23374.1
Available on line: 31.07.2024

Abstract

Bats are mammals with vital role played in numerous ecosystem services, however bats can be important reservoirs or hosts for several microorganisms. Rabies is a zoonosis caused by *Rabies lyssavirus* (RABV) that affects the central nervous system (CNS) of all mammals, including bats and humans. The action of IFN-stimulated genes (ISGs) could be responsible for inhibiting different stages of the viral replication cycle. A major family of ISGs are the*Interferon-induced proteins with tetrapeptide repeats* (IFITs) and your action against infections caused by viruses from different families was proven. This study describes the expression of *BatIFIT5* by RT-qPCR in different species of healthy and naturally infected with RABV bats. A total, of 36 bats were analyzed (18 positive and 18 negative for rabies) and 16 (44.44%) were positive for *BatIFIT5*. Here we analyzed fourteen species of bats with different eating and behavioral habits. Seven genetics lineages of RABV were evaluated and included in these 14 species of bats, no cases of RABV spillover were identified. In addition, we did not verified relationship between the bat species expression of *BatIFIT5* and RABV. Many points about immunology of bats are unknown and here we analyzed one of these points.

Keywords

bats, batIFIT5, chiroptera, interferon, IFIT5, rabies

Introduction

Bats are mammals belonging to the Chiroptera order, traditionally divided based on morphology and behavior into the suborders Mega and Microchiroptera. However, recent cytogenetic and molecular evidence supports a new classification into two suborders, Yangochiroptera and Yinpterochiroptera. The first consists of twelve microbat families, while the second suborder includes megabats and four microbat families (Teeling *et al*. 2000, Teeling *et al*. 2002, Springer *et al*. 2002, Teeling *et al.* 2005,). The microbats are globally distributed, except in Antarctica (Nowak *et al*. 1999). In Latin America, only members of microbats are found, with more than 290 species (Bat Conservation International, 2021). Although the vital role played in numerous ecosystem services, bats can be important reservoirs or hosts for several microorganisms, mainly viruses. Numerous viruses detected in bats could potentially affect humans, posing a major public health threat worldwide. The most recent example of a spillover event is*Severe acute respiratory syndrome coronavirus 2* (SARSCoV-2), the etiological agent of the current coronavirus disease 2019 (COVID-19) pandemic. On the other hand, rabies, an ancient fatal disease also maintained by bats, remains a serious public health concern in several countries (Kunz *et al*. 2011, Han *et al.* 2015, WHO, 2018, Bonilla-Aldana *et al*. 2021).

Rabies is a zoonosis caused by *Rabies lyssavirus* (RABV), a member of the *Lyssavirus* genus, that affects the central

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nervous system (CNS) of all mammals, including bats and humans (WHO, 2018, ICTV, 2018). Despite significant progress towards understanding RABV biology, the maintenance of the virus in nature is not completely elucidated. One of the most significant gaps is related to certain aspects of the pathogenesis of RABV infections in bats, the main reservoir of the virus. While RABV infects and causes death of some bats, previous studies have detected rabies antibodies in different species of these mammals, emphasizing that rabies is not always fatal in bats, unlike the other mammals (Jiang *et al*. 2010, O'shea *et al*. 2014, Costa *et al*. 2017, Almeida *et al.* 2019, Seetahal *et al*. 2020, Seidlova *et al*. 2020,).

The increase in viral load and efficient replication process that lead to a productive viral infection depends on intrinsic factors of the virus and the ability of the host immune system (IS) in controlling or not the disease. As viruses possess mechanisms to evade the IS, the host also has a coordinated defense network to prevent viral infection or at least make it less productive. The secretion of interferon (IFN) plays a critical role in the innate immune mechanism response to viral infections. There are different types of IFN, and their expression leads to activation of the JAK-STAT signalling cascate that culminates in expression of IFN-stimulated genes (ISGs), responsible for inhibiting different stages of the viral replication cycle. A major family of ISGs are the *Interferon-induced proteins with tetrapeptide repeats* (IFITs), a group of intracytoplasmic proteins without enzymatic activity with direct interaction capacity with viral RNAs. This family consists of four members in humans (IFIT1, IFIT2, IFIT3 and IFIT5), also expressed in other mammals (Stetson *et al*. 2006, Diamond *et al*. 2013, Goubau *et al*. 2013, Liu *et al*. 2013, Fensterl *et al.* 2015). The antiviral activity of IFIT5 in mammals demonstrated that IFIT5 levels were increased during viruses infections and lipopolysaccharides (Barber *et al*.2013, Li *et al*. 2020). The mechanism of this protein is attributed to interaction with nucleotides through 5'triphosphate portion from RNA (Feng *et al*. 2013, Santhakumar *et al*. 2018). The immune system of bats could have the same unknown particularities, therefore, studies about the underlying mechanism regarding antiviral activities could help to clarify important aspects in the maintenance of RABV in bats.

The action of IFITs against infections caused by viruses from different families was proven, nevertheless, studies regarding the role of these proteins during RABV infection are lacking in the scientific literature. Considering the significant role of ISGs against viral infection and the scarcity of studies related to the interaction between IFITs and RABV, in addition to the importance of bats as viruses reservoirs, this study describes the expression of *BatIFIT5* in different species of healthy and naturally infected with RABV bats.

Materials and Methods

Bats

Thirty-six bats collected between 2015 and 2020 in different municipalities of São Paulo state, Brazil, sent for rabies diagnosis, were selected. Samples from the central nervous system (CNS) and spleen were collected and stored at -80 ºC until use. This work complies with Protocol No. 04/2020 issued by the Ethics Committee of the Pasteur Institute of São Paulo (Brazil).

Rabies diagnosis

Routine identification of RABV antigens was performed by the direct fluorescent antibody test (DFAT), following standard diagnostic procedures (Dean *et al*. 1996).

RNA extraction

Total RNA of CNS and spleen were extracted with Trizol® (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Extracts from mice infected with the "fixed" strain Challenge Virus Standard (CVS-31) were included in all reactions as positive control and DEPC-treated water was included as negative control.

DNA extraction

Total DNA was extracted from the collected spleens using the Wizard® Genomic DNA Purification kit #TM050 (Promega Corporation) in accordance with the manufacturer's instructions. Ultrapure DNase/RNase-free water was used as negative control.

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Species identification

Positive bats for RABV were submitted to genetic and taxonomic identification (Vizzoto *et al*. 1973, Jones *et al.* 1976, Gregorin *et al.* 2002). Negatives bats for RABV were submitted only to genetic species identification. For genetic identification, total spleens DNA, extracted as described in 2.4 were submitted to amplification and sequencing of the mitochondrial cytochrome B and/or C (cytB or cytC) gene as described. The PCR and cytB sequencing were carried out with sense primer Bat 05A and antisense primer Bat 14A, for cytC was used sense primer LCO1490 and antisense primer HCO2198 (Table I).

Gene	Primer	Sense	Sequence (5′-3′)	Reference
cytB	Bat 05A	Forward	CGACTAATGACATGAAAAATCACCGTTG	Martins *et al.* 2009
	Bat 14A	Reverse	TATTCCCTTTGCCGGTTTACAAGACC	
cytC	LCO1490	Forward	GGTCAACAAATCATAAAGATATTGG	Folmer *et al.* 1994
	HCO2198	Reverse	TAAACTTCAGGGTGACCAAAAAATCA	

Table I. Primers used for PCR amplication and sequencing of cythcrome B (cytB) and C (cytC) for genetic identification of bat species.

Sequencing of RABV

The CNS of all positives bats were submitted to reverse transcription (RT) followed by polymerase chain reaction (RT-PCR), sequencing and phylogenetic analyses. For RT-PCR, extracted total RNA as described in 2.3 was used. The RT was carried out with primers that target the nucleoprotein gene (N) of RABV: sense primer Início_Posição1_F and antisense primer P784_Posição869_R (Table II). The reaction was performed in 50 volumes with aid of the SuperScript II reverse transcriptase kit (Invitrogen, Carlsbad, CA, USA), following the manufacturer's instructions. The reaction was incubated for 1 hour at 42 ºC followed by a final incubation at 72 ºC for 15 minutes. PCR was carried out as previously described (Carnieli *et al*. 2008), using the same primers. PCR amplicons were purified and sequenced with the same primers used for PCR. The RABV sequences were edited with the CHROMAS software (version 2.24 Copyright© 1998–2004 Technelysium Pty Ltd.). After editing, the output sequences were aligned with CLUSTAL/W using the BioEdit software (version 7.1.3.0). The putative amino acid (aa) sequences obtained were deduced with the BioEdit program (Hall, 1999). The phylogenetic tree was performed by the Maximum Likelihood method based on the Tamura 3-parameter model with Gamma distribution (Tamura *et al*. 1993), with MEGA 7 software (Tamura, 1992). A total of 591 nucleotides of the RABV *N* gene, nucleotide positions 71-592 in the PV strain of RABV (GenBank accession number: GB M13215.1), coding region aminoacids 1-174 were analyzed.

Gene	Primer	Sense	Sequence (5′-3′)	Reference
Nucleoprotein	Início_Posição1_F	Forward	ACGCTTAACAACAARATCARAG	Campos *et al.* 2011
(N)	P784_Posição869_R	Reverse	CCTCAAAGTTCTTGTGGAAGA	Soares *et al.* 2002

Table II. Primers used for PCR amplification of partial nucleoprotein gene of rabies virus.

Expression of BatIFIT5

Total RNA extracted of collected spleens as described in 2.3 was used to synthesize first strand cDNA using Oligo dT (18) following the manufacturer's instructions. To detect *BatFIT5* gene expression, cDNA was amplified in a 20 µL reaction using a SYBR® Premix Ex Taq™ II kit (TaKaRa, Dalian, China) on an Applied Biosystems 7500 real-time PCR system (1 cycle at 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and 60°C for 34 s). The RT-qPCR was carried out with human IFIT5 sense and antisense primers (Table 3). All cDNA samples were tested in duplicate and the results were normalized to positive and negative controls. *BatIFIT5* cloned into a pEFLink plasmid with FLAG peptide given by Dr. Muhammad Munir, from Lancaster University (UK) was used as a standard and positive control. Ultrapure DNase/RNase-free water was used as negative control.

Gene	Primer	Sense	Sequence (5′-3′)	Reference
IFIT5	Human IFIT5	Forward	TAAAAAAGGCCTTGGAGGTG	Zhang *et al.* 2013
		Reverse	CCAGGTCTGTGTAGGCAAAT	

Table III. Primers used for PCR amplification of partial IFIT5 gene.

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Results

Bats

Were collected in 17 municipalities from different regions of São Paulo State, southeast of Brazil. The exact location of each municipality is shown in Figure 1.

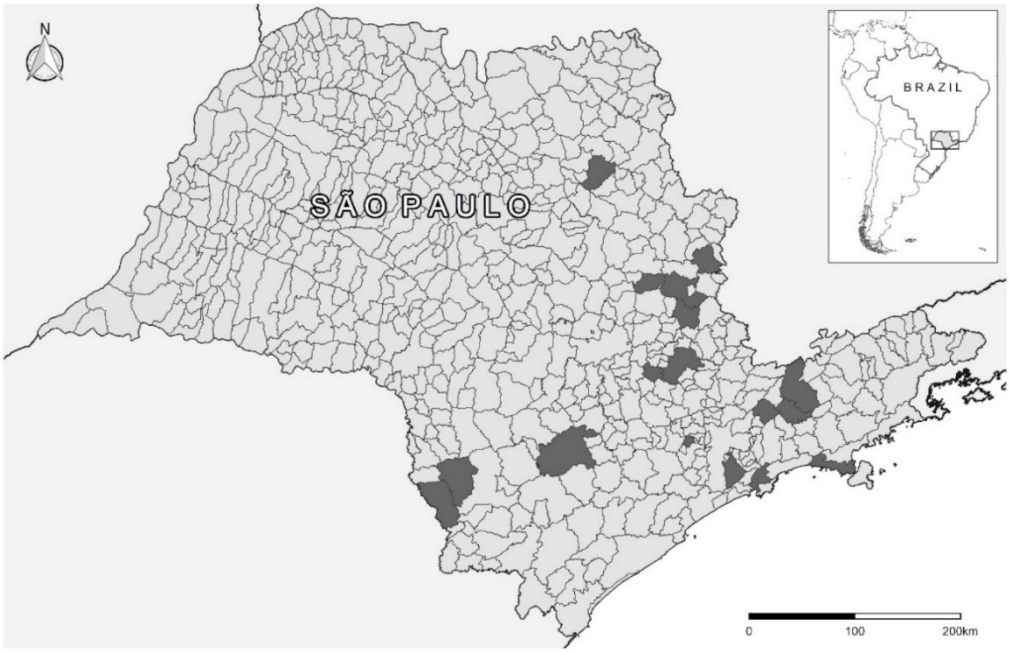

Figure 1. Map of São Paulo, state of southeastern Brazil, showing the spatial distribution of the municipalities (dark gray) where the bats were collected.

Rabies diagnosis

A total of 18 bats were positive for RABV and 18 bats were negative by DFAT. The species of each bat and result for rabies are described in Table IV.

Identification number	Specie	Total of animals	Rabies diagnosis
1270/20	*Myotis albescens*	1	Positive
130/19; 1026/19; 291/20 and 3007/20	*Eptesicus furinalis*	4	
700/19 and 645/20	*Histiotus velatus*	2	
1375/19; 278c/20 and 2824/20	*Artibeus lituratus*	3	
1421/18; 335/19; 765/19 and 182/20	*Nyctinomops laticaudatus*	4	
4458/19	*Lasiurus blossevillii*	1	
1094/19	*Tadarida brasiliensis*	1	
4704/19 and 181/20	*Desmodus rotundus*	2	
1695/15	*Artibeus lituratus*	1	Negative
1705/15; 2019/15; 2021/15 and 2047/15	*Glossophaga soricina*	4	
1709/15; 1741/15 and 2017/15	*Eumops glaucinus*	3	
1979/15	*Lasiurus ega*	1	
1992/15; 2040/15 and 2056/15	*Cynomops planirostris*	3	
2022/15; 2030/15; 2044/15;	*Molossus molossus*	6	

Table IV. Identification of each bat collected, species identified, number of animals and rabies diagnosis.

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Phylogenetic analysis of rabies virus

A phylogenetic tree was constructed utilizing the 18 samples of RABV sequenced in this work and additional 23 sequences of RABV recovered from GenBank (Figure 2). Eight clusters were formed: cluster 1 corresponds to sequences of viruses recovered from hematophagous bats *Desmodus rotundus* (DR); cluster 2 corresponds to sequences originated from bats *Tadarida brasiliensis* (TB); cluster 3 corresponds to sequences originated from bats *Myotis* sp. (Msp); cluster 4 corresponds to sequences originated from bats *Eptesicus furinalis* (EF); cluster 5 corresponds to sequences originated from bats *Histiotus velatus* (HV); cluster 6 corresponds to sequences originated from bats *Nyctinomops laticaudatus* (NL); cluster 7 corresponds to sequences originated from bats *Lasiurus* sp. (Lsp) from Brazil and cluster 8 corresponds to sequences originated from bats *Lasiurus* sp. (Lsp) from Americas. The species *T.brasiliensis*, *Myotis* sp., *E. furinalis*, *H.velatus*, *N. laticaudatus* and *Lasiurus* sp. were from non hematophagous bats.

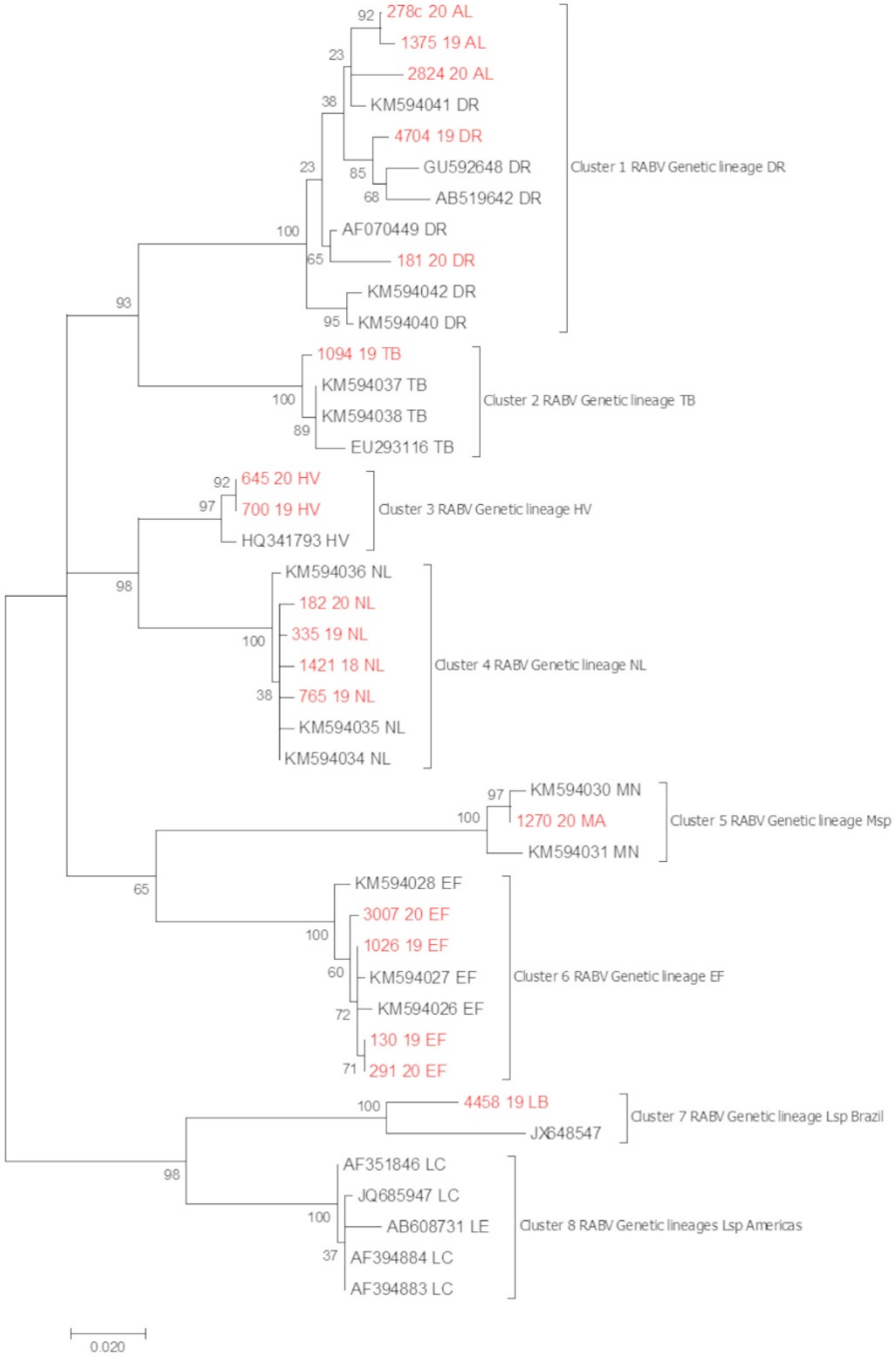

Figure 2. Phylogenetic analysis by Maximum Likelihood method. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model [37]. The tree with the highest log likelihood (-2455.0703) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and

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then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (4 categories (+G, parameter = 0.2542)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 42 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 522 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Thirumal Kumar et al. 2019). Samples sequenced in this work are in red.

Detection of BatIFIT5

A total of 36 bats were analyzed (18 positive and 18 negative for rabies) and 16 (44.44%) analyzed bats were positive for *BatIFIT5* in RT-qPCR. The description of detection of *BatIFIT5* according to rabies diagnosis, number and bats species are presented in Table V.

Detection of *BatIFIT5*	Rabies diagnosis	Number (%)	Identified bats species
Positive	Positive	12 (66.67%)	*Myotis albescens* (1270/20); *Eptesicus furinalis* (1026/19; 291/20 and 3007/20); *Histiotus velatus* (700/19 and 645/20); *Artibeus lituratus* (1375/19 and 278c/20); *Lasiurus blossevillii* (4458/19); *Tadarida brasiliensis* (1094/19); *Desmodus rotundus* (4704/19 and 181/20)
Negative	Positive	6 (33.33%)	*Nyctinomops laticaudatus* (1421/18, 335/19; 765/19 and 182/20); *Eptesicus furinalis* (130/19); *Artibeus lituratus* (2824/20)
Positive	Negative	4 (22.22%)	*Eumops glaucinus* (1741/15); *Glossophaga soricina* (2021/15); *Cynomops planirostris* (2040/15); *Molossus molossus* (2044/15)
Negative	Negative	14 (77.77%)	*Artibeus lituratus* (1695/15); *Glossophaga soricina* (1705/15; 2019/15 and 2047/15); *Eumops glaucinus* (1709/15 and 2017/15); *Lasiurus ega* (1979/15); *Cynomops planirostris* (1992/15 and 2056/15); Molossus molossus (2022/15; 2030/15; 2055/15; 2063/15 and 2064/15)

Table V. Detection of BatIFIT5, rabies diagnosis, number and percentage of bats and identified bats species.

Discussion and Conclusions

Currently, the majority of emerging infectious diseases are zoonoses originated from wildlife and bats constitute reservoirs of several high-impact viruses in nature (Jones *et al*. 2008, Wynne *et al*. 2013). Many biological aspects of these mammals are tough to be related to their capacity to carry several viral species, including longevity, capacity to fly and different eating and behavior habits. Here we investigated the transcript expression of *BatIFIT5* in different species of healthy and infected with RABV bats.

The highest prevalence of the protein was detected in positive animals for RABV (66.67%). Despite some cases of cure in humans, the illness causes serious impairment of CNS and for this is considered 100% fatal (Jackson, 2002). The expression of *BatIFIT5* in animals infected with RABV not scrap the antiviral activity of the protein and indicates activation of the innate response in presence of the virus. On the other hand, the virus can have mechanisms to counteract the action of *BatIFIT5*s, inhibiting therefore its clearance. Its should be consider, that all animals analyzed in this study were naturally infected and parameters as infectious dose, incubation period or previous immunological status are all unknown. Wang *et al*. 2015 developed a study that correlated the expression of duck IFIT5 (*duIFIT5*) in healthy ducks (*Anas platyrhynchos domesticus*) and ducks experimentally infected with *Duck hepatitis virus type 1* (DHV-1). In their study different organs and the authors also identified expression of IFIT5 in healthy animals. In our study were tested only the spleens of the bats and the expression of *BatIFIT5* was identified in a very low percentage of animals 4 (22.22%) not infected with RABV. It is important to emphasize that the animals were submitted only to rabies diagnosis, therefore these animals could be affected by other viruses or infectious pathogens as bacteria or fungi, which potentially induces IFITs.

Here we analyzed fourteen species of bats with different eating and behavioral habits. Seven genetics lineages of RABV were evaluated and included in these 14 species of bats, no cases of RABV spillover were identified (Oliveiræt *al*. 2010). In addition, we did not verified relationship between the bat species expression of *BatIFIT5* and RABV(except for the insectivorous bat *N. laticaudatus*). In fact expression of *BatIFIT5* was not identified in any animal *N. laticaudatus* analyzed, despite the infection with RABV. For *E. furinalis* and *A. lituratus* species the same situation happened (Positive for rabies and negative for *BatIFIT5*) in only one sample of each specie. However, three and two animals belonging to *E. furinalis* and *A. lituratus* were positive for RABV and *BatIFIT5* respectively. Therefore, the absence of *BatIFIT5* expression is not an exclusivity of the species. . Despite the low number of samples analyzed, our results could indicate any difference in the immunological system of *N. laticaudatus* bats.

Many points about immunology of bats are unknown and here we analyzed one of these points but this is the first

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study relating *BatIFIT5* and RABV in animals naturally infected and could help to better understand the relationship between viruses and reservoirs/hosts.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

We would like to thank Dr. Muhammad Munir, from Lancaster University (UK) for providing pEFLink plasmid with FLAG peptide and Marcela Mello Zamudio from Universidade de São Paulo (USP-FFLCH) for producing the map.

Funding data

This study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo FAPESP (Grant. No. 2019/03146-2) and Health State Secretary of São Paulo. Part of this work was supported by FINEP project number 1.10.0783.00.

References

Almeida, M. F., Rosa, A. R. D., Martorelli, L. F. A., Kataoka, A. P. A. G., & Aires, C. C. (2019). Rabies virus monitoring in bat populations in Rondônia state, Brazil. Revista da Sociedade Brasileira de Medicina Tropical, 52, e20180199. https://doi.org/10.1590/0037-8682-0199-2018

Barber, M. R., Aldridge, J. R., Jr, Fleming-Canepa, X., Wang, Y. D., Webster, R. G., & Magor, K. E. (2013). Identification of avian RIG-I responsive genes during influenza infection. Molecular immunology, 54(1), 89–97. https://doi.org/10.1016/j.molimm.2012.10.038

Bonilla-Aldana, D. K., Jimenez-Diaz, S. D., Arango-Duque, J. S., Aguirre-Florez, M., Balbin-Ramon, G. J., Paniz-Mondolfi, A., Suárez, J. A., Pachar, M. R., Perez-Garcia, L. A., Delgado-Noguera, L. A., Sierra, M. A., Muñoz-Lara, F., Zambrano, L. I., & Rodriguez-Morales, A. J. (2021). Bats in ecosystems and their Wide spectrum of viral infectious potential threats: SARS-CoV-2 and other emerging viruses. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases, 102, 87–96. https://doi.org/10.1016/j.ijid.2020.08.050

Bowen, R. A., O'Shea, T. J., Shankar, V., Neubaum, M. A., Neubaum, D. J., & Rupprecht, C. E. (2013). Prevalence of neutralizing antibodies to rabies virus in serum of seven species of insectivorous bats from Colorado and New Mexico, United States. Journal of wildlife diseases, 49(2), 367–374. https://doi.org/10.7589/2012-05-124

Campos, A. C., Melo, F. L., Romano, C. M., Araujo, D. B., Cunha, E. M., Sacramento, D. R., de Andrade Zanotto, P. M., Durigon, E. L., & Favoretto, S. R. (2011). One-step protocol for amplification of near full-length cDNA of the rabies virus genome. Journal of virological methods, 174(1-2), 1–6. https://doi.org/10.1016/j.jviromet.2011.03.030

Carnieli, P., Jr, Fahl, W.deO., Castilho, J. G., Oliveira, R.deN., Macedo, C. I., Durymanova, E., Jorge, R. S., Morato, R. G., Spíndola, R. O., Machado, L. M., Ungar de Sá, J. E., Carrieri, M. L., & Kotait, I. (2008). Characterization of Rabies virus isolated from canids and identification of the main wild canid host in Northeastern Brazil. Virus research, 131(1), 33–46. https://doi.org/10.1016/j.virusres.2007.08.007

Costa, L. J. C., Martorelli, L. F. A., Barone, G. T., Rosa, E. S. T., Vasconcelos, P. F. C., Pereira, A. S., & Fernandes, M. E. B. (2017). Seroprevalence of rabies virus antibodies in bats from high risk areas in Brazilian Amazonia between 2013 and 2015. Transactions of the Royal Society of Tropical Medicine and Hygiene, 111(8), 363–369. https://doi.org/10.1093/trstmh/trx069

Dean, D.J.; Abelseth, M.K.; Athanasiu, P. The fluorescent antibody test. In Laboratory techniques in rabies; Meslin, F.X., Kaplan, M.M., Koprowski, H., Eds.; World Health Organization: Geneva, 1996.

Diamond, M. S., & Farzan, M. (2013). The broad-spectrum antiviral functions of IFIT and IFITM proteins. Nature reviews. Immunology, 13(1), 46–57. https://doi.org/10.1038/nri3344

Feng, F., Yuan, L., Wang, Y. E., Crowley, C., Lv, Z., Li, J., Liu, Y., Cheng, G., Zeng, S., & Liang, H. (2013). Crystal structure and nucleotide selectivity of human IFIT5/ISG58. Cell research, 23(8), 1055–1058. https://doi.org/10.1038/cr.2013.80

Fensterl, V., & Sen, G. C. (2015). Interferon-induced Ifit proteins: their role in viral pathogenesis. Journal of virology, 89(5), 2462–2468. https://doi.org/10.1128/JVI.02744-14

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular marine biology and biotechnology, 3(5), 294–299.

Goubau, D., Deddouche, S., & Reis e Sousa, C. (2013). Cytosolic sensing of viruses. Immunity, 38(5), 855–869. https://doi.org/10.1016/j.immuni.2013.05.007

Gregorin, R.; Taddei, V.A. Chave Artificial Para a Identificação de Molossídeos Brasileiros (Mammalia, Chiroptera). Mastozoología Neotrop. 2002, 9, 13–32.

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In Nucleic acids symposium series, 41 (41), 95-98.

Han, H. J., Wen, H. L., Zhou, C. M., Chen, F. F., Luo, L. M., Liu, J. W., & Yu, X. J. (2015). Bats as reservoirs of severe emerging infectious diseases. Virus research, 205, 1–6. https://doi.org/10.1016/j.virusres.2015.05.006

ICTV Genus: Lyssavirus - Rhabdoviridae - Mononegavirales - International Committee on Taxonomy of Viruses (ICTV) Available online: https://talk.ictvonline.org/ictv-reports/ictv_online_report/negative-sense-rna viruses/mononegavirales/w/rhabdoviridae/795/genus-lyssavirus (accessed on 26 July 2021).

International, B.C. Conservation Progress in Latin America - Bat Conservation International Available online: https://www.batcon.org/article/conservation-progress-in-latin-america/ (accessed on 28 July 2021).

Jackson A. C. (2002). Rabies pathogenesis. Journal of neurovirology, 8(4), 267–269. https://doi.org/10.1080/13550280290100725

Jiang, Y., Wang, L., Lu, Z., Xuan, H., Han, X., Xia, X., Zhao, F., & Tu, C. (2010). Seroprevalence of rabies virus antibodies in bats from southern China. Vector borne and zoonotic diseases (Larchmont, N.Y.), 10(2), 177–181. https://doi.org/10.1089/vbz.2008.0212

Jones Jr, J. K., & Carter, D. C. (1976). Annotated checklist, with keys to subfamilies and genera. Biology of bats of the new world family Phyllostomidae, part I. Lubbock: Museum Texas Tech. University, 7-38.

Jones, K. E., Patel, N. G., Levy, M. A., Storeygard, A., Balk, D., Gittleman, J. L., & Daszak, P. (2008). Global trends in emerging infectious diseases. Nature, 451(7181), 990–993. https://doi.org/10.1038/nature06536

Kunz, T. H., Braun de Torrez, E., Bauer, D., Lobova, T., & Fleming, T. H. (2011). Ecosystem services provided by bats. Annals of the New York Academy of Sciences, 1223, 1–38. https://doi.org/10.1111/j.1749-6632.2011.06004.x

Li, J. J., Yin, Y., Yang, H. L., Yang, C. W., Yu, C. L., Wang, Y., Yin, H. D., Lian, T., Peng, H., Zhu, Q., & Liu, Y. P. (2020). mRNA expression and functional analysis of chicken IFIT5 after infected with Newcastle disease virus. Infection, genetics and evolution: journal of molecular epidemiology and evolutionary genetics in infectious diseases, 86, 104585. https://doi.org/10.1016/j.meegid.2020.104585

Liu, Y., Zhang, Y. B., Liu, T. K., & Gui, J. F. (2013). Lineage-specific expansion of IFIT gene family: an insight into coevolution with IFN gene family. PloS one, 8(6), e66859. https://doi.org/10.1371/journal.pone.0066859

Martins, F. M., Templeton, A. R., Pavan, A. C., Kohlbach, B. C., & Morgante, J. S. (2009). Phylogeography of the common vampire bat (Desmodus rotundus): marked population structure, Neotropical Pleistocene vicariance and incongruence between nuclear and mtDNA markers. BMC evolutionary biology, 9, 294. https://doi.org/10.1186/1471-2148-9-294

Nowak, R.M. Walkers' Mammals of the World; 6th ed.; Johns Hopkins University Press: Baltimore, 1999.

Oliveira, R. de N., de Souza, S. P., Lobo, R. S., Castilho, J. G., Macedo, C. I., Carnieli, P., Jr, Fahl, W. O., Achkar, S. M., Scheffer, K. C., Kotait, I., Carrieri, M. L., & Brandão, P. E. (2010). Rabies virus in insectivorous bats: implications of the diversity of the nucleoprotein and glycoprotein genes for molecular epidemiology. Virology, 405(2), 352–360. https://doi.org/10.1016/j.virol.2010.05.030

O'Shea, T. J., Bowen, R. A., Stanley, T. R., Shankar, V., & Rupprecht, C. E. (2014). Variability in seroprevalence of rabies virus neutralizing antibodies and associated factors in a Colorado population of big brown bats (Eptesicus fuscus). PloS one, 9(1), e86261. https://doi.org/10.1371/journal.pone.0086261

Santhakumar, D., Rohaim, M. A. M. S., Hussein, H. A., Hawes, P., Ferreira, H. L., Behboudi, S., Iqbal, M., Nair, V., Arns, C. W., & Munir, M. (2018). Chicken Interferon-induced Protein with Tetratricopeptide Repeats 5 Antagonizes Replication of RNA Viruses. Scientific reports, 8(1), 6794. https://doi.org/10.1038/s41598-018-24905-y

Seetahal, J. F. R., Greenberg, L., Satheshkumar, P. S., Sanchez-Vazquez, M. J., Legall, G., Singh, S., Ramkissoon, V., Schountz, T., Munster, V., Oura, C. A. L., & Carrington, C. V. F. (2020). The Serological Prevalence of Rabies Virus-Neutralizing Antibodies in the Bat Population on the Caribbean Island of Trinidad. Viruses, 12(2), 178. https://doi.org/10.3390/v12020178

Seidlova, V., Zukal, J., Brichta, J., Anisimov, N., Apoznański, G., Bandouchova, H., Bartonička, T., Berková, H., Botvinkin, A. D., Heger, T., Dundarova, H., Kokurewicz, T., Linhart, P., Orlov, O. L., Piacek, V., Presetnik, P., Shumkina, A. P., Tiunov, M. P., Treml, F., & Pikula, J. (2020). Active surveillance for antibodies confirms circulation of lyssaviruses in Palearctic bats. BMC veterinary research, 16(1), 482. https://doi.org/10.1186/s12917-020-02702-y

Soares, R. M., Bernardi, F., Sakamoto, S. M., Heinemann, M. B., Cortez, A., Alves, L. M., Meyer, A. D., Ito, F. H., & Richtzenhain, L. J. (2002). A heminested polymerase chain reaction for the detection of Brazilian rabies isolates from vampire bats and herbivores. Memorias do Instituto Oswaldo Cruz, 97(1), 109–111. https://doi.org/10.1590/s0074-02762002000100019

Springer, M. S., Teeling, E. C., Madsen, O., Stanhope, M. J., & de Jong, W. W. (2001). Integrated fossil and molecular data reconstruct bat echolocation. Proceedings of the National Academy of Sciences of the United States of America, 98(11), 6241–6246. https://doi.org/10.1073/pnas.111551998

Stetson, D. B., & Medzhitov, R. (2006). Type I interferons in host defense. Immunity, 25(3), 373–381. https://doi.org/10.1016/j.immuni.2006.08.007

Tamura K. (1992). Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G+C-content biases. Molecular biology and evolution, 9(4), 678–687. https://doi.org/10.1093/oxfordjournals.molbev.a040752

Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular biology and evolution, 10(3), 512–526. https://doi.org/10.1093/oxfordjournals.molbev.a040023

Teeling, E. C., Madsen, O., Van den Bussche, R. A., de Jong, W. W., Stanhope, M. J., & Springer, M. S. (2002). Microbat paraphyly and the convergent evolution of a key innovation in Old World rhinolophoid microbats. Proceedings of the National Academy of Sciences of the United States of America, 99(3), 1431–1436. https://doi.org/10.1073/pnas.022477199

Teeling, E. C., Scally, M., Kao, D. J., Romagnoli, M. L., Springer, M. S., & Stanhope, M. J. (2000). Molecular evidence regarding the origin of echolocation and flight in bats. Nature, 403(6766), 188–192. https://doi.org/10.1038/35003188

Teeling, E. C., Springer, M. S., Madsen, O., Bates, P., O'brien, S. J., & Murphy, W. J. (2005). A molecular phylogeny for bats illuminates biogeography and the fossil record. Science (New York, N.Y.), 307(5709), 580–584. https://doi.org/10.1126/science.1105113

Thirumal Kumar, D., Mendonca, E., Priyadharshini Christy, J., George Priya Doss, C., & Zayed, H. (2019). A computational model to predict the structural and functional consequences of missense mutations in O6-methylguanine DNA methyltransferase. Advances in protein chemistry and structural biology, 115, 351–369. https://doi.org/10.1016/bs.apcsb.2018.11.006

Vizzoto, L.; Taddei, V. Chave Para a Determinação de Quirópteros Brasileiros; Gráfica Francal: São José do Rio Preto, 1973.

Wang, B., Chen, Y., Mu, C., Su, Y., Liu, R., Huang, Z., Li, Y., Yu, Q., Chang, G., Xu, Q., & Chen, G. (2015). Identification and expression analysis of the interferon-induced protein with tetratricopeptide repeats 5 (IFIT5) gene in duck (Anas platyrhynchos domesticus). PloS one, 10(3), e0121065. https://doi.org/10.1371/journal.pone.0121065

WHO Expert Consultation on Rabies: Third Report; WHO technical report series;1012; World Health Organization, 2018.

Wynne, J. W., & Wang, L. F. (2013). Bats and viruses: friend or foe?. PLoS pathogens, 9(10), e1003651. https://doi.org/10.1371/journal.ppat.1003651

Zhang, B., Liu, X., Chen, W., & Chen, L. (2013). IFIT5 potentiates anti-viral response through enhancing innate immune signaling pathways. Acta biochimica et biophysica Sinica, 45(10), 867–874. https://doi.org/10.1093/abbs/gmt088

How to cite: Mosca Barboza et al. Activity of BatIFIT5 in different species of healthy and naturally infected with rabies virus bats. Veterinaria Italiana, Vol. 60 No. 2 (2024) DOI: 10.12834/VetIt.3427.23374.1