Molecular Characterization and Genomic Sequencing of Rabies in Northeast Argentina

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INTRODUCTION

In Argentina, the rabies virus (RABV; genus *Lyssavirus*, family *Rhabdoviridae*) circulates in two distinct epidemiological cycles: terrestrial and aerial, in various species of the order *Carnivora* and *Chiroptera*, with specific variants associated with different hosts. Thanks to vaccination and surveillance programs, there has been a reduction in cases of canine rabies; however, northeast Argentina presents a challenge as the AgV2 variant circulates in enzootic cycles among canines and wild species. The aim of this study is the molecular characterization and whole-genome sequencing (NGS) of positive RABV isolates in northeast Argentina.

METHODS

A total of 17 animal rabies samples were analyzed between August 2023 and August 2024, from the Specialized Zoonosis Center of the Ministry of Health of Chaco (Table 1). Five animal rabies samples characterized as AgV2 from other years were added to enhance the robustness of the phylogenetic analysis (CEZ454-20, CEZ455-20, CEZ471-20, CEZ507-21, CEZ631-22). The presence of viral RNA was confirmed by real-time PCR, a partial region of the viral nucleoprotein (N gene) was sequenced by Sanger, and an adapted Illumina COVIDseq protocol, *RabvPeru_primerSequences* designed by Brunker *et al*., 2020(1), and the Illumina platform were used for NGS.

Table 1. Animal rabies samples from the Specialized Zoonosis Center

Date	ID Malbrán	Species	Province
09/08/2023	Z270-24	Bovine	Formosa
20/09/2023	Z271-24	Bovine	Chaco
07/12/2023	Z272-24	Equine	Formosa
27/12/2023	Z273-24	Bovine	Chaco
27/12/2023	Z274-24	Bovine	Chaco
29/12/2023	Z275-24	Bovine	Formosa
11/01/2024	Z276-24	Bovine	Chaco
16/01/2024	Z277-24	Bovine	Chaco
31/01/2024	Z278-24	Bovine	Chaco
06/02/2024	Z189-24	Dog	Formosa
06/02/2024	Z279-24	Bat	Formosa
14/02/2024	Z280-24	Bovine	Chaco
23/02/2024	Z281-24	Bat	Corrientes
14/02/2024	Z282-24	Equine	Chaco
10/04/2024	Z283-24	Bat	Chaco
07/06/2024	Z291-24	Dog	Chaco
06/08/2024	Z554-24	Bat	Chaco

RESULTS
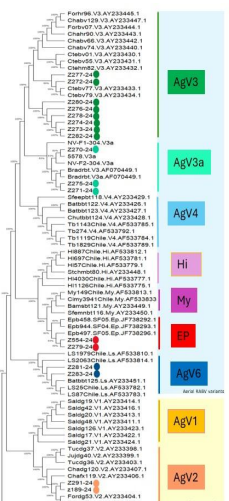
The terrestrial RABV variants (AgV2) and aerial variants (AgV3, AgV3a, AgV6, and *Eptesicus spp*) were identified from the characterization of a partial region of the N gene (320 bp) according to Cisterna *et al*., 2004 (Figure 1). Two complete RABV AgV2 genomes were obtained corresponding to canine samples from Formosa and Chaco, in addition to the added samples (n=5). Following the RABV Glue classification scheme (3), the maximum likelihood (ML) phylogenetic analysis revealed that the Argentine genomes obtained in this study grouped monophyletically in a lineage separate from lineages AM3a and AM3b corresponding to genomes from Brazil (Figure 2).
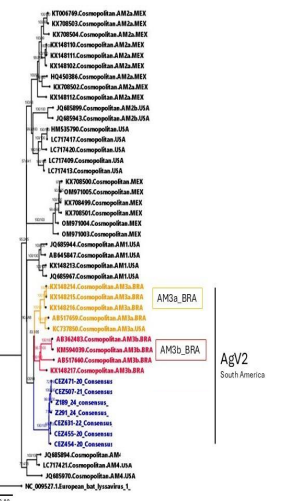
Figure 1: Neighbor Joining phylogeny produced by aligning the partial region of the N gene from Argentinean samples and representatives of different lineages, under the Kimura model and 1000 bootstrap. Samples this study are shown with point in different colours according to the corresponding lineage.

Figure 2. ML tree of 41 complete rabies genomes from America belonging to the Cosmopolitan clade (obtained from RABV Glue) and 7 complete genomes obtained in this study. The dataset was aligned using MAFFT, and the ML tree was built using IQtree under GTR + F + I +G4 and 1000 bootstrap. The Argentinian genomes are shown in blue.

CONCLUSION

Given the lack of genomic data in Argentina, our study provides the first genomic sequences of RABV and offers useful information for implementing control and prevention strategies for this disease, in line with the National Plan for the Prevention and Elimination of Human Rabies Transmitted by Dogs, representing a first step towards genomic surveillance in our country.

References: 1- Kirstyn Brunker, Josh Quick 2020. Rabies virus MinION sequencing protocol. **protocols.io** https://dx.doi.org/10.17504/protocols.io.ba4figtn. 2-Cisterna, D., Bonaventura, R., Caillou, S., Pozo, O., Andreau, M. L., Fontana, L. D., Echegoyen, C., de Mattos, C., de Mattos, C., Russo, S., Novaro, L., Elberger, D., & Freire, M. C. (2005). Antigenic and molecular characterization of rabies virus in Argentina. *Virus research*, 109(2), 139–147. https://doi.org/10.1016/j.virusres.2004.10.013. 3- GLUE: A flexible software system for virus sequence data. Joshua B Singer, Emma C Thomson, John McLauchlan, Joseph Hughes and Robert J Gifford. BMC Bioinformatics 2018 19:532. https://doi.org/10.1186/s12859-018-2459-9

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