IMMUNOFLUORESCENCE/PCR CORRELATION AND ITS RELEVANCE TO WHOLE GENOME SEQUENCING OF THE RABIES VIRUS

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INTRODUCTION

Currently, rabies is a fatal disease but preventable by vaccinating domestic animals, however, wildlife infections are a potential source among human and animal populations. Direct immunofluorescence as the gold standard test established by the World Health Organization (WHO) and the World Organisation for Animal Health (WOAH) for rabies virus is sensitive for qualitative identification, but to characterize field isolates and vaccine strains another method is needed to provide information on their genome.

The use of molecular techniques such as RT-qPCR and WGS complement the diagnosis of rabies-positive samples by providing complementary information for their characterization. For this type of tests, the quality controls established in CENASA protocols based on specific fluorescence intensity and amplification cycle are determinant for the success of sequencing and bioinformatics analysis.

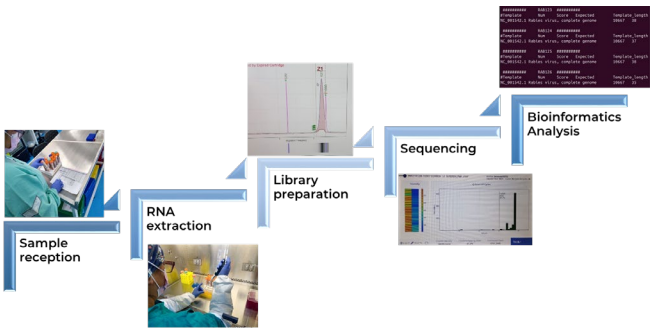
METHODS

Samples. Seventy-eight rabies-positive brains from four different groups of mammals were evaluated: chiroptera, bovines, equines and ovines.

Nucleic acid extraction and RT-qPCR. QIAamp® Viral RNA kit from Qiagen® and Ag-Path-ID™ One Step qPCR kit, for both the manufacturer's methodology was used.

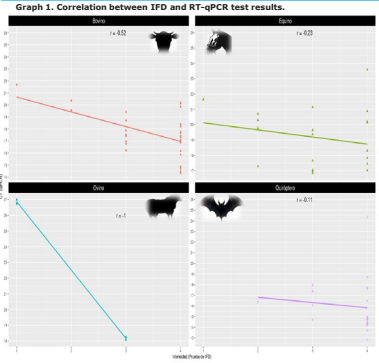
Whole Genome Sequencing. TruSeq® RNA Sample Prep from Illumina® according to the manufacturer's methodology.

Bioinformatic analysis. Use of software: fastQC, Trim_Galore, Bowtie2 and SPAdes. Finally, to identify the contigs obtained from the rabies virus, a local alignment was performed with the BLAST tool.



RESULTS

Graph 1. Correlation between IFD and RT-qPCR test results.



Graph 2. Correlation between IFD and WGS test results.

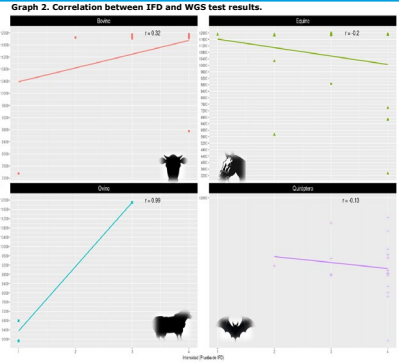


Table 1. Total samples analyzed by WGS. Sequences considered complete ranged in length from 11,800 to 11,998 bp.

Host	Intensity (+)	Genome	Total
Bovine	1	Partial	1
	2	Complete	2
	3	Complete	9
	4	Complete	17
		Partial	2
Equine	1	Complete	2
	2	Complete	2
		Partial	2
	3	Complete	7
		Partial	1
	4	Complete	5
		Partial	3
Sheep	1	Partial	2
	3	Complete	2
Chiroptera	2	Complete	1
	3	Complete	4
	4	Complete	16
		Total samples	78

The test results for each species show different types of correlation. Data analysis suggests an inverse correlation between IFD-PCR and PCR-WGS and a direct correlation between IFD-WGS. We obtained 85.9% of complete genomes (n=67) and 14.1% of partial sequences (n=11).

CONCLUSION

The results obtained in this study suggest that there is a negative correlation between the intensity of specific fluorescence and the Ct value, observing that the higher the number of crosses, the lower the Ct value of amplification of the samples. The correlation observed between the Ct value and the genome size tends to be negative in cattle, horses and sheep, where it is observed that the lower the Ct value, the higher the proportion of the rabies virus genome is formed. The correlation between the number of crosses and the size of the virus genome shows a positive trend for cattle and sheep, where it can be seen that the higher the fluorescence intensity, the higher the proportion of the virus genome; the opposite is the case for equines and chiroptera.

Likewise, this study suggests that external factors such as correct sample collection, preservation and shipment are determinant to maintain the integrity of the samples and, in turn, the success of the tests.

References: Servicio Nacional De Sanidad, Inocuidad y Calidad Agroalimentaria (SENASICA). (2023) Centro Nacional de Servicios de Diagnóstico en Salud Animal (CENASA). Available in: https://www.gob.mx/senasica/acciones-y-programas/el-centro-nacional-de-servicios-de-diagnostico-en-salud-animal

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