

Evolutionary Patterns of Rabies Virus Strains from Georgia Exhibit Risks of Their Transmission and Rabies Coinfections among Dogs and Cattle

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INTRODUCTION

Rabies is a zoonotic and neurotropic infectious disease caused by members of the *Lyssavirus* genus with rabies virus (RABV) as its prototype. In Georgia, rabies reflects endemic disease patterns, and has been officially documented since 1930. While RABV-infected dogs remain one of the main sources contributing significantly to the transmission of rabies across both humans and agricultural animals, one third of animal rabies cases in Georgia have been found to occur in agricultural animals, predominantly cattle (83 %). The genotypes and genetic relatedness of RABV isolates that infect dogs, cattle and wild animals in Georgia have been very poorly investigated and need to be urgently determined to gain greater and important insight into the risks of rabies transmission in these animals. Here, we provide an initial insight into the RABV genotypes associated predominantly with dogs and cattle, their population-based genetic structure and relationships, and certain trends of their evolutionary divergence in Georgia.

METHODOLOGY

A total of 73 RABV strains recovered from dogs and cattle (including one strain from jackals) in Georgia (2015-2016) were included in our phylogenetic analyses. The DNA sequences of a 1350-bp region of the nucleoprotein gene for these strains were subjected to molecular evolutionary analysis. These DNA sequences were obtained from the nucleotide database of the National Center for Biotechnology Information (NCBI). Using the Basic Local Alignment Search Tool (BLAST), the DNA sequences were blasted against the NCBI nucleotide database to obtain the respective DNA sequences of other RABV isolates recovered globally. The molecular evolutionary analyses were performed using MEGA X. The maximum likelihood (ML) algorithm was used to construct phylogenetic trees and to determine ancestral sequence types (STs). FEL (Fixed Effects Likelihood) and FUBAR (Fast, Unconstrained Bayesian AppRoximation for Inferring Selection) were used to determine the *dn/ds* ratios and trends of episodic positive/diversifying versus episodic negative/purifying selections, respectively. SplitsTree and SimPlot were employed to determine genetic recombination of nucleoprotein genes between the RABV strains.

RESULTS

The DNA sequence analysis of the nucleoprotein-encoding gene loci discriminated 73 RABV strains from Georgia into 41 STs. Certain strains from dogs and cattle were found to share the same STs (ST1, ST7, ST11, ST37). Two RABV strains (MT079902.1 and MT079888.1) recovered from dogs and jackals were deemed to share ST22. The genetically closest neighbours of the local RABV strains appeared to be those recovered in Turkey (with the ancestral strain determined for the closest relatives of the Georgian STs), Azerbaijan, Russia, Iran, Hungary (with the most recent ancestral strain determined for the closest relatives of the Georgian STs), Tajikistan and Iraq. The local RABV ST30 was determined to be the most recent ancestral ST for the entire subset of strains. The FEL analysis produced dn/ds ratios ≤ 0.05 ; FUBAR found evidence of episodic negative/purifying selection at 147 sites across the targeted gene loci of the local RABV STs. The SplitsTree analysis generated five parallelograms shared by multiple RABV strains, with the bootstrap values varying from 85 to 100 (fit: 100). SimPlot could identify recombination breakpoints in the RABV recombined gene loci.

DISCUSSION

Whereas rabies is the endemic zoonotic disease involving dogs and cattle, its transmission risk pathways in agricultural animals have been poorly understood in Georgia. The phylogenetic inferences from our analyses suggest that dogs can be one of the main sources of rabies transmission in cattle in Georgia. Besides, while a direction of rabies transmission between dogs and jackals could not be resolved in the analysis, the latter is assumed to be one of the possible sources of rabies transmission in dog populations in Georgia. Turkey Azerbaijan, Russia, Iran, Hungary, Tajikistan, Iraq and Georgia might be cross-border transmission hotspots for rabies, at least among these regions. The results of our recombination analyses are strongly suggestive of rabies co-infection risks in dog and cattle populations. A small sample size of the RABV strains and the fact that the DNA sequence data are limited to only a small section of their analysed genomes reflect collectively important uncertainties in this study. More in-depth and extensive investigations are required in order to gain greater insight into the rabies transmission risk pathways for cattle and other agricultural animals in Georgia.