

PHYLOGENETIC RELATIONSHIP BETWEEN RABIES VIRUS (*Lyssavirus rabies*) VARIANTS FROM TWO DIFFERENT SPECIES

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ABSTRACT

Rabies is a fatal zoonotic disease that affects several mammals. Hematophagous bats are recognized hosts of the rabies virus, and their main food source is the blood of other mammals, particularly cattle. During feeding, bats transmit the virus to cattle, which are victims of the disease, contributing to economic losses and increasing the risk of infection for humans. Based on this affinity in the rabies cycle between bats and cattle, the objective of this study was to analyze the phylogenetic relationships of rabies virus samples in cattle and bats. The G gene of the rabies virus was chosen for this study because it is directly related to the infection process. Nucleotide sequences of the viral G gene were selected from GenBank for samples obtained from infected cattle and bats. Maximum parsimony analyses were conducted using the Molecular Evolutionary Genetics Analysis software. The Maxima Parsimony tree indicated a phylogenetic relationship between the G genes of both hosts, indicating that the virus evolved from bats to cattle. Analysis of parsimoniously informative sites revealed that the viral G gene carried specific mutations in each host. Knowledge of the evolutionary relationships between the rabies virus and its hosts is critical for identifying potential new hosts and the possible routes of infection for humans.

Keywords Bat; Cattle; Evolution; Viral disease; Viral infection.

RELAÇÕES FILOGENÉTICAS DO VÍRUS DA RAIVA (*LYSSAVIRUS RABIES*) EM DUAS DIFERENTES ESPÉCIES

RESUMO

A Raiva é uma zoonose fatal que infecta várias espécies de mamíferos. Os morcegos hematófagos são reconhecidos como hospedeiros do vírus da Raiva e sua principal fonte de alimento é o sangue de outros mamíferos, especialmente os bovinos. Quando se alimentam, os morcegos transmitem o vírus para o bovino os quais são vítimas da doença, contribuindo para perdas econômicas e riscos de infecção para humanos. Baseado nesta afinidade do ciclo da Raiva entre morcegos e bovinos, o objetivo deste estudo foi analisar as relações filogenéticas de amostras do vírus da Raiva em ambos os hospedeiros, bovinos e morcegos. O gene G do vírus da Raiva foi escolhido para esta pesquisa porque ele está diretamente relacionado ao processo de infecção. Sequências de nucleotídeos do gene G viral foram selecionadas no GenBank a partir de amostras obtidas de bovinos e morcegos infectados. Análises de Máxima Parcimônia foram conduzidas utilizando o *software Molecular Evolutionary Genetics Analysis*. A árvore de Máxima Parcimônia indicou uma relação filogenética entre o gene G de ambos os hospedeiros, indicando que o vírus evoluiu dos morcegos para os bovinos. A análise dos sítios parcimoniosamente informativos revelou que o gene G viral apresentou mutações específicas em cada hospedeiro. O conhecimento sobre as relações evolutivas do vírus da Raiva e seus

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hospedeiros é crucial para identificar nos hospedeiros potenciais e novas rotas possíveis de infecção para humanos.

Palavras-chave: Morcegos. Bovinos. Evolução. Doença viral. Infecção viral

RELACIONES FILOGENÉTICAS DEL VIRUS DE LA RABIA (*LYSSAVIRUS RABIES*) EN DOS ESPECIES DIFERENTES

RESÚMEN

La rabia es una zoonosis fatal que infecta a varias especies de mamíferos. Los murciélagos hematófagos son reconocidos como huéspedes del virus de la rabia y su principal fuente de alimentación es la sangre de otros mamíferos, especialmente del ganado. Al alimentarse, los murciélagos transmiten el virus al ganado que es víctima de la enfermedad, contribuyendo a pérdidas económicas y riesgos de infección para los humanos. Basado en esta afinidad del ciclo de la rabia entre murciélagos y ganado, el objetivo de este estudio fue analizar las relaciones filogenéticas de las muestras de virus de la rabia tanto en huéspedes, ganado y murciélagos. El gen G del virus de la rabia fue elegido para esta investigación porque está directamente relacionado con el proceso de infección. Las secuencias de nucleótidos del gen G viral se seleccionaron en GenBank a partir de muestras obtenidas de bovinos y murciélagos infectados. Los análisis de parsimonia máxima se realizaron utilizando el software Molecular Evolutionary Genetics Analysis. El árbol de Máxima Parsimônia indicó una relación filogenética entre el gen G de ambos huéspedes, indicando que el virus evolucionó de murciélagos a bovinos. El análisis de los sitios parsimoniosamente informativos reveló que el gen G viral presentaba mutaciones específicas en cada huésped. El conocimiento sobre las relaciones evolutivas del virus de la rabia y sus huéspedes es crucial para identificar huéspedes potenciales y nuevas posibles rutas de infección para humanos.

Palabras clave: Murciélagos. Ganado. Evolución. Enfermedad viral. Infección viral

INTRODUCTION

Rabies is an invariably fatal zoonosis caused by an acute viral infection of the central nervous system in mammals caused by a virus. It is distributed worldwide, except in Antarctica, Australia, Japan, and New Zealand [1]. In Brazil, two main variants have been described: one associated with the urban cycle, isolated from dogs, cats, and humans, and the other associated with the sylvatic cycle, isolated from bats and cattle [1-4]. Sylvatic cycle variants are endemic to several regions of Brazil [1, 5-6].

The rabies virus belongs to the family Rhabdoviridae and the genus *Lyssavirus* [1, 7]. It is a single-stranded negative-sense RNA virus [8]. The viral genome contains five genes distributed in the order 3' N-P-M-G-L 5,' which encode five proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and viral-dependent RNA RNA polymerase (L) [1, 9]. This glycoprotein mediates the adsorption of the virus onto the host cell, assists in viral denudation, and catalyzes the fusion of the endocytic membrane. It is the major determinant of pathogenicity and is responsible for the induction of neutralizing antibodies [9-10].

Lyssavirus rabies (RABV) is one of 17 members of the genus *Lyssavirus*, 15 of which use members of Chiroptera as exclusive reservoirs, showing the importance of this order as a

reservoir for the genus [1, 11-12]. In addition to bats, the virus affects several mammalian species, some of which are of economic importance, including cattle [1, 13-14].

In South America, rabies in herbivores occurs cyclically [6]. It is usually transmitted by the vampire bat *Desmodus rotundus*, which is the most common and abundant vampire bat. They inhabit forests and deserts and take shelter in tree hollows, caves, culverts, abandoned mines, and even civil construction sites [15-16].

Given the cyclical relationship between cattle and bats in the transmission of the rabies virus, the objective of this study was to analyze the phylogenetic relationship of the virus from two different species, bats and cattle.

MATERIAL AND METHODS

Glycoprotein (G) RNA sequences obtained from the GenBank database (<https://www.ncbi.nlm.nih.gov/genbank/>) were selected using the following search parameters: “nucleotide, glycoprotein, rabies lyssavirus, bat, partial”, the same was done for cattle, replacing the term “bat” with “cattle”.

European bat lyssavirus 1 (EBLV-1) was used as the outgroup. It belongs to the genus *Lyssavirus* and causes an infection similar to that of classic rabies. To obtain the sequence, “nucleotide, glycoprotein, european bat lyssavirus 1, partial” were used as parameters, selecting a sequence with a size of 743 bp.

MEGA-X software [17] (available at <https://www.megasoftware.net/>) was used for the data analysis. Sequence alignment was performed using MUSCLE and manually corrected. Subsequently, Maxima Parsimony was analyzed to generate the phylogeny. The rooting of the outgroup was verified, and the support of the branches was calculated using the bootstrap method with 1000 repetitions. With the generated tree, a 50% cutoff value was chosen for data reliability in the consensus tree.

In addition, individual analyses of parsimoniously informative sites were performed to assess the uniformity and differences between sequences in relation to the host.

RESULTS

The analyses involved 44 sequences, corresponding to 12 sequences from bats and 32 sequences from cattle (Table 1), with sizes ranging between 805 and 1572 bp. A total of 1595 base pairs were identified. Of these, 871 remained conserved, 724 were variable, and 412 were informative. Ten trees with a maximum parsimony level of 1138 were generated. The consistency and retention indexes were 0.78 and 0.83, respectively.

Figure 1 presents the phylogeny generated from Maximum Parsimony analysis. Bootstrap values greater than 50% were reproduced in the branches, indicating data reliability.

Phylogenetic analyses of the G gene revealed that the virus evolved from bats to cattle. The viral strains isolated from bats had a basal position compared to the bovine strain, except for three branches (AB247429.2, AB247426.2, and AB247427.2).

Through the individual analysis of each of the 412 thrifty informative sites, uniformity was observed within and divergence between groups (virus extracted from bovine and bat) in 21 positions. Table 2 lists the substitutions that have occurred.

DISCUSSION

The virus-host relationship provides an extraordinary context for studying evolutionary processes. Viruses are obligate intracellular parasites, and their evolution is inexorably linked to the biology of the host [12]. The biological relationship between viruses and their hosts involves a delicate balance between the host's immune system and virus escape mechanisms.

The G gene is the main focus of studies involving genetic analysis because it is used to classify RABV isolates according to lineage, variant, host species, geographic origin, and/or distribution [9-10, 18]. Glycoproteins are responsible for viral adsorption and denudation, catalyze endocytic membrane fusion, and are the main antigens of rhabdoviruses [1, 9-10, 18].

Although many mammalian species are susceptible to *Lyssavirus* infection, the virus can only establish transmission networks in small numbers, indicating important barriers to transmission between species [19].

Bats are the main reservoirs of all *Lyssavirus* [20]. Members of this genus are thought to have evolved from bats into carnivores [21]. This study corroborated the finding that the same phenomenon may have occurred in cattle. Parsimonious analysis of informative sites suggested that some mutations are crucial for the virus to infect other hosts, such as those described in Table 1. Greater uniformity in the viral sequences of bovine origin was observed compared to those of the bat. The bat is the natural host of RABV; therefore, the virus must present greater genetic diversity in this host. Not all variants of this virus seem to be important in cattle infection, as can be seen in the phylogenetic tree (Figure 1) and in the mutations observed at the parsimoniously informative sites (Table 2).

Previous studies have shown that the rate of nucleotide substitution varies significantly in viruses that infect bats, reflecting a combination of host and environmental factors [19], corroborating the results of the present study (Table 2). Marston *et al.* [22] addressed the host specificities of *Lyssavirus*, arguing that infection of a new host requires adaptations of the virus and that several factors must be considered, both in the host and in the virus. Lissaviruses have co-evolved with specific bat species in restricted host reservoirs [22].

The vampire bat species, *D. rotundus* is distributed throughout Latin America, from northern Mexico to Argentina [15-16]. As *D. rotundus* feeds preferentially on the blood of cattle, the introduction and dissemination of herds provides food resources that are widely available in several regions of South America and have the potential for livestock production. Thus, the endemic character of rabies in cattle is maintained, resulting in sporadic disease [23]. When interventions such as vaccination and surveillance are not implemented or are unsuccessful, the virus can establish itself as a new host [22].

No studies have been conducted that relate the evolution of the virus from bats to cattle. However, the work conducted by Badrane *et al.* [24] showed that there is strong phylogenetic support indicating that *Lyssavirus* in bats occurred much earlier than in carnivores and that the change of hosts — bats to carnivores — occurred in the history of the *Lyssavirus* genus. The authors conducted studies on viral strains isolated from raccoons, skunks, and bats. They observed that the lineages were phylogenetically related, arguing that rabies may have an independent autochthonous origin maintained by bats, with spillover to skunks and raccoons [24-25].

The maximum parsimony tree (Figure 1) generated had a consistency and retention index of 0.78 and 0.83, respectively. A consistency index was proposed to measure the level of

homoplasy; the higher its value, the lower the number of homoplasies present. The retention index measures the number of real synaptormorphies and considers the maximum number of changes in a character [26]. The results indicated that the sequences used were suitable for analysis.

Lines AB247429.2, AB247426.2, and AB247427.2 (Figure 1) were phylogenetically closer to cattle, possibly due to mutations that occurred during the process of adaptation to the host (Table 1), indicating that these are viable lines for cattle infection.

CONCLUSION

The results of this study indicated that the virus (RABV) evolved from bats to cattle. In addition, the data showed great variation in the sequences of nitrogenous bases, and some variants of the virus seem to have greater importance in the infection of cattle. Studies on virus-host relationships are important for understanding viral infections, making it possible to promote control measures to prevent future outbreaks of the disease.

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