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Molecular epizootiology of rabies associated with terrestrial carnivores in Mexico

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Abstract

Epizootiological patterns of rabies are described, using antigenic and genetic analysis of samples obtained from infected domestic and wild mammals in 20 Mexican states during 1976–2002. Two independent origins are suggested for rabies in Mexican carnivores. One group shares ancestry with canine rabies, while the other group appears to share a common origin with bat rabies in North America. More than 12 sublineages were found in rabid dog populations, suggesting at least six major spatio-temporal foci. Coyote rabies was found as independent enzootic foci that probably emerged via spillover from dog rabies, translocated from major foci in the southcentral and western regions of Mexico. One focus of gray fox rabies was widely distributed in northwestern Mexico, overlapping with a focus in the same species in the southwestern United States. A skunk rabies focus distributed in the northcentral Mexican states appears to share a common origin with bat rabies foci in North America, and is a close relative of southcentral skunk and raccoon rabies in the United States. Two other skunk foci share a common ancestor with canine rabies and were distributed in northwest Mexico and Yucatan.

Keywords: Epizootiology; Rabies; Terrestrial carnivores

1. Introduction

Rabies has been recorded since the first human civilizations, with the dog as the main transmitter (Wilkinson, 2002). In developed countries, traditional descriptive approaches have been applied to infer patterns of disease transmission to humans (Krebs et al., 2003). This approach has been reinforced by the process of rabies virus typing at the antigenic and genetic level (Bourhy et al., 1993; Rupprecht et al., 1987; Smith et al., 1992). The detection and analysis of subtle differences within the rabies virus proteins and genes have permitted the identification of viral variants specifically maintained by different animals, such as dogs, foxes, raccoons, skunks, and bats. The combined use of those latter approaches, together with an efficient surveillance system for disease detection in animal populations, have allowed detailed descriptions of the distributions of major rabies foci, as well as the likely hosts responsible for maintenance (Bourhy et al., 1999; Nadin-Davis et al., 1999; Smith et al., 1995). Data provided by such molecular approaches have permitted insights to virus-reservoir relationships, patterns of transmission and dissemination, as well as viral evolution (Badrane and Tordo, 2001; Bourhy et al., 1993, 1999; Holmes et al., 2002; Nadin-Davis et al., 1999;

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Table 1
Standard antigenic reactivity of monoclonal antibodies against rabies viruses in Latin America

MAb[a] identification number reservoir associated	C1	C4	C9	C10	C12	C15	C18	C19	AgV[b]
CVS/ERA/SAD/PAST[c]	+	+	+	+	+	+	+	+	Control[d]
Dog/Mongoose	+	+	+	+	+	+	–	+	1
Dog	+	+	–	+	+	+	–	+	2
Vampire bat	–	+	+	+	+	–	–	+	3
Tadarida brasiliensis	–	+	+	+	+	–	–	–	4
Vampire bat	–	+	+/–	+	+	+/–	–	+/–	5
Lasiurus cinereus	+/–	+	+	+	+	–	–	–	6
Arizona fox	+	+	+	–	+	+	–	+	7
South central skunk	–	+	+	+	+	+	+	+	8
Tadarida brasiliensis mexicana	+	+	+	+	+	–	–	–	9
Baja California Sur skunk	+	+	+	+	–	+	–	+	10
Vampire bat	–	+	+	+	–	–	–	+	11

[a] Antigenic variant (specific variant type).
[b] Monoclonal antibody identification number.
[c] Laboratory fixed strains.
[d] Positive control for monoclonal antibody reactivity.

Smith et al., 1995). In contrast, developing countries are faced with less than ideal surveillance in animal populations. The reduced resources available are prioritized for diseases with overwhelming human morbidity and mortality. Often, sample availability is limited and wholly dependent upon human rabies occurrence or the perception of major outbreaks. During the past decade, different analytical approaches have been applied to Mexican rabies virus samples. These approaches have provided insights regarding rabies virus diversity and the likely reservoirs responsible for transmission and maintenance (De Mattos et al., 1999; Loza-Rubio et al., 1999; Velasco-Villa et al., 2002). However, previous studies have been limited by sample sizes or the approach applied, to address the deeper descriptive epizootiology of rabies in Mexico. In the present work, we incorporate 138 sequences comprising a 30-year period. The objective of this study was the molecular analysis of rabies viruses associated with terrestrial carnivores in Mexico, describing updated disease distributions, suggested trends of interspecies transmission, and predicted patterns of dissemination, by using 88 amino acids of the nucleoprotein C terminus.

2. Materials and methods

2.1. Antigenic characterization

The rabies virus N protein was characterized with a panel of eight monoclonal antibodies, previously used to infer rabies virus reservoir species associations in Latin America and the Caribbean (Diaz et al., 1994). This reduced panel was able to identify 11 reactivity patterns associated with different animals involved with rabies virus maintenance and transmission in Mexico and South America (Table 1).

2.2. Samples and sequences

In the present study, 138 sequences were analyzed, including the outgroup represented by the *Lyssavirus* species European Bat Lyssavirus 2 (EBL2) and Duvenhage. The analysis included 61 new samples gathered over a 4-year period (1999–2002), from 20 Mexican states (Table 2). These were chosen on the basis of the antigenic variant to encompass the greatest Mexican rabies virus diversity as possible. The remaining 77 correspond to historical samples, already sequenced and antigenically typed, used in context to support a greater analysis in space and time (Table 3). All samples represent major terrestrial carnivore rabies enzootic foci, associated with raccoons, skunks, foxes, dogs and coyotes in the United States (US), Canada and Mexico (De Mattos et al., 1999; Nadin-Davis et al., 1993, 1999; Smith et al., 1992, 1995). Within the 138 sequences, five sequences representing fox rabies in Yugoslavia and Israel were included (David et al., 2000), plus nine sequences associated with rabid bats, to make a distinction between Old World and New World fox rabies, as well as between bat and terrestrial carnivore rabies, respectively (De Mattos et al., 1999; Smith et al., 1992).

2.3. Partial amplification of the nucleoprotein gene and its sequencing

Total RNA was extracted from infected brain tissue by using Trizol (Invitrogen, San Diego, CA, formerly, GIBCO-BRL, Inc.), according to the manufacturer's instructions. Complementary DNA was produced by RT-PCR, using primers 304 sense and 1066 anti-sense, as described (Smith, 2002). Purified amplicons were sequenced on an Applied Biosystems 377 DNA automated sequencer, as described (De Mattos et al., 1999; Smith et al., 1995).

2.4. Nucleoprotein region used for construction of the phylogeny

Fragments of 264 bp of the rabies virus nucleoprotein genes from nucleotide 1157 to 1420, with respect to nucleotide positions in SAD B19 nucleoprotein gene, were used (Conzelmann et al., 1990; De Mattos et al., 1999; Smith et