

Molecular analysis of urban rabies case from vampire bat in Corrientes, Argentina

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Abstract

Miranda, A.O.; Núñez, S.E.; Martínez, L.; Gury–Dohmen, F.R.; Cisterna, D.M.: Molecular analysis of an urban rabies case caused by a vampire bat in Corrientes, Argentina. *Rev. vet.* 20: 2, 77–80, 2009. In April 2006, rabies virus (RABV) was detected in a domestic cat from Corrientes City, Argentina. Molecular characterization identified *Desmodus rotundus* as the reservoir species. Genetic analyses of 22 RABV strains obtained from *D. rotundus* between 1988 and 2006 in Argentina, revealed the co–circulation of two genetic lineages in Corrientes Province. Alteration of the ecosystem of vampire bats by human intervention in this region could explain such changes in their behavior, as well as rabies virus circulation.

Key words: cat, vampire bat, rabies, molecular epidemiology.

Resumen

Miranda, A.O.; Núñez, S.E.; Martínez, L.; Gury–Dohmen, F.R.; Cisterna, D.M.: Análisis molecular de un caso de rabia urbana causado por un murciélago vampiro en Corrientes, Argentina. *Rev. vet.* 20: 2, 77–80, 2009. En abril de 2006 se detectó virus de la rabia (RABV) en un gato doméstico de la ciudad de Corrientes, Argentina. La caracterización molecular identificó al murciélago *Desmodus rotundus* como la fuente viral. El análisis genético de 22 cepas de RABV aisladas de *D. rotundus* obtenidos entre 1998–2006 en Argentina, reveló la cocirculación de dos linajes genéticos en la Provincia de Corrientes. La alteración por parte del hombre, del ecosistema del murciélago en la región, podría explicar los cambios en su comportamiento y en la circulación del virus de la rabia.

Palabras clave: gato, vampiro, rabia, epidemiología molecular.

INTRODUCTION

The last case of terrestrial rabies transmitted by dogs or cats in Corrientes Province was in the late 80s. At this time, viral activity is related to rabies transmitted by more common vampire bat, *Desmodus rotundus*. Two cases of human rabies associated with that species have occurred in Isla del Cerrito, Chaco Province (1997), and San Luis del Palmar, Corrientes Province (2001)⁸.

D. rotundus is a non–migratory colonial species that roosts in natural shelters or in places associated with human beings. They may use several refuges and can share them with other bat species. Their home range was described between 10 and 20 km². As vampire bats feed on mammalian blood, the distribution of bat populations is affected by food availability, specially livestock^{4,9}.

In April 2006, a domestic cat from the suburbs of Corrientes City, with no history of rabies vaccination, showed a strange and aggressive behavior. It attacked two human beings and several dogs and cats. Two days later it died. After that, postmortem brain tissue samples were obtained and tested positive for rabies virus (RABV). Molecular research identified to *D. rotundus* as the species reservoir and revealed the circulation of a previously not recognized rabies lineage in that region².

The aim of this study was to analyze the molecular characteristics of an urban rabies case associated a *D. rotundus* bat occurred in Corrientes city in 2006.

MATERIAL AND METHODS

Virus isolates and sequences. A rabies isolate obtained from a cat from Corrientes City, and others five samples collected few months later within a radius of 10–20 km² from the place where the cat was detected,

Table 1. Rabies isolates from Argentina analyzed in this study

isolates	city, province	source	year	genetic variant
C0Ct–Cte06	Corrientes City, Corrientes	cat	2006	DR2
C1Bv–Cte06	Paso de la Patria, Corrientes	bovine	2006	DR1
C2BvCha06	Isla del Cerrito, Chaco	bovine	2006	DR1
C3Bv–Cte06	Isla del Cerrito, Chaco	bovine	2006	DR1
C4Hr–Cte06	Km 1023, Corrientes	horse	2006	DR2
C5Bv–Cte06	Empedrado, Corrientes	bovine	2006	DR1
CteBv5–03	General Paz, Corrientes	bovine	2003	DR1
655bv–Sal03	Unknown, Salta	bovine	2003	DR2
Chabv129–02	Gral San Martín, Chaco	bovine	2002	DR1
Ctehm82–01	San Luis del Palmar, Corrientes	human	2001	DR1
Chahr90–01	Colonia Mixta, Chaco	horse	2001	DR1
Chabv86–01	La Leonesa, Chaco	bovine	2001	DR1
Ctebv79–00	San Miguel, Corrientes	bovine	2000	DR1
Ctebv55–00	Colonia Romero, Corrientes	bovine	2000	DR1
Ctebv77–00	Concepción, Corrientes	bovine	2000	DR1
Chabv74–00	Colonia Mixta, Chaco	bovine	2000	DR1
Chabv72–00	Puerto Veloz, Chaco	bovine	2000	DR1
Chabv78–00	Colonia Mixta, Chaco	bovine	2000	DR1
Chabv66–00	San Martín, Chaco	bovine	2000	DR1
Ctebv01–99	Beron de Estrada, Corrientes	bovine	1999	DR1
5578bv–Sal98	Unknown, Salta	bovine	1998	DR2
4379bv–Juy98	Unknown, Jujuy	bovine	1998	DR2
Batbbt123	3 de febrero, Buenos Aires	<i>Tadarida brasiliensis</i> bat	2001	TB
Sfemnb116	Rosario, Santa Fe	<i>Myotis nigricans</i> bat	2001	MY
StcHmbat80	Rio Turbio, Santa Cruz	<i>Histiotus montanus</i> bat	2000	HM
Lc597–BA00	Villa Ballester, Buenos Aires	<i>Lasiurus cinereus</i> bat	2000	LA
Saldg04	Oran, Salta	dog	2002	TR1
Saldg146	Oran, Salta	dog	2002	TR1
Chadg120	Pampa Almirón, Chaco	dog	2001	TR2
Chafx119	Laguna Limpia, Chaco	fox	1999	TR2

were analyzed. Others 16 historical rabies sequences from vampire related–cases of Corrientes, Chaco, Jujuy and Salta provinces and eight nucleotide sequences from insectivorous and terrestrial species, were included in this study for comparison purposes only (Table 1).

RT–PCR and DNA sequencing. Viral RNA was extracted from samples using TRIzol® (Invitrogen, Carlsbad, CA, USA), following the manufacturer's instructions. Reverse transcription and PCR amplification were achieved with primers 10g and 304, as previously described¹⁴. The amplified product was sequenced using the Taq Big Dye Terminator Cycle Sequencing Ready Reaction Kit, according to the manufacturer's protocol on an Applied Biosystems 377 DNA automated sequencer (Applied Biosystems Inc. Foster City, California, USA).

Phylogenetic analysis. A 264–bp region corresponding to the nucleoprotein gene located between nucleotides 1157 and 1420 and amino acids 363 to 450,

according to positions for the fixed laboratory strain SAD B19, was analyzed. Complete alignment was performed with Clustal X 1.8 software¹⁶. Matrix data was analyzed using Kimura 2 parameters as a method of nucleotide substitution and *Neighbor–Joining* to reconstruct the phylogenetic tree (MEGA version 4.1)¹⁵. The statistical significance of the phylogenies constructed was estimated by bootstrap analysis with 1000 pseudoreplicate data sets. Bootstrap values above 60% were considered significant.

RESULTS

A total of 22 rabies sequences obtained from vampire–related cases segregated into a group identified as DR, well supported by high bootstrap values as shown in Figure 1. The remaining Argentinean samples, represented by others rabies reservoirs, grouped into different genetic groups: TB (*Tadarida brasiliensis*), MY (*Myotis sp.*), HM (*Histiotus montanus*), LA (*Lasiurus cinereus*), TR1 and TR2 (Terrestrial 1 and 2) showing

