

Phylogenetic analysis of rabies virus isolated from herbivores in Minas Gerais and São Paulo border (2000-2009), Brazil¹

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ABSTRACT- Estévez Garcia A.I., Peixoto H.C., Silva S.O., Polo G., Alves A.J., Brandão P.E., Cunha E.M. & Richtzenhain L.J. 2014. **Phylogenetic analysis of rabies virus isolated from herbivores in Minas Gerais and São Paulo border (2000-2009), Brazil.** *Pesquisa Veterinária Brasileira* 34(12):1196-1202. Departamento de Medicina Veterinária Preventiva e Saúde Animal, Faculdade de Veterinária e Zootecnia, Universidade de São Paulo, Av. Prof. Dr. Orlando Marques de Paiva 87, Cidade Universitária, São Paulo, SP 05508-270, Brazil. E-mail: isaestgar@yahoo.com

Rabies transmitted by the hematophagous bat *Desmodus rotundus* represents a public health concern and a burden for the Brazilian livestock industry. Current evidence suggests that rabies occurrence is related to landscape characteristics, topography, hydrography, animal production systems and land use. However, a few studies have analyzed the possible connections among geographic factors and the molecular diversity of the rabies virus, furthering the understanding of the spatial and temporal dynamics of outbreaks. A study reported that the latest rabies epizootics in herbivores reported in the eastern region of São Paulo (close to the Minas Gerais border) occurred in two epidemic waves; the first was before 1998, and the other occurred after 1999. Using this evidence, the aim of the present study was to analyze cases of rabies in herbivores in the southern region of Minas Gerais (2000-2009) and their possible relationship with the aforementioned epidemics, considering the geographic characteristics of the region. Partial sequences of glycoprotein (539 nt) and nucleoprotein genes (414 nt) were obtained from 31 rabies virus isolates from herbivores. A phylogenetic tree was proposed for each genomic region using the Neighbor joining method, fixing the Kimura 2-parameter evolution model with a bootstrap level of 1,000 replications. Genetic sublineages were plotted on maps, considering rabies risk areas for herbivores in São Paulo, as well as topographic characteristics and hydrographic basins, to visualize any apparent distribution pattern influenced by those features. The phylogenetic trees had concordant topologies, suggesting a possible common origin for rabies outbreaks in herbivores along the SP/MG border, surrounding the less elevated portions of the Serra da Mantiqueira and along the hydrographic basins of Piracicaba/Jaguari, Paranaíba do Sul, Grande, Pardo and Mogi-Guaçu rivers. The co-circulation of several viral lineages was observed in some municipalities, possibly due to an overlapping of rabies outbreaks. Inferred protein sequences of both genes showed synonymous mutations, except among residues 20 to 200, corresponding to the external domain of the glycoprotein. This information prompted cooperation among the animal health services of both states to reinforce rabies control in the border area.

INDEX TERMS: *Desmodus rotundus*, rabies, glycoprotein, nucleoprotein, sequence analysis, DNA.

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RESUMO.- [Análise filogenética de isolados do vírus da raiva de herbívoros na fronteira de Minas Gerais e São Paulo (2000-2009), Brasil.]

A raiva transmitida por morcegos hematófagos da espécie *Desmodus rotundus* representa uma preocupação de saúde pública e causa de importantes prejuízos para a pecuária brasileira. A evidência atual sugere que a ocorrência de raiva está relacionada às características da paisagem, topografia, hidrografia, sistemas de produção animal e usos da terra. Contudo, existem poucos estudos que analisem as possíveis conexões entre fatores geográficos e a diversidade molecular do vírus da raiva, permitindo a compreensão da dinâmica espacial e temporal dos focos de raiva. Um desses trabalhos estabeleceu que a última epizootia de raiva dos herbívoros registrada no leste do estado de São Paulo (na fronteira com Minas Gerais), aconteceu em duas ondas epidêmicas, sendo a primeira em 1998 e, em 1999, a segunda. Considerando esta evidência, o intuito do presente estudo foi analisar casos de raiva em herbívoros na região sudeste de Minas Gerais (2000-2009) e sua possível relação com a epidemia previamente mencionada, incluindo as características geográficas da região. Foram obtidas sequências parciais dos genes da glicoproteína (539 nt) e da nucleoproteína (414 nt) a partir de 31 isolados de vírus da raiva procedentes de herbívoros. Foi proposta uma árvore filogenética para cada região genômica usando o método de Neighbor joining, fixando o modelo evolutivo Kimura 2 - parâmetros com um nível de bootstrap de 1000 replicações. As sublinhagens genéticas foram localizadas sobre mapas, considerando as áreas de risco para raiva dos herbívoros em São Paulo, assim como as características topográficas e bacias hidrográficas com o intuito de visualizar qualquer padrão aparente de distribuição segundo essas características. As duas árvores filogenéticas mostraram topologias concordantes, sugerindo uma possível origem comum para os surtos que aconteceram ao longo da fronteira SP/MG, ao redor das porções menos elevadas da Serra da Mantiqueira e acompanhando as bacias hidrográficas dos rios Piracicaba/Jaguari, Paranaíba do Sul, Grande, Pardo e Mogi-Guaçu. Foi possível observar circulação de várias linhagens virais simultaneamente em alguns municípios, possivelmente por causa de sobreposição de surtos. As sequências de proteína inferidas a partir dos dois genes mostraram mutações sinônimas, excetuando aquelas encontradas entre os resíduos 20 a 200, correspondentes ao domínio externo da glicoproteína. Esta informação salienta a importância da cooperação entre as autoridades sanitárias de ambos os estados para reforçar o programa de controle da doença nas áreas limítrofes.

TERMOS DE INDEXAÇÃO: *Desmodus rotundus*, raiva, glicoproteína, nucleoproteína, análise de sequências, DNA.

INTRODUCTION

Rabies transmitted by hematophagous bat *Desmodus rotundus* represents a public health concern and has been a burden to the Brazilian livestock industry since 1911, when the first rabies outbreak in bovines and equines was officially reported in the south of the country. Since then, rabies has exhibited endemic, epidemic and sporadic presen-

tation in Brazil (Brasil 2009). Two studies have addressed rabies prevalence in Minas Gerais. The first consisted of a regression analysis of cases reported from 1976 to 1997. The study allowed the detection of the annual growing prevalence of rabies with outbreaks in 75% of the municipalities. Additionally, a geographic pattern was observed, spreading from "Triângulo Mineiro" to the center and south/southeast regions and from the "Zona da Mata" toward the North of the State (Silva et al. 2001). The second study included surveillance data from 1998 to 2006. During this period, the number of positive municipalities fluctuated, but there was an overall decreasing trend (Menezes et al. 2008).

It is believed that the drastic environmental transformations that occurred in the south and southeast regions of Minas Gerais due to the substitution of forests by pastures for livestock may have facilitated rabies dispersion in urban and rural ecosystems (Silva et al. 2001).

Regarding São Paulo, it is well documented that mountainous regions are more prone to shelter *D. rotundus*, due to favorable conditions, such as the higher rainfall index and milder temperatures (Gomes, 2011). Considering the existence of different degrees of rabies risk in livestock according to geographic areas, Animal Health authorities divided the State into epidemic, endemic and sporadic areas. The western region of São Paulo is classified as sporadic and the eastern region is epidemic, particularly the border between Minas Gerais and São Paulo along the Paraíba do Sul basin (Peres, 2008).

Current evidence suggests that rabies occurrence depends on several factors such as landscape characteristics, topography, hydrography, animal production systems and land use (Grenfell et al. 2004). River basins constitute one of the most important factors related to the presence of *D. rotundus* (Tadei et al. 1991).

In Brazil, few studies have explored the possible connections among geographic factors and the molecular diversity of the rabies virus. One study suggested that six main genetic lineages with molecular differences occur in São Paulo and Minas Gerais and that their distribution is apparently affected by rivers, chains of mountains and specific biomes (Kobayashi et al. 2008). Interestingly, an analysis of rabies virus sequences from both states, reveals that their viral subpopulations are closely related (Macedo et al. 2010). In contrast, other studies propose that geographic factors are not always involved in the genetic diversification of the rabies virus (Heinemann et al. 2002).

These types of studies are valuable because they help track the spatial and temporal dynamics of outbreaks by taking advantage of the mutation rate of the rabies virus. These mutations emerge as consequence of the interactions between the virus and its animal hosts, and because of ecological and geographical aspects that shape the distribution of the host populations (Nadin-Davis 2007, Real et al. 2005, Wunner 2007). Analyzing the phylogenetic relationships among rabies virus isolates also provides a more detailed way to comprehend epizootic dynamics; one example is a study by Carnieli et al. (2009); who examined evolutionary changes in glycoprotein and nucleoprotein

These sequences were included in the analysis to explore a possible genetic relationship of the Minas Gerais cases with previous and contemporary cases that occurred in São Paulo. Sequence alignments were performed with Clustal W (version 1.83) and trees were inferred using the neighbor-joining method with MEGA software (version 4) (Tamura et al. 2007), fixing the Kimura 2-parameter evolution model with a bootstrap level of 1,000 replications. Bootstrap values exceeding 70% were considered to indicate phylogenetic association. The cases in Minas Gerais studied here, in addition to all of the cases considered in both genetic analyses, were plotted on maps using ArcGis 10.2 (Environmental System Research Institute, Inc., Redlands, CA, USA), considering rabies risk areas for herbivores in São Paulo, as described by Peres (2008), and the topographic characteristics and hydrographic basins to visualize whether some genetic lineages had a trend to circulate in certain regions according the epidemiological status and key geographical features of the areas under study, as described by Kobayashi et al. (2008).

RESULTS

DNA amplicons of 914 nt and 1,478 nt of glycoprotein gene and nucleoprotein gene, respectively, were obtained after sequencing. In spite of several attempts to improve sequencing performance, it was not possible to use the full-length amplicons because of the poor-quality indexes obtained during the stringent evaluation process. The sequences were trimmed to consider only the reliable segments, as described above.

After editing the DNA sequences, two different genomic regions of rabies virus were obtained and analyzed independently: a glycoprotein gene fragment with a length of 539 nucleotides (positions from nucleotides 3,378 to 3,917 according to the Pasteur Virus complete genome; GenBank Accession number M13215), encoding for the glycoprotein external domain, and a 414 nucleotide fragment from the nucleoprotein gene (positions from nucleotides 299 to 712). The sequences were deposited into GenBank under accession numbers KF805574 to KF805627.

Figures 2 and 3 show the phylogenetic trees based on the glycoprotein and nucleoprotein gene analysis, respectively. Relevant information regarding the animal hosts, the chronology, the geographic origin and distribution of the rabies isolates is detailed below. Figure 2 shows the two main lineages according to the glycoprotein gene, labeled I and II. *Lineage I* is composed of Brazilian rabies viruses isolated from livestock and bats (hematophagous and non-hematophagous). *Lineage II* contains clusters of Brazilian rabies viruses from humans, dogs, cats and pigs. *Lineage I* showed several genetic sublineages designated as Ia to Ie as follows. *Sublineage Ia* contains rabies virus isolates from the epidemic area of São Paulo (1999-2008) and the southeastern border of Minas Gerais (2000-2009), including two from *Artibeus lituratus* in the sporadic area (the municipality of Dracena, in 2002, AB496680). *Sublineage Ib* includes bovine and vampire bat samples from the epidemic and endemic areas of São Paulo (1989-2004) and sequences from frugivorous bats from the sporadic (1998 and 2003) and endemic areas (1994). *Sublineage Ic* contains cases that occurred in the epidemic area of São Paulo (1995-2004), the endemic area (1992), the sporadic area

(1998), and two sequences from the Rio de Janeiro (1997 and 1998). *Sublineage Id* contains three cases from Tocantins and one from Maranhão. *Sublineage Ie* contains cases from Maranhão and Paraíba. *Lineage II* is comprised of

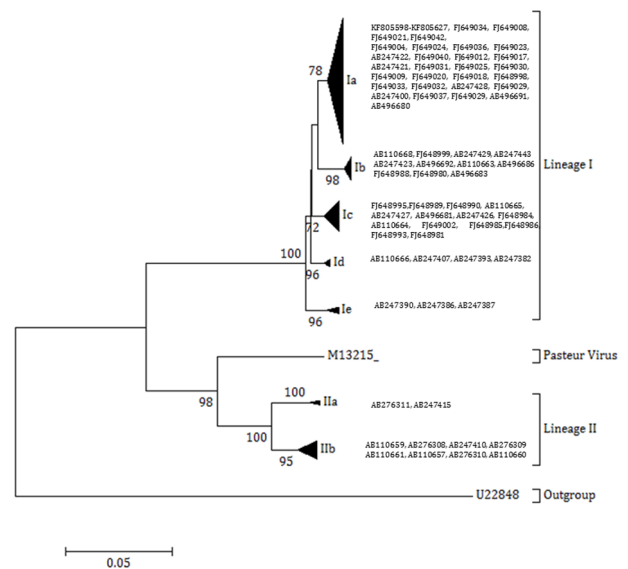


Fig.2. Phylogenetic tree based on partial sequence of the glycoprotein gene (539 nucleotides). Thirty-one partial sequences of glycoprotein gene from rabies cases in herbivores from several municipalities in the southern region of Minas Gerais from 2000 to 2009 (539 nucleotides). Also shown are the sequences retrieved from GenBank from herbivores and chiropterans (hematophagous and non-hematophagous) from different geographic regions of Brazil. The clades were condensed in order to help with visualization.

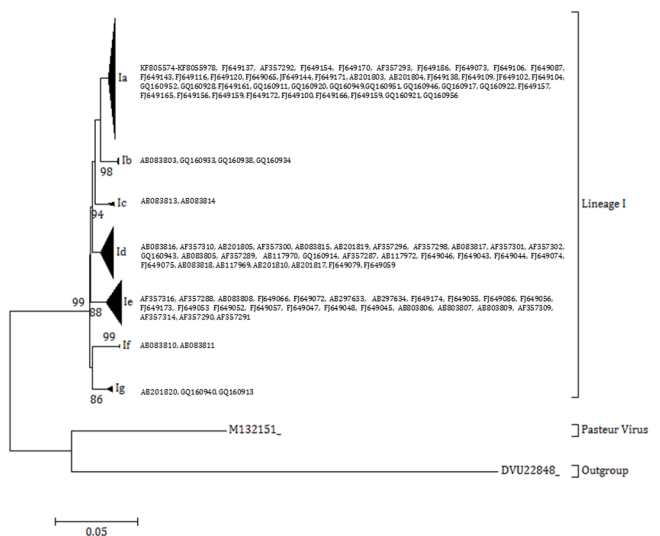


Fig.3. Phylogenetic tree based on a partial sequence of the nucleoprotein gene (414 nucleotides). Twenty-three partial sequences of the nucleoprotein gene from rabies cases in herbivores from several municipalities in the southern region of Minas Gerais, from 2000 to 2009 (414 nucleotides). Also shown are the sequences retrieved from GenBank from herbivores and chiropterans (hematophagous and non-hematophagous) from different geographic regions of Brazil. The clades were condensed in order to help with visualization.

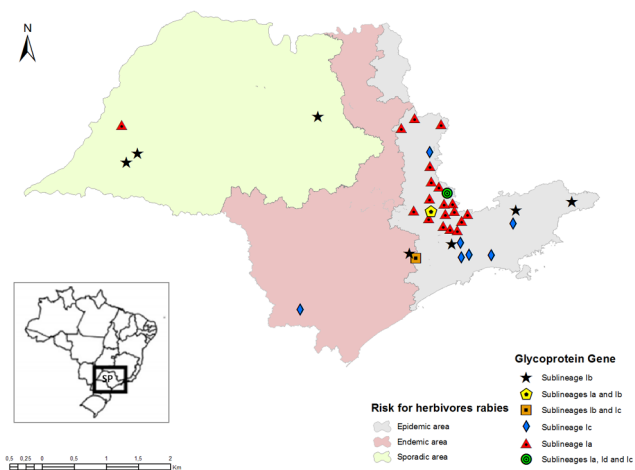


Fig.4. Geographic distribution of genetic sublineages of rabies virus according to risk areas in São Paulo state, glycoprotein gene.

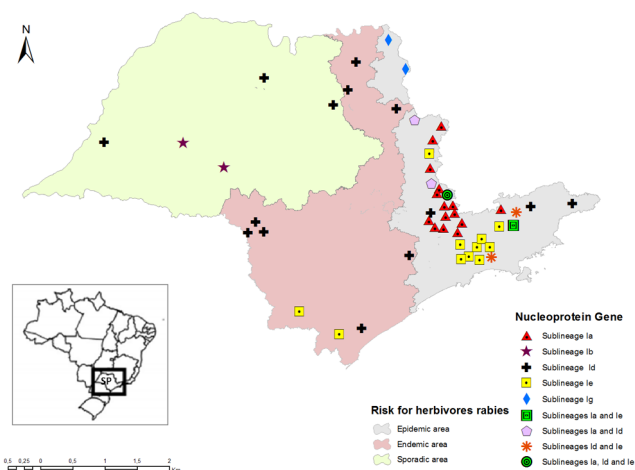


Fig.5. Geographic distribution of genetic sublineages of rabies virus according to risk areas in São Paulo state, nucleoprotein gene.

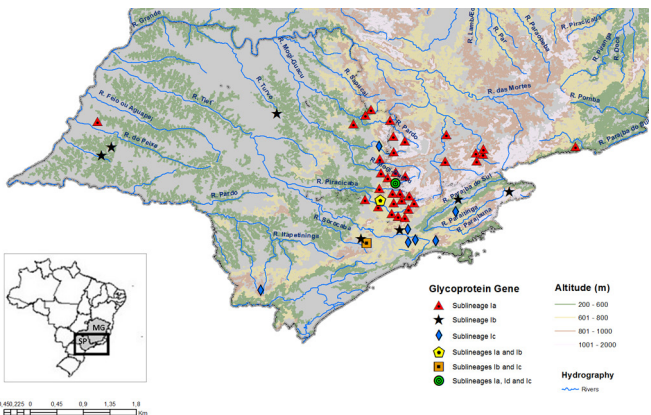


Fig.6. Geographic distribution of viral sublineages according to topography and hydrography of studied area, glycoprotein gene.

genetic sublineages *Ila* and *Ilb* as follows. Sublineage *Ila* contains dog (Maranhão) and human (Rio de Janeiro) viruses. Sublineage *Ilb* contains viruses from carnivores and

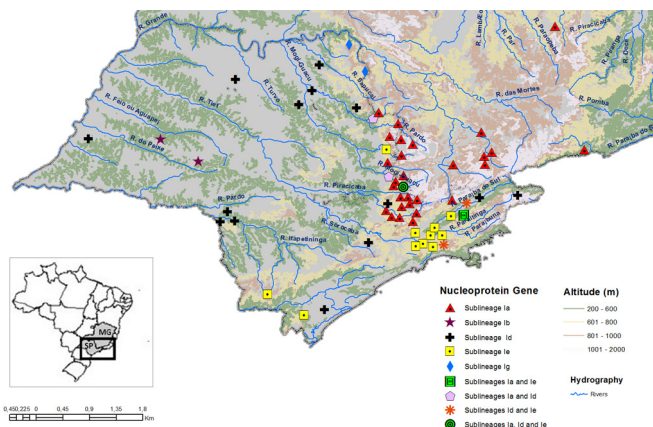


Fig.7. Geographic distribution of viral sublineages according to topography and hydrography of studied area, nucleoprotein gene

humans (unknown geographic origin), humans and dogs (Goiás), and pigs (Paraná).

Figure 3 shows the three main genetic divisions according to the nucleoprotein gene. *Lineage I* resembles the *Lineage I* based on the glycoprotein gene and is composed of Brazilian rabies virus isolates from herbivores and bats. *Lineage I* can be divided into seven sublineages from *Ia* to *Ig*. Sublineage *Ia* includes isolates from São Paulo (1998-2008) and Minas Gerais (2000-2009) from the epidemic area. Sublineage *Ib* incorporates two isolates from the sporadic region for rabies in São Paulo that occurred in 2008 and one isolate from Goiás (1998). Sublineage *Ic* contains sequences from Mato Grosso. Sublineage *Id* contains sequences from the epidemic area, and the endemic and sporadic areas for rabies in São Paulo (1988-2001). Sublineage *Ie* contains sequences from the epidemic area (1992-1998) and the endemic area of São Paulo (1986, 1992, 1999) and two isolates from Rio de Janeiro. Sublineage *If* contains two isolates from Tocantins. Sublineage *Ig* contains one sequence from Goiás and two from the epidemic area of São Paulo (2007-2008).

Figures 4 and 5 show the geographical distribution of the genetic sublineages described in the phylogenetic analysis for glycoprotein and nucleoprotein genes, respectively, according to the risk areas for rabies in São Paulo. In these maps, only the information concerning the area under study (São Paulo/Border of Minas Gerais with São Paulo) is illustrated. No data were available regarding the risk areas in Minas Gerais.

The amino acid sequences were inferred from the nucleotide sequences with BioEdit software. Several non-synonymous substitutions were observed in the glycoprotein sequence among residues 20 to 200 and in the nucleoprotein sequences among residues 77 to 214.

The cases analyzed in the present study occurred along southern border of Minas Gerais with São Paulo, coinciding with a chain of mountains named Serra da Mantiqueira. This region presents a topographic mosaic with altitudes ranging from 200 to 1500 meters. Figures 6 and 7 show that all of the cases studied here occurred in the less elevated portions of the Serra da Mantiqueira (600-1,000m

above sea level), which belongs to an extensive area of the Atlantic forest biome that is irrigated primarily by the Piracicaba-Jaguari, Paranaíba do Sul, Grande, Pardo and Mogi-Guaçu river basins.

DISCUSSION

Both phylogenetic trees suggested that the cases from Minas Gerais (2000-2009) most likely have the same genetic origin as the São Paulo epizootics. Phylogeny based on the glycoprotein gene establishes a closer relationship between the sequences obtained in the present study and the cases that occurred after 1999 because they are included in the same clade. The topologies of both phylogenetic trees are mutually consistent, reinforcing the hypothesis of a possible common origin; moreover, the glycoprotein and nucleoprotein genes of

the rabies virus circulating in the livestock and bats of this geographic area are highly conserved, as previously stated by Carnieli et al (2009).

Regarding the polymorphism analysis, only seven, non-synonymous amino acid substitutions in residues 20 to 200 were detected in the external domain of the glycoprotein. This region is the most conserved portion of the glycoprotein, primarily because of structural restrictions (Benmansour et al. 1992, Kissi et al. 1995). All mutations detected in the nucleoprotein gene were synonymous.

Although *sublineage Ia* (according to the glycoprotein and nucleoprotein genes) is predominant in the epidemic area, there is spatial and chronological overlapping with other subpopulations of rabies viruses. This observation was previously reported by Kobayashi et al. (2008). In addition, in some municipalities, there are two or three viral lineages circulating simultaneously (e.g., Itapira, Mococa, Morungaba, Pindamonhangaba, Salesópolis, Socorro, Taubaté and Vargem Grande Paulista). A similar behavior in rabies virus isolates from different species of non-hematophagous bat was found in Argentina, where the municipalities of Santa Fé, and Rosario, among others, showed co-circulation of multiple virus lineages (Piñero et al. 2012).

Conversely, the opposite situation can be observed when the same genetic sublineage is found at distant geographic points. One example is *sublineage Ia* (based on the glycoprotein gene analysis); this lineage was circulating in bovine and *D. rotundus* from municipalities in the epidemic area and was found in one frugivorous bat (*Artibeus lituratus*) in Dracena in the sporadic area, 690 km away from the epidemic area. Although there is a close phylogenetic relationship among *D. rotundus* and *A. lituratus* rabies virus isolates in some cases, according to Shoji et al. (2004), new evidence suggests that it is possible that the presence of species-specific genetic lineages can adapt to certain hosts. The occurrence of the same lineages of rabies virus isolates from non-hematophagous bats circulating in non-adjacent locations reported by Kobayashi et al. (2005) supports this idea and was subsequently confirmed by Oliveira et al. (2010) and Fahl et al. (2012). *Sublineage Id*, showed a similar behavior (based on the nucleoprotein gene analysis).

Regarding to maps of the geographic distribution of genetic lineages according to risk areas, only *Sublineage Ia*

(glycoprotein and nucleoprotein) showed an apparent tendency to circulate in epidemic regions.

The distribution of several clusters is separated by geographic boundaries such as mountain ranges. Another interesting finding is that several cases involving the same sublineage were distributed along the same river (Kobayashi et al. 2008). The results of the present study are discordant with the first statement and compatible with the second one because *sublineage Ia* was observed across the Serra da Mantiqueira. A possible explanation is that bat colonies could have moved between São Paulo and Minas Gerais using the less elevated areas as a corridor. The presence of rabies cases along Piracicaba-Jaguari, Paranaíba do Sul, Grande, Pardo and Mogi-Guaçu river basins agrees with other researchers, who reported a similar epidemiological behavior for this region (Tadei 1991, Trajano 1996).

The spatial and chronological overlapping of rabies cases is most likely related to the mobility of *D. rotundus*, which frequently circulates among shelters and caves in a radius of 2-3 km with a maximum distance of 20 km (Delpietro et al. 1992, Vieira et al. 2013). This behavioral characteristic enhances the spread of rabies among migrating bat populations, with the concomitant involvement of livestock in the transmission cycle as an accidental host (Trajano 1996, Real et al. 2005).

Major environmental changes (i.e., a sudden shift from agriculture to livestock farming, construction of highways and railways and hydroelectric plants) are among the factors that can force bat colonies to change their migration patterns, roosts and habitats (Estrada & Coates-Estrada 2002, Evelyn & Stiles 2003, Quesada et al. 2004).

CONCLUSIONS

São Paulo and Minas Gerais rabies virus isolates are more genetically related compared with sequences from other geographic regions.

Phylogenetic analysis of two genes suggested that Minas Gerais cases (2000-2009) most likely have the same genetic origin as the São Paulo epizootics (1997-2002).

The glycoprotein gene analysis showed a closer relationship of the Minas Gerais cases with rabies virus isolates involved in the second epidemic wave in São Paulo that occurred after 1999.

Sublineage Ia (according to the glycoprotein and nucleoprotein genes) had a tendency to circulate in the epidemic area of São Paulo.

A chronological and spatial overlapping of viral subpopulations was observed primarily in the epidemic area of São Paulo could be related to bat colonies migration and most of the mutations found in both genes were synonymous except those in residues 20 to 200, which correspond to the external domain of glycoprotein.

This information prompted cooperation among the animal health services of both states to reinforce rabies control in the border area of São Paulo and Minas Gerais.

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REFERENCES

- Benmansour A., Brahim M., Tuffereau C., Coulon P., Lafay F. & Flamand A. 1992. Rapid sequence evolution of street rabies glycoprotein is related to the highly heterogeneous nature of the viral population. *Virology* 187:33-45.
- Bourhy H. & Kissi B.T. 1993. Taxonomy and evolutionary studies on lyssaviruses with special reference to Africa. *Onderstepoort J. Vet. Res.* 60(4):277-282.
- Brasil 2009. Manual de Controle da Raiva em Herbívoros. 2ª ed. Ministério da Agricultura Pecuária e Abastecimento, Brasília, DF.
- Brinkman F.S.L. & Leipe D.D. 2001. Phylogenetic analysis, p.349. In: Baxevanis A. & Ouelette B.F. (Ed.), *Bioinformatics: a practical guide to the analysis of genes and proteins* 2nd ed. Wiley Interscience, New York.
- Carnieli P., Castilho J.G., Fahl W.O., Verás N.M.C. & Timenetsky M. 2009. Genetic characterization of rabies virus isolated from cattle between 1997 and 2002 in an epizootic area in the state of São Paulo, Brazil. *Virus Res.* 144:215-245.
- Delpietro H.A., Marchevsky N. & Simonetti E. 1992. Relative population densities and predation of the common vampire bat (*Desmodus rotundus*) in natural and cattle-raising areas in north-east Argentina. *Prev. Vet. Med.* 14:13-20.
- Estrada A. & Coates-Estrada R. 2002. Bats in continuous forest, forest fragments and in an agricultural mosaic habitat-island at Los Tuxtlas, Mexico. *Biol. Conserv.* 103:237-245.
- Evelyn M.J. & Stiles D.A. 2003. Roosting requirements of two frugivorous bats (*Sturnira lilium* and *Artibeus intermedius*) in fragmented Neotropical forest. *Biotropica* 35:405-418.
- Gomes N.M. & Monteiro A.M.V. 2011. Raiva bovina no estado de São Paulo e sua distribuição espacial entre 1992 e 2003 [Bovine rabies in São Paulo state and its spatial distribution between 1992 and 2003]. *Arq. Bras. Med. Vet. Zootec.* 63:279-286.
- Grenfell B.T., Pybus O.G., Gog J.R., Wood J.L.N., Janet M., Mumford J.A. & Holmes E.C. 2004. Unifying the epidemiological and evolutionary dynamics of pathogens. *Science* 303:327-333.
- Fahl W.O., Carnieli Jr P., Castilho J.G., Carrieri M.L., Kotait I., Yamamoto K., Oliveira R.N. & Brandão P.E. 2012. *Desmodus rotundus* and *Artibeus* spp bats might present distinct rabies virus lineages. *Braz J. Infect. Dis.* 16:545-551.
- Hall T. 1999. BioEdit: a user friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* 41:95-98.
- Heinemann M.B., Fernandes-Matioli F.M., Cortez A., Soares R.M., Sakamoto S.M., Bernardi F., Ito F.H., Madeira A.M. & Richtzenhain L.J. 2002. Genealogical analyses of rabies virus strains from Brazil based on N gene alleles. *Epidemiol. Infect.* 128:503-511.
- Kissi B., Tordo N. & Bourhy H. 1995. Genetic polymorphism in the rabies virus nucleoprotein gene. *Virology* 209:526-537.
- Kobayashi Y., Hirano S., Mochizuki N., Itou T., Ito F.H. & Sakai T. 2008. Molecular and geographic analyses of vampire bat-transmitted cattle rabies in central Brazil. *BMC Vet. Res.* 4:44-52.
- Kobayashi Y., Sato G., Shoji Y., Sato T., Itou T., Cunha E.M., Samar S.I., Carvalho A.A., Nociti D.P., Ito F.H. & Sakai T. 2005. Molecular epidemiological analysis of bat rabies viruses in Brazil. *J. Vet. Med. Sci.* 67:647-652.
- Ito M., Itou T., Shoji Y., Sakai T., Ito F.H., Arai Y., Takasaki T. & Kurane I. 2003. Discrimination between dog-related and vampire bat-related rabies viruses in Brazil by strain-specific reverse transcriptase-polymerase chain reaction and restriction fragment length polymorphism analysis. *J. Clin. Virology* 26:317-330.
- Macedo C.I., Carnieli P., Fahl W.O., Oliveira J., Oliveira R., Achkar S.M., Castilho J.G., Carrieri M.L. & Kotait I. 2010. Genetic characterization of rabies virus isolated from bovines and equines between and 2008 in the states of São Paulo and Minas Gerais. *Revta Soc. Bras. Med. Trop.* 43:116-120.
- Menezes F.L., Silva J.A., Moreira E.C., Meneses J.N.C., Magalhães D.F., Barbosa A.D. & Oliveira C.S.F. 2008. Distribuição espaço-temporal da raiva bovina em Minas Gerais, 1998 a 2006. *Arq. Bras. Med. Vet. Zootec.* 60:566-573.
- Miyaki C.Y., Russo C.A. & Pereira S.L. 2012. Capítulo 13: reconstrução filogenética, métodos geométricos, p.124. In: Matioli S. & Fernandes F.M. (Eds), *Biologia Molecular e Evolução*. 2ª ed. Holos Editora, Riberão Preto, SP.
- Nadin-Davis S. 2007. Molecular epidemiology, p.69-122. In: Jackson A.C. & Wunner W.H. (Eds), *Rabies*. Elsevier, Ontario, Canada.
- Oliveira R.N., Souza S.P., Vaz Lobo R.S., Castilho J.G., Macedo C.I., Carnieli Jr P., Fahl W.O., Achkar S.M., Scheffer K.C., Kotait I., Carrieri M.L. & Brandão P.E. 2010. Rabies virus insectivorous bats: implication of the diversity of the nucleoprotein and glycoprotein genes for molecular epidemiology. *Virology* 405:352-360.
- Orciari L.A., Niezgoda M., Hanlon C.A., Shaddock J.H., Sanderlin J.H., Yager P.A. & Rupprecht C.E. 2001. Rapid clearance of SAG-2 rabies virus from dogs after oral vaccination. *Vaccine* 19:4511-4518.
- Peres N.F. 2008. Profilaxia e controle da raiva dos herbívoros domésticos no Estado de São Paulo, Sudeste do Brasil no período 1997-2007. *Dissertação de Mestrado*, Universidade de São Paulo, São Paulo, SP.
- Piñero C., Dohmen F.G., Beltrán F., Martínez L., Novaro L., Russo S., Palacios G. & Cisterna D.M. 2012. High diversity of rabies viruses associated with insectivorous bats in Argentina: presence of several independent enzootics. *PLOS Negl. Trop. Dis.* 6:e1635.
- Quesada M., Stoner K.E., Lobo J.A., Herrerías-Diego Y., Palacios-Guevara C., Munguía-Rosas M.A., O-Salazar K.A. & Rosas-Guerrero V. 2004. Effects of forest fragmentation on pollinator activity and consequences for plant reproductive success and mating patterns in bat-pollinated bombacaceous trees. *Biotropica* 36:131-138.
- Real L.A., Henderson J.C., Biek R., Snaman J., Jack T.L., Childs J.E., Stahl E., Waller L., Tinline R. & Nadin-Davis S. 2005. Unifying the spatial population dynamics and molecular evolution of epidemic rabies virus. *Proc. Natl Acad. Sci. USA* 102:12107-12111.
- Sato G., Itou T., Shoji Y., Miura Y., Mikami T., Ito M., Kurane I., Samara S.I., Carvalho A.A., Nociti D.P., Ito F.H. & Sakai T. 2004. Genetic and phylogenetic analysis of glycoprotein of rabies virus isolated from several species in Brazil. *J. Vet. Med. Sci.* 66:747-753.
- Sato G., Kobayashi Y., Shoji Y., Sato T., Itou T. & Ito F.H. 2006. Molecular epidemiology of rabies from Maranhão and surrounding states in the northeastern region of Brazil. *Arch. Virol.* 151:2243-2251.
- Sato G. Determination of phylogenetic and genetic feature of the glycoprotein gene of the human rabies virus strains in Brazil <<http://www.ncbi.nlm.nih.gov/nuccore/AB276308>> (Unpublished)
- Shoji Y., Kobayashi Y., Sato G., Itou T., Miura Y., Mikami T., Cunha E., Samara S., Carvalho A.B., Nociti D., Ito F.H., Kurane I. & Sakai T. 2004. Genetic characterization of rabies viruses isolated from frugivorous bat (*Artibeus* spp) in Brazil. *J. Vet. Med. Sci.* 66(10):1271-1273.
- Silva J.A., Moreira E.C., Haddad J.P.A., Modena C.M. & Tubaldini M.A.S. 2001. Distribuição temporal e espacial da raiva bovina em Minas Gerais, 1976 a 1997. *Arq. Bras. Med. Vet. Zootec.* 53. Available from <<http://dx.doi.org/10.1590/S0102-09352001000300001>> Access on 19 Sept. 2014.
- Tadei V., Gonçalves C.A., Pedro W.A., Tadei W.J., Kotait I. & Arieta C. 1991. Distribuição do Morcego Vampiro, *Desmodus rotundus* (Chiroptera, Phyllostomidae) no Estado de São Paulo e a raiva dos animais domésticos. *Secretaria de Agricultura e Abastecimento de São Paulo, São Paulo, SP.*
- Tamura K., Dudley J., Nei M. & Kumar S. 2007. MEGA 4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol. Biol. Evol.* 24:1596-1599.
- Tordo N., Poch O., Ermine A., Keith G. & Rougeon F. 1986. Walking along the rabies genome: is the large G-L intergenic region a remnant gene? *Proc. Natl Acad. Sci. USA* 83:3914-3918.
- Trajano E. 1996. Movements of cave bats in Southeastern Brazil, with emphasis on the population ecology of the common vampire *Desmodus rotundus* (Chiroptera). *Biotropica* 28:121-129.
- Vieira L.F.P., Pereira S.R.F.G., Carnieli P., Tavares L.C.B. & Kotait I. 2013. Phylogeography of rabies virus isolated from herbivores and bats in the Espírito Santo State, Brazil. *Virus Genes* 46:330-336.
- Wunner W.H. 2007. Rabies virus, p.23-68. In: Jackson A.C. & Wunner W.H. (Eds), *Rabies*. Elsevier, Ontario, Canada.