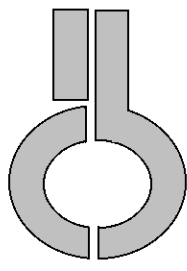


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Clinical & Molecular Virology Laboratory
Institute of Biomedical Sciences
University of São Paulo (USP), Brazil



Antigenic and Genetic Studies of the Rabies Virus Isolated in Brazil

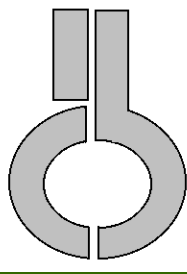
Silvana Regina Favoretto

Scientific Researcher, Pasteur Institute of São Paulo
Rabies Research Group



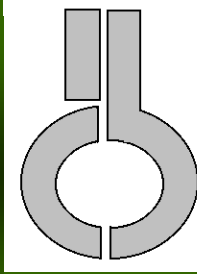
Rabies Transmitted by Vampire Bats
in the Amazon Region: Expert Consultation
(Brasília, 10-11 October 2006)

Historical Context



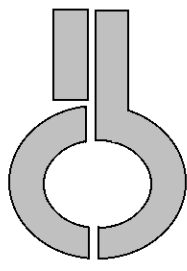
- 1996
 - Study of the molecular epidemiology of rabies virus isolated from Latin America (PAHO/CDC/Health Ministry)
 - Methodology
 - ▣ Antigenic characterization
 - ▣ Genetic characterization
 - Objective
 - ▣ Knowledge of the rabies virus variants present in Latin America and the probable reservoirs

Antigenic Typing of Brazilian Rabies Virus Samples Isolated from Animals and Humans, 1989–2000



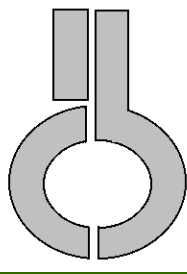
- Six antigenic variants previously defined in the monoclonal antibody panel.
- Six different profiles that were not compatible with those in the panel: five in non vampire bats and one in humans and marmoset.
- *AgV3-Desmodus rotundus* was isolated from most of the studied animal species, showing the importance of *D. rotundus* as reservoir of the rabies virus in Brazil.

Antigenic Characterization of Rabies Virus Isolates in Brazil, Using a Panel of Monoclonal Antibodies



AgV		Host
2	Dog	Cat, dog, <i>C. thous</i> , human, <i>P. cancrivorous</i> sheep
3	Vampire bat	Cat, cattle, dog, goat, hematophagous and no hematophagous bats, horse, human, sheep, swi
4	<i>T. brasiliensis</i>	Insectivorous bat
5	Vampire bat from Venezuela	Bat, cat, <i>C. thous</i>
6	<i>L. cinereus</i>	Insectivorous bat
	Lab	Coati, Human, PV, CVS

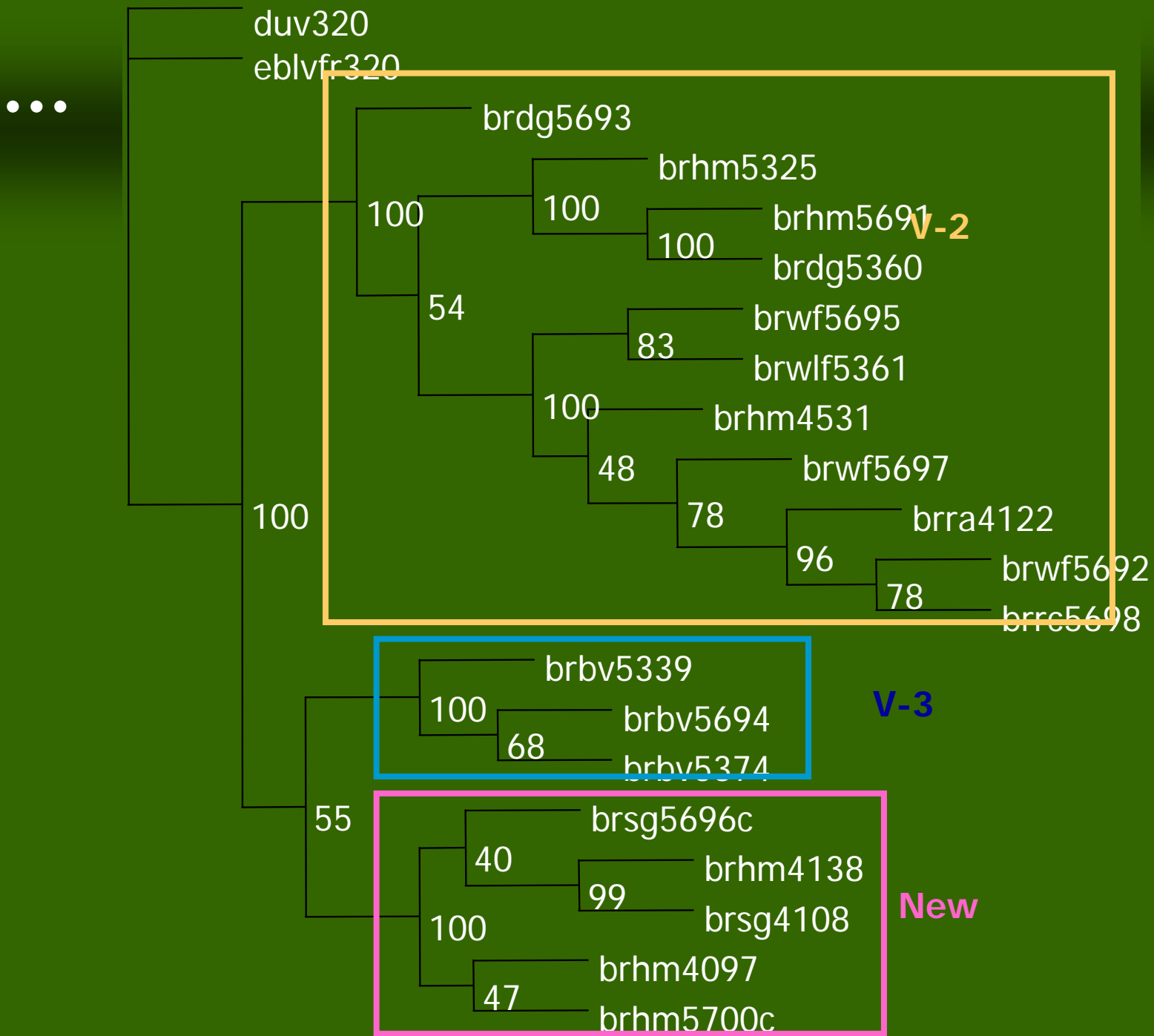
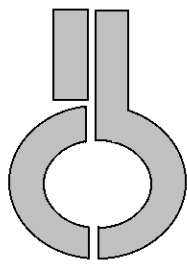
Rabies in Marmosets (*Callithrix jacchus*), Ceará, Brazil



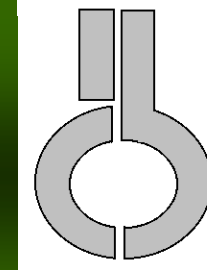
- The high degree of genetic homology among the Ceará rabies viruses, in conjunction with surveillance data
 - Epidemiologic linkage between the viruses
 - Confirmed that they represent a unique and independent rabies endemic cycle.
 - Evidence of *C. jacchus* acting as a rabies reservoir.



→ See examples in next 2 slides



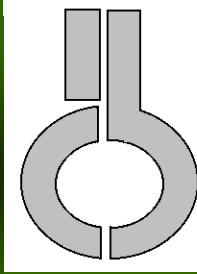
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	C1	C4	C9	C10	C12	C15	C18	C19	AgV
CVS/ERA-SAD/PAS	+	+	+	+	+	+	+	+	Lab
Perro/ Mang	+	+	+	+	+	+	-	+	1
Perro	+	+	-	+	+	+	-	+	2
Vampiro	-	+	+	+	+	-	-	+	3
Tad brasilie	-	+	+	+	+	-	-	-	4
Vampiro V	-	+	v	+	+	v	-	v	5
Lasiu ciner	v	+	+	+	+	-	-	-	6
Zorr de Ariz	+	+	+	-	+	+	-	+	7
Zorri cen/ su	-	+	+	+	+	+	+	+	8
Tad br mex	+	+	+	+	+	-	-	-	9
Baja CS zor	+	+	+	+	-	+	-	+	10
Vampiro	-	+	+	+	-	-	-	+	11
<i>C. jacchus</i>	-	-	+	+	-	-	-	-	*

* Pre established antigenic pattern in Brazil (Favoretto et al., 2002)

Source: Favoretto, SR; Matos, CC; Moraes, NB; Araújo, FAA; Matos, CA. 2001.

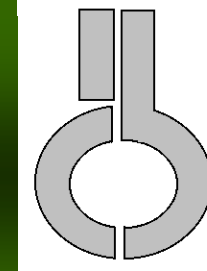


Serologic and Molecular Epidemiology of the Rabies Virus Isolated in a Bat Colony from *Histiotus velatus*, São Paulo City, Brazil

- The rabies virus positivity was 12%.
- 42% of bats showed titers up to 0.5 IU/ml
 - 62,5% of these bats had titers above 1.07 IU/ml
- The antigenic characterization showed a different profile, not established in the panel
- The genetic rabies variant profile identified a monophyletic group indicating that species as the most probable reservoir of this strain.

→ See examples in next 2 slides

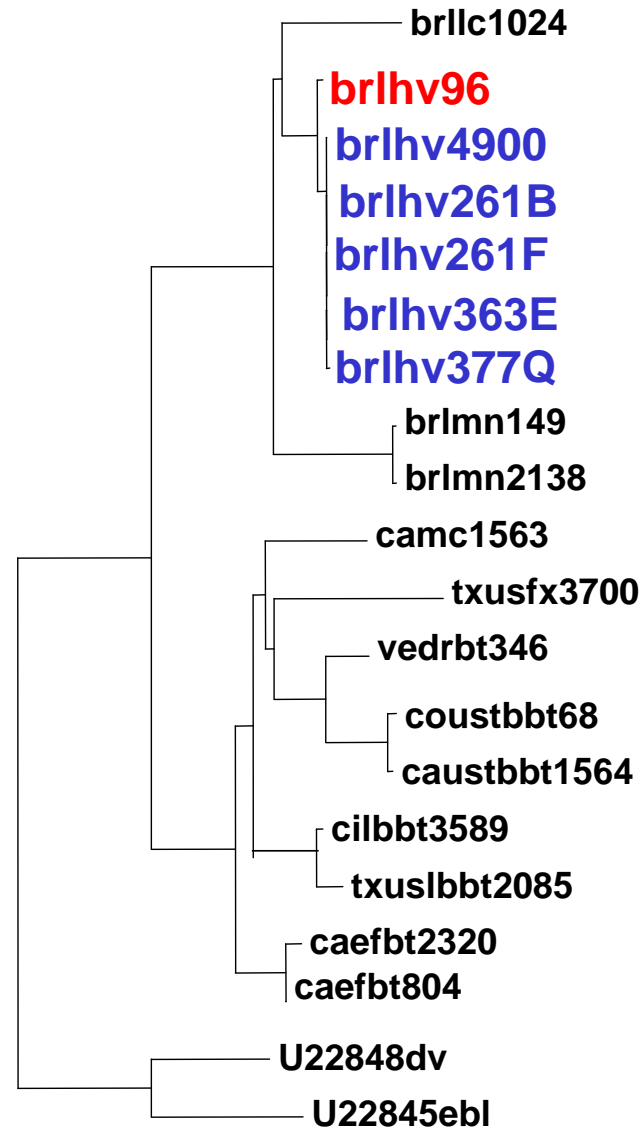
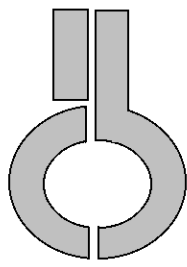
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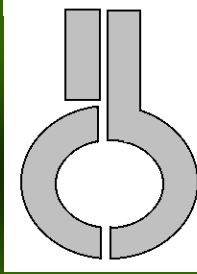
	C1	C4	C9	C10	C12	C15	C18	C19	AgV
CVS/ERA-SAD/PAS	+	+	+	+	+	+	+	+	Lab
Perro/Mangusto	+	+	+	+	+	+	-	+	1
Perro	+	+	-	+	+	+	-	+	2
Vampiro	-	+	+	+	+	-	-	+	3
<i>T. brasiliensis</i>	-	+	+	+	+	-	-	-	4
Vampiro V	-	+	V	+	+	V	-	V	5
<i>L. cinereus</i>	V	+	+	+	+	-	-	-	6
Zorri de Arizona	+	+	+	-	+	+	-	+	7
Zorri centro/sul	-	+	+	+	+	+	+	+	8
<i>T. brasiliensis</i>	+	+	+	+	+	-	-	-	9
Baja CS Zorri	+	+	+	+	-	+	-	+	10
Vampiro	-	+	+	+	-	-	-	+	11
Marmoset	-	-	+	+	-	-	-	-	*
<i>Histiopus velatus</i>	-	-	-	-	+	-	-	-	*

CDC/Atlanta - USA

* Pre established antigenic pattern in Brazil (Favoretto et al., 2002)

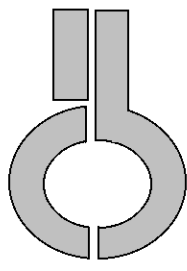


Genetic and Antigenic Characterization of the Rabies Virus Isolated from Insectivorous Bats, *Nyctinomops sp.*



- The antigenic profile showed was not pre-established in the monoclonal antibodies panel.
- The *Nyctinomops sp.* samples segregated alone showing this species as the possible reservoir of that strain.
- The isolation of the virus shows that the virus is nearby and present in the population of bats.
- The existence of rabid bats in urban area represents a risk of rabies infection for humans and domestic animals.

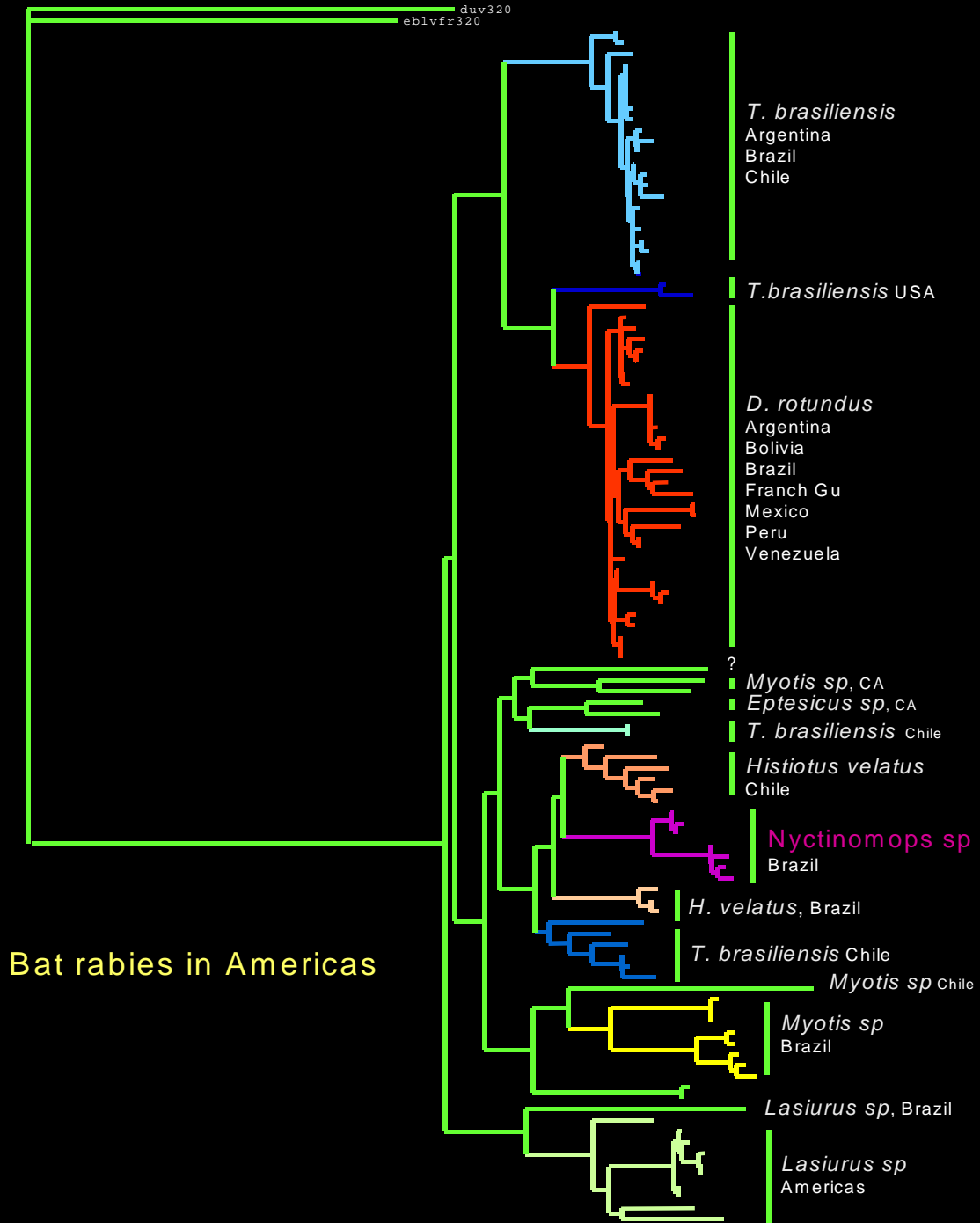
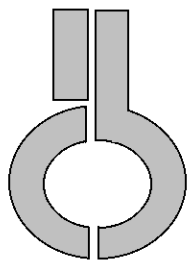
→ See examples in next 2 slides



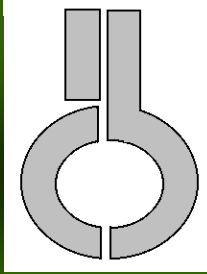
	C1	C4	C9	C10	C12	C15	C18	C19	AgV
CVS/ERA - SAD/PAST	+	+	+	+	+	+	+	+	Lab
Dog / Mongoose	+	+	+	+	+	+	-	+	1
Dog	+	+	-	+	+	+	-	+	2
Vampire bat	-	+	+	+	+	-	-	+	3
Tadarida brasiliensis	-	+	+	+	+	-	-	-	4
Vampire Venezuela	-	+	V	+	+	V	-	V	5
Lasiurus cinereus	V	+	+	+	+	-	-	-	6
Fox Arizona	+	+	+	-	+	+	-	+	7
Little Fox SC	-	+	+	+	+	+	+	+	8
T.brasiliensis (Mex.)	+	+	+	+	+	-	-	-	9
Little Fox (SC - Mex)	+	+	+	+	-	+	-	+	10
Vampire bat (Mex.)	-	+	+	+	-	-	-	+	11
Marmoset	-	-	+	+	-	-	-	-	*
Histiotus velatus	-	-	-	-	+	-	-	-	*
Nyctinomops sp	-	+	-	+	+	-	-	-	*
Nyctinomops sp	-	+	-	+	+	-	+	+	*

CDC/Atlanta - USA

* Pre established antigenic pattern in Brazil (Favoretto et al., 2002)

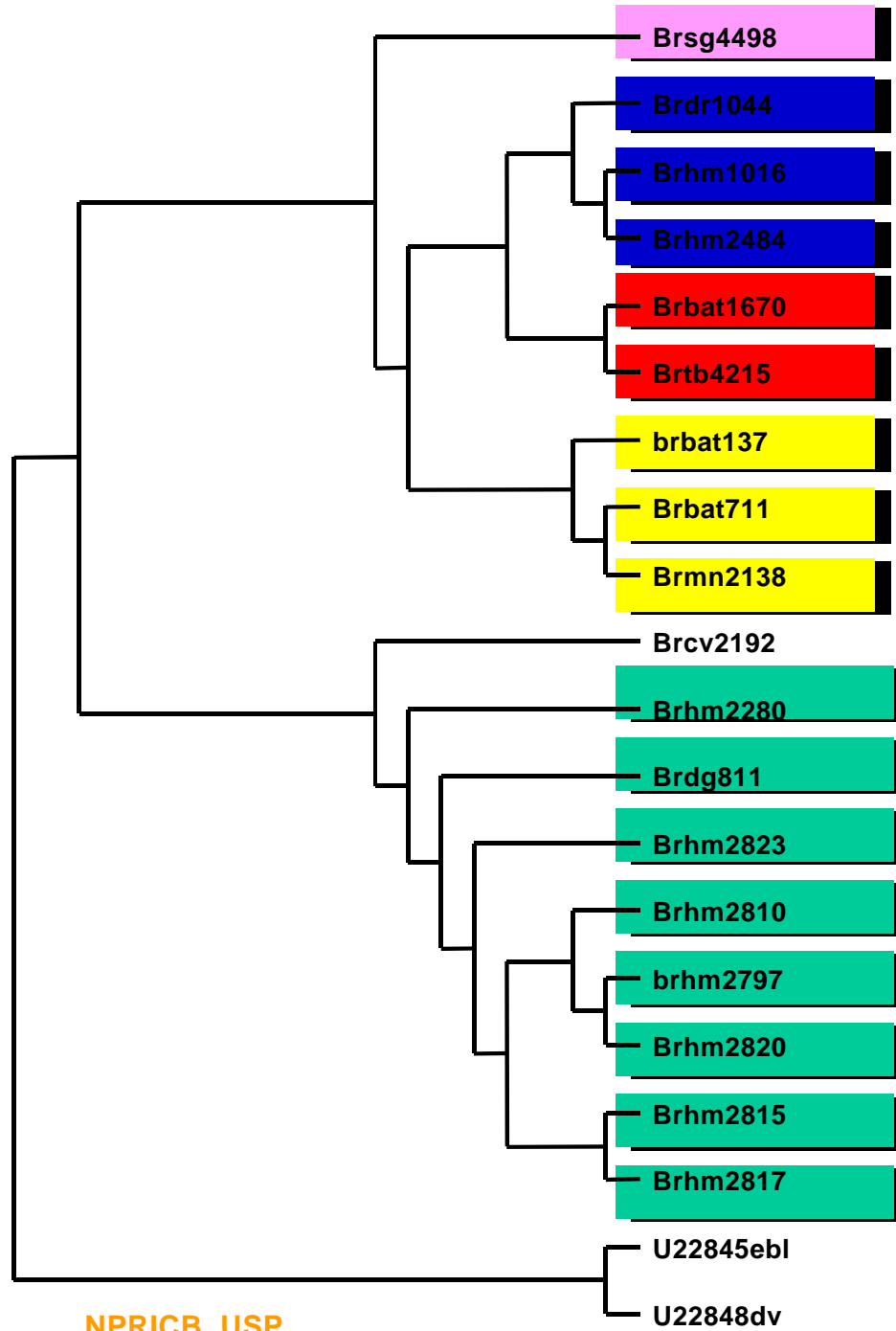


Rabies Virus Detection and Phylogenetic Studies in Samples from an Exhumed Human

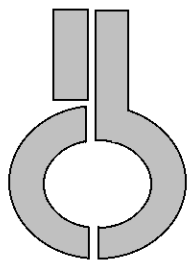


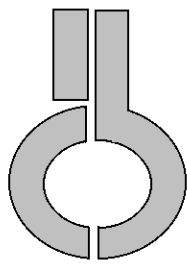
- The sample segregated with isolates obtained from another human rabies sample variant 3 and the *Desmodus rotundus* sample.
- These results demonstrated the importance of the RT-PCR technique for rabies virus detection, especially in cases where the sample is not suitable for all laboratory assays, like decomposed ones.

→ See examples in next 2 slides



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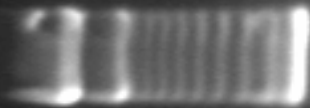


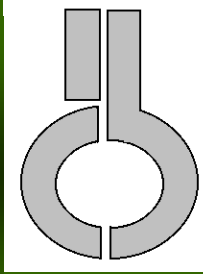


Positive Sample Brhm1016

Negative Sample

100pb DNA ladder





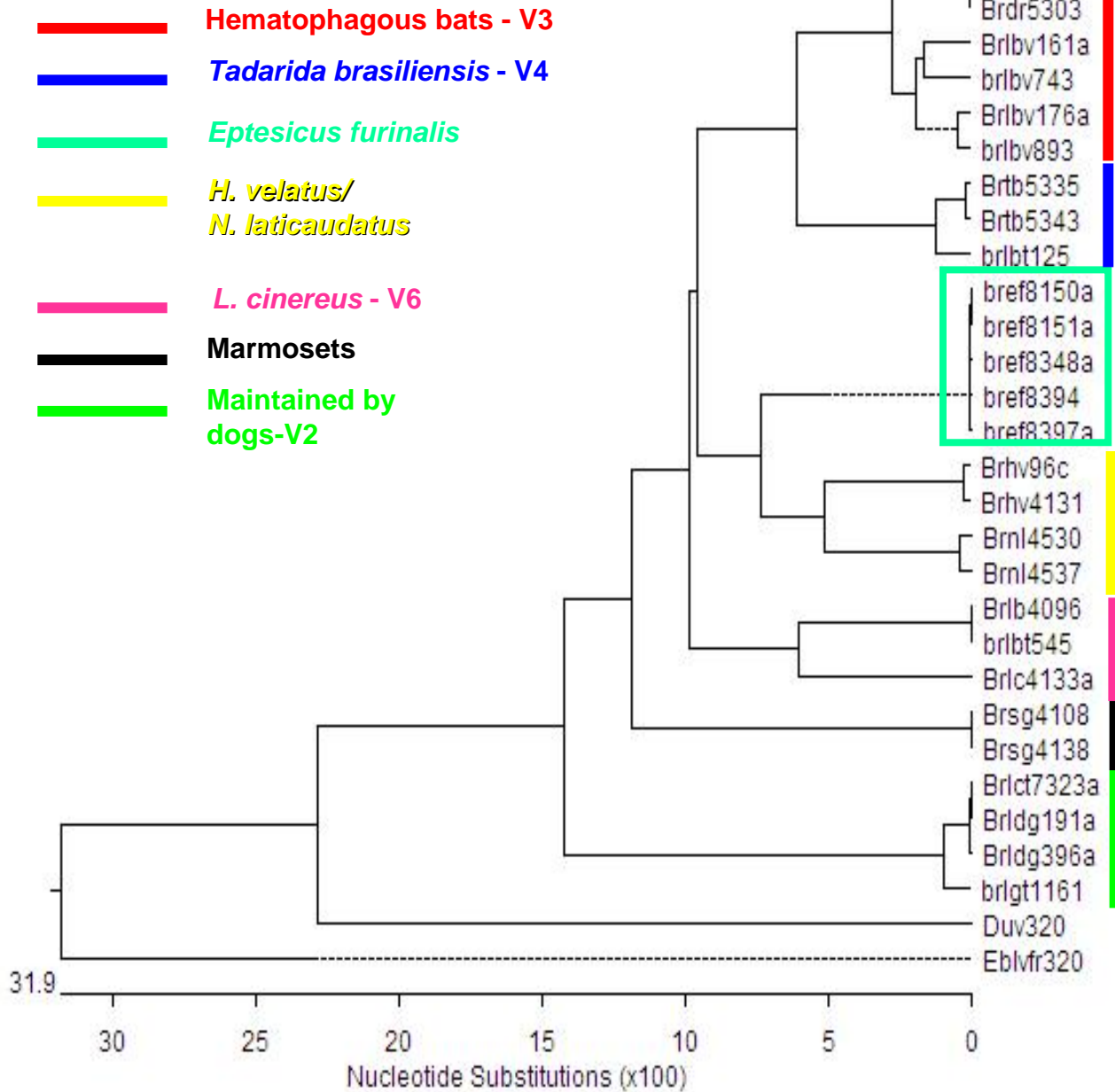
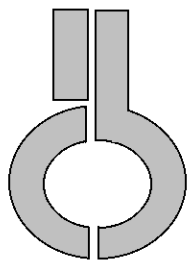
Rabies Virus Isolated from an Insectivorous Bats Colony *Eptesicus furinalis*: Virus Characterization and Epidemiological Study

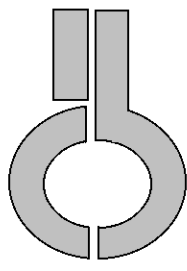
- A different profile, not previously established in the monoclonal panel.
- The viruses segregated in an independent branch, indicating this specie as the probable reservoir of this strain in this area.
- The presence of rabies virus in insectivorous bats in urban areas.

→ See examples in next 2 slides

Sources: Favoretto et al, 2002

Almeida, MF; Favoretto, SR; Amatuzzi, E; Martorelli, LFA; Trezza-Netto, J; Campos, ACA; Ozahata, CH; Silva, ALC; Sodré, MM; Durigon, EL. 2005.

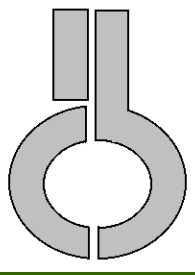




Reaction patterns of Brazilian Rabies Virus Isolates to Monoclonal Antibodies of Possible Different Antigenic Variants

Year isolation	Host	C1	C4	C9	C1 0	C1 2	C1 5	C1 8	C1 9
1996	<i>H. velatus</i>	-	-	-	-	+	-	-	-
1999	<i>H. velatus</i>	-	-	-	-	+	-	-	-
2000	<i>H. velatus</i>	-	-	-	-	+	-	-	-
1995	<i>L. borealis</i>	-	+	+	+	-	-	-	-
1999	<i>M. abrasus</i>	-	-	-	-	+	-	-	-
1999	<i>M. albecens</i>	-	-	-	-	+	-	-	-
1997	<i>N. macrotis</i>	-	+	-	+	+	-	+	+
1997	<i>N. laticaudatus</i>	-	-	-	-	+	-	-	-
1998	<i>N. laticaudatus</i>	-	+	-	+	-	-	-	+
1999	<i>N. laticaudatus</i>	-	-	-	-	+	-	-	-
1996	<i>Cat</i>	-	+	-	+	+	-	-	-
1998	<i>E. furinalis</i>	-	-	-	-	+	-	-	-
1998	<i>E. auripendulus</i>	-	-	-	-	+	-	-	-
2000	<i>E. furinalis</i>	-	-	-	-	+	-	-	-
2000	<i>C. thous</i>	-	-	-	-	+	-	-	-
1998	Human	-	-	+	+	-	-	-	-
1998	Human	-	-	+	+	-	-	-	-
1998	<i>Callithrix jacchus</i>	-	-	+	+	-	-	-	-

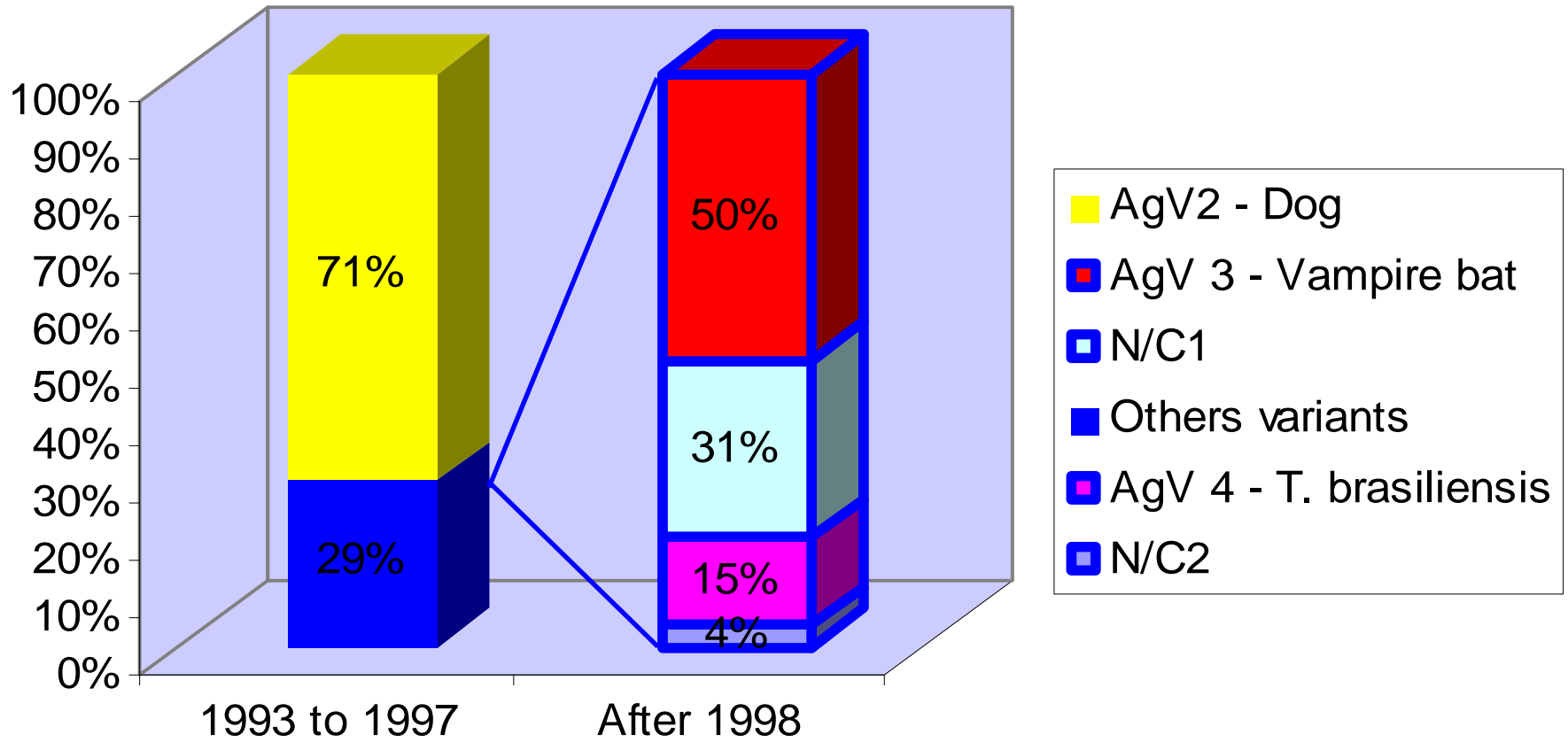
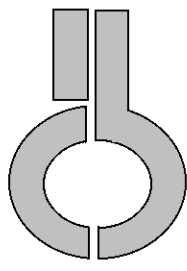
Source: Favoretto et al, 2002

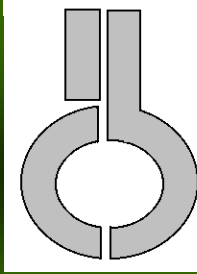


Rabies in Northwestern São Paulo State, Brazil: Antigenic and Genetic Characterization of Virus Isolates

- *From 1993 to 1997*
 - 71% rabies cases (dogs, cats, cattle, horses) related to AgV2-dog
 - 29% rabies cases (cattle from rural areas with the presence of vampire bats attacks)
- *After 1998*
 - No rabies cases related to AgV2-dog
 - 50% rabies cases related to Agv3-*D. rotundus*
 - 50% rabies cases related to insectivorous bats variants.

See graph on next page →

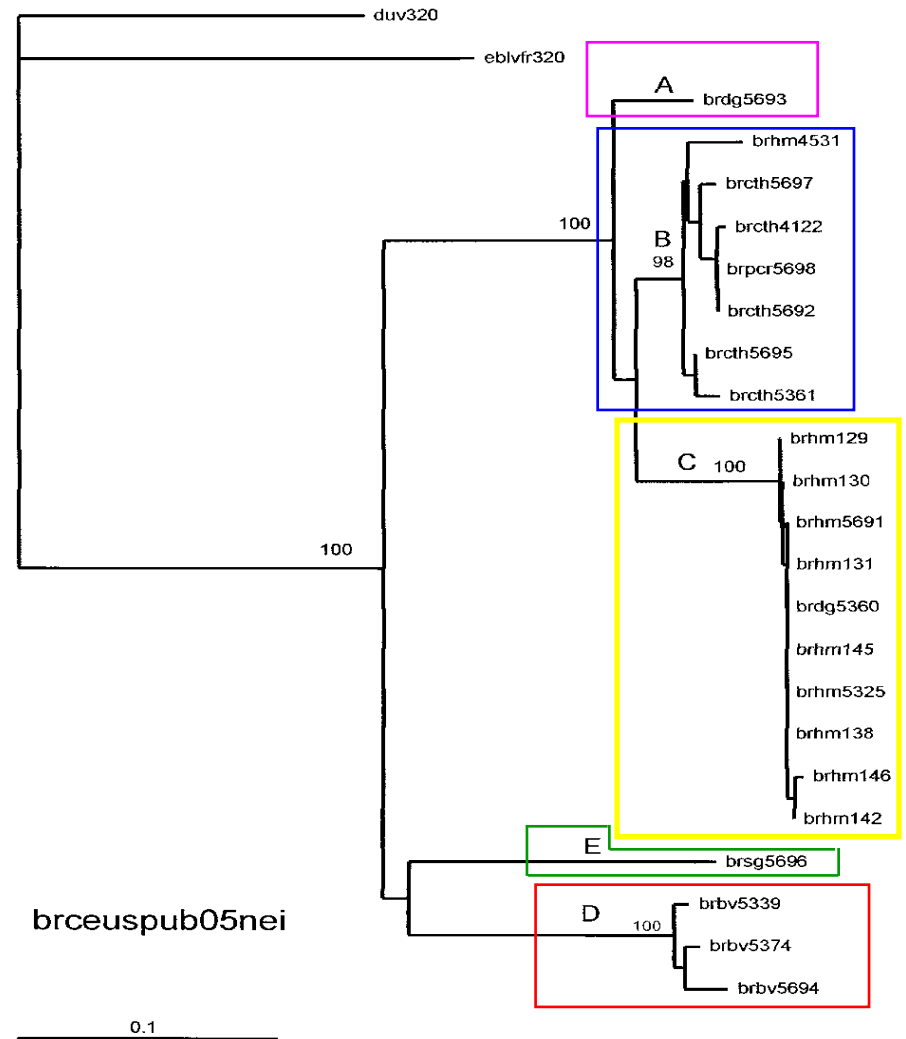
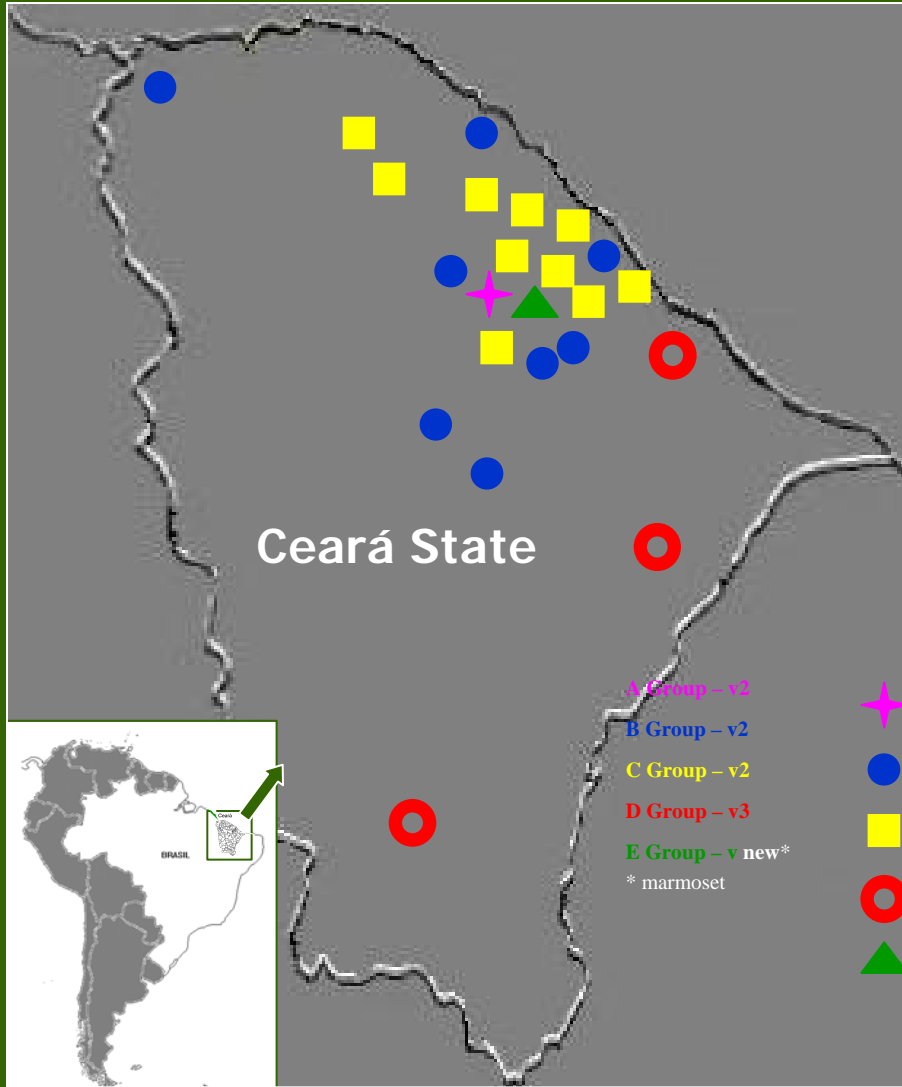
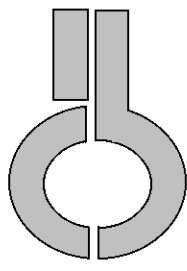




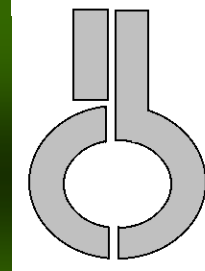
Emergence of the Rabies Virus Maintained by Dogs in Humans and Terrestrial Wildlife and the Different Endemic Cycles of the Rabies Virus in Ceará State, Brazil

- The phylogenetic analyses revealed segregation in 5 lineages:
 - One lineage related to vampire bat *D. rotundus*.
 - One lineage related to marmoset *C. j. jacchus*.
 - Three lineages imply in the existence of at least three overlapping endemic cycles related to AgV2-dogs
 - Two of these maintained by dogs.
 - One by wildlife.

See map and chart on next 2 pages →

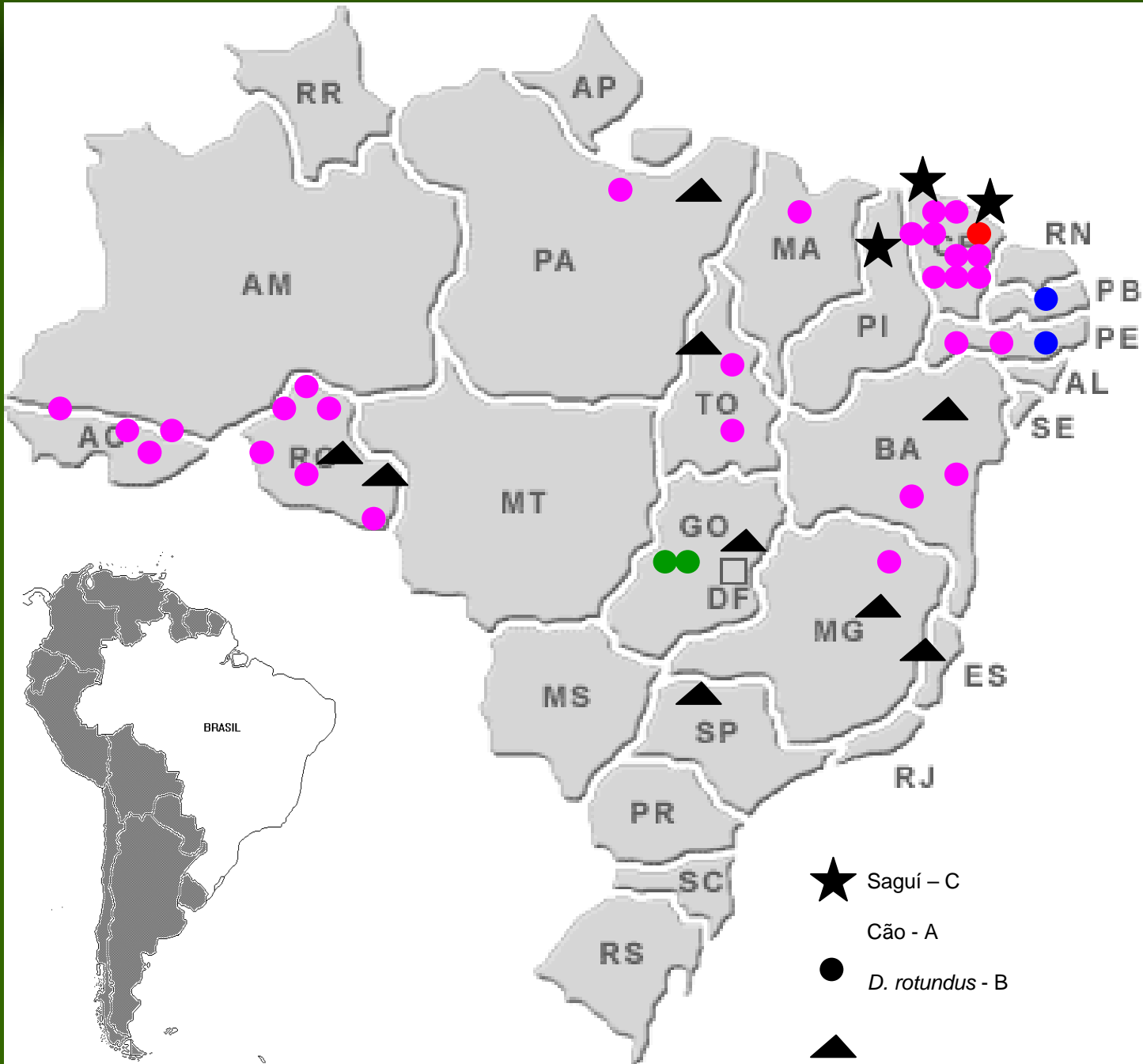
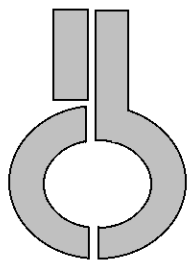


Antigenic and Genetic Studies of the Rabies Virus Isolated from Humans in Brazil



- Presence of multiple endemic cycles maintained by distinct animal populations .
- Inter-species transmission in different regions in Brazil.
- 4 different endemic cycles related to the Rabies virus (AgV2 - dog).

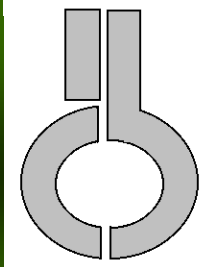
See map and chart on next 2 pages →



- ★ Sagú - C
- Cão - A
- *D. rotundus* - B
- ▲

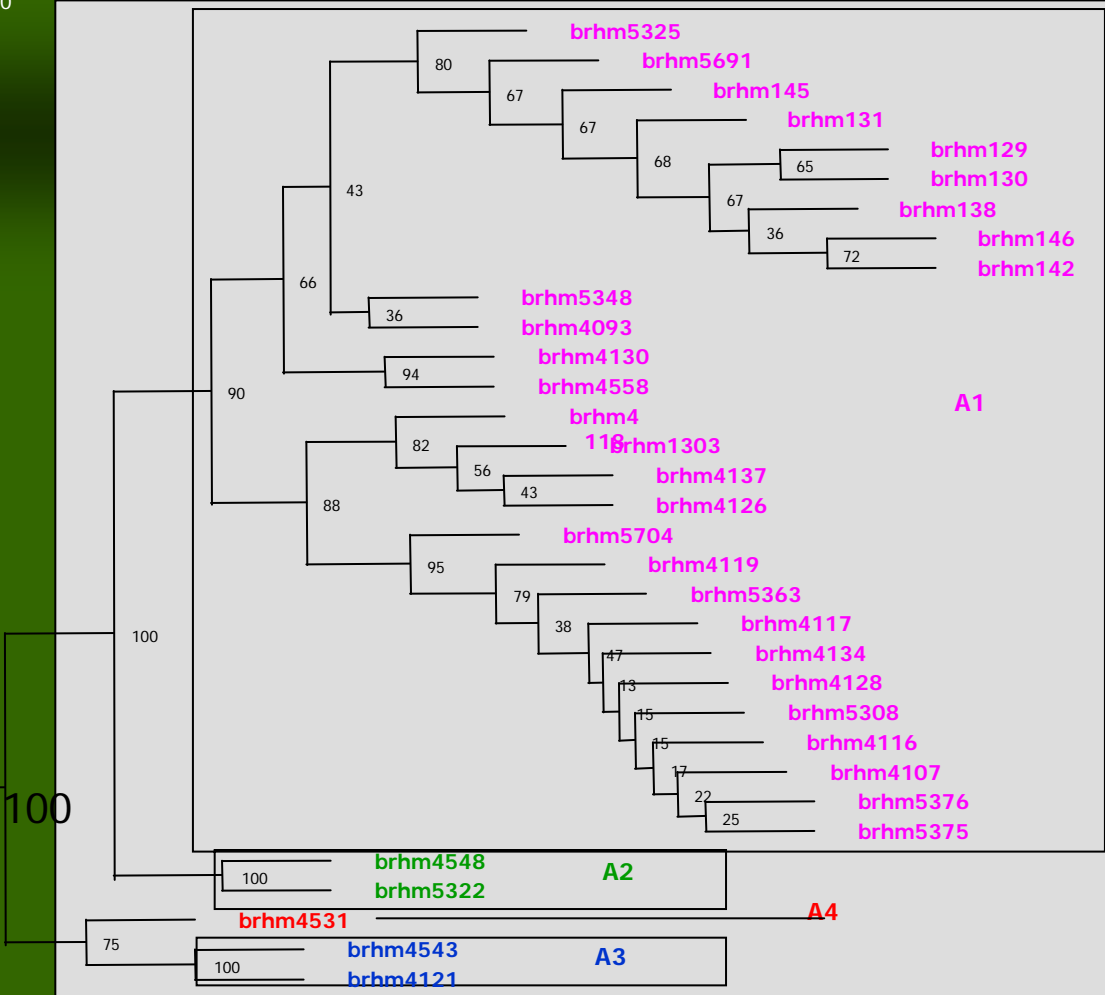
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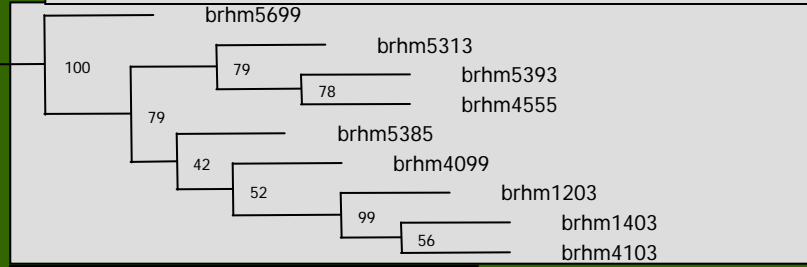


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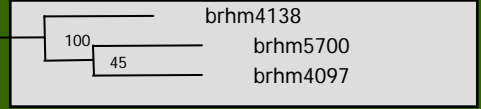
A



B



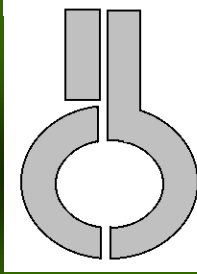
C



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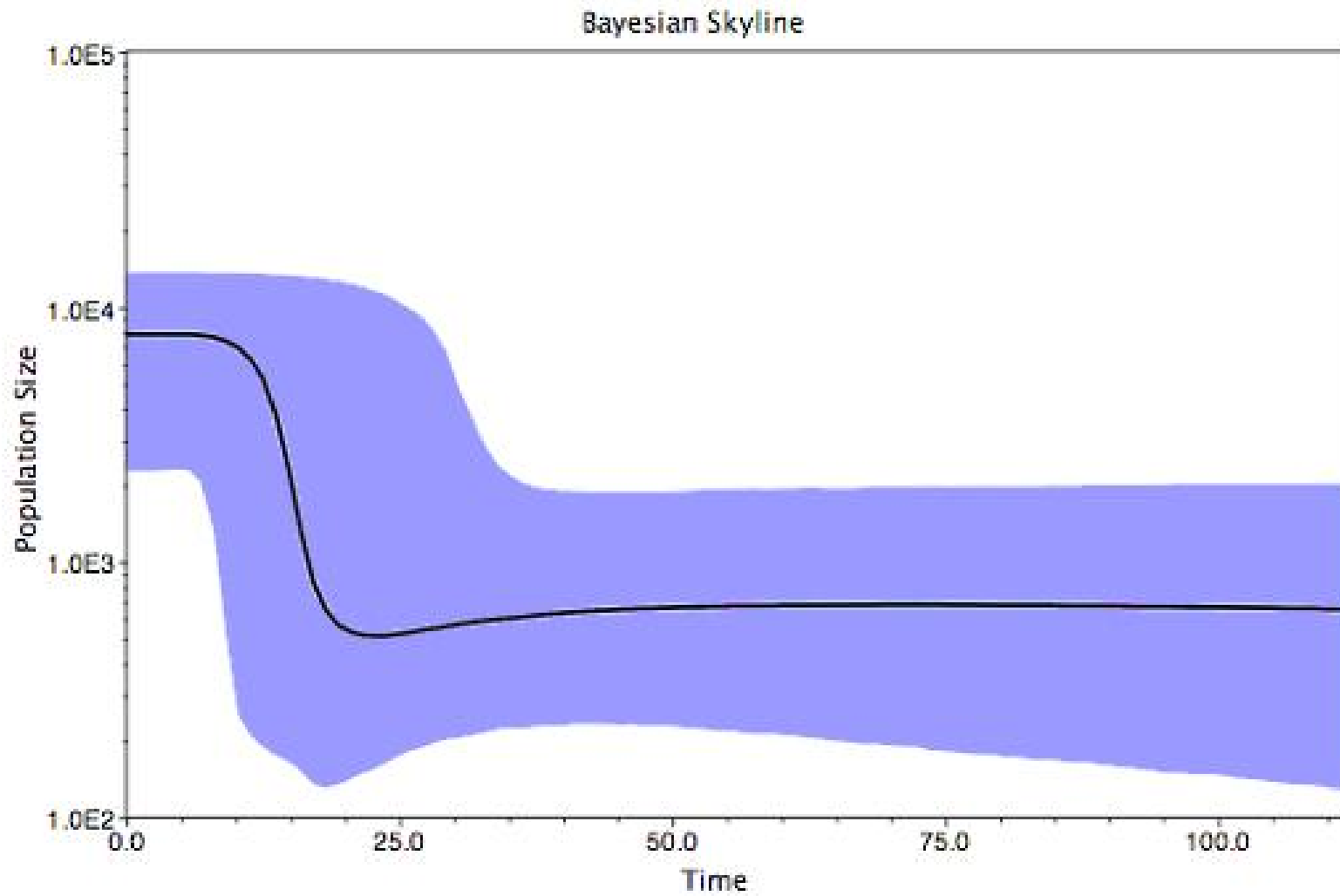
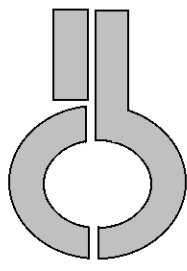
Demography



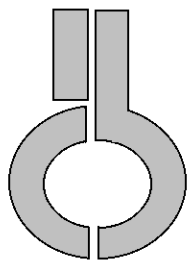
- Phylodynamic Analysis
- Nucleoprotein N amino-terminal analysis in samples isolates from America, European and Asia
- This Delay suggests that the aerial cycle supplies the terrestrial cycle, and so furthers the maintenance of the virus in nature

See examples of aerial and terrestrial cycles in next 2 slides →

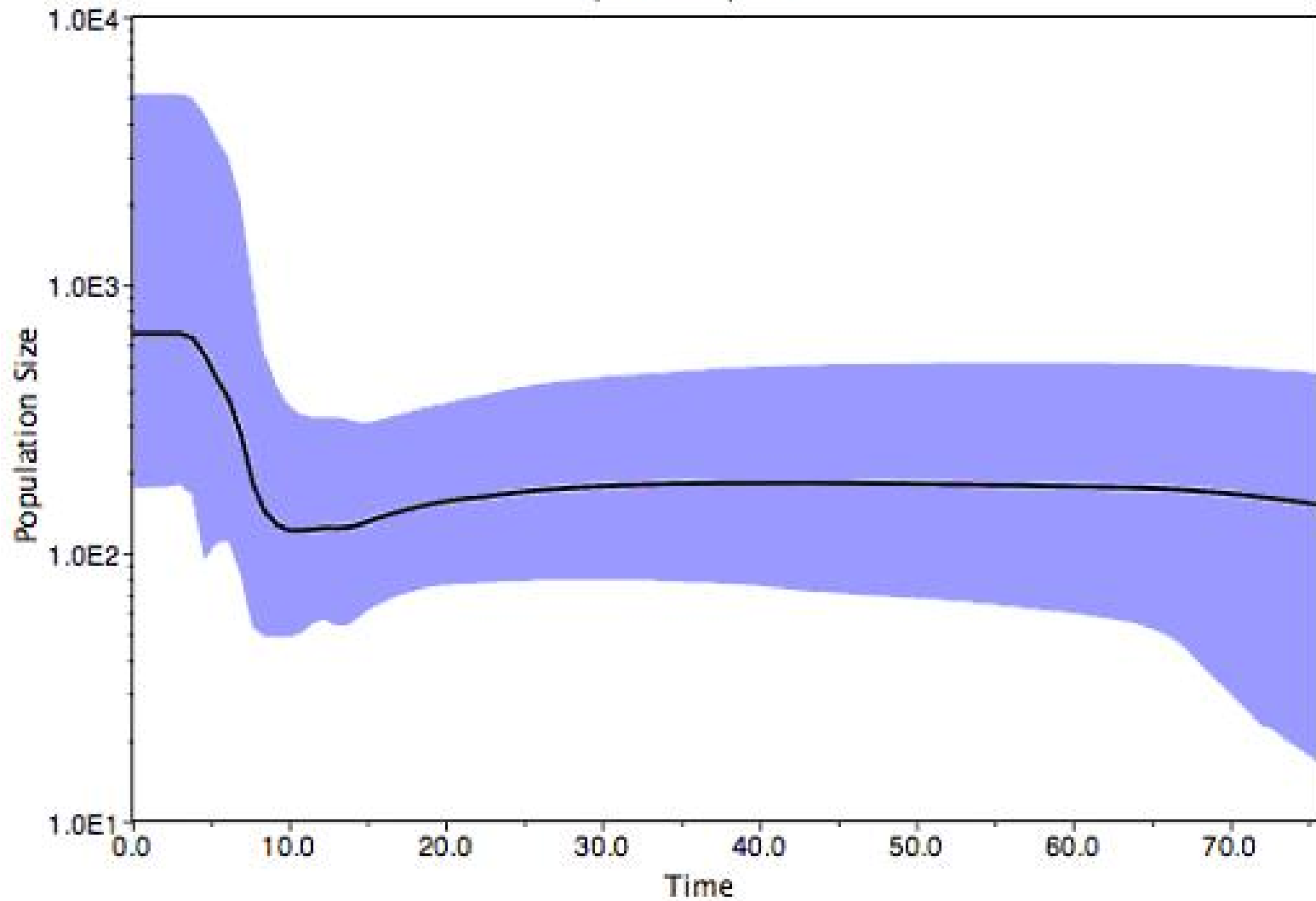
Aerial Cycle



Terrestrial Cycle

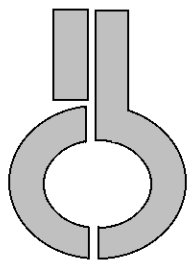


Bayesian Skyline



Rabies Research Group
Clinical & Molecular Virology Lab
Institute of Biomedical Sciences
University of São Paulo

Núcleo de Pesquisas em Raiva
Laboratório de Virologia Clínica e Molecular
Instituto de Ciências Biomédicas - ICB
Universidade de São Paulo - USP



Silvana Regina Favoretto - Instituto Pasteur de São Paulo & USP

Luzia Fátima A. Martorelli - Lab. de Zoonoses e Doenças Transmitidas por Vetores, PMSP & USP

Débora Regina Veiga Sacramento - Núcleo Viral da Genomic Engenharia Molecular Ltda & USP

Luzia Helena Queiroz da Silva - Faculdade de Medicina Veterinária (UNESP-Araçatuba) & USP

Marilene F. Almeida - Laboratório de Zoonoses e Doenças Transmitidas por Vetores, PMSP

Elenice Sequetin Cunha - Instituto Biológico de São Paulo

Caroline Cotrim Aires - USP - Museu de Zoologia / Laboratório Biológico Evolução e Conservação de Vertebrados

Angélica Cristine de Almeida Campos - USP - Núcleo de Pesquisas em Raiva

Roberta Zveibel Toporovski - USP - Núcleo de Pesquisas em Raiva

USP - Instituto de Ciências Biomédicas - **Paolo Marinho de Andrade Zanotto**

USP - Faculdade de Medicina Veterinária e Zootecnia - **Marcelo Labruna**

CDC - Centers for Disease Control and Prevention, (USA) - **Charles E. Rupprecht**

USP - Instituto de Ciências Biomédicas - **Cecília C. de Mattos**

USP - Instituto de Ciências Biomédicas - **Carlos A. de Mattos**

Universidade Thomas Jefferson - PA (USA) - **Hilary Koprowski**

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