Epidemiology of vampire bat-transmitted rabies virus in Goiás, central Brazil: re-evaluation based on G-L intergenic region

Shinji Hirano, Takuya Itou*, Adolorata AB Carvalho, Fumio H Ito, Takeo Sakai

Abstract

Background: Vampire bat related rabies harms both livestock industry and public health sector in central Brazil. The geographical distributions of vampire bat-transmitted rabies virus variants are delimited by mountain chains. These findings were elucidated by analyzing a high conserved nucleoprotein gene. This study aims to elucidate the detailed epidemiological characters of vampire bat-transmitted rabies virus by phylogenetic methods based on 619-nt sequence including unconserved G-L intergenic region.

Findings: The vampire bat-transmitted rabies virus isolates divided into 8 phylogenetic lineages in the previous nucleoprotein gene analysis were divided into 10 phylogenetic lineages with significant bootstrap values. The distributions of most variants were reconfirmed to be delimited by mountain chains. Furthermore, variants in undulating areas have narrow distributions and are apparently separated by mountain ridges.

Conclusions: This study demonstrates that the 619-nt sequence including G-L intergenic region is more useful for a state-level phylogenetic analysis of rabies virus than the partial nucleoprotein gene, and simultaneously that the distribution of vampire bat-transmitted RABV variants tends to be separated not only by mountain chains but also by mountain ridges, thus suggesting that the diversity of vampire bat-transmitted RABV variants was delimited by geographical undulations.

Background

Rabies is a zoonosis that kills infected mammals, including humans, and is mainly transmitted by carnivores. In the Americas, chiropterans (insectivorous, frugivorous and hematophagous bat) are another reservoir of this disease. Although dog-transmitted rabies in central Brazil has been reduced by aggressive vaccination programs [1], chiroptera (particularly the common vampire bat, Desmodus rotundus)-transmitted rabies remains endemic in this region, and harms both the livestock industry and the public health sector [2,3].

To date, vampire bat-transmitted rabies in livestock has been controlled by reducing the population of vampire bats and by vaccinating livestock [3,4]. However, the depopulation of vampire bats has limitations and the effects are temporary, while vaccination of livestock is only carried out for some animals and is ineffective in decreasing rabies levels in vampire bats.

For the sustainable and effective control of vampire bat rabies, further knowledge of epidemiological features, such as vampire bat ecology and the dynamics of vampire bat-transmitted rabies, is necessary. Molecular epidemiological analysis of vampire bat-transmitted cattle rabies cases using the partial nucleoprotein gene, which is the most conserved gene in the rabies virus (RABV) genome, has suggested that the distribution of variants in Brazil is delimited by mountain chains and clustered in tens of thousands of square kilometers [5]. However, the vampire bats migrate several kilometers from their nests [6]. To elucidate a more detailed genetic clustering and geo-distribution of genetic clades of vampire bat-transmitted RABV, 204 isolates from Goiás, which includes the 185 isolates analyzed previously, were employed and analyzed by a phylogenetic method based on a nucleotide sequence encompassing the G-L intergenic region locating between glycoprotein...
(G) and polymerase (L) gene loci, which is the most divergent region in the RABV genome and is used for monitoring epidemiological changes in the evolution of RABV [7,8].

**Results**

**Phylogenetic analysis**
The 204 RABV isolates divided into 8 phylogenetic lineages in the previous nucleoprotein gene analysis were divided into 10 phylogenetic lineages with significant bootstrap values (Figure 1; details shown in Table 1). Isolates of the C-5 and C-6 lineages designated by Kobayashi et al. belonged to the A-lineage, while the B-lineage consisted of some isolates of C-5. The C-, D-, F- and J-lineages included isolates belonging to C-12, C-1, C-22 and C-3, respectively. Isolates of C-20 belonged to the I-lineage, and C-21 was divided into three lineages; G-, H- and I-lineages. Two isolates not belonging to any lineages in previous studies were assigned to the E-lineage.

**Geographical plotting**
In this study, geographical areas in Goiás were divided by mountain chains into the Northwest, North central, Northeast and South regions (Figure 2). Most isolates of the A-lineage were distributed in the South region. The B-lineage was likely to exist in the Northeast and North central regions. The isolates belonging to the C-, E-, F-, G-, H- and I-lineages were distributed in the Northwest region. The G-, H- and I-lineages have narrow distributions and are apparently separated by mountain ridges (Figure 2; Area I), while the A-lineage was distributed in a wide range throughout a Southeastern basin (Figure 2; Area II). The isolates of the D-lineage were plotted on an eastern edge of the North central region. The J-lineage had a wide geographical distribution in Goiás.

**Discussion**
Previous studies had elucidated that the distributions of vampire bat and transmitted RABV are delimited by mountain chains [5]. In the present study, it was reconfirmed that mountain chains divide the distribution patterns of each viral lineage. Furthermore, the isolates belonging to C-5 having a wide range in Goiás were divided into the A- and B-lineages, and were found to be distributed in the South and North regions on either side of a mountain chain. This finding supports Kobayashi’s hypothesis that distribution of vampire bat-transmitted RABV is affected by mountain chains.

The same variants of vampire bat-transmitted RABV were spread widely in flat low lands (< 800 m), but at higher elevations (800-1600 m), they had a narrower distribution [9]. However, the G-, H- and I-lineages were found to be separated by mountain ridges in low areas (400-800 m) located in a southern undulating area of the Northwestern region (Figure 2; Area I). Furthermore, the A-lineage was located in an eastern basin of the South region (> 800 m; Figure 2; Area II). Considering that the higher lands showed an undulating
Table 1 Isolates from Goiás

| Sample | Species     | Location            | Year | This study | Previous study | N203-nt | Partial G & GL |
|--------|-------------|---------------------|------|------------|----------------|---------|----------------|
| BRbv371| Cattle      | Caldas Novas        | 2002 | A          | C-5            | AB307182 | AB544082       |
| BRbv372| Cattle      | Água Limpa          | 2002 | A          | C-5            | AB307183 | AB544083       |
| BRbv373| Cattle      | Caldas Novas        | 2002 | A          | C-5            | AB307184 | AB544084       |
| BRbv375| Cattle      | Santa Cruz de Goias | 2002 | A          | C-5            | AB307186 | AB544085       |
| BRbv379| Cattle      | Corumbaíba          | 2002 | A          | C-5            | AB307190 | AB544089       |
| BRbv380| Cattle      | Corumbaíba          | 2002 | A          | C-5            | AB307191 | AB544090       |
| BRbv381| Cattle      | Cristalina          | 2002 | A          | C-5            | AB307192 | AB544091       |
| BRbv386| Cattle      | Ipameri             | 2002 | A          | C-5            | AB307197 | AB544095       |
| BRbv391| Cattle      | Caldas Novas        | 2002 | A          | C-5            | AB307201 | AB544099       |
| BRbv392| Cattle      | Água Limpa          | 2002 | A          | C-5            | AB307202 | AB544100       |
| BRbv393| Cattle      | Itumbiara           | 2002 | A          | C-5            | AB307203 | AB544101       |
| BRbv395| Cattle      | Itumbiara           | 2002 | A          | C-5            | AB307205 | AB544102       |
| BRbv396| Cattle      | Itumbiara           | 2002 | A          | C-5            | AB307206 | AB544103       |
| BRbv397| Cattle      | Buriti Alegre       | 2002 | A          | C-5            | AB307207 | AB544104       |
| BRbv398| Cattle      | Itumbiara           | 2002 | A          | C-5            | AB307208 | AB544105       |
| BRbv400| Cattle      | Buriti Alegre       | 2002 | A          | C-5            | AB307210 | AB544107       |
| BRbv404| Cattle      | Buriti Alegre       | 2002 | A          | C-5            | AB307214 | AB544111       |
| BRbv405| Cattle      | Buriti Alegre       | 2002 | A          | C-5            | AB307215 | AB544112       |
| BRbv407| Cattle      | Goiandira           | 2002 | A          | C-5            | AB307217 | AB544114       |
| BRbv412| Cattle      | Itapaci             | 2002 | A          | C-5            | AB307222 | AB544118       |
| BRbv413| Cattle      | Caldas Novas        | 2002 | A          | C-5            | AB307223 | AB544119       |
| BRbv416| Cattle      | Morrinhos           | 2002 | A          | C-5            | AB307226 | AB544122       |
| BRbv421| Cattle      | Nova Aurora         | 2002 | A          | C-5            | AB307231 | AB544124       |
| BRbv429| Cattle      | Corumbaíba          | 2002 | A          | Ud*            | AB307238 | AB544129       |
| BRbv432| Cattle      | Ipameri             | 2002 | A          | C-5            | AB307241 | AB544132       |
| BRbv438| Cattle      | Buriti Alegre       | 2002 | A          | C-5            | AB307247 | AB544137       |
| BRhr441| Horse       | Buriti Alegre       | 2002 | A          | Ud             | AB307251 | AB544140       |
| BRbv442| Cattle      | Buriti Alegre       | 2002 | A          | C-5            | AB307252 | AB544141       |
| BRbv447| Cattle      | Urutaí              | 2002 | A          | C-6            | AB307255 | AB544143       |
| BRbv449| Cattle      | Caldas Novas        | 2002 | A          | C-5            | AB307256 | AB544145       |
| BRbv451| Cattle      | Ipameri             | 2002 | A          | C-5            | AB307258 | AB544147       |
| BRbv453| Cattle      | São Luís de Montes Belos | 2002 | A          | C-5            | AB307260 | AB544149       |
| BRbv456| Cattle      | Orízona             | 2002 | A          | Ud             | AB307263 | AB544150       |
| BRbv457| Cattle      | Água Limpa          | 2002 | A          | C-5            | AB307264 | AB544151       |
| BRbv458| Cattle      | Buriti Alegre       | 2002 | A          | C-5            | AB307265 | AB544152       |
| BRbv466| Cattle      | Itumbiara           | 2002 | A          | C-5            | AB307273 | AB544157       |
| BRbv469| Cattle      | Ipameri             | 2002 | A          | C-6            | AB307275 | AB544159       |
| BRbv471| Cattle      | Santa Cruz de Goias | 2002 | A          | C-6            | AB307277 | AB544161       |
| BRbv472| Cattle      | Ipameri             | 2002 | A          | C-6            | AB307278 | AB544162       |
| BRbv473| Cattle      | Itumbiara           | 2002 | A          | C-5            | AB307279 | AB544163       |
| BRbv475| Cattle      | Caldas Novas        | 2002 | A          | C-5            | AB307281 | AB544165       |
| BRbv477| Cattle      | Itumbiara           | 2002 | A          | C-5            | AB307283 | AB544166       |
| BRbv478| Cattle      | Itumbiara           | 2002 | A          | C-5            | AB307284 | AB544167       |
| BRhr483| Horse       | Panamá              | 2002 | A          | C-5            | AB307288 | AB544170       |
| BRbv486| Cattle      | Itumbiara           | 2002 | A          | Ud             | AB307291 | AB544171       |
| BRbv489| Cattle      | Itumbiara           | 2002 | A          | Ud             | AB307295 | AB544174       |
| BRbv493| Cattle      | Ipameri             | 2002 | A          | C-6            | AB307298 | AB544177       |
| BRbv495| Cattle      | Panamá              | 2002 | A          | C-5            | AB307300 | AB544179       |
| BRbv496| Cattle      | Panamá              | 2002 | A          | C-5            | AB307301 | AB544180       |

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| Isolates from Goiás (Continued) |
|-------------------------------|
| BRbv497 Cattle | Itumbiara | 2002 | A | C-S | AB307302 | AB544181 |
| BRbv498 Cattle | Bela Vista de Goiás | 2002 | A | C-S | AB307303 | AB544182 |
| BRbv502 Cattle | Itumbiara | 2002 | A | C-S | AB307306 | AB544186 |
| BRbv503 Cattle | Orizona | 2002 | A | Ud | AB307307 | AB544187 |
| BRbv509 Cattle | Buriti Alegre | 2002 | A | C-S | AB307311 | AB544192 |
| BRbv514 Cattle | Panamã | 2002 | A | C-S | AB307316 | AB544196 |
| BRbv516 Cattle | Orizona | 2002 | A | Ud | AB307318 | AB544198 |
| BRbv521 Cattle | Urutai | 2002 | A | Nd | Nd | AB544201 |
| BRbv524 Cattle | Urutai | 2002 | A | C-6 | AB307323 | AB544204 |
| BRbv526 Cattle | Panamã | 2002 | A | C-S | AB307325 | AB544206 |
| BRbv527 Cattle | Campos Belos | 2002 | A | C-S | AB307326 | AB544207 |
| BRbv684 Cattle | Ipameri | 2001 | A | C-S | AB307412 | AB544211 |
| BRbv685 Cattle | Corumbaiba | 2001 | A | Nd | Nd | AB544212 |
| BRbv686 Cattle | Ipameri | 2001 | A | C-S | AB307413 | AB544213 |
| BRbv687 Cattle | Corumbaiba | 2001 | A | C-S | AB307414 | AB544214 |
| BRbv690 Cattle | Ipameri | 2001 | A | C-S | AB307417 | AB544215 |
| BRbv691 Cattle | Ipameri | 2001 | A | C-S | AB307418 | AB544216 |
| BRbv694 Cattle | Ipameri | 2001 | A | Nd | Nd | AB544219 |
| BRbv697 Cattle | Campo Alegre de Goiás | 2001 | A | C-S | AB307423 | AB544221 |
| BRbv698 Cattle | Campo Alegre de Goiás | 2001 | A | C-S | AB307424 | AB544222 |
| BRbv699 Cattle | Campo Alegre de Goiás | 2001 | A | C-S | AB307425 | AB544223 |
| BRbv700 Cattle | Caldas Novas | 2001 | A | Nd | Nd | AB544224 |
| BRbv701 Cattle | Caldas Novas | 2001 | A | C-S | AB307426 | AB544225 |
| BRbh704 Horse | Corumbaiba | 2001 | A | Nd | Nd | AB544227 |
| BRbv705 Cattle | Ipameri | 2001 | A | C-S | AB307428 | AB544228 |
| BRbv707 Cattle | Corumbaiba | 2001 | A | Nd | Nd | AB544229 |
| BRbh708 Sheep | Ouvidor | 2001 | A | Nd | Nd | AB544230 |
| BRbv710 Cattle | Morrinhos | 2001 | A | C-S | AB307431 | AB544232 |
| BRbv711 Cattle | Morrinhos | 2001 | A | C-S | AB307432 | AB544233 |
| BRbv717 Cattle | Ipameri | 2001 | A | C-S | AB307438 | AB544235 |
| BRbv718 Cattle | Ipameri | 2001 | A | C-S | AB307439 | AB544236 |
| BRbv741 Cattle | Marzagão | 2001 | A | C-S | AB307450 | AB544244 |
| BRbv742 Cattle | Ipameri | 2001 | A | C-S | AB307451 | AB544245 |
| BRbv745 Cattle | Buriti Alegre | 2001 | A | C-S | AB307454 | AB544248 |
| BRbv747 Cattle | Corumbaiba | 2001 | A | Ud | AB307456 | AB544250 |
| BRbv748 Cattle | Campo Alegre de Goiás | 2001 | A | C-S | AB307457 | AB544251 |
| BRbv752 Cattle | Catalão | 2001 | A | Ud | AB307461 | AB544254 |
| BRbh755 Horse | Cumari | 2001 | A | Nd | Nd | AB544257 |
| BRbv770 Cattle | Ipameri | 2002 | A | C-S | AB307476 | AB544269 |
| BRbv771 Cattle | Caldas Novas | 2002 | A | C-S | AB307477 | AB544270 |
| BRbv780 Cattle | Nova Crixás | 2002 | A | C-S | AB307484 | AB544274 |
| BRbv785 Cattle | Corumbaiba | 2002 | A | C-S | AB307486 | AB544277 |
| BRbv786 Cattle | Buriti Alegre | 2002 | A | Ud | AB307487 | AB544278 |
| BRbv796 Cattle | Fires do Rio | 2002 | A | C-6 | AB307495 | AB544283 |
| BRbv797 Cattle | Cristalina | 2002 | A | C-6 | AB307496 | AB544284 |
| BRbv402 Cattle | Divinópolis de Goiás | 2002 | B | C-S | AB307212 | AB544109 |
| BRbv406 Cattle | Posse | 2002 | B | C-S | AB307216 | AB544113 |
| BRbv459 Cattle | Monte Alegre de Goiás | 2002 | B | C-S | AB307266 | AB544153 |
| BRbv461 Cattle | Monte Alegre de Goiás | 2002 | B | C-S | AB307268 | AB544155 |
| BRbv462 Cattle | Monte Alegre de Goiás | 2002 | B | C-S | AB307269 | AB544156 |
| BRbv479 Cattle | Colinas do Sul | 2002 | B | C-S | AB307285 | AB544168 |
| BRbv481 Cattle | Serrandópolis | 2002 | B | C-S | AB307287 | AB544169 |
| Isolates from Goiás (Continued) |
|----------------------------------|
| **BRbv490** Cattle | Colinas do Sul | 2002 | B | C-S | AB307296 | AB544175 |
| **BRbv494** Cattle | Divinópolis de Goiás | 2002 | B | C-S | AB307299 | AB544178 |
| **BRbv500** Cattle | Colinas do Sul | 2002 | B | C-S | AB307304 | AB544184 |
| **BRbv505** Cattle | Colinas do Sul | 2002 | B | C-S | AB307309 | AB544188 |
| **BRhr506** Horse | Colinas do Sul | 2002 | B | Nd | Nd | AB544189 |
| **BRhr507** Horse | Colinas do Sul | 2002 | B | Nd | Nd | AB544190 |
| **BRbv510** Cattle | Monte Alegre de Goiás | 2002 | B | C-S | AB307312 | AB544193 |
| **BRbv511** Cattle | Divinópolis de Goiás | 2002 | B | C-S | AB307313 | AB544194 |
| **BRbv518** Cattle | Campos Belos | 2002 | B | C-S | AB307319 | AB544199 |
| **BRbv525** Cattle | Panamá | 2002 | B | C-S | AB307324 | AB544205 |
| **BRbv753** Cattle | Monte Alegre de Goiás | 2001 | B | C-S | AB307462 | AB544255 |
| **BRbv762** Cattle | Divinópolis de Goiás | 2001 | B | C-S | AB307470 | AB544263 |
| **BRbv387** Cattle | Doverlândia | 2002 | C | C-12 | AB307198 | AB544096 |
| **BRbv401** Cattle | Mineiros | 2002 | C | C-12 | AB307211 | AB544108 |
| **BRbv424** Cattle | Doverlândia | 2002 | C | C-12 | AB307234 | AB544125 |
| **BRbv692** Cattle | Mineiros | 2001 | C | C-12 | AB307419 | AB544217 |
| **BRbv712** Cattle | Doverlândia | 2001 | C | C-12 | AB307433 | AB544234 |
| **BRbv751** Cattle | Mineiros | 2001 | C | C-12 | AB307460 | AB544253 |
| **BRbv383** Cattle | Nova Amérlica | 2002 | D | C-1 | AB307194 | AB544092 |
| **BRbv384** Cattle | Nova Amérlica | 2002 | D | C-1 | AB307195 | AB544093 |
| **BRbv409** Cattle | Rubiataba | 2002 | D | C-1 | AB307219 | AB544115 |
| **BRbv411** Cattle | Morinhos | 2002 | D | C-1 | AB307221 | AB544117 |
| **BRbv452** Cattle | Rubiataba | 2002 | D | C-1 | AB307259 | AB544148 |
| **BRbv767** Cattle | Rubiataba | 2002 | D | C-1 | AB307474 | AB544267 |
| **BRbv793** Cattle | Uruaí | 2002 | D | C-1 | AB307493 | AB544282 |
| **BRbv440** Cattle | Nova Crixás | 2002 | E | Ud | AB307249 | AB544139 |
| **BRbv519** Cattle | Mundo Novo | 2002 | E | Ud | AB307320 | AB544200 |
| **BRhr389** Horse | Nova Crixás | 2002 | F | Nd | Nd | AB544098 |
| **BRbv434** Cattle | Itaparanga | 2002 | F | Ud | AB307243 | AB544133 |
| **BRbv439** Cattle | Mundo Novo | 2002 | F | C-22 | AB307248 | AB544138 |
| **BRbv488** Cattle | Carmo do Rio Verde | 2002 | F | Ud | AB307294 | AB544173 |
| **BRbv749** Cattle | Nova Crixás | 2001 | F | C-22 | AB307458 | AB544252 |
| **BRbv757** Cattle | Monte Alegre de Goiás | 2001 | F | C-22 | AB307465 | AB544259 |
| **BRbv763** Cattle | Aruanã | 2002 | F | C-22 | AB307471 | AB544264 |
| **BRbv773** Cattle | Aruanã | 2002 | F | C-22 | AB307479 | AB544272 |
| **BRbv774** Cattle | Mozarlândia | 2002 | F | C-22 | AB307480 | AB544273 |
| **BRpv787** Sheep | Ipameri | 2002 | F | Nd | Nd | AB544279 |
| **BRbv378** Cattle | Novo Brasil | 2002 | G | C-21 | AB307189 | AB544088 |
| **BRbv425** Cattle | Novo Brasil | 2002 | G | C-21 | AB307235 | AB544126 |
| **BRbv445** Cattle | Morinhos | 2002 | G | Ud | AB307254 | AB544142 |
| **BRbv448** Cattle | Buriti de Goiás | 2002 | G | C-21 | AB307256 | AB544144 |
| **BRhr467** Horse | Buriti de Goiás | 2002 | G | Nd | Nd | AB544158 |
| **BRbv474** Cattle | Moisporá | 2002 | G | C-21 | AB307280 | AB544164 |
| **BRbv501** Cattle | Anicuns | 2002 | G | C-21 | AB307305 | AB544185 |
| **BRbv515** Cattle | Córrego do Ouro | 2002 | G | C-21 | AB307317 | AB544197 |
| **BRbv522** Cattle | Buriti de Goiás | 2002 | G | Nd | Nd | AB544202 |
| **BRbv523** Cattle | Córrego do Ouro | 2002 | G | C-21 | AB307322 | AB544203 |
| **BRbv764** Cattle | Córrego do Ouro | 2002 | G | C-21 | AB307472 | AB544265 |
| **BRbv385** Cattle | Ivolândia | 2002 | H | C-21 | AB307196 | AB544094 |
| **BRbv414** Cattle | Ivolândia | 2002 | H | C-21 | AB307224 | AB544120 |
| **BRbv415** Cattle | Ivolândia | 2002 | H | C-21 | AB307225 | AB544121 |
| **BRbv426** Cattle | Amorinópolis | 2002 | H | C-21 | AB307236 | AB544127 |
| Isolates                  | Source          | Year  | Series | Genotype | Accession 1 | Accession 2 |
|--------------------------|-----------------|-------|--------|-----------|-------------|-------------|
| BRbv427                  | Cattle          | 2002  | H      | C-21      | AB307237    | AB544128    |
| BRbv431                  | Cattle          | 2002  | H      | Ud        | AB307240    | AB544131    |
| BRbv437                  | Cattle          | 2002  | H      | Ud        | AB307246    | AB544136    |
| BRbv693                  | Cattle          | 2001  | H      | C-21      | AB307420    | AB544218    |
| BRhr703                  | Horse           | 2001  | H      | Nd        | Nd          | AB544226    |
| BRbv736                  | Cattle          | 2001  | H      | C-21      | AB307446    | AB544242    |
| BRbv744                  | Cattle          | 2001  | H      | C-21      | AB307453    | AB544247    |
| BRbv370                  | Cattle          | 2002  | I      | C-21      | AB307181    | AB544081    |
| BRbv376                  | Cattle          | 2002  | I      | C-21      | AB307187    | AB544086    |
| BRbv377                  | Cattle          | 2002  | I      | C-21      | AB307188    | AB544087    |
| BRbv388                  | Cattle          | 2002  | I      | Ud        | AB307199    | AB544097    |
| BRbv399                  | Cattle          | 2002  | I      | C-21      | AB307209    | AB544106    |
| BRbv403                  | Cattle          | 2002  | I      | C-20      | AB307213    | AB544110    |
| BRbv410                  | Cattle          | 2002  | I      | C-21      | AB307220    | AB544116    |
| BRbv420                  | Cattle          | 2002  | I      | C-21      | AB307230    | AB544123    |
| BRbv430                  | Cattle          | 2002  | I      | C-21      | AB307239    | AB544130    |
| BRbv435                  | Cattle          | 2002  | I      | C-20      | AB307244    | AB544134    |
| BRbv436                  | Cattle          | 2002  | I      | Ud        | AB307245    | AB544135    |
| BRbv450                  | Cattle          | 2002  | I      | C-21      | AB307257    | AB544146    |
| BRbv460                  | Cattle          | 2002  | I      | C-20      | AB307267    | AB544154    |
| BRbv470                  | Cattle          | 2002  | I      | C-20      | AB307276    | AB544160    |
| BRbv491                  | Cattle          | 2002  | I      | C-20      | AB307297    | AB544176    |
| BRbv499                  | Cattle          | 2002  | I      | Nd        | Nd          | AB544183    |
| BRbv513                  | Cattle          | 2002  | I      | C-20      | AB307315    | AB544195    |
| BRbv681                  | Cattle          | 2001  | I      | Nd        | Nd          | AB544208    |
| BRbv682                  | Cattle          | 2001  | I      | Nd        | Nd          | AB544209    |
| BRbv683                  | Cattle          | 2001  | I      | C-21      | AB307411    | AB544210    |
| BRbv696                  | Cattle          | 2001  | I      | C-21      | AB307422    | AB544220    |
| BRbv709                  | Cattle          | 2001  | I      | C-21      | AB307430    | AB544231    |
| BRbv720                  | Cattle          | 2001  | I      | C-21      | AB307440    | AB544237    |
| BRbv721                  | Cattle          | 2001  | I      | C-21      | AB307441    | AB544238    |
| BRbv722                  | Cattle          | 2001  | I      | C-21      | AB307442    | AB544239    |
| BRbv734                  | Cattle          | 2001  | I      | C-21      | AB307444    | AB544240    |
| BRbv735                  | Cattle          | 2001  | I      | Ud        | AB307445    | AB544241    |
| BRbv738                  | Cattle          | 2001  | I      | C-20      | AB307448    | AB544243    |
| BRbv743                  | Cattle          | 2001  | I      | C-20      | AB307452    | AB544246    |
| BRbv746                  | Cattle          | 2001  | I      | C-20      | AB307453    | AB544249    |
| BRbv756                  | Cattle          | 2001  | I      | C-21      | AB307464    | AB544258    |
| BRbv758                  | Cattle          | 2001  | I      | C-21      | AB307466    | AB544260    |
| BRbv759                  | Cattle          | 2001  | I      | C-21      | AB307467    | AB544261    |
| BRbv760                  | Cattle          | 2001  | I      | C-21      | AB307468    | AB544262    |
| BRbv765                  | Cattle          | 2002  | I      | C-20      | AB307473    | AB544266    |
| BRbv769                  | Cattle          | 2002  | I      | C-21      | AB307475    | AB544268    |
| BRbv772                  | Cattle          | 2002  | I      | C-21      | AB307478    | AB544271    |
| BRhr782                  | Horse           | 2002  | I      | Nd        | Nd          | AB544275    |
| BRbv784                  | Cattle          | 2002  | I      | C-20      | AB307485    | AB544276    |
| BRbv790                  | Cattle          | 2002  | I      | C-21      | AB307490    | AB544280    |
| BRbv508                  | Cattle          | 2002  | J      | C-3       | AB307310    | AB544191    |
| BRbv754                  | Cattle          | 2001  | J      | C-3       | AB307463    | AB544256    |
| BRbv792                  | Cattle          | 2002  | J      | C-3       | AB307492    | AB544281    |

*Undefined; †No data; ‡Kobayashi et al. (2008)
landscape, the results suggest that the distribution patterns of vampire bat-transmitted RABV variants depend on such undulations. On the other hand, the distribution of common vampire bats in a valley is limited by the ridges that form the valley [10], thus supporting the notion that the distribution of RABV variants is affected by smaller topography than mountain chains.

Conclusions
The present study analyzed the epidemiology of vampire bat-transmitted RABV using a 619-nt region containing the partial glycoprotein gene and the G-L intergenic region, and indicated that the isolates can be further divided into several phylogenetic lineages with significant bootstrap values when compared to characterization based on the 203-nt partial N gene. Furthermore, the phylogenetic lineages were divided by both mountain chains and mountain ridges. In future studies, it will be important to analyze samples from different time points and to elucidate the dynamics of vampire bat-transmitted rabies in order to establish effective and sustainable control measures for preventing rabies circulation among vampire bats.

Methods
Samples
A total of 204 samples obtained from 192 cattle, 10 horses and 2 sheep in Goiás from October 2001 to August 2002 were employed, which had been confirmed as rabies positive through fluorescent antibody test and mouse inoculation test (Table 1). Viral RNA was extracted from the brain as described previously [11]. Lineages of the 164 cattle isolates, C-1, C-3, C-5, C-6, C-12, C-20, C-21 and C-22, were previously characterized based on a 203-nt sequence of the nucleoprotein gene [5], and are shown in Table 1.

Determination of nucleotide sequences
RT-PCR and direct sequencing with the HmG5-1302 \( _{4615}TGTGAGATTCACCCTCCGTG_{4642} \) positions
relative to PV strain genome (Accession No. M13215) and
RVLa-1 (5325ATRGGTTACATCAAAACCTG5414) primer
pair were performed as described previously [11]. The tar-
get sequence includes the partial glycoprotein gene and G- L
intergenic region. Nucleotide sequences were deter-
mined using the ATGC program version 4.0 (GENETYX
Co., Tokyo, Japan).

Phylogenetic analysis
Multiple nucleotide sequence alignments of the partial gly-
coprotein gene and G-L intergenic region were generated
by the ClustalW package in MEGA ver. 4.0 [12]. A phylo-
genetic tree was constructed by the neighbor joining (NJ)
method with bootstrap analysis (1000 pseudoreplicates)
under the p-distance model. Phylogenetic clustering sup-
ported by a bootstrap value exceeding 70% was regarded
as a reliable lineage [13]. Results were validated by the
maximum likelihood method using PhyML [14]. In order
to reconfirm the shape of the NJ tree, the ML tree was
constructed under HKY substitution model justified by
MODELETEST packaged in Hyphy program [15].

Geographical plotting
The 204 RABV isolates were plotted onto a geographical
map described using the DIVA-GIS program [16] with
GIS data from Instituto Brasileiro de Geografia e Estatis-	ica [17] and DIVA-GIS gData [18].

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Author details
1Nihon University Veterinary Research Center, 1866 Kameino, Fujisawa 252-
0880, Kanagawa, Japan. *Faculty of Agriculture and Veterinary Science,
Department of Preventive Veterinary Medicine, UNESP, Via de Acesso Prof.
Paulo Donato Castellano, Jaboricabal, São Paulo 14884-900, Brazil.
2Department of Preventive Veterinary Medicine and Animal Health, Faculty
of Veterinary Medicine and Zootchny, University of São Paulo, Av. Prof. Dr.
Orlando Marques de Paiva, 87, Cidade Universitária, São Paulo 05508-000,
Brazil.

Authors’ contributions
SH participated in the design of the study, performed the experimental
procedures and the data analysis, and wrote the manuscript. TI, AABC, FHI
and TS elaborated the study design, management, coordination, and
drafting the manuscript. The authors have read and approved the final
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Competing interests
The authors declare that they have no competing interests.

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