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Source: Journal of Wildlife Diseases, 37(3) : 582-593

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-37.3.582

Journal of Wildlife Diseases, 37(3), 2001, pp. 582–593

SURVEY OF BAT POPULATIONS FROM MEXICO AND PARAGUAY FOR RABIES

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ABSTRACT: A mammalian survey was conducted in Mexico (October 1994–January 1996) and in Paraguay (August 1996-March 1997); a complete specimen was collected for each bat in the survey, including primary voucher specimen, ectoparasites, karyotype, and various frozen tissues. The surveys combined provided 937 brain samples (65 bat species) for rabies diagnosis. One male *Lasiurus ega*, collected in Paraguay, tested positive for the rabies virus (overall prevalence rate of 0.1%). Nucleotide sequence from a 300 bp region of the rabies nucleoprotein gene was compared with sequence obtained from representative rabies virus samples in the repository at the Centers for Disease Control and Prevention (Atlanta, Georgia, USA). Rabies virus extracted from the brain material of *L. ega* differed by only one nucleotide from a 300 bp consensus sequence (>99% homology) derived from samples for the variant of rabies virus transmitted by *Lasiurus cinereus*. *Lasiurus ega* differed by approximately 15% for the variant transmitted by *Desmodus rotundus*. Phylogenetic analysis found no evidence to suggest *L. ega* is a reservoir for rabies antigenic variant 6. The most likely explanation for rabies in *L. ega* was infection following contact with a rabid *L. cinereus*.

Key words: Bats, epizootiology, *Lasiurus ega*, rabies, reservoir, survey.

INTRODUCTION

Information on the prevalence of rabies in Latin American bat populations is sparse. Usually reports of rabies in bats are individual cases submitted to public health laboratories because of contact with humans or domestic animals. The submitted bats are rarely identified to species and case summaries do not include information on the total number of bats tested (Organizacion Panamericana de la Salud, 1997). Additionally, bats collected by the public are often sick or injured, and therefore represent a biased subpopulation of bats more likely to be infected with rabies.

Museum specimens could provide valuable information on the prevalence of rabies in free flying bat populations; however, bats collected for this purpose are not often examined for evidence of rabies infection. Access to the brain tissue necessary for rabies diagnosis can destroy cranial characteristics used for species identification and lessens the value of the specimen to a collection. A method developed by Greenhall (1965) extracts brain tissue through the foramen magnum without causing damage to the skull, thus allowing rabies diagnosis on previously unsampled bat populations.

Decreasing bias in the surveillance of rabies is essential to understanding the role of bats in the epidemiology of the disease. Using a modified brain extraction method, mammalian surveys in Mexico and in Paraguay provided a large collection of bats for rabies diagnosis, which provided an opportunity to examine infection in several bat populations of Latin America. Previously, most bat rabies studies in this region have focused on two bat species (*Tadarida brasiliensis, Desmodus rotundus*) because of their association with human and domestic animal rabies cases. Those studies collected numerous individuals from roosting sites (Constantine et al., 1968; Favi and Catalán, 1986; Steece and Altenbach, 1989; Tadei et al., 1991). The present study is distinct from others in that the sample contains numerous individuals from a large and taxonomically diverse assemblage of species, collected during flight.

MATERIALS AND METHODS

Mammalian surveys were conducted in the Mexican states of Michoacán and Colima and

throughout Paraguay; the surveys increased the number of specimens in museum collections as well as documented the mammalian species of these areas. A complete specimen was collected for each individual animal in the survey, including primary voucher specimen (generally skin, skull, and skeleton), ectoparasites, karyotype, and various frozen tissues (brain, heart, liver, kidney, and muscle). The combined surveys provided 937 brain samples (65 bat species) for rabies diagnosis (Table 1).

Twenty-one localities (October 1994–January 1996) and twenty-seven localities (August 1996–March 1997) were sampled in Mexico and Paraguay, respectively (Appendix A). Bats were collected using mist nets placed in a variety of habitats at each locality. In addition, hand nets were used in culverts, buildings, or other roosts. The nets were attended, and bats were removed promptly and placed in individual containers. Each bat was euthanized by inhalation of ether, and then processed as a complete specimen. Brain tissue was extracted through the foramen magnum using a saline syringe adapted from the method of Greenhall (1965). The brain tissue was placed into a cryovial and stored at the vapor phase of liquid nitrogen. Tissues were later transferred to a freezer at −80 C for permanent storage. Voucher specimens were collected for deposition in mammal collections of The Museum Texas Tech University (Lubbock, Texas, USA); Instituto de Biología, Universidad Nacional Autónoma de México (México, D.F., México); Universidad Autónoma Metropolitana-Iztapalapa (México, D.F., México); Universidad Autónoma del Estado de Morelos (Cuernavaca, Morelos, México); Universidad Michoacana de San Nicolás de Hidalgo (Morelia, Michoacán, México); and Museo Nacional de Historia Natural del Paraguay (San Lorenzo, Paraguay). C. López-Gonzáles, R. López-Wilchis, Salvador Gaona, C. Sánchez-Hernández, and M. L. Romero-Almaraz confirmed field identifications of bats.

Thin smears were prepared in duplicate by transferring brain tissue to a glass microscope slide with an applicator stick and blotting the excess on absorbent paper toweling. After air drying for 20 min, the slides were fixed in acetone at −20 C from 2 to 4 hr to overnight. The fixed smears were stained with fluorescein isothiocyanate-labeled rabies diagnostic reagents prepared from hyperimmune equine serum (BBL Microbiology Systems, Cockeysville, Maryland, USA, 1:80 dilution) and/or from monoclonal antibodies (Centocor Inc., Malvern, Pennsylvania, USA, 1:80 dilution) using standard methods (Velleca and Forrester, 1981).

Any positive tissues were prepared for rabies virus typing. Duplicate smears were incubated with a panel of 19 monoclonal antibodies using previously described methods (Diaz et al., 1994). Visualization of bound antibody was achieved by staining with fluorescein-conjugated goat antibody to mouse immunoglobulin G (Organon Teknika Corp., Durham, North Carolina, USA). Additionally, RNA extracted from brain tissue was reverse transcribed and amplified by polymerase chain reaction (RT/PCR). The RNA was extracted with TRIzol (GIBCOBRL Inc., Grand Island, New York, USA) according to manufacturer's instructions. The RT/PCR was achieved with primers 10g and 304 as previously described (Smith et al., 1992; Smith, 1995). A band of appropriate molecular weight was visualized in an agarose gel stained with ethidium bromide. The band was excised from a low melting point agarose gel, purified using the Wizard TM Minipreps DNA purification system (Promega, Madison, Wisconsin, USA) and sequenced using the ABI PRISM DNA Sequencing Kit (PE Applied Biosystems, Foster City, California, USA) according to manufacturer's instructions.

Automated fluorescence sequencing was performed on an Applied Biosystems 377 DNA sequencer (PE Applied Biosystems, Foster City, California, USA). Nucleotide sequence from a 300 bp region of the rabies nucleoprotein was compared with sequence obtained from 403 bat rabies virus samples (23 species) in the repository at the Centers for Disease Control and Prevention (Atlanta, Georgia, USA). A phylogenetic analysis of the data was conducted using the programs DNADIST, NEIGHBOR, SEQBOOT, and CONSENSE in the PHYLIP package, version 3.5 (Felsenstein, 1993). GenBank sequence for an Australian bat lyssavirus (Af081020) was used as the outgroup. Graphical representation of the phylogenetic analysis was obtained with the program TREEVIEW (Page, 1996).

RESULTS

Brain tissue from 65 bat species (937 individuals) was tested for the presence of the rabies virus (Table 1). Of the 937 individuals, one male *Lasiurus ega* (TK 62880; The Museum Texas Tech University) was positive for rabies virus. Although many *Artibeus lituratus, Eumops bonariensis, Molossus molossus,* and *Sturnira lilium* were collected, sample sizes of most species were not large enough for independent analysis. Therefore, all species

TABLE 1. Bat species collected from Mexico and Paraguay. Twenty-one localities (October 1994–January 1996) and twenty-seven localities (August 1996–March 1997) were sampled in Mexico and Paraguay, respectively. See Appendix A for specific locality information.

	Mexico		Paraguay		
	F[a]	M[b]	F	M	Total
Anoura geoffroyi	1	9	0	0	10
Artibeus fimbriatus	0	0	13	7	20
Artibeus hirsutus	1	1	0	0	2
Artibeus intermedius	10	12	0	0	22
Artibeus jamaicensis	7	11	2	3	23
Artibeus lituratus	3	0	45	51	99
Balantiopteryx plicata	1	5	0	0	6
Carollia perspicillata	0	0	10	10	20
Chiroderma doriae	0	0	0	1	1
Chiroderma salvini	1	0	0	0	1
Dermanura azteca	3	3	0	0	6
Dermanura phaeotis	3	2	0	0	5
Dermanura sp.	1	0	0	0	1
Dermanura tolteca	7	5	0	0	12
Desmodus rotundus	11	19	2	2	34
Diaemus youngii	0	0	2	0	2
Enchistenes hartii	2	1	0	0	3
Eptesicus brasiliensis	0	0	2	0	2
Eptesicus furinalis	0	0	19	7	26
Eptesicus sp.	0	0	2	0	2
Eumops dabbenei	0	0	0	2	2
Eumops glaucinus	0	0	12	9	21
Eumops bonariensis	0	0	97	63	160
Eumops perotis	0	0	0	1	1
Glossophaga leachii	0	3	0	0	3
Glossophaga mexicana	1	0	0	0	1
Glossophaga morenoi	0	3	0	0	3
Glossophaga soricina	7	11	3	3	24
Glossophaga sp.	0	2	0	0	2
Histiotus macrotus	0	0	0	4	4
Idionycteris phyllotis	1	0	0	0	1
Lasiurus borealis	0	2	0	0	2
Lasiurus ega	0	0	3	8	11
Leptonycteris curasoae	1	2	0	0	3
Leptonycteris nivalis	2	7	0	0	9
Macrotus watershousii	2	8	0	0	10
Micronycteris sp.	0	1	0	0	1
Molossops abrasus	0	0	3	2	5
Molossops planirostris	0	0	5	1	6
Molossops rufus	0	1	0	0	1
Molossops temminckii	0	0	21	20	41
Molossus ater	0	0	13	7	20
Molossus molossus	0	0	48	17	65
Molossus sinaloe	4	0	0	0	4
Mormoops megalophylla	5	4	0	0	9
Myotis albescens	0	0	8	6	14
Myotis nigricans	0	0	14	6	20
Myotis riparius	0	0	0	1	1
Myotis sp.	0	0	3	2	5
Myotis velifer	1	0	0	0	1
Natalus straminus	1	0	0	0	1
Noctilio leporinus	0	0	4	5	9
Nyctinomops laticaudatus	0	0	2	1	3

TABLE 1. Continued.

	Mexico		Paraguay		
	F[a]	M[b]	F	M	Total
Plecotus mexicanus	7	2	0	0	9
Promops centralis	0	0	1	3	4
Promops nasutus	0	0	1	6	7
Pteronotus davyi	1	0	0	0	1
Pteronotus parnelli	8	1	0	0	9
Pteronotus personatus	1	0	0	0	1
Pygoderma bilabiatum	0	0	10	8	18
Sturnira lilium	14	4	31	34	83
Sturnira ludovici	5	4	0	0	9
Sturnira occidentalis	1	0	0	0	1
Tadarida brasiliensis	2	14	0	0	16
Vampyrops lineatus	0	0	12	7	19
Grand Total	115	137	388	297	937

[a] Female.
[b] Male.

were treated as one sample resulting in a prevalence rate of 0.1%. The rabies positive *L. ega* was captured by mist net over water of the Pilcomayo river in Paraguay (22°27.16′; 62°20.65′) during early spring (18 August 1996). The bat was an adult male of average size in cranial and body measurements, but weighed 9 g, which is below average body weight (11–14 g).

Virus typing by reaction of brain material with monoclonal antibodies detected antigenic variant 6, a variant of rabies virus associated with the lasiurine bats of the USA (Smith, 1989; Diaz et al., 1994). Pairwise analysis of the rabies virus RNA extracted from the brain material of the Paraguayan *L. ega* (identified as PAle3513 in Table 2) differed by only one nucleotide (>99% homology) from the 300 bp consensus sequence for virus from *Lasiurus cinereus* collected in the USA (Table 2). Seven additional rabies virus samples from *L. ega,* all collected in the USA (Arizona, Texas), varied in their nucleotide homology with the Paraguayan sample and with the *L. cinereus* consensus sequence. One Arizona *L. ega* differed significantly from all other samples in the analysis, sharing <90% homology with the other *L. ega* and *L. cinereus* samples. Two Arizona *L. ega* shared >99% homology with the consensus sequence for the virus samples from *L. cinereus.* The four remaining *L. ega* (three from Arizona and one from Texas) shared five nucleotide substitutions, setting them apart from the consensus sequence of *L. cinereus.*

The rabies variant found in *L. ega* from Paraguay and in *L. cinereus* from the USA differed by approximately 15% from rabies virus in *Desmodus rotundus* collected in Argentina (Table 2). The rabies variant from *L. ega* also differed 15% from published rabies virus sequence from *D. rotundus* of Brazil, Paraguay, and Venezuela (Smith et al., 1992; de Mattos et al., 1996).

A phylogenetic analysis of rabies virus from 403 bat samples (representing 23 species) grouped 22/23 samples of *L. cinereus* in a single clade that included a minority of samples from seven other species (Fig. 1) (Smith et al., 1995). The minority of samples in this clade included 3/7 *L. ega,* 1/9 *Antrozous pallidus,* 2/114 *Eptesicus fuscus,* 1/12 *Lasiurus intermedius,* 2/26 *Lasionycteris noctivagans,* 1/6 *Myotis velifer,* and 1/2 *Plecotus townsendii.* All samples in this clade show high homology with the consensus sequence for virus samples from *L. cinereus.* No internal clustering associated with any one species was identified within this clade. Monophy-

TABLE 2. Nucleotide sequence of a 300 bp region of the rabies nucleoprotein gene from *Lasiurus cinereus, Lasiurus ega,* and *Desmodus rotundus.* Genome position indicates the 5′ nucleotide in reference to the sequence of SADB19 (Conzelman et al., 1990). Samples are identified as follows: The first four letters indicate the state and country of collection (AZ, Arizona; CA, California; FL, Florida; IN, Indiana; MI, Missiones; NY, New York; PA, Paraguay; TN, Tennessee; TX, Texas; US, United States), the fifth and sixth letters are an abbreviation for the bat species (dr, *Desmodus rotundus*; lc, *L. cinereus*; le, *L. ega*;), the number is a unique identifier for each sample in the virus repository at the Centers for Disease Control and Prevention. The sequence for ten identical virus samples from hoary bats collected in Arizona, California, Colorado, Georgia, Indiana, Tennessee, Texas, and Wisconsin are not shown individually, but are identified as the consensus sequence for *L. cinereus* (CONSENSUS).

	1157				1206
Consensus	TGAAGCAGCT	GAACTGACCA	AGACAGAAGT	GGCTTTGGCA	GATGACGGAA
INUSlc1199	----------	----------	----------	----------	----------
TXUSlc4272	----------	----------	----------	----------	----------
AZUSle3046	----------	----------	----------	----------	----------
AZUSle3870	----------	----------	----------	----------	--------g-
INUSlc3178	----------	----------	----------	----------	----------
INUSlc3193	----------	----------	----------	----------	----------
INUSlc3206	----------	----------	----------	----------	----------
INUSlc4642	----------	----------	----------	----------	----------
INUSlc4647	----------	----------	----------	----------	----------
NYUSlc46	----------	----------	----------	----------	----------
TNUSlc3313	----------	----------	----------	----------	----------
FLUSlc884	----------	----------	----g-----	----------	----------
INUSlc3197	----------	----------	----------	------a---	----------
__PAle3513	----------	----------	----------	----------	----------
AZUSle3050	----------	----------	----------	----------	----------
AZUSle3347	----------	----------	----------	----------	----------
AZUSle3284	----------	----------	----------	----------	----------
TXUSle4266	----------	----------	----------	----------	----------
AZUSle3285	---g------	---tca--t-	----t-----	a--c-----t	----------
MIARdr893	---g------	---t-a--a-	-ag-t---ac	---cc-----	----------

	1207				1256
Consensus	CCGTCAACTC	TGATGATGAG	GACTACTTCT	CAAGTGAAAC	CAGGAGTCCT
INUSlc1199	----------	----------	----------	----------	----------
TXUSlc4272	----------	----------	----------	----------	----------
AZUSle3046	----------	----------	----------	----------	----------
AZUSle3870	----------	----------	----------	----------	----------
INUSlc3178	----------	----------	----------	----------	----------
INUSlc3193	----------	----------	----------	-------g--	----------
INUSlc3206	----------	----------	----------	----------	----------
INUSlc4642	----------	----------	----------	----------	----------
INUSlc4647	----------	----------	----------	----------	----------
NYUSlc46	----------	----------	----------	----------	----------
TNUSlc3313	----------	----------	----------	----------	----------
FLUSlc884	----------	----------	----------	----------	----------
INUSlc3197	----------	----------	----------	----------	----------
__PAle3513	----------	---c------	----------	----------	----------
AZUSle3050	-a--------	---c------	----------	-------g--	----------
AZUSle3347	-a--------	---c------	----------	-------g--	----------
AZUSle3284	----------	----------	----------	-------g--	----------
TXUSle4266	----------	----------	----------	-------g--	----------
AZUSle3285	-------t--	----------	----------	-t-----g--	---------g
MIARdr893	-a-----t--	----------	----------	-c--------	---------a

TABLE 2. Continued.

	1257				1306
Consensus	GAGGCGGTTT	ACACCCGAAT	CATGATGAAT	GGAAGTCGAT	TGAAAAGATC
INUSlc1199	----------	-t--------	---------c	----------	----------
TXUSlc4272	----------	----------	---------c	----------	----------
AZUSle3046	----------	----------	----------	----------	----------
AZUSle3870	----------	----------	----------	----------	----------
INUSlc3178	----------	----------	----------	----------	----------
INUSlc3193	----------	----------	----------	----------	----------
INUSlc3206	----------	----------	----------	----------	----------
INUSlc4642	----------	----------	----------	----------	----------
INUSlc4647	----------	----------	----------	----------	----------
NYUSlc46	----------	----------	----------	----------	----------
TNUSlc3313	----------	----------	----------	----------	----------
FLUSlc884	----------	----------	----------	----------	----------
INUSlc3197	----------	----------	----------	----------	----------
__PAle3513	----------	----------	----------	----------	----------
AZUSle3050	----------	-t--------	----------	---g--a---	----------
AZUSle3347	----------	-t--------	----------	---g--a---	----------
AZUSle3284	----------	-t--------	----------	---g--a---	----------
TXUSle4266	-----a----	-t--------	----------	---g------	----------
AZUSle3285	-----a--c-	----t-----	----------	---g--a--c	----------
MIARdr893	--a--a----	----a-----	----------	---g--a--c	-a--------

	1307				1356
Consensus	ACACATAAGG	AGGTATGTCT	CAGTCAGCTC	CAATCATCAG	GCCCGTCCTA
INUSlc1199	----------	----------	----------	----------	----------
TXUSlc4272	----------	----------	----------	----------	----------
AZUSle3046	----------	----------	----------	----------	----------
AZUSle3870	----------	----------	----------	----------	----------
INUSlc3178	----------	----------	----------	----------	----------
INUSlc3193	----------	----------	----------	----------	----------
INUSlc3206	----------	----------	----------	----------	----------
INUSlc4642	----------	------a---	----------	----------	----------
INUSlc4647	----------	----------	----------	----------	----------
NYUSlc46	----------	----------	----------	----------	----------
TNUSlc3313	----------	----------	----------	----------	--a-------
FLUSlc884	----------	----------	----------	----------	----------
INUSlc3197	----------	----------	----------	----------	----------
__PAle3513	----------	----------	----------	----------	----------
AZUSle3050	----------	----------	----------	----------	--a-------
AZUSle3347	----------	----------	----------	----------	--a-------
AZUSle3284	----------	----------	----------	----------	--a-------
TXUSle4266	----------	----------	----------	----------	--a-------
AZUSle3285	----------	----------	----------	---------a	--t--c--c-
MIARdr893	----------	----------	----------	t--------a	a-t--c----

TABLE 2. Continued.

	1357				1406
Consensus	ACTCATTCGC	TGAATTTTTG	AACAAGACAT	ACTCAAGTGA	TTCTTAAAGA
INUSlc1199	----------	----------	----------	----------	----------
TXUSlc4272	----------	----------	----------	----------	----------
AZUSle3046	----------	----------	----------	----------	----------
AZUSle3870	----------	----------	----------	----------	----------
INUSlc3178	----------	-------c--	----------	----------	----------
INUSlc3193	----------	----------	----------	----------	----------
INUSlc3206	----------	----------	----------	----------	----------
INUSlc4642	----------	----------	----------	----------	----------
INUSlc4647	----------	------c---	----------	----------	----------
NYUSlc46	----------	----------	----------	----------	----------
TNUSlc3313	----------	----------	----------	----------	----------
FLUSlc884	----------	----------	----------	----------	----------
INUSlc3197	----------	----------	----------	----------	----------
__PAle3513	----------	----------	----------	----------	----------
AZUSle3050	----------	----------	----------	----------	----------
AZUSle3347	----------	----------	----------	----------	----------
AZUSle3284	----------	----------	----------	----------	----------
TXUSle4266	----------	----------	----------	----------	----------
AZUSle3285	----------	c--g-----a	----------	----g-----	---g----a-
MIARdr893	----g--t--	g--g-----a	-----a----	----g-----	c--g-g-g--

	1407				1456
Consensus	GTAGAACAAC	GAGATGGTAA	ACATCAATAA	ATTATGTACA	TCCTTCATGA
INUSlc1199	----------	----------	----------	----------	----------
TXUSlc4272	----------	----------	----------	----------	----------
AZUSle3046	----------	----------	----------	----------	----------
AZUSle3870	----------	----------	----------	----------	----------
INUSlc3178	----------	----------	----------	----------	----------
INUSlc3193	----------	----------	----------	----------	----------
INUSlc3206	----------	----------	----------	----c-----	----------
INUSlc4642	----------	----------	----------	----------	----------
INUSlc4647	----------	----------	----------	----------	----------
NYUSlc46	----------	-------c--	----------	----------	----------
TNUSlc3313	----------	----------	----------	----------	----------
FLUSlc884	----------	----------	----------	--g-------	----------
INUSlc3197	----------	----------	----------	---g------	----------
__PAle3513	----------	----------	----------	-c--------	--t-------
AZUSle3050	-----g----	----------	----------	----------	----------
AZUSle3347	-----g----	----------	----------	----------	----------
AZUSle3284	-----g----	----------	----------	----------	----------
TXUSle4266	-----g----	----------	----------	----------	---c------
AZUSle3285	--t-------	a----t----	---ct-----	---g------	-------c--
MIARdr893	--t-------	a----t----	---c---cg-	ga-g-a----	--tc------

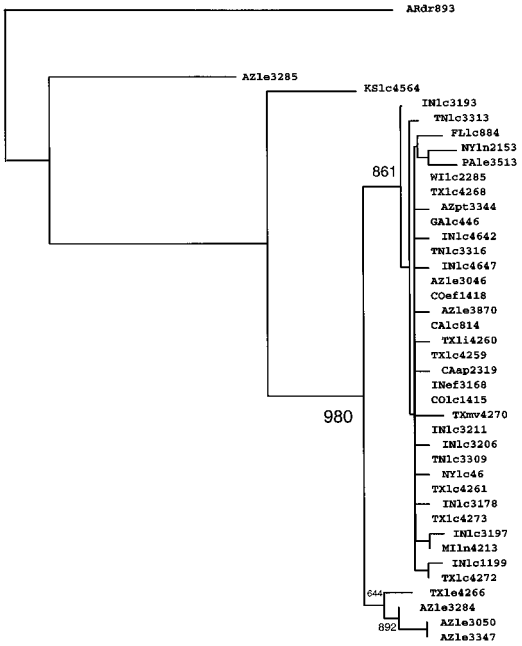

FIGURE 1. Neighbor-joining tree for nucleotide sequence of a 300 bp region of the rabies nucleoprotein gene found in 40 bats. GenBank sequence for an Australian bat lyssavirus (Af081020) was used as the outgroup to root the tree (not shown). Bootstrap values obtained from 1000 iterations of the data are shown on the cladogram. Samples are identified as follows: first two letters represent the state or country of collection (AR, Argentina; AZ, Arizona; CA, California; CO, Colorado; FL, Florida; GA, Georgia; IN, Indiana; KS, Kansas; MI, Michigan; NY, New York; PA, Paraguay; TN, Tennessee; TX, Texas; WI, Wisconsin;), third and fourth letters represent the bat species (ap, *Antrozous pallidus;* dr, *Desmodus rotundus;* ef, *Eptesicus fuscus;* 1c, *Lasiurus cinereus;* 1e, *Lasiurus ega;* 1i, *Lasiurus intermedius;* 1n, *Lasionycteris noctivagans;* mv, *Myotis velifer;* pt, *Plecotus townsendii*), and the number is a unique identifier for the sample in the virus repository at the Centers for Disease Control and Prevention (Atlanta, Georgia, USA).

ly of this sample cluster and exclusion of five *L. ega* samples was supported in 861 of 1,000 bootstrap iterations of the tree construction.

A second clade (supported in 892 of the 1,000 bootstrap iterations) included three virus samples from *L. ega* (all from Arizona). A single virus sample from *L. ega* collected in Texas was less resolved in the analysis, clustering with the clade comprising of the Arizona *L. ega* samples in 644 of the 1,000 bootstrap iterations. The remaining sample, a *L. ega* collected in Arizona, differed significantly from all other samples in the analysis forming an independent lineage.

DISCUSSION

Bats of the genus *Lasiurus* are distributed from Canada to Argentina. The genus includes seven species, all of which are aerial insectivores. Lasiurine bats have been studied in North America, but little is known about their natural history or habits in South America (Redford and Eisenberg, 1992). In the northern region of their distribution, lasiurine bats are migratory and/or hibernate during the winter months. Members of the genus are thought to be primarily solitary but may collect in small clusters, which may represent nursery roost groups or migratory aggregations. Interspecific interactions or aggregations in lasiurine bats are unknown.

The southern yellow bat, *Lasiurus ega,* is distributed from southern California and Arizona to northern Argentina and is considered common throughout its range (Eisenberg, 1989). It roosts in palm trees, ornamental trees, and under roofs in buildings, and is usually collected in nets over water (Barques et al., 1993). This bat does not hibernate, but becomes torpid in cooler weather. Although poorly documented, this bat most likely is migratory, seeking warmer roosts and more consistent food supplies in winter (Eisenberg, 1989).

The body weight of the rabies positive *L. ega* was less than normal, possibly as a result of its rabies infection. Redford and Eisenberg (1992) reported the weight of male *L. ega* from Paraguay to range from 11 to 14 g with a mean of 12.3 g and Barquez et al. (1993) reported a range of 13 to 20 g for specimens from Argentina. Constantine (1988) stated that an ill, rabid bat is often thin and dehydrated. Signs of rabies in laboratory studies include reluc-

tance to eat and refusal of water (Brass, 1994). The inability to fly also is associated with a late stage rabies infection, which was not true of this rabid bat.

Previous studies have reported rabies prevalence data similar to that reported in this study. Yancey et al. (1997) reported 0/171 for a sample containing 12 different bat species; Steece and Altenbach (1989) reported 4/750 adults and 14/600 juveniles for *Tadarida brasiliensis;* Favi and Catalán (1986) reported 1/619 for *T. brasiliensis;* Constantine et al. (1968) reported 5/2192 at one locality and 5/652 at another locality for *T. brasiliensis.* In studies where presumed healthy bats are screened for the rabies virus, prevalence rates are consistently reported as <0.1%.

Information about the distribution of rabies virus variants in wildlife reservoirs of Latin America is sparse. Limited amounts of sequence data are available for South American bats with the exception of virus samples from *D. rotundus* and *T. brasiliensis.* An important inference from this analysis is that the cycle maintaining the rabies variant in *L. ega* appears to be independent of rabies in the common vampire bat (*D. rotundus*), which has been implicated as the major vector in livestock rabies outbreaks of Latin America (Fig. 1). All 34 specimens of *D. rotundus* collected in Mexico and Paraguay were negative for the rabies virus.

Determining the relationship between rabies variants and their hosts can be problematic when species are represented with only a few samples or from only a small part of their range. In the USA, *L. cinereus* is presumed to be the reservoir for antigenic variant 6. This analysis identified the same rabies variant in the Paraguayan *L. ega.* The most likely explanation of this rabies infection is contact with a rabid *L. cinereus.* The infection in *L. ega* is believed to be a spillover event; although the adaptive mechanism is unknown, populations of virus selected by intraspecific contact are less efficiently transmitted after interspecific contact. Isolated spillover cases occur, however serial transmission in a second species is not often observed.

Infection of multiple Lasiurus species (with the same rabies variant) could indicate a generalized lasiurine reservoir, with infection occurring throughout the area of sympatry (much of North and South America) by sustained interspecific transmission. Although no evidence of a generalized reservoir is supported in this analysis, the possibility that *L. ega* may serve as a reservoir for the variant in Latin America cannot be excluded.

While indicating the presence of antigenic variant 6 in Latin America, the data does not identify a reservoir outside the USA. Identification of bat reservoirs in Latin America and clarification of issues related to spillover infection, awaits accumulation of additional data. Virus typing, bat species identification, and additional information about the natural history of bat species are needed to understand the role of bats in the epidemiology of rabies in the western hemisphere.

ACKNOWLEDGMENTS

The authors thank the staffs of the Rabies Laboratories in the Arizona, California, Colorado, Florida, Georgia, Indiana, Kansas, Michigan, New York, Tennessee, Texas, and Wisconsin Departments of Health for contributing the bat rabies samples used in this study. We thank C. López-Gonzáles, C. Sánchez-Hernández, M. L. Romero-Almaraz, R. López-Wilchis, A. Núñez-Garduño, R. D. Owen, and M. R. Willig, and their colleagues and students for valuable assistance in collection and identification of bats. For laboratory assistance, we thank P. Yager for help with the monoclonal antibody analysis and L. Orciari for help with the nucleotide sequencing. C. Rupprecht made a substantial contribution by allowing the use of the rabies laboratory.

Funding was provided for collaborative research investigating the mammals of Michoacán by the Mexican Consejo Nacional de Ciencia y Technología (CONACyT grant 400-355-5-1361N, awarded to C. Sánchez-Hernández, and grant 1369-N9206, awarded to R. López-Wilchis), and by the National Science Foundation (NSF grant INT-9301940, awarded to R. D. Owen). The Paraguayan specimens were collected as part of a project investigating small

mammals (NSF grant DEB-9400926, awarded to R. D. Owen and M. R. Willig). Travel Assistance to CDC was provided by the Department of Biological Sciences, Texas Tech University, and the CDC Rabies Section.

LITERATURE CITED

BARQUES, R. M., N. P. GIANNINI, AND M. A. MARES. 1993. Guide to the Bats of Argentina. Oklahoma Museum of Natural History, Norman, Oklahoma, 119 pp.

BRASS, D. A. 1994. Rabies in Bats. Livia Press, Ridgefield, Connecticut, 335 pp.

CONSTANTINE, D. G., E. S. TIERKEL, M. D. KLECKNER, AND D. M. HAWKINS. 1968. Rabies in New Mexico cavern bats. Public Health Reports 83: 303–316.

———. 1988. Health precautions for bat researchers. *In* Ecological and behavioral methods for the study of bats, T. H. Kunz (ed.). Smithsonian Institution Press, Washington, D.C., pp. 491–526.

CONZELMANN, K. K., J. H. COX, L. G. SCHNEIDER, AND H. J. THIEL. 1990. Molecular cloning and complete nucleotide sequence of the attenuated rabies virus SADB19. Virology 175: 485–499.

DE MATTOS, C. A., C. C. DE MATTOS, J. S. SMITH, E. T. MILLER, S. PAPO, A. UTRERA, AND B. I. OSBURN. 1996. Genetic characterization of rabies field isolates from Venezuela. Journal Clinical Microbiology 34: 1553–1558.

DIAZ, A. M., S. PAPO, A. RODRIGUEZ, AND J. S. SMITH. 1994. Antigenic analysis of rabies-virus isolates from Latin America and the Caribbean. Journal of Veterinarian Medicine Series B 41: 153–160.

EISENBERG, J. F. 1989. Mammals of the Neotropics, Vol. 1, The Northern Neotropics: Panama, Colombia, Venezuela, Guyana, Suriname, French Guiana. University of Chicago Press, Chicago, Illinois, 550 pp.

FAVI, M., AND R. CATALÁN. 1986. Rabia en murcielagos en Chile. Avances en Ciencias Veterinarias 1: 73–76.

FELSENSTEIN, J. 1993. PHYLIP Inference Package. Version 3.5c. Department of Genetics, University of Washington, Seattle, Washington, 93 pp.

GREENHALL, A. M. 1965. Technique for the removal of the brain in bats. Nature 208: 1014–1015.

ORGANIZACION PANAMERICANA DE LA SALUD. 1997. Vigilancia epidemilogica de la rabia en las Americas. Boletin de Vigilancia epidemilogica de la rabia en las Americas. Boletin de Vigilancia Epidemiologica de la Rabia en las Americas XXIX. Instituto Panamericano de Proteccion de Alimentos y Zoonosis, Martínez, Buenos Aires, Argentina, 29 pp.

PAGE, R. D. 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. Computer Applied Bioscience 12: 357–358.

REDFORD, K. H., AND J. F. EISENBERG. 1992. Mammals of the Neotropics, Volume 2, The Southern Cone: Chile, Argentina, Uruguay, Paraguay. University of Chicago Press, Chicago, Illinois, 430 pp.

SMITH, J. S. 1989. Rabies virus epitopic variation: Use in ecologic studies. Advances in Virus Research 36: 215–253.

———, L. A. ORCIARI, P. A. YAGER, H. D. SEIDEL, AND C. K. WARNER. 1992. Epidemiologic and historical relationships among 87 rabies virus isolates as determined by limited sequence analysis. Journal of Infectious Disease 166: 296–307.

———. 1995. Rabies Virus. *In* Manual of clinical microbiology, 6th Edition, P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.). American Society for Microbiology Press, Washington, D.C., pp. 997–1003.

———, L. A. ORCIARI, AND P. A. YAGER. 1995. Molecular epidemiology of rabies in the United States. Seminars in Virology 6: 387–400.

STEECE, R., AND S. ALTENBACH. 1989. Prevalence of rabies specific antibodies in the Mexican free-tailed bat (*Tadarida brasiliensis mexicana*) at Lava Cave, New Mexico. Journal of Wildlife Diseases 25: 490–496.

TADEI, V., C. A. GONCALVES, W. A. PEDRO, W. J. TADEI, I. KOTAIT, AND C. ARIETA. 1991. Distribuicao do morcego vampiro *Desmodus rotundus* (Chiroptera, Phyllostomidae) no estado de São Paulo e a raiva dos animais domesticos. Governo do Estado de São Paulo Secretaria de Agricultura e Abastecimento Coordenadoria de Assistencia Tecnica Integral, Campinas, São Paulo, Brazil, 107 pp.

VELLECA, W. M., AND F. T. FORRESTER. 1981. Laboratory Methods for Detecting Rabies. US Government Printing Office, Washington, D.C., 159 pp.

YANCEY, F. D., P. RAJ, S. U. NEILL, C. JONES. 1997. Survey of rabies among free-flying bats from the Big Bend region of Texas. Occasional Papers Museum Texas Tech University 165: 1–5.

Received for publication 25 January 2000.

APPENDIX A.

Gazetteer of chiropteran collection localities from Michoacan and Colima, Mexico during October 1994–January 1996, and from Paraguay during August 1996–March 1997. Collecting localities are listed in alphabetical order for each country, then by date of collection period. Collection periods were from 1 to 3 nights in duration. Locality information is followed by scientific names of the bats collected at each site. Species are listed in alphabetical order. Description of collection localities and bat species includes (in the following format):

Site reference name, State or Department, Country. Latitude (decimal minutes), Longitude (decimal minutes), first day of collection period (month/day/year). Bat species, number of males, number of females.

Apatzingan, Michoacan, Mexico. 19°3.80′, 102°19.64′, 1/07/95. *Artibeus intermedius* 2,1. *Artibeus jamaicensis* 1,1. *Glossophaga leachii* 1,0.

Aquililla, Michoacan, Mexico. 18°45.65′, 102°45.94′, 1/27/95. *Dermanura azteca* 0,1. *Dermanura sp.* 0,1. *Dermanura tolteca* 1,0. *Glossophaga leachii* 1,0. *Leptonycteris curasoae* 1,1.

Arteaga Platanito, Michoacan, Mexico. 18°38.22′ 101°55.64′, 5/04/95. *Macrotus waterhousii* 4,2. *Pteronotus parnelli* 0,1.

Benito Juarez, Michoacan, Mexico. 19°19.23′, 100°29.50′, 10/27/94. *Chiroderma salvini* 0,1. *Dermanura azteca* 1,0. *Dermanura phaeotis* 2,1. *Dermanura tolteca* 3,6. *Desmodus rotundus* 1,0. *Glossophaga soricina* 6,2. *Glossophaga sp.* 1,0. *Sturnira lilium* 1,2.

Carapan, Michoacan, Mexico. 19°51.17′, 102°1.96′, 1/4/95. *Sturnira ludovici* 2,1. *Tardarida brasiliensis* 0,1.

Cenobio Moreno, Michoacan, Mexico. 19°5.92′, 102°29.99′, 1/9/95. *Glossophaga morenoi* 1,0. *Glossophaga soricina* 1,0.

Cenobio Moreno/Apatzingan, Michoacan, Mexico. 19°3.34′, 102°13.47′, 1/9/95. *Artibeus intermedius* 1,0. *Dermanura tolteca* 0,1. *Glossophaga leachii* 1,0. *Macrotus waterhousii* 1,0.

Cerro Colorado, Michoacan, Mexico. 19°19.00′, 100°27.80′, 10/25/94. *Artibeus intermedius* 6,6. *Artibeus jamaicensis* 3,1. *Artibeus lituratus* 0,2. *Dermanura tolteca* 1,0. *Desmodus rotundus* 0,1. *Pteronotus parnelli* 0,1. *Sturnira lilium* 2,7.

Coalcoman, Michoacan, Mexico. 18°52.04′, 103°8.25′, 6/16/95. *Enchistenes hartii* 0,1. *Sturnira lilium* 1,2.

Cueva Los Ortices, Colima, Mexico. 19°4.80′, 103°43.64′, 1/17/96. *Desmodus rotundus* 10,2. *Glossophaga soricina* 2,1. *Mormoops megalophylla* 1,1. *Natalus straminus* 0,1. *Pteronotus davyi* 0,1. *Pteronotus parnelli* 0,3. *Pteronotus personatus* 0,1.

Dos Aguas, Michoacan, Mexico. 18°49.38′, 102°56.08′, 2/5/95. *Lasiurus borealis* 1,0. *Plecotus mexicanus* 2,5. *Pteronotus parnelli* 1,1.

Hidalgo Centro Recreativo, Michoacan, Mexico. 19°36.84′, 100°41.89′, 11/1/94. *Leptonycteris nivalis* 1,0. *Mormoops megalophylla* 3,3.

Hidalgo Cerro el Ventero, Michoacan, Mexico. 19°37.70′, 100°41.70′, 11/1/94. *Idionycteris phyllotis* 0,1. *Myotis velifer* 0,1. *Plecotus mexicanus* 0,2.

Huetamo La Yerbabuena, Michoacan, Mexico. 18°31.38′, 100°56.29′, 5/11/95. *Glossophaga morenoi* 1,0. *Macrotus waterhousii* 1,0. *Pteronotus parnelli* 0,1.

La Concha Lago de Chandio, Michoacan, Mexico. 19°5.62′, 102°24.39′, 1/5/95. *Artibeus hirsutus* 1,0. *Artibeus intermedius* 2,2. *Artibeus jamaicensis* 4,0. *Balantiopteryx plicata* 4,1. *Dermanura phaeotis* 0,1. *Glossophaga mexicana* 0,1. *Macrotus waterhousii* 1,0. *Molossus sinaloe* 0,4.

Las Grutas, Michoacan, Mexico. 19°38.34′, 100°30.10′, 10/29/94. *Anoura geoffroyi* 9,1. *Artibeus hirsutus* 0,1. *Dermanura azteca* 2,2. *Desmodus rotundus* 5,4. *Glossophaga morenoi* 1,0. *Leptonycteris nivalis* 6,2. *Mormoops megalophylla* 0,1. *Sturnira lilium* 0,2. *Sturnira ludovici* 2,6. *Sturnira occidentalis* 0,1. *Tardarida brasiliensis* 4,0.

Nueva Italia, Michoacan, Mexico. 18°50.97′, 102°8.02′, 5/7/95. *Artibeus intermedius* 1,0. *Artibeus jamaicensis* 0,1. *Lasiurus borealis* 1,0. *Macrotus waterhousii* 1,0. *Micronycteris sp.* 1,0. *Pteronotus parnelli* 0,1.

Palos Marias, Michoacan, Mexico. 18°48.84′, 103°32.24′, 1/14/96. *Artibeus jamaicensis* 2,3. *Dermanura phaeotis* 0,1. *Desmodus rotundus* 0,3. *Glossophaga soricina* 0,2. *Leptonycteris curasoae* 1,0. *Molossops rufus* 1,0. *Sturnira lilium* 0,1.

Quiroga, Michoacan, Mexico. 19°40.60′, 101°34.53′, 12/31/94. *Tardarida brasiliensis* 10,1.

Villa Victoria, Michoacan, Mexico. 18°47.54′, 103°25.38′, 6/23/95. *Glossophaga soricina* 1,2.

Villa Victoria/Cueva Las Playitas, Michoacan, Mexico. 18°44.78′, 103°22.89′, 6/20/95. *Artibeus intermedius* 0,1. *Artibeus jamaicensis* 1,1. *Artibeus lituratus* 0,1. *Balantiopteryx plicata* 1,0. *Desmodus rotundus* 3,1. *Enchistenes hartii* 1,1. *Glossophaga soricina* 1,0. *Glossophaga sp.* 1,0.

Base Naval Pedro P. Pena, Boqueron, Paraguay. 22°27.16′, 62°20.65′, 8/16/96. *Eptesicus furinalis* 1,2. *Eumops glaucinus* 1,4. *Eumops patagonicus* 4,19. *Histiotus macrotus* 4,0. *Lasiurus ega* 1,0. *Molossops planirostris* 0,2. *Molossops temminckii* 3,4. *Molossus molossus* 6,12. *Myotis albescens* 1,2. *Myotis nigricans* 1,1. *Noctilio leporinus* 0,2. *Promops nasutus* 4,1.

Estancia Canada Mil, Boqueron, Paraguay. 22°22.68′, 62°15.57′, 8/21/96. *Eptesicus furinalis* 3,4. *Eumops patagonicus* 3,4. *Molossops temminckii* 9,6. *Molossus molossus* 3,1. *Myotis nigricans* 1,4. *Promops nasutus* 2,0.

Estancia Chacoite, Boqueron, Paraguay. 21°11.40′, 61°41.81′, 3/22/97. *Eptesicus furinalis* 0,2. *Eptesicus sp.* 0,2. *Eumops patagonicus* 13,26. *Molossus molossus* 0,4. *Myotis nigricans* 0,1.

Estancia Golondrina, Caazapa, Paraguay. 25°32.30′, 55°29.02′, 10/11/96. *Artibeus lituratus* 4,5. *Sturnira lilium* 0,1.

Estancia Golondrina, Caazapa, Paraguay. 25°40.95′, 55°31.80′, 11/1/96. *Artibeus fimbriatus* 1,0. *Sturnira lilium* 1,0.

Estancia Itabo, Canindeyu, Paraguay. 24°26.18′, 54°39.76′, 2/1/97. *Artibeus fimbriatus* 2,4. *Artibeus lituratus* 7,5. *Carollia perspicillata* 2,2. *Eumops glaucinus* 1,1. *Eumops patagonicus* 1,1. *Pygoderma bilabiatum* 3,1. *Sturnira lilium* 3,5.

Estancia Loma Pora, Presidente Hayes, Paraguay. 23°29.92′, 57°32.92′, 1/21/97. *Diaemus youngii* 0,2. *Eumops patagonicus* 4,0. *Lasiurus ega* 0,2. *Molossops temminckii* 1,0. *Molossus ater* 2,5. *Molossus molossus* 2,7. *Myotis albescens* 3,3. *Myotis nigricans* 0,2. *Myotis sp.* 1,3. *Vampyrops lineatus* 1,0.

Estancia Rivas, Canindeyu, Paraguay. 24°30.43′, 54°38.25′, 9/29/96. *Artibeus fimbriatus* 0,1. *Artibeus lituratus* 0,1. *Eumops glaucinus* 2,2. *Molossus molossus* 1,0. *Myotis sp.* 1,0. *Sturnira lilium* 3,2.

Estancia Rivas, Canindeyu, Paraguay. 24°30.63′, 54°37.12′, 10/1/96. *Artibeus lituratus* 3,7.

Estancia Rivas, Canindeyu, Paraguay. 24°26.23′, 54°39.98′, 10/3/96. *Artibeus fimbriatus* 1,3. *Carollia perspicillata* 3,2. *Promops centralis* 1,0. *Pygoderma bilabiatum* 1,0. *Sturnira lilium* 2,0.

Estancia Samaklay, Presidente Hayes, Paraguay. 23°28.81′, 59°48.43′, 2/25/97. *Eptesicus furinalis* 1,2. *Eumops glaucinus* 2,1. *Eumops patagonicus* 18,26. *Lasiurus ega* 3,1. *Molossops planirostris* 0,2. *Molossops temminckii* 1,2. *Molossus ater* 2,2. *Molossus molossus* 4,6. *Myotis albescens* 1,2. *Noctilio leporinus* 1,2.

Estancia Samaklay, Presidente Hayes, Paraguay. 23°29.47′, 59°49.18′, 2/26/97. *Eumops dabbenei* 1,0. *Eumops glaucinus* 1,0. *Lasiurus ega* 2,0. *Molossops temminckii* 2,0. *Molossus molossus* 0,1. *Noctilio leporinus* 4,0.

Estancia Sombrero, Cordillera, Paraguay. 25°4.20′, 56°36.13′, 2/13/97. *Artibeus fimbriatus* 1,1. *Artibeus jamaicensis* 1,1. *Artibeus lituratus* 5,7. *Carollia perspicillata* 1,0. *Chiroderma doriae* 1,0. *Eptesicus furinalis* 0,1. *Glossophaga soricina* 3,3. *Molossus ater* 1,2. *Sturnira lilium* 2,4. *Vampyrops lineatus* 0,1.

Estancia Yacare, Neembucu, Paraguay. 26°35.44′, 58°6.05′, 1/6/97. *Eptesicus furinalis* 1,2. *Eumops dabbenei* 1,0. *Eumops patagonicus* 2,4. *Lasiurus ega* 2,0. *Molossops abrasus* 2,0. *Molossops planirostris* 0,1. *Molossus ater* 2,4. *Myotis albescens* 0,1. *Myotis nigricans* 0,1. *Promops centralis* 1,0.

Militar Gabino Mendoza, Alto Paraguay, Paraguay. 20°5.30′, 61°47.22′, 9/3/96. *Eumops patagonicus* 17,10. *Eumops perotis* 1,0. *Molossops temminckii* 3,3. *Molossus molossus* 0,2. *Myotis nigricans* 2,5. *Nyctinomops laticaudatus* 1,2.

Parque Nacional Serrania San Luis, Concepcion, Paraguay. 22°36.81′, 57°21.17′, 12/6/96. *Artibeus lituratus* 1,0. *Eumops glaucinus* 0,1. *Eumops patagonicus* 1,3. *Molossops abrasus* 0,1. *Molossus molossus* 0,1. *Vampyrops lineatus* 0,1.

Parque Nacional Serrania San Luis, Concepcion, Paraguay. 22°37.91′, 57°21.35′, 12/7/96. *Artibeus jamaicensis* 1,0. *Artibeus lituratus* 3,0. *Eptesicus brasiliensis* 0,1. *Eptesicus furinalis* 1,2. *Eumops glaucinus* 2,0. *Eumops patagonicus* 0,3. *Molossops abrasus* 0,1. *Molossus molossus* 1,9. *Promops centralis* 1,0. *Pygoderma bilabiatum* 1,1. *Sturnira lilium* 1,4. *Vampyrops lineatus* 1,3.

Parque Nacional Serrania San Luis, Concepcion, Paraguay. 22°40.34′, 57°20.96′, 12/8/96. *Artibeus jamaicensis* 1,1. *Artibeus lituratus* 4,5. *Eptesicus brasiliensis* 0,1. *Eptesicus furinalis* 0,3. *Eumops glaucinus* 0,3. *Eumops patagonicus* 0,1. *Molossops abrasus* 0,1. *Molossops planirostris* 1,0. *Molossops temminckii* 0,3. *Molossus molossus* 0,4. *Pygoderma bilabiatum* 1,0. *Sturnira lilium* 2,1. *Vampyrops lineatus* 4,6.

Parque Nacional Teniente Enciso, Boqueron, Paraguay. 21°12.59′, 61°39.60′, 3/17/97. *Desmodus rotundus* 1,1. *Eumops patagonicus* 0,1. *Molossops temminckii* 0,1. *Molossus molossus* 0,1. *Myotis albescens* 1,0. *Myotis nigricans* 1,0.

Parque Nacional Ybycui, Paraguari, Paraguay. 26°4.64′, 56°50.98′, 11/12/96. *Artibeus lituratus* 2,0. *Desmodus rotundus* 1,0. *Eptesicus furinalis* 0,1. *Molossops temminckii* 1,2. *Myotis nigricans* 1,0. *Promops centralis* 0,1. *Pygoderma bilabiatum* 0,1. *Sturnira lilium* 1,2.

Parque Nacional Ybycui, Paraguari, Paraguay. 26°5.65′, 56°50.38′, 11/14/96. *Artibeus lituratus* 2,3. *Desmodus rotundus* 0,1. *Pygoderma bilabiatum* 1,3. *Sturnira lilium* 12,6.

Reserva Natural del Bosque Mbaracayu, Canindeyu. 24°7.69′, 55°30.34′, 11/23/96. *Artibeus lituratus* 1,3. *Carollia perspicillata* 2,0. *Pygoderma bilabiatum* 1,0.

Reserva Natural del Bosque Mbaracayu, Canindeyu. 24°8.75′, 55°19.14′, 11/26/96. *Artibeus lituratus* 2,1. *Sturnira lilium* 1,0. *Vampyrops lineatus* 0,1.

Reserva Natural del Bosque Mbaracayu, Canindeyu. 24°8.06′, 55°23.13′, 11/28/96. *Artibeus lituratus* 5,2. *Carollia perspicillata* 1,1. *Pygoderma bilabiatum* 0,3. *Sturnira lilium* 6,2. *Vampyrops lineatus* 1,0.

Reserva Natural Privado Itabo, Canindeyu, Paraguay. 24°27.47′, 54°39.85′, 9/24/96. *Artibeus fimbriatus* 1,3. *Artibeus lituratus* 3,3. *Carollia perspicillata* 1,5. *Myotis riparius* 1,0. *Sturnira lilium* 0,4.

Reserva Natural Privado yPeti, Caazapa, Paraguay. 25°32.90′, 55°28.53′, 11/3/96. *Artibeus lituratus* 7,3.

Reserva Natural Privado yPeti, Caazapa, Paraguay. 25°32.90′, 55°28.53′, 11/3/96. *Artibeus fimbriatus* 1,1. *Artibeus lituratus* 0,1. *Pygoderma bilabiatum* 0,1.