

2014

Bat Rabies in Guatemala

James E. Ellison
Auburn University, JEllison@cdc.gov

Amy T. Gilbert
United States Department of Agriculture, Amy.T.Gilbert@aphis.usda.gov

Sergio Recuenco
Division of High-Consequence Pathogens and Pathology

David Moran
Universidad del Valle de Guatemala

Danilo A. Alvarez
Universidad del Valle de Guatemala

See next page for additional authors

Follow this and additional works at: https://digitalcommons.unl.edu/icwdm_usdanwrc

 Part of the [Life Sciences Commons](#)

Ellison, James E.; Gilbert, Amy T.; Recuenco, Sergio; Moran, David; Alvarez, Danilo A.; Kuzmina, Natalia; Garcia, Daniel L.; Peruski, Leonard F.; Mendonca, Mary T.; Lindblade, Kim A.; and Rupprecht, Charles E., "Bat Rabies in Guatemala" (2014). *USDA National Wildlife Research Center - Staff Publications*. 1497.
https://digitalcommons.unl.edu/icwdm_usdanwrc/1497

This Article is brought to you for free and open access by the U.S. Department of Agriculture: Animal and Plant Health Inspection Service at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in USDA National Wildlife Research Center - Staff Publications by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Authors

James E. Ellison, Amy T. Gilbert, Sergio Recuenco, David Moran, Danilo A. Alvarez, Natalia Kuzmina, Daniel L. Garcia, Leonard F. Peruski, Mary T. Mendonca, Kim A. Lindblade, and Charles E. Rupprecht



Bat Rabies in Guatemala

James A. Ellison^{1,2*}, Amy T. Gilbert³, Sergio Recuenco¹, David Moran⁴, Danilo A. Alvarez⁴, Natalia Kuzmina¹, Daniel L. Garcia^{5,6}, Leonard F. Peruski^{5,6}, Mary T. Mendonça², Kim A. Lindblade^{5,6}, Charles E. Rupprecht⁷

1 Division of High-Consequence Pathogens and Pathology, Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America, **2** Department of Biological Sciences, Auburn University, Auburn, Alabama, United States of America, **3** United States Department of Agriculture, National Wildlife Research Center, Ft. Collins, Colorado, United States of America, **4** Center for Health Studies, Universidad del Valle de Guatemala, Guatemala City, Guatemala, **5** Centers for Disease Control and Prevention Regional Office for Central America, Guatemala City, Guatemala, **6** Division of Health Protection, Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America, **7** Ross University School of Veterinary Medicine, Basseterre, St. Kitts, West Indies

Abstract

Rabies in bats is considered enzootic throughout the New World, but few comparative data are available for most countries in the region. As part of a larger pathogen detection program, enhanced bat rabies surveillance was conducted in Guatemala, between 2009 and 2011. A total of 672 bats of 31 species were sampled and tested for rabies. The prevalence of rabies virus (RABV) detection among all collected bats was low (0.3%). Viral antigens were detected and infectious virus was isolated from the brains of two common vampire bats (*Desmodus rotundus*). RABV was also isolated from oral swabs, lungs and kidneys of both bats, whereas viral RNA was detected in all of the tissues examined by hemi-nested RT-PCR except for the liver of one bat. Sequencing of the nucleoprotein gene showed that both viruses were 100% identical, whereas sequencing of the glycoprotein gene revealed one non-synonymous substitution (302_{T,S}). The two vampire bat RABV isolates in this study were phylogenetically related to viruses associated with vampire bats in the eastern states of Mexico and El Salvador. Additionally, 7% of sera collected from 398 bats demonstrated RABV neutralizing antibody. The proportion of seropositive bats varied significantly across trophic guilds, suggestive of complex intraspecific compartmentalization of RABV perpetuation.

Citation: Ellison JA, Gilbert AT, Recuenco S, Moran D, Alvarez DA, et al. (2014) Bat Rabies in Guatemala. PLoS Negl Trop Dis 8(7): e3070. doi:10.1371/journal.pntd.0003070

Editor: Maya Williams, U.S. Naval Medical Research Unit No. 2, Indonesia

Received: February 10, 2014; **Accepted:** June 23, 2014; **Published:** July 31, 2014

This is an open-access article, free of all copyright, and may be freely reproduced, distributed, transmitted, modified, built upon, or otherwise used by anyone for any lawful purpose. The work is made available under the Creative Commons CC0 public domain dedication.

Funding: This work was supported in part by the CDC Global Disease Detection Program, Technical Support Corps. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* Email: JEllison@cdc.gov

Introduction

Bats (Order: Chiroptera) have been implicated as hosts and reservoirs for numerous emerging infectious diseases, and are considered one of the most relevant groups of mammals in the study of disease ecology [1]. Guatemala is home to some of the world's richest bat biodiversity, with over 104 extant species [2–4]. As one representative global disease detection site established by the U.S. Centers for Disease Control and Prevention, enhanced rabies surveillance and pathogen discovery over the past five years targeting bats has facilitated the discovery of numerous novel viral and bacterial agents [5–8]. Considering the diversity and zoonotic potential of pathogens detected to date, the most pressing zoonotic threat from bats in Guatemala is *Rabies virus* (RABV), the only *Lyssavirus* documented in the New World [9]. Rabies is defined clinically in humans that present with an acute progressive encephalitis dominated by hyperactivity or paralytic syndromes that eventually deteriorate towards coma and death in nearly 100% of cases [10].

Rabies epizootiology is well appreciated in countries with an established laboratory-based surveillance network. Combined with molecular epidemiology, enhanced and passive surveillance are used to define the geographic distribution of RABV variants, infer the temporal and spatial spread of infections associated with

diverse reservoir hosts, identify spillover infections into humans, and to devise relevant prevention and control based upon such information [11–13]. Generally, RABV can be divided into two major clades: one comprising variants associated with carnivores around the globe, and another containing variants associated with bats, raccoons and skunks in the New World. In Latin America, RABV is classically recognized as two broadly distinct epizootiological forms or 'cycles', one 'urban', in which dogs may serve as the primary reservoir host and vector, and the other as a so-called rural or 'sylvatic' cycle, involving wildlife [13].

RABV transmitted by vampire bats (*Desmodus rotundus*) represents the most apparent economic and public health threat associated with bats [14]. Vampire bats exist only in Latin America, and range from the Tropic of Cancer in Mexico, to the Tropic of Capricorn in Argentina and Chile [15]. Vampire bats include three monotypic genera, and all three species derive nutrition from feeding on the blood of other vertebrates. A consequence of this unique biological adaptation is that vampire bats are highly effective at transmitting RABV to a wide diversity of mammals, primarily livestock, but also humans, if preferred prey are not widely available [16]. In Latin America, human rabies fatalities have been associated with RABV spillover from frugivorous and insectivorous bats. However, the burden of

Author Summary

In this study we provide results of the first active and extensive surveillance effort for rabies virus (RABV) circulation among bats in Guatemala. The survey included multiple geographic areas and multiple species of bats, to assess the broader public and veterinary health risks associated with rabies in bats in Guatemala. RABV was isolated from vampire bats (*Desmodus rotundus*) collected in two different locations in Guatemala. Sequencing of the isolates revealed a closer relationship to Mexican and Central American vampire bat isolates than to South American isolates. The detection of RABV neutralizing antibodies in 11 species, including insectivorous, frugivorous, and sanguivorous bats, demonstrates viral circulation in both hematophagous and non-hematophagous bat species in Guatemala. The presence of bat RABV in rural communities requires new strategies for public health education regarding contact with bats, improved laboratory-based surveillance of animals associated with human exposures, and novel techniques for modern rabies prevention and control. Additionally, healthcare practitioners should emphasize the collection of a detailed medical history, including questions regarding bat exposure, for patients presenting with clinical syndromes compatible with rabies or any clinically diagnosed progressive encephalitis.

mortality appears negligible compared to the human rabies burden associated with the common vampire bat [17–19].

Laboratory-based surveillance is recommended by the World Health Organization in all RABV enzootic countries. Accurate ongoing prevalence measures are essential in rabies prevention and control, to estimate the burden of disease, to monitor trends to evaluate the effectiveness of case intervention, and to ensure appropriate management of outbreaks [20]. The surveillance system for rabies in Guatemala is passive, where samples from suspected human and animal cases are collected, sent, and tested by the direct fluorescent antibody (DFA) test at one of two laboratories: the Laboratorio Nacional de Salud in Villa Nueva (main facility), or Laboratorio de Ministerio de Agricultura, Ganadería y Alimentación (MAGA) in Quetzaltenango. The number of reported human rabies cases has decreased over the past two decades. Dogs remain the primary RABV vector, and are associated with approximately 73% (44 of 60) of human cases from 1994 to 2012. However, 23% (14 of 60) of these cases were due to an unspecified or unknown exposure [21,22]. In the U.S., numerous reports link insectivorous bats to the majority of human rabies cases without a history of conventional exposure to RABV [23]. During the same time period in Guatemala, despite detection of over 200 cases of rabies in livestock, and suspected association of such cases with vampire bat rabies, there were no reported cases in bats.

Why RABV has not been detected in Guatemalan bats is unclear, considering RABV has been detected in the majority of bats species tested throughout North America [24]. Very few bats are tested based upon the existing passive surveillance system, and monoclonal antibody or genetic characterization of RABV variants infecting humans, domestic animals and wildlife is not performed. Given the nocturnal and somewhat cryptic nature of bats, transmission dynamics are difficult to study in natural populations, and many critical gaps remain for a basic understanding the epizootiology of different RABV variants maintained in specific bat taxa. In the present study, we report

results from field studies conducted in Guatemala from 2009–2011. The objective of the study was to test whether enhanced surveillance would: (1) complement passive surveillance, specifically regarding the presence of RABV in bats, and (2) extend information on the geographic distribution of RABV circulation among bats to assess the public and veterinary health risks associated with bats in Guatemala.

Methods

Ethics statement

All animals were captured and handled in accordance with national guidelines (Guide for the Care and Use of Laboratory Animals) [25]. Protocols for animal capture and use were approved by the CDC Animal Care and Use Committee (USA) protocol number 1843 and 2096, and the Animal Care and Use Committee of the Universidad del Valle de Guatemala (Guatemala).

Enhanced surveillance sampling

Guatemala was selected as one major comparative New World study location as part of the U.S. Centers for Disease Control and Prevention's (CDC) Global Disease Detection (GDD) program among ten international locations. The objective of the CDC GDD program is to develop and strengthen global capacity to rapidly detect, accurately identify, and promptly contain emerging infectious threats. Nineteen field sites for sampling bats in Guatemala were selected on the basis of historical outbreaks of rabies, contemporary national surveillance data, known or suspected vampire bat depredation upon human populations, or neurological illness reported in livestock. Figure 1a illustrates the geographical distribution of field sites used in the present study. Bats were collected using mist nets set near fruit trees, confined livestock, or near the entrance of caves. Nets were opened between 19:00 to 0:00, and checked every 30 minutes. Bats were removed from mist nets, placed individually in cloth bags, and transported to a nearby temporary field station, where they were sedated by a 0.05 to 0.1 mg intramuscular injection of ketamine hydrochloride, oral/fecal swabs obtained, and terminal blood samples were collected by cardiac puncture under heavy anesthesia. Following euthanasia, bats were identified to the level of species following a key for bats of Costa Rica [26]. Standard morphological measurements were also collected (e.g. gender, age, mass, and forearm length). A complete necropsy was then performed on all bats, and samples were stored immediately on dry ice in the field, and maintained thereafter at -70°C in the laboratory at the Universidad del Valle de Guatemala until shipment to the CDC Rabies Laboratory in Atlanta, GA. Carcasses were banded and fixed in 10% buffered formalin for several days, then permanently transferred to 70% ethanol, for archival purposes.

Direct fluorescent antibody testing

Brain impressions prepared from frozen bat tissues were fixed in acetone at -20°C , and RABV antigens were detected by the DFA test, using fluorescein isothiocyanate (FITC)-labeled monoclonal antibody (mAb) conjugate (Fujirebio Diagnostics, Inc., Malvern, PA, USA), as described [27].

Serology

The presence of RABV neutralizing antibodies (rVNA) was determined by the rapid fluorescent focus inhibition test (RFFIT) or a modified micro-RFFIT test on sera collected from ten field sites [28,29]. The rVNA titers of individual bats were calculated by

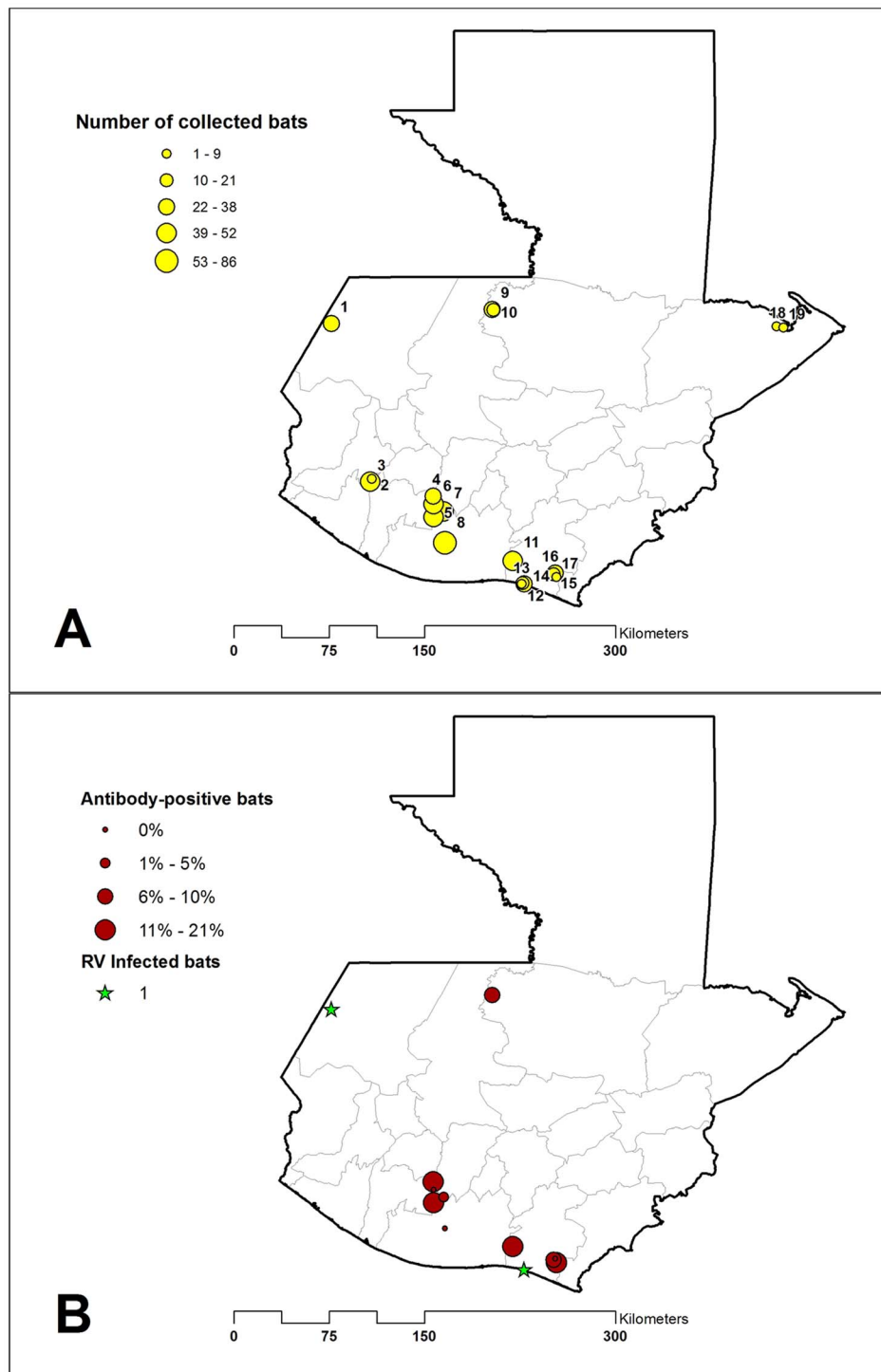


Figure 1. Map of selected field sites, Guatemala 2009–2011. (A) Yellow circles indicate the location 19 sites where bats were tested for RABV antigen and circle size is proportional to the number of bats collected. (B) Red circles indicate the location of ten sites where sera were available for rVNA testing and circle size is proportion to the level of rVNA seroprevalence among tested bats. The location of two rabies infected vampire bats (*D. rotundus*) is indicated by green stars. doi:10.1371/journal.pntd.0003070.g001

the Reed-Muench method, and were converted to international units (IU/mL) by comparison to a standard rabies immune globulin (SRIG) control containing 2 IU/mL [30]. The SRIG titer was generally higher in the micro-RFFIT test compared to the standard RFFIT. For the objective of this study, positive rVNA

titers (≥ 0.06 IU/mL) were defined by at least 50% neutralization of the RABV challenge virus dose (50 focus forming doses) at a 1:5 dilution (RFFIT) or 1:10 dilution (micro-RFFIT). Final titers less than 0.06 IU/mL were considered negative for the presence of rVNA for the purposes of this investigation.

Table 1. Bats collected for rabies testing from 19 field sites in Guatemala, 2009–2011.

Family	Species	Frequency (%)
Molossidae		
	<i>Molossus sinaloae</i>	2 (0.3)
Mormoopidae		
	<i>Pteronotus davyi</i>	20 (3.0)
Noctilionidae		
	<i>Noctilio leporinus</i>	1 (0.1)
Phyllostomidae		
	<i>Artibeus jamaicensis</i>	128 (19.0)
	<i>Artibeus lituratus</i>	35 (5.2)
	<i>Artibeus phaeotis</i>	10 (1.5)
	<i>Artibeus toltecus</i>	2 (0.3)
	<i>Carollia brevicauda</i>	2 (0.3)
	<i>Carollia castanea</i>	2 (0.3)
	<i>Carollia perspicillata</i>	30 (4.5)
	<i>Carollia sowelli</i>	2 (0.3)
	<i>Centurio senex</i>	1 (0.1)
	<i>Chiroderma salvini</i>	6 (0.9)
	<i>Chiroderma villosum</i>	1 (0.1)
	<i>Desmodus rotundus</i>	200 (29.8)
	<i>Glossophaga soricina</i>	47 (7.0)
	<i>Lasiurus ega</i>	1 (0.1)
	<i>Macrophyllum macrophyllum</i>	2 (0.3)
	<i>Micronycteris microtis</i>	25 (3.7)
	<i>Phyllostomus discolor</i>	14 (2.1)
	<i>Platyrrhinus helleri</i>	18 (2.7)
	<i>Sturnira lilium</i>	96 (14.3)
	<i>Sturnira ludovici</i>	2 (0.3)
	<i>Trachops cirrhosus</i>	1 (0.1)
	<i>Uroderma bilobatum</i>	5 (0.7)
	<i>Vampyressa pusilla</i>	2 (0.3)
	<i>Vampyrodes caraccioli</i>	1 (0.1)
Vespertilionidae		
	<i>Eptesicus fuscus</i>	5 (0.7)
	<i>Myotis elegans</i>	6 (0.9)
	<i>Myotis keaysi</i>	1 (0.1)
	<i>Myotis nigricans</i>	4 (0.6)
	Total	672 (100)

doi:10.1371/journal.pntd.0003070.t001

Antigenic characterization

Antigenic characterization was performed by indirect immunofluorescence using eight mAbs directed against the RABV nucleoprotein (N) antigens (C1, C4, C9, C10, C12, C15, C18, C19), supplied by EMD Millipore Corporation (Billerica, MA, USA), as previously described [31]. Positive reactivity pattern results were analyzed using previous described antigenic variant patterns [32,33].

Virus isolation

Virus isolation was attempted on oral and fecal swabs stored in growth medium (MEM-10), and organs, obtained from

DFA-positive bats. Homogenates (10% w/v) were prepared in MagnaNA lyser tissue homogenizer tubes containing 1.4-mm (diameter) ceramic beads (Roche Applied Science, Penzberg, Germany), using 1.0 mL of MEM-10 as a diluent. The entire solution from the oral swab, and 0.5 ml from the fecal swab was used to inoculate cells for isolation. For virus recovery, 100 μ L of test inoculum was added to 1 mL of MEM-10 containing 5×10^6 mouse neuroblastoma cells (MNA) in a T-25 tissue culture flask (Corning, NY). Tissue culture flasks were incubated at 0.5% CO₂ at 37°C for 72 hours. All cultures were sub-passaged a minimum of four times. For infectivity assessments, Teflon-coated four well slides were seeded with 30 μ L of MEM-10 containing 0.5×10^6 cells per mL, and incubated in a humid chamber at 0.5% CO₂ at

37°C for 24 hours. The slides were then rinsed with phosphate-buffered saline (PBS 4550), and fixed in cold acetone at -20°C for one hour. RABV antigens were visualized by use of the DFA test, using optimal working dilutions of FITC-labeled anti-RABV mAb conjugate (Fujirebio Diagnostics, Inc., Malvern, PA, USA) after each passage.

RNA extraction, reverse transcription, and PCR

Total RNA was extracted from fecal swabs and organ tissues of RABV-infected bats using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Fecal swabs were stored in 1 ml of MEM-10 in the field, and 200 µl of the swab suspension was mixed with 1 ml of TRIzol for RNA extraction. Primers were selected within the coding region of the N gene and the initial reaction was performed with sense primer 1066F, GARAGAAGATTCTT-CAGRGA (positions 1157–1173), which was also used for reverse transcription and antisense primer 304B, TTGACGAA-GATCTTGCTCAT (positions 1514–1533). The hemi-nested reaction was performed with sense primer 1087F, GAGAAR-GAACTTCARGA (positions 1157–1173). The glycoprotein (G) gene was amplified as two overlapping fragments using primer combinations umf2/994b and 760f/308b, as previously described [34]. All positions are given according to the Street Alabama Dufferin RABV strain genome sequence (GenBank accession number M31046). The RT-PCR was performed as described elsewhere [35]. Positive results were confirmed by nucleotide sequencing. RT-PCR products were purified with Wizard PCR Preps DNA Purification System (Promega, Madison, WI, USA), according to the manufacturer's recommendations and sequenced in forward and reverse directions as described by [35], using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit, version 1.1 on an ABI3770 sequencer (Applied Biosystems, Carlsbad, CA, USA).

Phylogenetic analysis

The complete N and G sequences were assembled and translated to amino acid sequences using the Bio Edit program [36]. The dataset was supplemented with complete and partial gene sequences available from GenBank and aligned in ClustalX [37]. Table S1 describes details of all sequences used in this study, including accession numbers, country of origin, year, and specimen source. The GTR+I+G model were selected based on the Bayesian factor and Akaike criterion evaluated in MEGA, version 5.1. No molecular clock evaluation was implemented. The analysis was performed using the Bayesian skyline population prior, and two independent Markov Chain Monte Carlo (MCMC) runs were performed with 1,000,000 iterations each. The results were combined in Log Combiner, and the resulting maximum clade credibility (MCC) tree generated with Tree Annotator using 20% for burn in and visualized in Fig Tree, version 1.4.0 [38].

Statistical analysis

All statistical analyses were performed using JMP version 9.0.2 or SAS version 9.2 (SAS Institute Inc., Cary, NC). The 95% confidence intervals (CI) were calculated for proportions of seropositive bats by species and location of capture. A nested mixed logistic model was used to test the effect of trophic guild on seroprevalence. Species nested within trophic guild was treated as a random effect and trophic guild was treated as a fixed effect. As only a single sanguivorous species was tested, we also compared the model outputs with a data set excluding vampire bats. The antibody prevalence between sex were also compared using χ^2 tests, and the level of significance was evaluated at $\alpha = 0.05$.

Results

Geographic distribution of bat sampling

During 2009–2011, a total of 672 bats of 31 species were collected from Guatemala. Bats were collected during 2009 (n=220; Table S1), 2010 (n=135; Table S2), and 2011 (n=317; Table S3). Table 1 provides the details on the cumulative frequency of capture by species. Among all captures, 56.3% were male. The most frequently captured species was *D. rotundus* (n=200; 30%), followed by the Jamaican fruit bat, *Artibeus jamaicensis* (n=128; 19%) (Table 1).

Serologic evidence of RABV circulation in bats

From sera available for testing (n=398), 28 bats demonstrated detectable rVNA for an antibody prevalence of 7% (95% CI 5–10%). Seroprevalence was highest for insectivorous species (21%), followed by omnivorous (8%) and sanguivorous (9%) taxa. The proportion of rVNA seropositive bats varied significantly across trophic guilds in a complete data set ($F_{3,20} = 4.65$, $p = 0.04$; $Species (Diet) = 3.4 \times 10^{-21}$) and a data set without vampire bats ($F_{2,20} = 6.97$, $p = 0.005$; $Species (Diet) = 4.4 \times 10^{-19}$). In both models, pairwise contrasts revealed a significant difference between frugivorous and insectivorous bats. Insectivorous bats were 8.5 times as likely to be seropositive compared to frugivorous bats. No other pairwise contrasts in rVNA seroprevalence between trophic guild levels were significant. Among capture locations, the proportion of seropositive bats was highest for Naranjo (21%), followed by El Jobo (19%), and El Penate (11%). Table 2 provides the details for individual bat species sampled by location and trophic guild. Antibody prevalence between sexes was similar (3.8% of males, 3.3% of females; $P = 0.77$). Species composition of bats captured varied across sites (Table 2).

Detection of RABV and antigenic typing

RABV antigens were detected in the brain from two common vampire bats. The bats were collected in El Pumpo, near the Pacific Coast in the Department of Santa Rosa, and Palo Seco, near the border with Mexico in the Department of San Marcos (Figure 1b). The antigenic reaction pattern derived from the brain specimens were consistent with RABV antigenic variant V3 associated with *Desmodus rotundus* (Table 3). Upon capture, one of the two rabid bats demonstrated clinical signs consistent with rabies infection. Additionally, this bat was dehydrated, in poor physical condition, and had evidence of several bite wounds to its body.

Isolation of RABV and detection of viral RNA by hemi-nested reverse transcription-PCR

RABV was isolated in MNA cells 24 h after inoculation from the kidney of one bat, and from oral swabs of both rabid bats. Additional sub-passages revealed the presence of RABV in the spleen of one bat, and the kidney and lung for both bats. RABV was not isolated from the heart, liver, intestine, and fecal swabs. Viral RNA was detected by hemi-nested RT-PCR in all specimens examined, except the liver of Bat 321. The results of RABV isolation and nucleic acid detection from tissues of rabid bats are presented in Table 4.

GenBank accession numbers

In total, two RABV G sequences and two RABV N sequences were generated from the brain tissues of infected bats in this study and deposited into GenBank (accession numbers: KF656696–99).

Table 2. Bat sera tested for rabies virus neutralizing antibodies (positive/tested) from ten collection sites in Guatemala, 2009–2011.

Species	Collection Sites										Subtotal	95% CI	
	Agüero	El Jobo	El Penate	Finca San Julian	La Viña	Los Hilos	Los Tarrales	Montañas Azules	Naranjo	Salacuim			
Frugivorous													
<i>Artibeus jamaicensis</i>	(0/22)	(0/6)	(0/17)	(0/17)	(1/4)	(0/1)	(0/4)	(0/4)	(0/4)	(0/2)	0.03 (6/199)	0.01–0.06	
<i>Artibeus lituratus</i>	(0/2)	(1/1)	(0/3)	(0/3)	(0/2)	(0/2)	(0/3)	(0/3)	(0/4)	(0/4)	0.02 (1/60)	0.00–0.09	
<i>Artibeus phaeotis</i>	(0/1)						(0/1)	(0/1)			0.07 (1/15)	0.01–0.30	
<i>Artibeus toltecus</i>	(0/6)	(1/11)	(0/1)	(0/3)	(0/3)	(0/3)	(0/3)	(0/1)	(0/1)	(0/1)	0.0 (0/1)	-	
<i>Carollia castanea</i>	(0/6)	(1/11)	(0/1)	(0/3)	(0/3)	(0/3)	(1/2)	(0/1)	(0/1)	(0/1)	0.0 (0/2)	-	
<i>Carollia perspicillata</i>	(0/6)	(1/11)	(0/1)	(0/3)	(0/3)	(0/3)	(1/2)	(0/1)	(0/1)	(0/1)	0.0 (0/2)	-	
<i>Centurio senex</i>	(0/6)	(1/11)	(0/1)	(0/3)	(0/3)	(0/3)	(1/2)	(0/1)	(0/1)	(0/1)	0.07 (2/29)	0.02–0.22	
<i>Chiroderma salvini</i>	(0/1)										0.0 (0/1)	-	
<i>Platyrrhinus helleri</i>	(0/1)										0.0 (0/15)	-	
<i>Sturnira lilium</i>	(0/15)	(1/4)	(0/5)	(0/9)	(0/3)	(0/2)	(0/14)	(0/14)	(1/1)	(0/4)	0.03 (2/66)	0.01–0.10	
<i>Sturnira ludovici</i>	(0/1)										0.0 (0/2)	-	
<i>Uroderma bilobatum</i>	(0/1)										0.0 (0/3)	-	
Insectivorous													
<i>Vampyressa pusilla</i>	(0/3)										0.21 (8/38)	0.11–0.36	
<i>Eptesicus fuscus</i>	(0/2)										0.0 (0/2)	-	
<i>Macrophyllum macrophyllum</i>	(0/2)										0.0 (0/4)	-	
<i>Molossus sinaloae</i>	(0/2)										0.0 (0/2)	-	
<i>Myotis elegans</i>	(0/1)										0.17 (1/6)	0.03–0.56	
<i>Myotis keaysi</i>	(0/1)										0.0 (0/1)	-	
<i>Myotis nigricans</i>	(0/1)										0.33 (1/3)	0.06–0.79	
<i>Pteronotus davyi</i>	(0/1)										0.30 (6/20)	0.15–0.52	
Omnivorous													
<i>Glossophaga soricina</i>	(0/8)	(2/6)	(0/1)	(0/1)	(0/1)	(0/1)	(0/8)	(0/8)	(0/5)	(0/2)	0.08 (6/73)	0.04–0.17	
<i>Micronycteris microtis</i>	(1/3)										0.08 (3/37)	0.03–0.21	
<i>Phyllostomus discolor</i>	(0/2)										0.04 (1/25)	0.01–0.20	
<i>Trachops cirrhosus</i>	(0/2)										0.20 (2/10)	0.06–0.51	
Sanguivorous													
<i>Desmodus rotundus</i>	(0/22)	(2/10)	(0/2)	(0/5)	(0/6)	(1/4)	(0/5)	(0/5)	(2/13)	(3/21)	0.09 (8/88)	0.05–0.17	
Total	0.0 (0/86)	0.19 (8/42)	0.11 (1/9)	0.0 (0/43)	0.07 (1/15)	0.0 (0/30)	0.11 (4/38)	0.02 (1/50)	0.21 (10/48)	0.08 (3/37)	0.07 (28/398)	0.05–0.10	
95% CI	-	0.10–0.33	0.02–0.44	-	0.01–0.30	-	0.04–0.24	0.00–0.10	0.12–0.34	0.03–0.21			

doi:10.1371/journal.pntd.0003070.t002

Table 3. Antigenic patterns of two bat rabies virus isolates from Guatemala.

Patterns of reaction (N-mAbs)										Antigenic Variant
C1	C4	C9	C10	C12	C15	C18	C19			
Bat 258	-	+	+	+	-	-	+			<i>Desmodus rotundus</i> (V3)
Bat 321	-	+	+	+	-	-	+			<i>Desmodus rotundus</i> (V3)

doi:10.1371/journal.pntd.0003070.t003

Table 4. Cell culture isolation and primary/hemi-nested (nRT-PCR) results from rabid bat specimens collected in Guatemala.

Bat 258												Bat 321											
Specimen Source	Virus Isolation				RNA detection ¹				Virus Isolation				RNA detection										
	Passage 1	Passage 2	Passage 3	Passage 4	Passage 1	Passage 2	Passage 3	Passage 4	Passage 1	Passage 2	Passage 3	Passage 4	Passage 1	Passage 2	Passage 3	Passage 4	Primary	Hemi-nested					
Fecal swab	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+					
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
Spleen	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
Heart	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
Liver	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-					
Lung	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
Intestine	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+					
Oral Swab	+	+	+	+	NT ²	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT					

Sequences from positive RNA detection in this table were not published in Genbank.

¹Primary (primers 1066:304), Hemi-nested (primers 1087:304).

²NT, not tested.

doi:10.1371/journal.pntd.0003070.t004

However, there is little evidence that this method actually targets rabid bats, and the apparent positive effect of culling on seroprevalence, combined with demographic and behavioral responses, may actually increase the proportion of susceptible bats [41,48]. A recent report also suggests that it may be more economically beneficial to support pre-exposure vaccination of cattle, than rely on vampire bat culling [49].

Rabies surveillance at the national level in Guatemala falls under the jurisdiction of the Ministry of Public Health and Social Welfare [50]. Rabies control efforts in Guatemala are focused primarily on mass vaccination of domestic dogs, which has led to a significant decrease in human rabies cases, though it remains unclear whether current efforts can achieve ultimate elimination of canine rabies [21,51]. Indirect vaccination of vampire bats with recombinant RABV vaccines have proven immunogenic and efficacious in experimental infection models of *D. rotundus*, though this strategy has not been tested in the field [52]. Despite possibly reducing RABV infection of cattle, this strategy would not eliminate the behavior or health and economic consequences (e.g. secondary bacterial infections) of vampire bat depredation on cattle. Interventions at the human-bat interface should be directed at decreasing the risk of human exposure to bats by improved educational campaigns on the risk of rabies associated with bats and the importance of laboratory testing and prophylactic treatment following exposures, as well as bat-proofing dwellings where feasible [53]. Routine laboratory-based RABV surveillance is necessary to properly evaluate any intervention strategy and should include the submission and testing of all human and animal cases involving bite contact with bats and other reservoir hosts. For Guatemala, overcoming cold chain and transportation barriers should be a priority to increase the proportion of contact cases that are tested. To further elucidate the relative impacts of the urban or sylvatic cycles on public health and agriculture, routine typing of all positive rabies cases should be implemented. Rapid exchange of information between sectors involved in human and animal rabies surveillance and control is essential.

Unfortunately, despite significant progress in the prevention and control of rabies, once clinical signs manifest in humans the case fatality rate approaches 100%, even with intensive supportive care [47]. However, appropriate post-exposure prophylaxis, including immediate washing/flushing and disinfection of the wound and prompt administration of RIG, and modern cell-culture vaccines according to recommended vaccination schedules assures prevention of rabies if bitten by a rabid bat [54]. Fortunately, modern cell culture vaccines are readily available for both humans and animals in Guatemala, and national authorities should provide pre-exposure prophylaxis guidelines for persons or animals with regular exposure to bats or other potential wildlife reservoirs (e.g. carnivores).

As the first focused rabies study in Guatemala, gaps were evident in our study. Limitations to the current study included a lack of more focal seasonal sampling, a broader spatial scale, and a need for greater engagement of the Ministries of Health and

Agriculture in a One Health context to improve routine laboratory based surveillance. The presence of bat RABV in rural communities likely requires new strategies for joint public health and veterinary education and outreach, increased availability for diagnostic laboratory testing in remote areas, and continued enhanced surveillance for rabies prevention and control, as suggested for other developing countries [55,56]. Additional studies at the human-bat interface would be useful to obtain information on demographic characteristics (e.g. age, gender, education) of persons exposed to bats, circumstances of bat exposures (e.g. bites, scratches, skin contact), actions taken following exposure, and knowledge of bat-borne zoonoses. Previously, based upon ignorance or inaction, preventable human rabies cases have occurred from bat exposure [57–59]. Given the implications when such recommendations are not operative, localities with a high risk for exposure to bats, and bat-borne zoonoses, should be targeted in laboratory based surveillance activities for the evaluation of robust, long-term prevention and control strategies.

Supporting Information

Table S1 Bats collected for rabies testing from eight field sites in Guatemala, 2009.

(DOCX)

Table S2 Bats collected for rabies testing from two field sites in Guatemala, 2010.

(DOCX)

Table S3 Bats collected for rabies testing from nine field sites in Guatemala, 2011.

(DOCX)

Table S4 List of sequences included in the data set from Central and South American vampire bat RABV.

(DOCX)

Acknowledgments

The authors thank M. Morales-Betoulle, R. Cirraiz, J.R. Martinez, R. Medrano, F. Villatoro, L. Escobar, M. L Müller, M.R. Lopez, N. Rosales and A. Estevez for excellent field and technical assistance.

Disclaimer. Use of trade names and commercial sources are for identification only and do not imply endorsement by the US Department of Health and Human Services. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of their institutions.

Author Contributions

Conceived and designed the experiments: JAE ATG SR DM DAA KAL CER. Performed the experiments: JAE ATG SR DM DAA NK DLG CER. Analyzed the data: JAE ATG SR NK MTM CER. Contributed reagents/materials/analysis tools: JAE ATG SR DM DAA NK DLG LFP MTM KAL CER. Wrote the paper: JAE ATG SR DM DAA NK DLG LFP MTM KAL CER.

References

- Luis AD, Hayman DT, O'Shea TJ, Cryan PM, Gilbert AT, et al. (2013) A comparison of bats and rodents as reservoirs of zoonotic viruses: are bats special? *Proceedings of the Royal Society B: Biological Sciences* 280: 20122753.
- Mickleburgh SP, Hutson AM, Racey PA (2002) A review of the global conservation status of bats. *Oryx* 36: 18–34.
- McCarthy TJ, Davis WB, Hill JE, Jones JK, Cruz GA (1993) Bat Mammalia:(Chiroptera) records, early collectors, and faunal lists for Northern Central America. *Annals of Carnegie Museum* 62: 191–228.
- Pérez SG, López JE (2012) Five new records of bats for Guatemala. *Chiroptera Neotropical* 18: 1106–1110.
- Tong S, Li Y, Rivailler P, Conrardy C, Castillo DA, et al. (2012) A distinct lineage of influenza A virus from bats. *Proc Natl Acad Sci U S A* 109: 4269–4274.
- Bai Y, Kosoy M, Recuenco S, Alvarez D, Moran D, et al. (2011) *Bartonella* spp. in Bats, Guatemala. *Emerg Infect Dis* 17: 1269–1272.
- Quan PL, Firth C, Conte JM, Williams SH, Zambrana-Torrel CM, et al. (2013) Bats are a major natural reservoir for hepaciviruses and pegiviruses. *Proc Natl Acad Sci U S A* 110: 8194–8199.
- Tao Y, Shi M, Conrardy C, Kuzmin IV, Recuenco S, et al. (2013) Discovery of diverse polyomaviruses in bats and the evolutionary history of the Polyomaviridae. *J Gen Virol* 94: 738–748.

9. Rupprecht CE, Hanlon CA, Hemachudha T (2002) Rabies re-examined. *Lancet Infect Dis* 2: 327–343.
10. Pan American Health Organization (2000) Norms and standards in epidemiology: case definitions Anthrax, Brucellosis and Rabies *Epidemiol Bull.* pp. 13–14.
11. Dyer JL, Wallace R, Orciari L, Hightower D, Yager P, et al. (2013) Rabies surveillance in the United States during 2012. *J Am Vet Med Assoc* 243: 805–815.
12. Rosatte RC (1987) Bat Rabies in Canada: History, Epidemiology and Prevention. *Can Vet J* 28: 754–756.
13. De Mattos CC, De Mattos CA, Loza-Rubio E, Aguilar-Setien A, Orciari LA, et al. (1999) Molecular characterization of rabies virus isolates from Mexico: implications for transmission dynamics and human risk. *Am J Trop Med Hyg* 61: 587–597.
14. Schneider MC, Romijn PC, Uieda W, Tamayo H, da Silva DF, et al. (2009) Rabies transmitted by vampire bats to humans: an emerging zoonotic disease in Latin America? *Rev Panam Salud Publica* 25: 260–269.
15. Greenhall AM, Joermann G, Schmidt U, Seidel MR (1983) *Desmodus rotundus*. *Mammalian species* 202: 1–6.
16. Mayen F (2003) Haematophagous bats in Brazil, their role in rabies transmission, impact on public health, livestock industry and alternatives to an indiscriminate reduction of bat population. *Journal of Veterinary Medicine, Series B* 50: 469–472.
17. Favoretto SR, Carrieri ML, Cunha EM, Aguiar EA, Silva LH, et al. (2002) Antigenic typing of Brazilian rabies virus samples isolated from animals and humans, 1989–2000. *Rev Inst Med Trop Sao Paulo* 44: 91–95.
18. Goncalves MA, Sa-Neto RJ, Brazil TK (2002) Outbreak of aggressions and transmission of rabies in human beings by vampire bats in northeastern Brazil. *Rev Soc Bras Med Trop* 35: 461–464.
19. de Mattos CA, Favi M, Yung V, Pavletic C, de Mattos CC (2000) Bat rabies in urban centers in Chile. *J Wildl Dis* 36: 231–240.
20. World Health Organization (2010) WHO expert consultation on rabies: first report: World Health Organization.
21. Pan American Health Organization (2013) SIEPI Epidemiological Information System. Washington, DC.
22. Schneider MC, Belotto A, Ade MP, Leanes LF, Correa E, et al. (2005) Epidemiologic situation of human rabies in Latin America in 2004. *Epidemiol Bull* 26: 2–4.
23. Messenger SL, Smith JS, Rupprecht CE (2002) Emerging epidemiology of bat-associated cryptic cases of rabies in humans in the United States. *Clin Infect Dis* 35: 738–747.
24. Constantine D (2009) Bat rabies and other lyssavirus infections. Bat rabies and other lyssavirus infections. USGS circular 1329, Reston, VA, USA.
25. Janet C, Barbee R, Bielitzki J, Clayton L, Donovan J, et al. (2011) Guide for the care and use of laboratory animals. Washington, DC: The National Academies Press.
26. Timm RM, LaVal RK (1998) A field key to the bats of Costa Rica. Occasional Publication Series, University of Kansas Center of Latin American Studies 22: 1–30.
27. Dean DJ, Ableseth MK, Atanasiu P (1996) The fluorescent antibody test In: FX. Meslin, MM. Kaplan and H. Koprowski, editors. *Laboratory techniques in rabies*: World Health Organization, Geneva, Switzerland. pp. 88–95.
28. Smith J, Yager P, Baer G (1996) A rapid fluorescent focus inhibition test (RFFIT) for determining rabies virus-neutralizing antibody. *Laboratory techniques in rabies* 4: 181–192.
29. Kuzmin IV, Niezgodna M, Franka R, Agwanda B, Markotter W, et al. (2008) Lagos bat virus in Kenya. *J Clin Microbiol* 46: 1451–1461.
30. Reed LJ, Muench H (1938) A simple method of estimating fifty per cent endpoints. *American Journal of Epidemiology* 27: 493.
31. Smith JS, Reid-Sanden F, Roumillat L, Trimarchi C, Clark K, et al. (1986) Demonstration of antigenic variation among rabies virus isolates by using monoclonal antibodies to nucleocapsid proteins. *Journal of clinical microbiology* 24: 573–580.
32. Dyer JL, Niezgodna M, Orciari LA, Yager PA, Ellison JA, et al. (2013) Evaluation of an indirect rapid immunohistochemistry test for the differentiation of rabies virus variants. *J Virol Methods* 190: 29–33.
33. Smith JS (1989) Rabies virus epitopic variation: use in ecologic studies. *Adv Virus Res* 36: 215–253.
34. Ellison JA, Johnson SR, Kuzmina N, Gilbert A, Carson WC, et al. (2013) Multidisciplinary approach to epizootiology and pathogenesis of bat rabies viruses in the United States. *Zoonoses Public Health* 60: 46–57.
35. Kuzmin IV, Orciari LA, Arai YT, Smith JS, Hanlon CA, et al. (2003) Bat lyssaviruses (Aravan and Khujand) from Central Asia: phylogenetic relationships according to N, P and G gene sequences. *Virus Res* 97: 65–79.
36. Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. pp. 95–98.
37. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, et al. (2007) Clustal W and Clustal X version 2.0. *Bioinformatics* 23: 2947–2948.
38. Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol Biol* 7: 214.
39. Nadin-Davis SA, Loza-Rubio E (2006) The molecular epidemiology of rabies associated with chiropteran hosts in Mexico. *Virus Res* 117: 215–226.
40. Condori-Condori RE, Streicker DG, Cabezas-Sanchez C, Velasco-Villa A (2013) Enzootic and epizootic rabies associated with vampire bats, Peru. *Emerg Infect Dis* 19. doi: 10.3201/eid1909.130083
41. Streicker DG, Recuenco S, Valderrama W, Gomez Benavides J, Vargas I, et al. (2012) Ecological and anthropogenic drivers of rabies exposure in vampire bats: implications for transmission and control. *Proc Biol Sci* 279: 3384–3392.
42. Bowen RA, O'Shea TJ, Shankar V, Neubaum MA, Neubaum DJ, et al. (2013) Prevalence of neutralizing antibodies to rabies virus in serum of seven species of insectivorous bats from Colorado and New Mexico, United States. *J Wildl Dis* 49: 367–374.
43. Costa LJ, Andrade FA, Uieda W, Martorelli LF, Kataoka AP, et al. (2013) Serological investigation of rabies virus neutralizing antibodies in bats captured in the eastern Brazilian Amazon. *Trans R Soc Trop Med Hyg* 107: 684–689.
44. Streicker DG, Franka R, Jackson FR, Rupprecht CE (2013) Anthropogenic roost switching and rabies virus dynamics in house-roosting big brown bats. *Vector Borne Zoonotic Dis* 13: 498–504.
45. Turmelle AS, Jackson FR, Green D, McCracken GF, Rupprecht CE (2010) Host immunity to repeated rabies virus infection in big brown bats. *J Gen Virol* 91: 2360–2366.
46. Gilbert AT, Fooks AR, Hayman DT, Horton DL, Muller T, et al. (2013) Deciphering Serology to Understand the Ecology of Infectious Diseases in Wildlife. *EcoHealth* 10: 298–313.
47. Petersen BW, Rupprecht CE (2011) Human rabies epidemiology and diagnosis. In: Tkachev S, editor. *Non-Flavivirus Encephalitis*. Rijeka, Croatia: InTech. pp. 247–278.
48. Rupprecht CE, Willoughby R, Slate D (2006) Current and future trends in the prevention, treatment and control of rabies. *Expert Rev Anti Infect Ther* 4: 1021–1038.
49. Anderson A, Shwiff S, Gebhardt K, Ramirez AJ, Shwiff S, et al. (2012) Economic Evaluation of Vampire Bat (*Desmodus rotundus*) Rabies Prevention in Mexico. *Transbound Emerg Dis* 61: 140–146.
50. Pan American Health Organization (2004) Country profiles. Guatemala. *Epidemiol Bull*: Pan American Health Organization. pp. 8–11.
51. Forde T (2009) Rabies Surveillance and Control in Guatemala: Université de Montréal.
52. Almeida MF, Martorelli LF, Aires CC, Barros RF, Massad E (2008) Vaccinating the vampire bat *Desmodus rotundus* against rabies. *Virus Res* 137: 275–277.
53. Lopez A, Miranda P, Tejada E, Fishbein DB (1992) Outbreak of human rabies in the Peruvian jungle. *Lancet* 339: 408–411.
54. Rupprecht CE, Briggs D, Brown CM, Franka R, Katz SL, et al. (2010) Use of a reduced (4-dose) vaccine schedule for postexposure prophylaxis to prevent human rabies. *MMWR Recomm Rep* 59: 1–9.
55. Lembo T, Niezgodna M, Velasco-Villa A, Cleaveland S, Ernest E, et al. (2006) Evaluation of a direct, rapid immunohistochemical test for rabies diagnosis. *Emerg Infect Dis* 12: 310–313.
56. Durr S, Naissengar S, Mindekem R, Diguimbye C, Niezgodna M, et al. (2008) Rabies diagnosis for developing countries. *PLoS Negl Trop Dis* 2: e206.
57. van Thiel P-PA, de Bie RM, Efimov F, Tepaske R, Zaaijer HL, et al. (2009) Fatal human rabies due to Duvenhage virus from a bat in Kenya: failure of treatment with coma-induction, ketamine, and antiviral drugs. *PLoS neglected tropical diseases* 3: e428.
58. Johnson N, Fooks A, McColl K (2008) Reexamination of Human Rabies Case with Long Incubation, Australia. *Emerging Infectious Diseases* 14: 1950–1951.
59. Liu Y, Zhang S, Zhao J, Zhang F, Hu R (2013) Isolation of Irkut virus from a *Murina leucogaster* bat in China. *PLoS Negl Trop Dis* 7: e2097.

Table S1. Bats collected for rabies testing from eight field sites in Guatemala, 2009.

Species	El Jobo	El Penate	La Viña	Los Hilos	Los Tarrales	Montañas Azules	Naranjo	Salacuim	Subtotal
<i>Artibeus jamaicensis</i>	6		4	1			4	2	17
<i>Artibeus lituratus</i>		1			2			4	7
<i>Artibeus toltecus</i>					1				1
<i>Carollia castanea</i>					1		1		2
<i>Carollia perspicillata</i>	11	1		3	3				18
<i>Centurio senex</i>								1	1
<i>Desmodus rotundus</i>	10	2	6		4		13	21	56
<i>Glossophaga soricina</i>	6		1	1	5		5	2	20
<i>Micronycteris microtis</i>	3			22					25
<i>Myotis elegans</i>	1						5		6
<i>Myotis nigricans</i>	1								1
<i>Phyllostomus discolor</i>					10				10
<i>Platyrrhinus helleri</i>					2				2
<i>Pteronotus davyi</i>				1			19		20
<i>Sturnira lilium</i>	4	5	3	2	9	1	1	4	29
<i>Sturnira ludovici</i>			1		1				2
<i>Trachops cirrhosus</i>								1	1
<i>Vampyressa pusilla</i>								2	2
Total	42	9	15	30	38	1	48	37	220

Table S2. Bats collected for rabies testing from two field sites in Guatemala, 2010.

Species	Agüero	Montañas Azules	Subtotal
<i>Artibeus jamaicensis</i>	22	4	26
<i>Artibeus lituratus</i>	2	3	5
<i>Artibeus phaeotis</i>		1	1
<i>Artibeus toltecus</i>	1		1
<i>Carollia perspicillata</i>	6	2	8
<i>Desmodus rotundus</i>	22	5	27
<i>Eptesicus fuscus</i>	3	1	4
<i>Glossophaga soricina</i>	8	8	16
<i>Macrophyllum macrophyllum</i>	2		2
<i>Molossus sinaloae</i>	2		2
<i>Myotis keaysi</i>		1	1
<i>Myotis nigricans</i>	1	1	2
<i>Platyrrhinus helleri</i>	1	10	11
<i>Sturnira lilium</i>	15	13	28
<i>Uroderma bilobatum</i>	1		1
Total	86	49	135

Table S3. Bats collected for rabies testing from nine field sites in Guatemala, 2011.

Species	Don Israel	Don Neto, El Pumpo	Finca Don Hugo	Finca Guadalupe	Finca Las Conchas	Finca Las Pavas	Finca San Julian	Palo Seco	Finca El Pumpo	Subtotal
<i>Artibeus jamaicensis</i>		12	2	10	5	1	17	18	20	85
<i>Artibeus lituratus</i>	3	2	2	6			3	1	6	23
<i>Artibeus phaeotis</i>		2		3		1			3	9
<i>Carollia brevicauda</i>				2						2
<i>Carollia perspicillata</i>					1		3			4
<i>Carollia sowelli</i>		2								2
<i>Chiroderma salvini</i>		1	3	1			1			6
<i>Chiroderma villosum</i>				1						1
<i>Desmodus rotundus</i>		3	24	30	1	24	5	10	20	117
<i>Eptesicus fuscus</i>						1				1
<i>Glossophaga soricina</i>	1	2	1	3			1		3	11
<i>Lasiurus ega</i>									1	1
<i>Myotis nigricans</i>									1	1
<i>Noctilio leporinus</i>									1	1
<i>Phyllostomus discolor</i>		4								4
<i>Platyrrhinus helleri</i>			1	1			2		1	5
<i>Sturnira lilium</i>		8	9	11			9		2	39
<i>Uroderma bilobatum</i>				1		1	2			4
<i>Vampyroides caraccioli</i>				1						1
Total	4	36	42	70	7	28	43	29	52	317

Table S4. List of nucleoprotein sequences included in the data set from Central and South American vampire bat RABV.

Sequence ID	Country	Year	Specimen Source
A13-0396 (KF656697)	Guatemala	2012	Vampire bat
A13-0465 (KF656696)	Guatemala	2012	Vampire bat
AB083809	Brazil	1998	Cattle
AB297627	Brazil	2002	Neotropical fruit bat
AB297632	Brazil	2002	Vampire bat
AB519641	Brazil	1998	Great fruit-eating bat
AB519642	Brazil	2000	Vampire bat
AF045166	Peru	1996	Human
AF070449	Brazil	unknown	Vampire bat
AF351852	Trinidad	1995	Vampire bat
AF352671	Paraguay	1994	Vampire bat
AY854587	Mexico	1996	Vampire bat
AY854589	Mexico	1990	Dog
AY854592	Mexico	2000	Cow
AY877433	Mexico	1990	Cow
AY877434	Mexico	1995	Horse
AY877435	Mexico	1993	Cow
EF363728	Ecuador	2005	Human
EF363733	Brazil	2005	Human
EF428582	Brazil	2006	Vampire bat
EU293113	Guyana	1990	Dog
FJ228492	Salvador	2002	Human
FJ228493	Mexico	2003	Cow
GU991824	Mexico	2002	Vampire bat
HM368179	Ecuador	2007	Cow
JF693469	Columbia	2002	Horse
JF693473	Columbia	1996	Cat
JF693475	Columbia	1995	Human
JQ685936	Mexico	2009	Vampire bat
JQ685953	Mexico	2009	Vampire bat
JX648424	Peru	2002	Horse
KC758860	Argentina	1996	Cow
KC758861	Argentina	2001	Cow
U22478	French Guiana	1994	Dog