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Journal

Global Change and Human Health, 13(4)

Authors

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Publication Date

2016-12-01

DOI

10.1007/s10393-016-1183-z

Peer reviewed

ECOHEALTH

CrossMark

Original Contribution

Viral Diversity, Prey Preference, and *Bartonella* Prevalence in *Desmodus rotundus* in Guatemala

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Abstract: Certain bat species serve as natural reservoirs for pathogens in several key viral families including henipa-, lyssa-, corona-, and filoviruses, which may pose serious threats to human health. The Common Vampire Bat (*Desmodus rotundus*), due to its abundance, sanguivorous feeding habit involving humans and domestic animals, and highly social behavioral ecology, may have an unusually high potential for interspecies disease transmission. Previous studies have investigated rabies dynamics in *D. rotundus*, yet the diversity of other viruses, bacteria, and other microbes that these bats may carry remains largely unknown. We screened 396 blood, urine, saliva, and fecal samples from *D. rotundus* captured in Guatemala for 13 viral families and genera. Positive results were found for rhabdovirus, adenovirus, and herpesvirus assays. We also screened these samples for *Bartonella* spp. and found that 38% of individuals tested positive. To characterize potential for interspecies transmission associated with feeding behavior, we also analyzed cytochrome B sequences from fecal samples to identify prey species and found that domestic cattle (*Bos taurus*) made up the majority of blood meals. Our findings suggest that the risk of pathogen spillover from *Desmodus rotundus*, including between domestic animal species, is possible and warrants further investigation to characterize this microbial diversity and expand our understanding of foraging ecology in their populations.

Keywords: bats, Chiroptera, *Bartonella*, pathogen discovery, disease ecology, feeding preference, adenovirus, herpesvirus, rhabdovirus, viral

INTRODUCTION

In recent years, bats have received growing attention as reservoirs for emerging infectious diseases (Olival et al. 2012; Wong et al. 2007; Wibbelt et al. 2010). A number of

zoonotic viruses with significant human and animal morbidity and mortality, including SARS, Ebola, and Nipah Virus, have been linked to bat reservoirs (Chua et al. 2000; Li et al. 2005; Leroy et al. 2005; Halpin et al. 2000; Swanepoel et al. 2007; Rahman et al. 2010). Fifteen viral families are known to infect 75 bat genera, with Rhabdo-, Flavi-, Bunya-, Corona-, and Togaviridae representing those viral families found most often in multiple bat genera (Olival et al. 2012). Serological evidence of influenza A viruses, such as the H17N10 strain from Guatemala and H18N11 strain from Peru, have also been discovered among Neotropical bats including *Desmodus rotundus* (Tong et al. 2012, 2013). Bacteria from the genus *Bartonella*, which includes bacteria known to cause several diseases in humans and other animals, are highly diverse in bats and have been previously detected in *Desmodus rotundus* from Guatemala (Bai et al. 2011).

The Common Vampire Bat *(Desmodus rotundus)* is a highly social and abundant Neotropical species that frequently comes into contact with humans and domestic animals (Mayen 2003; Johnson et al. 2014; Favoretto et al. 2013). These bats are nearly ubiquitous throughout Guatemala (McCarthy et al. 1993; Pérez and López 2012), where they are known carriers of rabies (Arellano-Sota

1988). While it is difficult to estimate their contribution towards the disease on a large scale, recent surveillance efforts suggest a low (0.3%) rabies prevalence among bats, including *D. rotundus* as well as other bat species, in Guatemala (Ellison et al. 2014). *Desmodus rotundus*, along with the White-winged Vampire Bat (*Diphylla ecaudata*) and the Hairy-legged Vampire Bat (*Diaemus youngii*), are the only known parasitic sanguivorous mammals (Nowak 1994). While both *Diphylla ecaudata* and *Diaemus youngii* feed on avian hosts (Hoyt and Altenbach 1981; Greenhall and Schutt 1996), *Desmodus rotundus* feeds mainly on the blood of mammals (Greenhall et al. 1971; Greenhall 1972), with a preference for livestock including domestic cattle and horses (Bahlman and Kelt 2007).

Unlike the two other comparatively rare vampire species, *D. rotundus* has larger population and a wider geographic range, with recent population increases associated with the expansion of livestock farming (Voigt and Kelm 2006). Unique social behaviors, including reciprocal altruism via bloodmeal sharing and social grooming, have also been well documented among *D. rotundus* (Wilkinson 1984, 1986; Carter and Wilkinson 2013), which have obvious implications for disease transmission. Additionally, co-species roosting with *Phyllostomus discolor* and *Sturnira*
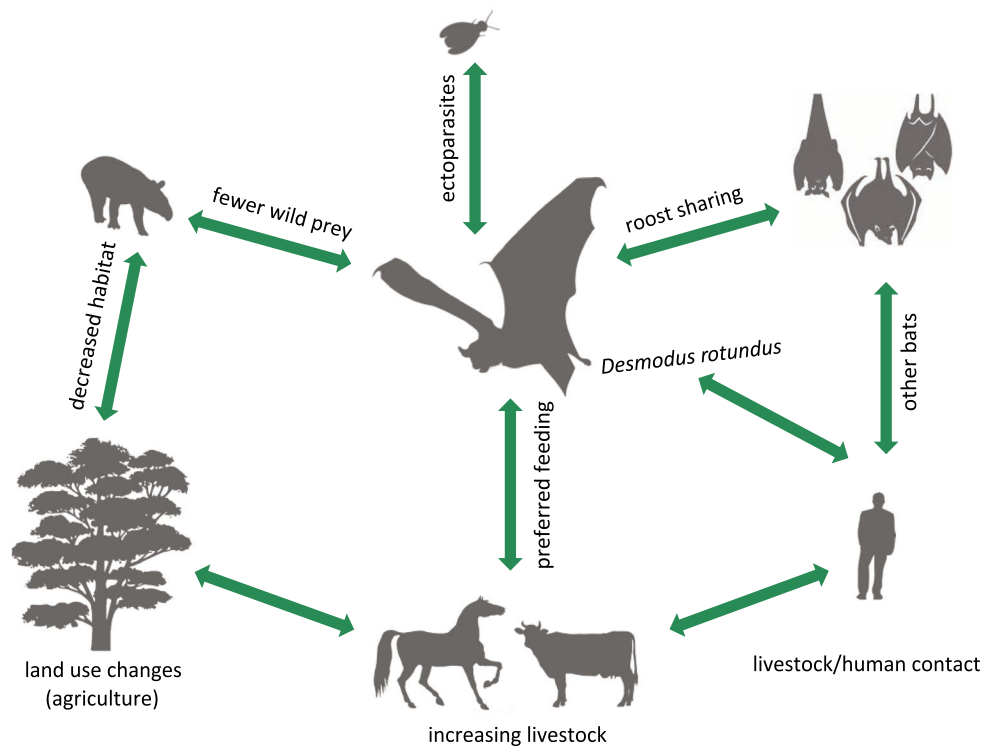

Figure 1. Schematic of *Desmodus rotundus* ecology and interspecies interactions

lilium has also been reported (Wohlgenant 1994), as well as with *Chrotopterus auritus*, *Trachops cirrhosus*, and *Glossophaga soricina* (N.B. Simmons, pers. observation). Due to these behavioral and ecological factors, *Desmodus rotundus* occurs at a unique interface between humans, livestock, and other bat species, where it may act as a key species for infectious disease transmission (Figure 1).

Although *D. rotundus* has been studied to investigate rabies dynamics and spread in Latin America (Da Rosa et al. 2006; Velasco-Villa et al. 2006, Moran et al. 2015), very little is known about other potential pathogens that these bats might carry, or the likelihood of spillover to humans and domestic animals. One of the few non-rabies studies on this species described a novel coronavirus from Brazil (Brandão et al. 2008), a finding of potential interest considering the bat origin of the SARS pandemic (Li et al. 2005), and the widespread geographic distribution of MERS-related coronaviruses (Annan et al. 2013; Anthony et al. 2013a; Ithete et al. 2013; Memish et al. 2013).

Viral and bacterial surveillance and discovery is useful for evaluating the possibility of microbe sharing with other animals and humans that come into contact with *D. rotundus*. Identifying bloodmeals from bat fecal samples can also reveal ecological connections of epidemiological importance such as host prey preference (Carter et al. 2006), and understanding the foraging patterns and movement of both *D. rotundus* and their prey may give further insights into patterns of microbe sharing and potential routes of disease transmission. In this study, we collect and analyze clinical specimens from *D. rotundus* in Guatemala to examine their viral diversity and host species feeding preferences in order to better understand the potential for pathogen spillover.

MATERIALS AND METHODS

Sampling and Study Site

Bats were captured and specimens were collected between April and May 2013 in two departments in Guatemala. One hundred *Desmodus rotundus* individuals were captured at four sites in Finca Los Tarrales, Patalul in the Suchitepequz department (14.519°, −91.136; 14.523°, −91.136;

Figure 2. Location of bat sampling sites in Guatemala: Finca Los Tarrales in the Suchitepequz department (*n* = 100) and Cubilgüitz in the Alta Verapaz department (*n* = 3)

Table 1. Summary of Viral and Bacterial Assays.

Assay	Target	Primer	Citation
Alphavirus	NSP4	Alpha1F: GAYGCITAYYTIGAYATGGTIGAIGG Alpha1R: KYTCYTCIGTRTGYTTIGTICCIGG Alpha2F: GIAAYTGYAAYGTIACICARATG Alpha2R: GCRAAIARIGCIGCIGCYTYIGGICC	Sánchez-Seco et al. (2001)
Adenovirus	Polymerase	FLTR: TIMGNGGIGGIMGNTGYTAYCC RTR: GTDGCRAAISHICCRTABARIGMRTT FNR: GTITWYGAYATHTGYGGHATGTAYGC RNR: CCAICCBCDRTTRTGIARIGTRA	Wellehan et al. (2004)
Coronavirus	RdRp	CoV-FWD1:CGTTGGIACWAAYBTVCCWYTICARBTRGG CoV-RVS1:GGTCATKATAGCRTCAVMASWWGCNACATG CoV-FWD2: GGCWCCWCCHGGNGARCAATT CoV-RVS2: GGWAWCCCCAYTGYTGWAYRTC	Quan et al. (2010)
Enterovirus	VP4/2	EVRV1: CTCCGGCCCCTGAATRYGGCTAA EVRV2:TCIGGIARYTTCCACCACCAICC EVRV3: ACCRASTACTTTGGGTGTCCGTG EVRV2anew: CCGGYAAYTTCCASCACCA	Unpublished
Flavivirus	NS5	Flavi-FWD: TGYRBTTAYAACATGATGGG Flavi-RVS: GTGTCCCAICCNGCNGTRTC	Moureau et al. (2007)
Hantavirus	L Segment	HAN-L-F1:ATGTAYGTBAGTGCWGATGC HAN-L-R1: AACCADTCWGTYCCRTCATC HAN-L-F2: TGCWGATGCHACIAARTGGTC HAN-L-R2: GCRTCRTCWGARTGRTGDGCAA	Klempa et al. (2006)
Hepacivirus	Helicase	HGLV-ak1: TACGCIACNGCIACNCCICC HGLV-ak2: TCGAAGTTCCCIGTRTANCCIGT HGLV-ak3: GACIGCGACICCICCIGG HGLV-ak4: TCGAAGTTCCCIGTRTAICCIGT	Kapoor et al. (2013)
Herpesvirus	Polymerase	DFA: GAYTTYGCNAGYYTNTAYCC ILK: TCCTGGACAAGCAGCARNYSGCNMTNAA KG1: GTCTTGCTCACCAGNTCNACNCCYTT TGV: TGTAACTCGGTGTAYGGNTTYACNGGNGT IYG: CACAGAGTCCGTRTCNCCRTADAT	VanDevanter et al. (1996)
Influenza A	Matrix	FLUAV-MU44: GTCTTCTAACCGAGGTCGAAACG FLUAV-M-L287: GCATTTTGGACAAAGCGTCTACG	Anthony et al. (2012)
Orbivirus	VP1	VP1/F2494/1,TCTGAGATGTAYGTYGGAGATGATA VP1/F2494/2,TCTGAGATGTAYGTYGGTGATGACA VP1/F2494/3,TCGGAACARTAYGTVGGNGAYGATA VP1/F2494/4,TCNGARCARTAYGTKGGNGAYGACA VP1/R2682,CCYTGYTTNGCRTGNGTYTGYGTYTTYTC	Palacios et al. (2011)
Paramyxovirus	Polymerase	PAR-F1: GAAGGITATTGTCAIAARNTNTGGAC PAR-R: GCTGAAGTTACIGGITCICCDATRTTNC PAR-F2: GTTGCTTCAATGGTTCARGGNGAYAA PAR-R: GCTGAAGTTACIGGITCICCDATRTTNC	Tong et al. (2008)

Table 1. continued

Assay	Target	Primer	Citation
Pegivirus	Helicase	AK4340F1: GTACTTGCTACTGCNACNCC	Kapoor et al. (2013)
		AK4630R1:TACCCTGTCATAAGGGCRTC	
		AK4340F2: CTTGCTACTGCNACNCCWCC	
		AK4630R2: TACCCTGTCATAAGGGCRTCNGT	
Rhabdovirus	L gene	PVO3: CCADMCBTTTTGYCKYARRCCTTC	Unpublished
		PVO4: RAAGGYAGRTTTTTYKCDYTRATG	
		PVO3: CCADMCBTTTTGYCKYARRCCTTC	
		PVOnstF: AARTGGAAYAAYCAYCARMG	
Bartonella bacteria	ribC	BARTON-1: TAACCGATATTGGTTGTGTTGAAG	Johnson et al. (2003)
		BARTON-2: TAAAGCTAGAAAGTCTGGCAACATAACG	

14.519°, −91.134°; 15.524°, −91.141°), and 3 individuals were captured at one site in Cubilgüitz, Cobán in the Alta Verapaz department (15.675°, −90.424°) (Figure 2). Project protocols for animal capture and use were approved by the Ethics and Animal Care and Use Committee of the Universidad del Valle de Guatemala (Guatemala City, Guatemala), and by the Guatemala National Protected Areas Council (research license I-025-2009, transport and export license N. 1559/2013). Bats were captured in mist nets near roost sites following standard methods (e.g., Kunz and Parsons 1988). After removal from the mist net, the bats were kept in clean cloth bags for 1–4 h until sampling. All bats were identified to species, and morphological data were collected including mass, forearm length, age class, sex, physical condition, and pregnancy or lactation status. Whole blood ($n = 89$), serum ($n = 89$), oral swabs ($n = 103$), urine ($n = 12$), and fecal swabs ($n = 103$) were collected from 103 individual bats using non-lethal, minimally invasive protocols. Small amounts of blood were collected in the field from the brachial vein and diluted with a 1:10 ratio of phosphate buffered saline. Oropharyngeal swabs, fecal swabs, and urine samples were collected in viral transport media and frozen immediately with dry ice in the field. Samples were maintained at −80°C after field collection and were shipped frozen with dry ice to the Center for Infection and Immunity at Columbia University in New York.

PCR Screening

Total nucleic acid was extracted from all samples ($n = 396$) using the EasyMag (bioMérieux, Inc.) platform, and cDNA synthesis was performed using SuperScript II first-strand synthesis supermix (Invitrogen) according to the manufacturer's protocols. Viral discovery was performed using consensus PCR assays with degenerate primers, targeting adenoviruses, herpesviruses, rhabdoviruses, influenza A viruses, coronaviruses, paramyxoviruses, hantaviruses, flaviviruses, orbiviruses, enteroviruses, alphaviruses, hepaciviruses, and pegiviruses (Table 1, see Anthony et al. 2015 for detailed assay conditions). Screening for *Bartonella* spp. bacteria was also performed (Table 1). Additional herpesvirus and *Bartonella* spp. assays were performed for terminase and gltA gene regions, respectively, but these attempts were unsuccessful. All PCR products of expected size were cloned into Strataclone PCR cloning vector, and 8 white colonies for each PCR product were sequenced using standard M13R primers.

Trace sequences were analyzed and edited using Geneious 7.1.5. Sequences were aligned using ClustalW and alignment confidence was assessed using GUIDANCE (Penn et al. 2010). Models of evolution were selected using jModelTest, and phylogenetic trees were constructed with Mega 5.2.2 using maximum-likelihood methods with 2000 bootstraps. Unrooted ML trees are presented.

Cytochrome B Analysis

Prey species were identified and field identification of *Desmodus rotundus* was confirmed by sequencing cytochrome B from bat fecal samples using CytB forward (GAGGMCAAATATCATTCTGAGG) and CytB reverse (TAGGGCVAGGACTCCTCCTAGT) primers (Townzen et al. 2008). All PCR products of expected size were cloned into Strataclone PCR cloning vector, and 24 white colonies for each PCR product were sequenced using standard

M13R primers. Trace sequences were analyzed and edited using Geneious 7.1.5. Host identities were confirmed and prey identities detected by NCBI BLAST comparison with sequences of known identity.

RESULTS

A total of 5120 consensus PCR assays were performed for the detection of viruses from 13 different families and genera, including herpesviruses (HVs; $n = 396$), rhabdoviruses (RVs; $n = 396$), influenza A viruses (IFAVs; $n = 396$), coronaviruses (CoVs; $n = 396$), paramyxoviruses (PMVs; $n = 396$), hantaviruses (HTVs; $n = 396$), flaviviruses (FLVs; $n = 396$), orbiviruses (OVs; $n = 396$), enteroviruses (EVs; $n = 396$), alphaviruses (AVs; $n = 396$), hepaciviruses (HCV; $n = 92$), and pegiviruses (PGV; $n = 92$). HCV and PGV assays were performed on serum samples only. None of these samples were positive for IFAVs, CoVs, PMVs, HTVs, FLVs, OVs, EVs, AVs, HCVs, or PGVs, despite recent evidence of detection in other bat species (Quan et al. 2013; Tong et al. 2013; Sumibcay et al. 2012; Guo et al. 2013). In total, 50 PCR positives were detected in these samples, corresponding to herpesviruses ($n = 35$), adenoviruses ($n = 14$), and rhabdoviruses ($n = 1$). Adenoviruses and a single rhabdovirus were only found in fecal samples; herpesviruses were found in oral, fecal, and blood samples; and *Bartonella* was found in serum, blood, and fecal samples (Table 2). Among all individuals, HV and AdV prevalences were 32 and 12.6%, respectively. Consensus PCR assays were also performed for *Bartonella* bacteria ($n = 396$), detecting *Bartonella* spp. sequences in a total

of 43 samples from 39 individuals, yielding a prevalence of 37.9%.

When analyzed phylogenetically, the 320-bp adenovirus sequences ($n = 14$) separated into two clades within the genus *Mastadenovirus*, which we refer to as *Desmodus rotundus* Adenovirus 1 and 2 (DrAdV-1 and 2; accession numbers KX774295-KX774308)

These two viruses showed 95 and 89% shared identities, respectively, with a *D. rotundus* adenovirus previously reported from Brazil (Lima et al. 2013), suggesting a degree of host specificity for this clade (Figure 3). A single rhabdovirus (DrRV) was identified, and phylogenic analyses of the 260-bp L segment of the polymerase gene placed it among the genus *Vesiculovirus*, with the closest BLAST match sharing 76% identity with Pike fry Rhabdovirus (Supplementary Figure A); however, we qualify that the fragment amplified is very short and therefore may not provide accurate phylogenetic placement within the family. Two herpesviruses (DrHV-1 and DrHV-2) were also detected, and analyses of the 180-bp herpesvirus polymerase gene placed DrHV-1 among the subfamily Gammaherpesvirinae, with a 99% shared identity with *Pteropus giganteus* Herpesvirus-5. DrHV-2 was placed among the subfamily Betaherpesvirinae, with a 70% identity match with *P. giganteus* Herpesvirus-10 (Figure 4).

Cytochrome B sequencing of fecal samples detected *D. rotundus* DNA in 94 out of 103 samples, with 97–99% identity to *D. rotundus* sequences that have been published. CytB sequences from prey were detected in 52 out of 103 bats. *Bos taurus* sequences were detected in 47 out of the 52 individuals that yielded positive bloodmeal host identification (90.38%), *Bos indicus* sequences were detected in

Table 2. Summary of Positive Results from Pathogen Discovery Performed on *Desmodus rotundus*.

Assay	Blood clot	Serum	Fecal swab	Oral swab	Urine	Total
Herpesvirus						
DrHV-1	3/89	1/89	1/103	29/103	0/12	34/396
DrHV-2	0/89	0/89	0/103	1/103	0/12	1/396
Total	3/89	1/89	1/103	30/103	0/12	35/396
Adenovirus						
DrAdV-1	0/89	0/89	9/103	0/103	0/12	9/396
DrAdV-2	0/89	0/89	5/103	0/103	0/12	5/396
Total	0/89	0/89	14/103	0/103	0/12	14/396
Rhabdovirus						
DrRV-1	0/89	0/89	1/103	0/103	0/12	1/396
Bartonella	35/89	3/89	5/103	0/103	0/12	43/396

four individuals (7.69%), and *Equus* spp. sequences were detected in 5 individuals (9.61%). Among these results, co-detection of CytB sequences from two different domestic cattle species, *Bos taurus* and *Bos indicus*, in the same bat were detected in three individuals (5.77%), and CytB sequences from both *Bos taurus* and *Equus* spp. were also detected in one individual (1.92%).

To analyze the relationships between study site, age, sex, pregnancy status, prey species, and presence or absence of viruses and bacteria, we constructed contingency tables using Fisher's exact and Chi squared tests. None of these characteristics were significant predictors of the presence of viral or bacterial sequences. Chi squared tests indicated that sample type was a significant predictor of adenovirus, herpesvirus, and *Bartonella* spp. presence ($P < 0.001$, $df = 4$). A generalized linear model corroborated the Chi squared test and demonstrated that Herpesvirus detection was most likely in oral swabs ($P < 0.001$, $df = 395$), and Adenovirus detection from fecal swabs ($P < 0.001$, $df = 395$) (Table 3). CytB prey sequences and microbial sequences were detected in 31 out of 103 individuals, with several instances of coinfection by multiple microbes (Figure 5).
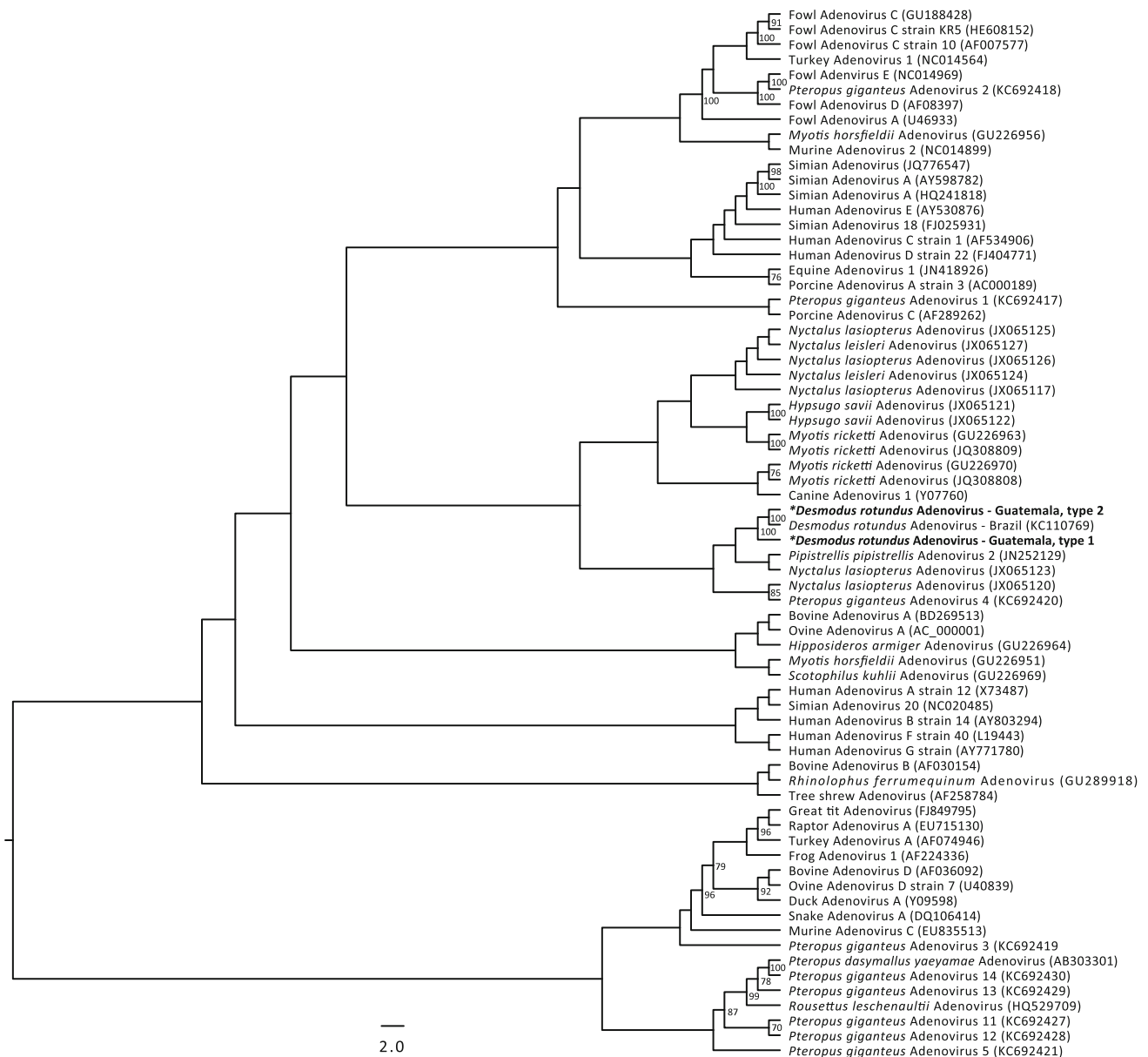

Figure 3. Phylogenetic (ML) tree of Adenovirus polymerase. Target gene: polymerase. Positive samples: 13 fecal. Amplicon: 320 bp. Closest BLAST match: *D. rotundus* Adenovirus from Brazil. (Dr AdV1 Query cover = 84%, Identity = 95%; Dr AdV2 Query cover = 79%, Identity = 89%)
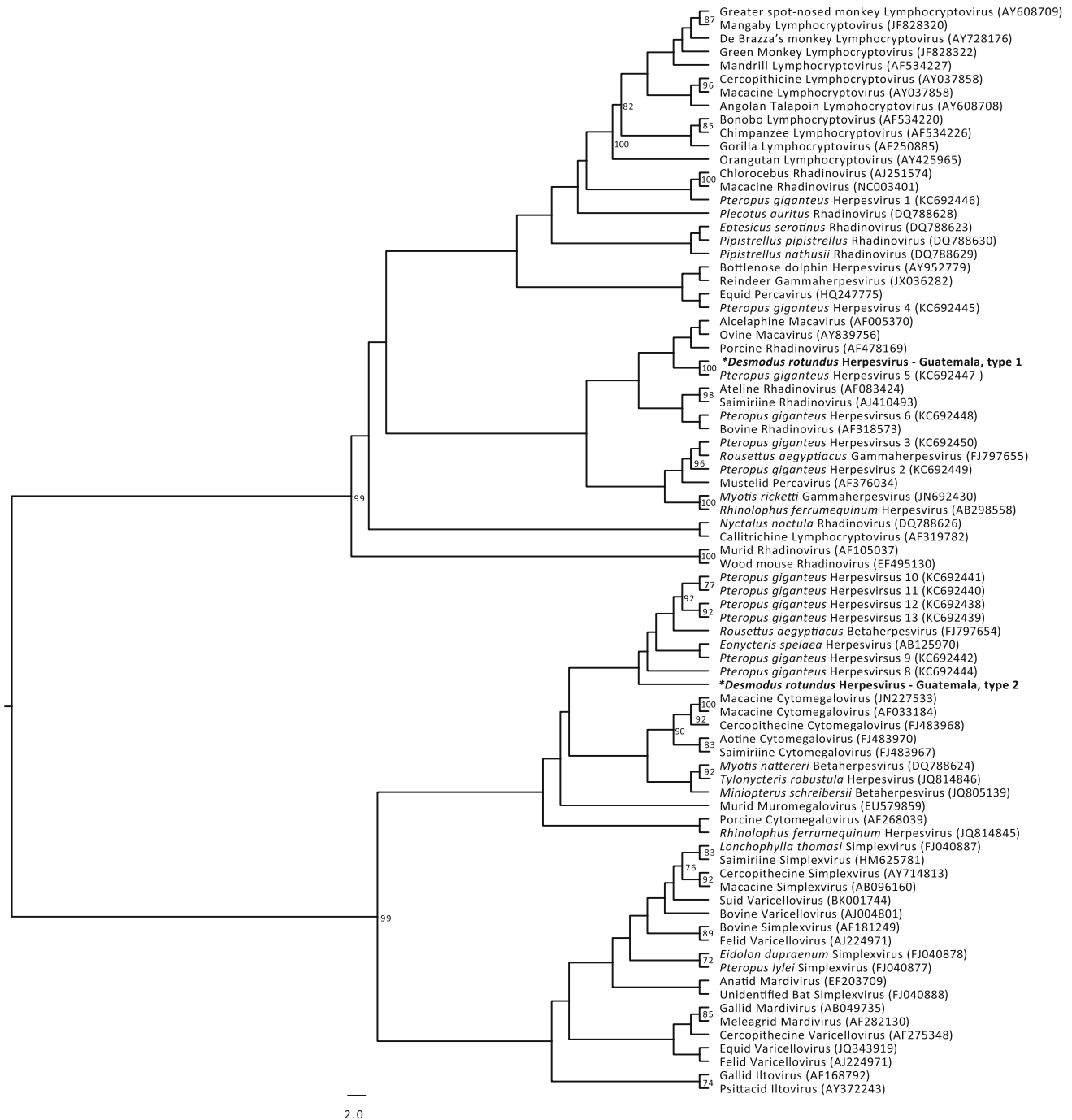
Figure 4. Phylogenetic (ML) tree of Herpesvirus polymerase. Positive samples: 1 serum, 3 blood clot, 30 oral swab, 1 fecal swab (35 total). Amplicon: 180 bp. Closest BLAST matches: *P. giganteus* HV-5; *P. giganteus* HV-10 (Dr HV1 Query cover = 94%, Identity = 99%; Dr HV2 Query cover = 95%, Identity = 70%)

DISCUSSION

Here we demonstrate that *Desmodus rotundus* from Guatemala harbor both unique and previously described viruses from the adenovirus ($n = 14/396$), rhabdovirus ($n = 1/396$), and her-pesvirus ($n = 35/396$) families, as well as bacteria from the genus *Bartonella* ($n = 43/396$). Our analyses of feeding prefer-ences also emphasize the potential for microbe sharing via direct contact between *D. rotundus* and domestic livestock species.

Table 3. Statistical Associations Between Field Characteristics and Viral or Bacterial Presence.

	Site	Sample type*	Sex	Age	Pregnant	*Bos taurus*	*Bos indicus*	*Equus*	*Bartonella*	AdV	HV	RV
Site		1.0	0.24	0.23	1.0	0.59	1.0	1.0	1.0	0.34	1.0	1.0
Sample type*	1.0		1.0	0.97	0.93	1.0	1.0	1.0	**<2.2e−16**	**1.00e−07**	**1.06e−14**	1.0
Sex	0.24	1.0		0.09	0.06	0.43	0.62	1.0	0.63	1.0	0.72	1.0
Age	0.23	0.97	0.09		0.06	0.57	1.0	0.14	1.0	0.41	0.80	1.0
Pregnant	1.0	0.93	0.06	0.06		1.0	1.0	0.22	0.71	1.0	0.71	1.0
Bos taurus	0.59	1.0	0.43	0.57	1.0		0.33	0.37	0.26	0.25	0.29	0.46
Bos indicus	1.0	1.0	0.62	1.0	1.0	0.33		1.0	1.0	1.0	0.65	1.0
Equus	1.0	1.0	1.0	0.14	0.22	0.37	1.0		1.0	1.0	0.16	1.0
Bartonella	1.0	**<2.2e−16**	0.63	1.0	0.71	0.26	1.0	1.0		1.0	0.40	1.0
AdV	0.34	**1.00e−07**	1.0	0.41	1.0	0.25	1.0	1.0	1.0		0.62	**0.03**
HV	1.0	**1.06e−14**	0.72	0.80	0.71	0.29	0.65	0.16	0.40	0.62		1.0
RV	1.0	1.0	1.0	1.0	1.0	0.46	1.0	1.0	1.0	**0.03**	1.0	

Results from Fisher's exact tests between variables. Viral and bacterial presence was coded based on presence/absence. Bos taurus, Bos indicus, and Equus represent presence/absence of prey CytB sequences. Statistically significant associations are in bold. ⋆ Associations with sample type were determined using a Chi squared test based on sample types (blood, serum, fecal swab, oral swab, and urine).
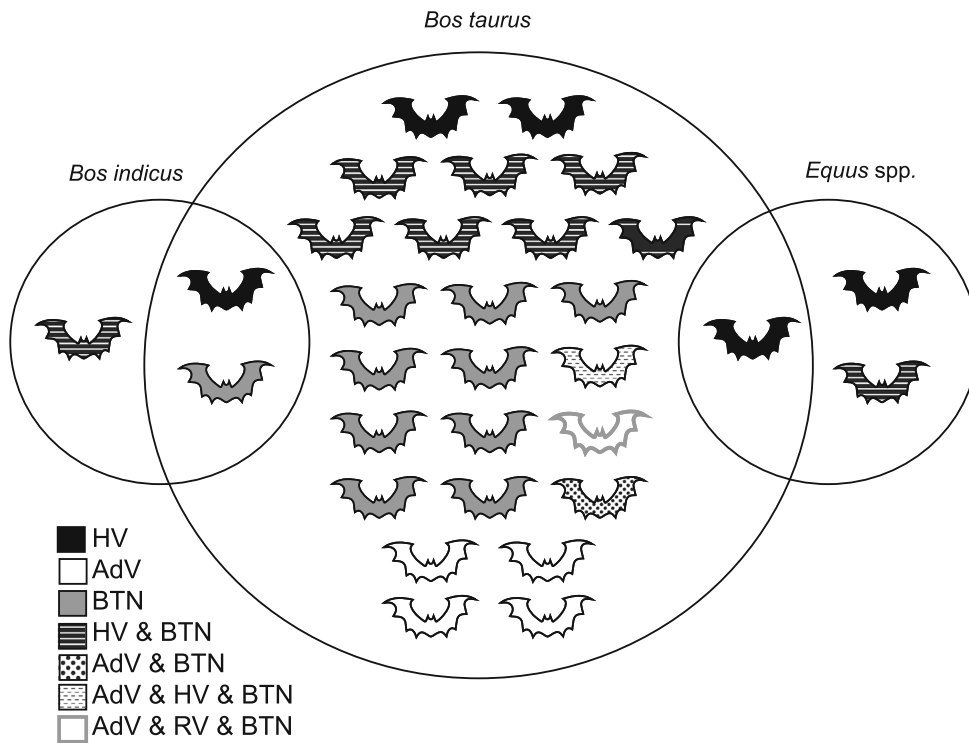

Figure 5. Distribution of viral and bacterial sequence presence among individual bats for which prey sequences were also detected (*HV* Herpesvirus; *AdV* Adenovirus; *RV* Rhabdovirus; *BTN* Bartonella)

Two discrete adenoviruses, DrAdV-1 and DrAdV-2, were detected in 13 fecal samples from 13 individual bats. These adenoviruses were highly similar in sequence identity to an adenovirus known from Brazil, which was also detected in *Desmodus rotundus* from a pool of spleen, liver, lungs, and kidney samples (Lima et al. 2013, GenBank

accession: KC110769). Other bat adenoviruses have been found among the Vespertillionidae, Rhinolophidae, Pteropodidae, and Hipposideridae in areas including Hungary (Jánoska et al. 2011), China (Li et al. 2010), Japan (Maeda et al. 2008), Germany (Drexler et al. 2011), and Spain (unpublished, GenBank accession: AFO66606-AFO66618). Phylogenetic analyses place all of these various bat adenoviruses in the genus *Mastadenovirus*, which also includes all known mammalian adenoviruses. The phylogenetic placement of DrAdV-1 and DrAdV-2 among viruses from the same host species yet distant from other bat adenoviruses suggests some form of host specificity, however, the relatively small number of known bat adenoviruses precludes a thorough test of co-phylogeny. While originally thought to co-speciate with their hosts (as apparent in the case of mastadenoviruses and mammalian species as well as aviadenoviruses and bird species), the detection of atadenoviruses among various bird, reptile, and mammalian clades suggests that host switching events among Adenoviridae may also have occurred in multiple viral lineages (Harrach 2000; Wellehan et al. 2004).

A single rhabdovirus, DrRV, was detected in one fecal sample and demonstrated 65–75% identity matches with known insect-borne, mammalian, and fish rhabdoviruses belonging to the genus *Vesiculovirus*. The classification of DrRV among the *Vesiculovirus* genus suggests that it could be an insect-borne virus, as most known mammalian Vesiculoviruses are arboviruses (King et al. 2012). However, in some cases, viruses in this genus may also be transmitted by direct contact between mammals, including livestock such as in the case of Vesicular Stomatitis Virus (Stallknecht et al. 2001; Mead et al. 2004). Since the fecal sample in which this viral sequence was found also had CytB sequences from *Bos taurus*, the virus may have been taken up via the bloodstream of the prey or via accidental ingestion of an insect while feeding. *Desmodus rotundus* is not known to opportunistically feed on insects, although insect remains have occasionally been found in the guts of *D. rotundus* during necropsies (G.G. Carter, pers. comm.). While the conserved L segment of the rhabdovirus polymerase gene we targeted is useful for viral detection and discovery, it is known to give a poor phylogenetic signal and inconsistent branching or polytomies (Bourhy et al. 2005, Kuzmin et al. 2006). Therefore, the lack of resolution in the gene tree for this rhabdovirus polymerase suggests that further sequence analysis is required for resolving the classification of this novel virus. We note that given our study design, the detection of certain CNS rhabdoviruses—

including rabies—is unlikely among non-lethal samples from apparently healthy animals.

Herpesviruses were the most commonly detected viruses in our study. Two herpesviruses, DrHV-1 and DrHV-2, were detected in 35 samples from 33 individual bats. DrHV-1 was detected in a single oral sample, and DrHV-2 was detected among oral ($n = 30$), blood ($n = 3$), serum ($n = 1$), and fecal ($n = 1$) samples. The closest identity match for DrHV-2 was a 70% shared identity with a *Pteropus giganteus* herpesvirus from Bangladesh, PgHV-10. For DrHV-1, the closest identity match was a 99% shared identity with a different *P. giganteus* Herpesvirus, PgHV-5. Both PgHV-10 and PgHV-5 were detected in samples from Bangladesh (Anthony et al. 2013b). We cross-referenced this sequence with the PREDICT HealthMap database and found an identical viral sequence from a *Desmodus rotundus* sample that was collected and processed in Brazil in September 2011 (www.healthmap.org/predict). A possible explanation for the similarity between PgHV-5 and DrHV-1 may be that the viruses are actually divergent and the targeted polymerase fragment is highly conserved and therefore may yield an unreliable phylogenetic signal. In a previous study, different bat species from the family Vespertilionidae were also infected with herpesviruses that were apparently the same even based on 370aa glycoprotein B and 760aa DPOL gene targets (Wibbelt et al. 2007). Follow-up PCR assays were performed for the herpesvirus terminase gene region, but these attempts did not successfully amplify the targeted region.

In the event that DrHV-1 and PgHV-5 are in fact closely related, our finding of similar herpesviruses in disparate regions and in distantly related bat hosts could be attributed to a widespread nature of the virus in question. Domestic pigs are known to come into contact with excrement from *Pteropus* spp., which was subsequently found to play a key role in cross-species transmission of Nipah Virus in Malaysia (Field et al. 2001). *Desmodus rotundus* also comes into contact with domestic species other than cattle and horses, which may include pigs, goats, and even poultry (Greenhall et al. 1971). Ecological niche studies also suggest that the expansion of livestock has facilitated—and will likely continue to facilitate—range expansion and population growth in *D. rotundus* (Lee et al. 2012). As international animal trade and transport is known to play a role in microbe transmission (Fèvre et al. 2006), we propose that an alternative explanation for the results of this study could be that the reservoirs of DrHV-1 or PgHV-10 are not necessarily bats, but possibly a

widespread domestic species or another taxa that also may come into contact with both *D. rotundus* and *Pteropus giganteus* across their respective geographic ranges.

Six discrete *Bartonella* spp. sequences were detected in 43 samples from 39 individuals, yielding a prevalence of 37.9% among all individuals (*n* = 103). Nucleotide identities ranged from 77.95 to 98.61% (8–124 raw nucleotide differences). Previous studies have also demonstrated a similarly high prevalence (∼33%) of *Bartonella* spp. in bats in Guatemala (Bai et al. 2011). The ectoparasites of *Desmodus rotundus* include blood-feeding batflies from the family Streblidae, which carry bacteria including potentially pathogenic strains of *Bartonella* (Morse et al. 2012). *Bartonella* spp. are found among many mammalian species, and lineages from different *Bartonella bovis* strains have been shown to be associated with cattle breed origins, including *Bos taurus* and *Bos indicus* which are often kept as mixed breeds in Guatemala (Bai et al. 2013). A previous study on Puerto Rican bat communities suggested that vector specificity and parasite load may influence differences in *Bartonella* spp. prevalence between bat hosts (Olival et al. 2015). However, in terms of possible interspecies *Bartonella* spp. transmission, interactions between *Desmodus rotundus*, ectoparasites, prey, and other co-roosting bats—as well as humans in terms of direct (via bites) or indirect (via contact with domestic animal species) contact—remain largely uncharacterized. PCR assays were also performed to amplify the gltA gene region, however, these attempts were unsuccessful and therefore phylogenetic comparison with other studies remains limited.

D. rotundus feeding preference was characterized by detecting cytochrome B sequences from digested blood meals via fresh fecal samples. Positive PCR products were cloned in order to obtain separate *D. rotundus* and prey sequences, as well as to detect the presence of sequences from different prey species. Half of the fecal specimens we tested (52/103) yielded CytB sequences from prey, and demonstrated preferential feeding on *Bos taurus*, with occasional feeding on *Bos indicus* and *Equus* spp. These results are consistent with previous evidence based on stable isotope analyses, which also demonstrated preferential feeding by *Desmodus rotundus* on livestock over native mammals (Voigt and Kelm 2006). However, other recent studies have shown variations in *D. rotundus* feeding behavior based on different prey availability, which included preferential feeding on chickens and pigs in the absence of high density cattle ranching (Bobrowiec et al. 2015). We found no evidence of human blood meals from the bats that we sampled. While we recognize that there may be geographic and temporal differences in vampire feeding behavior based on prey availability, our results suggest that frequent contact between *D. rotundus* and domestic species, particularly cattle and horses, may increase the probability of viral and bacterial transmission.

CONCLUSION

Multi-disciplinary microbial ecology studies, such as our efforts to characterize the microbe diversity hosted by *Desmodus rotundus* in Guatemala combined with analysis of ecological feeding preference, can answer questions about human–bat public health interfaces and have additional value for conservation and ecology. The results of our study demonstrate that *D. rotundus* carries both novel and known microbes, and that the populations in this region appear to display an overwhelming preference for feeding on domestic livestock species. As populations of *D. rotundus* continue to expand in Latin America with the increasing production of livestock, better understanding of their infectious disease ecology and diversity will become even more critical. Subsequent studies are necessary to further resolve the phylogeny and further investigate the actual pathogenicity of the novel viruses detected in this study. Additional research efforts involving microbial discovery and surveillance among domestic animals may also prove useful for investigating whether microbe sharing occurs between *D. rotundus* and their prey, as well as how viral and bacterial diversity may change seasonally and geographically. Gaining a better understanding of the role of *D. rotundus* as a potential source and reservoir of infectious diseases will help better inform public health and wildlife management policies aimed at preventing or mitigating future disease spillover events in the face of ecosystem changes.

ACKNOWLEDGEMENTS

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