Epizootiology of Tacaribe Serocomplex Viruses (Arenaviridae) Associated with Neotomine Rodents (Cricetidae, Neotominae) in Southern California

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Abstract

The objective of this study was to advance our knowledge of the epizootiology of Bear Canyon virus and other Tacaribe serocomplex viruses (Arenaviridae) associated with wild rodents in California. Antibody (immunoglobulin G [IgG]) to a Tacaribe serocomplex virus was found in 145 (3.6%) of 3977 neotomine rodents (Cricetidae: Neotominae) captured in six counties in southern California. The majority (122 or 84.1%) of the 145 antibody-positive rodents were big-eared woodrats (*Neotoma macrotis*) or California mice (*Peromyscus californicus*). The 23 other antibody-positive rodents included a white-throated woodrat (*N. albigula*), desert woodrat (*N. lepida*), Bryant's woodrats (*N. bryanti*), brush mice (*P. boylii*), cactus mice (*P. eremicus*), and deer mice (*P. maniculatus*). Analyses of viral nucleocapsid protein gene sequence data indicated that Bear Canyon virus is associated with *N. macrotis* and/or *P. californicus* in Santa Barbara County, Los Angeles County, Orange County, and western Riverside County. Together, analyses of field data and antibody prevalence data indicated that *N. macrotis* is the principal host of Bear Canyon virus. Last, the analyses of viral nucleocapsid protein gene sequence data suggested that the Tacaribe serocomplex virus associated with *N. albigula* and *N. lepida* in eastern Riverside County represents a novel species (tentatively named "Palo Verde virus") in the genus *Arenavirus*.

Key Words: Bear Canyon virus—Palo Verde virus—Arenaviridae—Tacaribe serocomplex—Arenavirus—Cricetidae—*Neotoma albigula*—*Neotoma lepida*—*Neotoma macrotis*—*Peromyscus californicus*.

Introduction

THE NORTH AMERICAN MEMBERS of the Tacaribe serocomplex (Arenaviridae) include Bear Canyon virus (BCNV), Big Brushy Tank virus (BBTV), Catarina virus (CTNV), Middle Pease River virus (MPRV), Ocozocoautla de Espinosa virus (OCEV), Real de Catorce virus (RCTV), Skinner Tank virus (SKTV), Tamiami virus (TAMV), Tonto Creek virus (TTCV), and Whitewater Arroyo virus (WWAV) (Cajimat et al. 2011, 2012, 2013). The results of a previous study (Milazzo et al. 2011) suggested that WWAV or are-

naviruses that are antigenically closely related to WWAV are etiological agents of severe febrile illnesses in humans in the United States. Woodrats and other members of the Cricetidae: Neotominae (Musser and Carleton 2005) are natural hosts of Tacaribe serocomplex viruses in the western United States (Cajimat et al. 2011).

Specific knowledge of the Tacaribe serocomplex viruses associated with neotomine rodents in California is limited to the isolation of BCNV from seven rodents—a big-eared woodrat (*Neotoma macrotis*, previously *Neotoma fuscipes macrotis*; Matocq 2002) captured in the Santa Monica

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Mountains in Los Angeles County, four California mice (*Peromyscus californicus*) captured in the Santa Ana Mountains in Orange County, and a big-eared woodrat and California mouse captured in the Santa Ana Mountains in Riverside County (Fulhorst et al. 2002, Cajimat et al. 2007). The purpose of this study was to extend and refine our knowledge of the epizootiology of BCNV and other Tacaribe serocomplex viruses associated with neotomine rodents in southern California.

Materials and Methods

Rodents

Blood or serum samples from 3977 neotomine rodents from six counties in southern California were tested for anti-arenavirus antibody (Table 1). The rodents were captured in August, 1998–January, 2013, by personnel from The California Department of Public Health; Los Angeles County, Department of Public Health; San Gabriel Valley Mosquito and Vector Control District; County of Riverside, Department of Environmental Health; Orange County Vector Control District; and County of San Diego, Department of Environmental Health during surveillance for the plague bacillus (*Yersinia pestis*), Sin Nombre virus, and other rodent-associated agents of human disease. The rodents were from two sites in Kern County, six sites in Santa Barbara County, 49 sites distributed across Los Angeles County, 24 sites in Orange County, 32 sites in the western one-third of Riverside County, three sites in far eastern Riverside County, and 28 sites in San Diego County. Traps were set one night at 91 sites, two consecutive nights at 3 sites, and one night on two or more occasions at 50 sites. The skins, skeletons, and frozen samples of kidney and other solid tissues from 708 (17.8%) of the 3977 rodents were deposited into the Museum of Texas Tech University.

Assay for antibody

The blood and serum samples were tested for immunoglobulin G (IgG) to WWAV strain AV 9310135 (Fulhorst et al. 1996), using an enzyme-linked immunosorbent assay (ELISA) (Bennett et al. 2000). We note that IgG to BCNV in naturally infected big-eared woodrats and naturally infected California mice is reactive to AV 9310135 in ELISA (Fulhorst et al. 2002). The antibody titers in the positive samples were 320, 1280, or ≥ 5120 .

Assay for arenavirus

Samples of brain, liver, and kidney from 109 antibody-positive rodents and 337 antibody-negative rodents were assayed for arenavirus by cultivation in monolayers of Vero E6 cells (Fulhorst et al. 2001a). The antibody-positive rodents (one white-throated woodrat [*Neotoma albigula*], 13 Bryant's woodrats [*Neotoma bryanti*], 40 big-eared woodrats, one desert woodrat [*Neotoma lepida*], two brush mice [*Peromyscus boylii*], 47 California mice, three cactus mice [*Peromyscus eremicus*], two deer mice [*Peromyscus maniculatus*]) were from 28 sites: KRN-1 ($n=1$), SB-1 ($n=4$), SB-2 ($n=2$), SB-3 ($n=3$), LA-1 ($n=1$), LA-2 ($n=2$), LA-3 ($n=2$), LA-4 ($n=1$), LA-5 ($n=1$), LA-6 ($n=5$), LA-7 ($n=1$), LA-8 ($n=1$), LA-9 ($n=2$), LA-10 ($n=1$), LA-11 ($n=1$), ORA-1 ($n=1$), ORA-3 ($n=5$), ORA-4 ($n=5$), ORA-5 ($n=1$), ORA-6 ($n=15$), RIV-1 ($n=2$), RIV-2 ($n=4$), RIV-3 ($n=5$), RIV-4 ($n=24$), RIV-5 ($n=5$), RIV-6 ($n=7$), RIV-7 ($n=4$), and SD-5 ($n=3$) (Table 2). The antibody-negative rodents (32 Bryant's woodrats, 33 big-eared woodrats, 19 brush mice, 193 California mice, 30 cactus mice, 20 deer mice, one pinyon mouse [*Peromyscus truei*], nine western harvest mice [*Reithrodontomys megalotis*]) were from 17 sites: SB-1 ($n=2$), LA-1 ($n=13$), LA-2 ($n=2$), LA-3 ($n=15$), LA-4 ($n=9$), LA-6 ($n=50$), LA-7 ($n=4$), LA-8 ($n=11$), LA-9 ($n=4$), LA-10 ($n=8$), LA-11 ($n=8$), ORA-3 ($n=58$), ORA-4

TABLE 1. PREVALENCE OF ANTI-ARENAVIRUS ANTIBODY IN 3977 NEOTOMINE RODENTS CAPTURED IN SOUTHERN CALIFORNIA IN 1998–2013, BY SPECIES AND COUNTY

Species ^a	County ^{b,c}						Total
	KRN	SB	LA	ORA	RIV	SD	
<i>Neotoma albigula</i>					1/2	0/1	1/3
<i>Neotoma bryanti</i>			4/177	6/301	0/55	3/28	13/561
<i>Neotoma cinerea</i>	0/1						0/1
<i>Neotoma lepida</i>					1/1		1/1
<i>Neotoma macrotis</i>	1/1	8/12	11/84	10/120	21/101	10/104	61/422
<i>Onychomys torridus</i>					0/2		0/2
<i>Peromyscus boylii</i>		3/33	0/36	0/129	0/318		3/516
<i>Peromyscus californicus</i>		1/17	1/55	21/376	38/379		61/827
<i>Peromyscus crinitus</i>					0/49		0/49
<i>Peromyscus eremicus</i>			0/45	3/420	0/166		3/631
<i>Peromyscus maniculatus</i>		0/8	2/162	0/285	0/361		2/816
<i>Peromyscus truei</i>			0/2		0/27		0/29
<i>Reithrodontomys megalotis</i>		0/7	0/22	0/70	0/20		0/119
Total	1/2	12/77	18/583	40/1,701	61/1,481	13/133	145/3,977

^a*Neotoma bryanti* was distinguished from *N. lepida* on the basis of knowledge of the geographical ranges of these species (Patton et al. 2008).

^bKRN, Kern (two sites); SB, Santa Barbara (six sites); LA, Los Angeles (49 sites); ORA, Orange (24 sites); RIV, Riverside (35 sites); SD, San Diego (28 sites).

^cNumber positive/number of animals tested for immunoglobulin G (IgG) to Whitewater Arroyo virus strain AV 9310135.

TABLE 2. PREVALENCE OF ANTI-ARENAVIRUS ANTIBODY IN 2069 NEOTOMINE RODENTS CAPTURED AT 33 SITES IN SOUTHERN CALIFORNIA IN 1998–2013, BY STUDY SITE AND SPECIES^a

Site ^b	Species ^{c,d}										Total
	Nalb	Nbry	Nlep	Nmac	Pboy	Pcal	Pere	Pman	Rmeg	Other	
KRN-1				1/1							1/1
SB-1				4/6							4/6
SB-2				3/3	1/9	1/1					5/13
SB-3				1/1	2/11	0/11		0/1			3/24
LA-1		1/5			0/1	0/1		0/7			1/14
LA-2		0/1				0/1		2/14	0/1		2/17
LA-3				2/4	0/1	0/6		0/3	0/2	0/1	2/17
LA-4				0/4		1/4			0/2		1/10
LA-5		1/1									1/1
LA-6		1/14		4/7	0/1	0/14	0/17	0/2			5/55
LA-7				1/3		0/1			0/1		1/5
LA-8		1/3		0/1		0/3	0/4	0/1			1/12
LA-9				2/2		0/1		0/3			2/6
LA-10		0/1		1/2		0/2	0/4				1/9
LA-11				1/2		0/3			0/4		1/9
ORA-1		1/12		0/5		0/4			0/2		1/23
ORA-2				2/14	0/2	1/27	0/5		0/1		3/49
ORA-3		0/3		0/5	0/3	5/52					5/63
ORA-4		0/7		3/6	0/31	8/36	0/2	0/1	0/3		11/86
ORA-5		0/2		0/1	0/9	1/13	0/2				1/27
ORA-6		5/184		5/45	0/54	6/179	3/319	0/57	0/10		19/848
RIV-1	1/1		1/1				0/53	0/13		0/25	2/93
RIV-2		0/7		2/3	0/45	2/21	0/13	0/12		0/1	4/102
RIV-3		0/6		2/12	0/60	5/19	0/9	0/15		0/1	7/122
RIV-4		0/3		1/4	0/3	25/87	0/2			0/1	26/100
RIV-5		0/1		5/12	0/35	1/35		0/7	0/2		6/92
RIV-6				5/18	0/7	5/102	0/6				10/133
RIV-7		0/3		6/14	0/34	0/39	0/1	0/11			6/102
SD-1				1/1							1/1
SD-2				1/1							1/1
SD-3				1/3							1/3
SD-4				4/12							4/12
SD-5		3/7		3/6							6/13
Total	1/1	13/260	1/1	61/198	3/306	61/662	3/437	2/147	0/28	0/29	145/2,069

^aThe 1908 rodents from 111 other sites in southern California were negative for anti-arenavirus antibody.

^bKRN, Kern County; SB, Santa Barbara County; LA, Los Angeles County; ORA, Orange County; RIV, Riverside County; SD, San Diego County. KRN-1, Grapevine Canyon, Tehachapi Mountains; SB-1, Vandenberg Air Force Base (34°36'30"N, 120°38'02"W); SB-2, Santa Ynez Mountains (34°27'47"N, 119°42'51"W); SB-3, Romero Canyon, Santa Ynez Mountains (34°26' 38"N, 119°35'41"W); LA-1, Hungry Valley (34°43'52"N, 118°48'18"W); LA-2, Portal Ridge, north of the Sierra Pelona Mountains (34°40'27"N, 118°26'18"W); LA-3, western San Fernando Valley (34°13'56"N, 118°37'35"W); LA-4, near Bell Canyon, Simi Hills (34°11'45"N, 118°39'45"W); LA-5, San Gabriel Valley (34°09'00"N, 117°55'48"W); LA-6, near Dry Canyon, Santa Monica Mountains (34°8'00"N, 118°38'16"W); LA-7, foothills of the Santa Monica Mountains in western Los Angeles County; LA-8, Lachusa Canyon, Santa Monica Mountains (34°03'59"N, 118°52'30"W); LA-9, Solstice Canyon, Santa Monica Mountains (34°02'17"N, 118°44'51"W); LA-10, Ramirez Canyon, Santa Monica Mountains (34°02'02"N, 118°47'57"W); LA-11, Zuma Canyon, Santa Monica Mountains (34°01'54"N, 118°48'44"W); ORA-1, Anaheim Hills (33°51'31"N, 117°42'48"W); ORA-2, Silverado Canyon, Santa Ana Mountains (33°44'56"N, 117°34'55"W); ORA-3, El Cariso #1, Santa Ana Mountains (33°39'50"N, 117°25'11"W); ORA-4, El Cariso #2, Santa Ana Mountains (33°39'44"N, 117°25'37"W); ORA-5, San Juan Hot Springs, Santa Ana Mountains (33°36'54"N, 117°31'38"W); ORA-6, Lucas Canyon, Santa Ana Mountains (33°33'53"N, 117°31'29"W); RIV-1, near the Palo Verde Diversion Dam (33°43'31"N, 114°30'08"W); RIV-2, San Jacinto Valley (33°43'20"N, 116°48'03"W); RIV-3, San Jacinto Valley (33°42'31"N, 116°45'40"W); RIV-4, San Jacinto Valley (33°42'23"N, 116°45'19"W); RIV-5, Morrell Potrero, Santa Ana Mountains (33°36'55"N, 117°21'13"W); RIV-6, Bear Canyon Trailhead, Santa Ana Mountains (33°36'45"N, 117°25'35"W); RIV-7, Santa Rosa Plateau (33°32'59"N, 117°16'20"W); SD-1, Montezuma Valley (33°12'07"N, 116°28'37"W); SD-2, Quana Canyon (33°05'55"N, 116°39'56"W); SD-3, Blue Sky Canyon (33°00'52"N, 117°00'35"W); SD-4, Los Peñasquitos Canyon (32°56'01"N, 117°08'21"W); SD-5, base of El Cajon Mountain (32°53'07"N, 116°48'47"W). The geographical coordinates of KRN-1 are not known with certainty; the geographical coordinates of LA-7 were omitted to preserve the privacy of the persons who live at this locality. The Santa Ana Mountains extend from eastern Los Angeles County, along the Riverside–Orange county line, into northern San Diego County; the Santa Rosa Plateau is a southeastern extension of the Santa Ana Mountains in Riverside County; and the Palo Verde Diversion Dam (RIV-1) spans the Colorado River from western La Paz County, Arizona, to eastern Riverside County.

^cNalb, *Neotoma albigula*; Nbry, *Neotoma bryanti*; Nlep, *Neotoma lepida*; Nmac, *Neotoma macrotis*; Pboy, *Peromyscus boylii*; Pcal, *Peromyscus californicus*; Pere, *Peromyscus eremicus*; Pman, *Peromyscus maniculatus*; Rmeg, *Reithrodontomys megalotis*; Other, *Onychomys torridus* (RIV-1, *n* = 1; RIV-2, *n* = 1), *Peromyscus crinitus* (RIV-1, *n* = 24), and *Peromyscus truei* (LA-3, *n* = 1; RIV-3, *n* = 1; RIV-4, *n* = 1). The 19 antibody-positive rodents and 257 (31.0%) of the 829 antibody-negative rodents from ORA-6 were captured in 18 nights in 16 months in February, 1999–October, 2002.

^dNumber positive/number of animals tested for immunoglobulin G (IgG) to *Whitewater Arroyo virus* strain AV 9310135.

($n=23$), ORA-6 ($n=12$), RIV-4 ($n=61$), RIV-5 ($n=12$), and RIV-6 ($n=45$). Cells harvested from the monolayers on day 13 or 14 postinoculation were tested for arenaviral antigen using an indirect fluorescent antibody test in which the primary antibody was a hyperimmune mouse ascitic fluid raised against WWAV strain AV 9310135.

Genetic characterization of arenavirus AV B0300052

The BCNV strain AV B0300052 was isolated from woodrat TK91001 (Table 3). The complete nucleotide sequence of the nucleocapsid (N) protein gene of AV B0300052 was determined in a previous study (Cajimat et al. 2011).

Assay for arenaviral RNA

Samples of kidney from 42 antibody-positive, culture-negative rodents from 17 sites were tested for arenaviral N protein gene RNA: KRN-1, *N. macrotis* ($n=1$); SB-1, *N. macrotis* ($n=4$); SB-3, *N. macrotis* ($n=1$) and *P. boylii* ($n=2$); LA-1, *N. bryanti* ($n=1$); LA-3, *N. macrotis* ($n=2$); LA-5, *N. bryanti* ($n=1$); LA-6, *N. bryanti* ($n=1$) and *N. macrotis* ($n=2$); LA-8, *N. bryanti* ($n=1$); LA-9, *N. macrotis* ($n=1$); ORA-1, *N. bryanti* ($n=1$); ORA-6, *N. bryanti* ($n=5$),

N. macrotis ($n=2$), *P. californicus* ($n=2$), and *P. eremicus* ($n=3$); RIV-1, *N. albigula* ($n=1$) and *N. lepida* ($n=1$); RIV-3, *N. macrotis* ($n=2$); RIV-4, *P. californicus* ($n=1$); RIV-6, *N. macrotis* ($n=3$); RIV-7, *N. macrotis* ($n=1$); and SD-5, *N. bryanti* ($n=3$). Collectively, these 42 rodents represented the geographical distribution of the antibody-positive animals archived in the Museum of Texas Tech University.

First-strand cDNA was synthesized by using SuperScript™ III Reverse Transcriptase (Invitrogen Corp., Carlsbad, CA) with oligonucleotide 19C-cons (Cajimat et al. 2007). The first-round PCR assays used MasterTaq Kit (5 PRIME, Inc., Gaithersburg, MD) in conjunction with oligonucleotides NW1696R (Bowen et al. 1997) and 19C-cons; AVNP13 (Cajimat et al. 2007), AVNP39 (5'-GTTGKTCTCWWGGY-TYCTGAAGAG-3'), and 19C-cons; or AVNP13, AVNP169 (5'-GTWGTCTCTGGTTCTCTRAAGAG-3'), and 19C-cons. The second-round PCR assays used MasterTaq Kit in conjunction with AVNP13, AVNP169, and AVNP171 (5'-TGYCTCTCTGGAGARGGATGGCC-3'); AVNP170 (5'-CATGATACARTCCATCAAWGCACARTG-3') and AVNP171; AVNP172 (5'-CCTTTCTTYTTGCTYTTRATCAC-3') and AVNP171; or AVNP1 (Fulhorst et al. 2001b), AVNP172, and 1010C (Bowen et al. 1996). Oligonucleotides AVNP170

TABLE 3. CALIFORNIA ARENAVIRUSES INCLUDED IN THE ANALYSES OF NUCLEOCAPSID PROTEIN GENE SEQUENCE DATA, BY STUDY SITE

Virus ^a	Strain	N protein ^b	Rodent			
			Voucher ^c	Species ^d	Date ^e	Site ^f
BCNV	AV C0900009	KC807193	TK113612	Nmac	2/16/2001	SB-1
BCNV	AV D1100015	KC807194	TK160419	Pboy	5/21/2002	SB-3
BCNV	AV D1100036	KC807195	TK160422	Nmac	5/21/2002	SB-3
BCNV	AV B0160054	KC807184	TK90192	Nmac	1/11/2000	LA-6
BCNV	AV B0300019	KC807183	TK90226	Nmac	2/8/2000	LA-9
BCNV	AV B0300052	FJ907244	TK91001	Nmac	1/19/2000	LA-11
BCNV	AV A0060207	FJ907245	TK90779	Pcal	6/11/1998	ORA-3
BCNV	AV A0060209	AF512833	TK90599	Pcal	6/11/1998	ORA-4
BCNV	AV C0120030	KC807187	TK93458	Pcal	6/4/1998	ORA-6
BCNV	AV C0120039	KC807188	TK93461	Pcal	4/23/1999	ORA-6
BCNV	AV A0060071	KC807185	TK90837	Nmac	6/6/1996	ORA-HJC
BCNV	AV A0060083	KC807186	TK90798	Nmac	7/2/1998	ORA-SC
BCNV	AV B0170044	KC807190	TK90919	Pcal	1/11/2000	RIV-4
BCNV	AV 98470029	AY924392	TK83707	Nmac	9/22/1998	RIV-5
BCNV	AV A0070039	AY924391	TK90438	Pcal	11/13/1998	RIV-6
BCNV	AV B0640008	KC807189	TK92349	Nmac	6/6/2000	RIV-7
PVEV	AV F0190005	KC807191	TK179749	Nalb	3/24/2004	RIV-1
PVEV	AV F0190012	KC807192	TK179750	Nlep	3/24/2004	RIV-1

^aBCNV, Bear Canyon virus; PVEV, Palo Verde virus.

^bNucleocapsid (N) protein gene sequence (GenBank accession number). The nucleotide sequences in FJ907244, KC807183, KC807184, and KC807187-KC807195 were from rodents tested for anti-arenavirus antibody in this study. The 6 other N protein gene sequences were from rodents that were positive for anti-arenavirus antibody in previous studies (Bennett et al. 2000, Fulhorst et al. 2002).

^cMuseum of Texas Tech University.

^dNalb, *Neotoma albigula*; Nlep, *Neotoma lepida*; Nmac, *Neotoma macrotis*; Pboy, *Peromyscus boylii*; Pcal, *Peromyscus californicus*. The species identities of the 11 woodrats (*Neotoma* spp.) were confirmed by analysis of cytochrome *b* gene sequences.

^eDate captured (month/day/year).

^fSB-1, Vandenberg Air Force Base; SB-3, Romero Canyon, Santa Ynez Mountains; LA-6, near Dry Canyon, Santa Monica Mountains; LA-9, Solstice Canyon, Santa Monica Mountains; LA-11, Zuma Canyon, Santa Monica Mountains; ORA-3, El Cariso #1, Santa Ana Mountains; ORA-4, El Cariso #2, Santa Ana Mountains; ORA-6, Lucas Canyon, Santa Ana Mountains; ORA-HJC, Holy Jim Canyon, Santa Ana Mountains; ORA-SC, near the city of San Clemente; RIV-1, near the Palo Verde Diversion Dam; RIV-4, San Jacinto Valley; RIV-5, Morrell Potrero, Santa Ana Mountains; RIV-6, Bear Canyon Trailhead, Santa Ana Mountains; RIV-7, Santa Rosa Plateau. Rodents TK90837, TK90798, and TK93458 were positive for antibody to *Whitewater Arroyo virus* strain AV 9310135 in a previous study (Bennett et al. 2000) and positive for N protein gene RNA in previously unpublished work. None of the rodents tested for anti-arenavirus antibody in this study were from ORA-HJC (33°40'39"N, 117°31'03"W) or ORA-SC (33°24'35"N, 117°35'24"W).

and AVNP171 were derivatives of NW1696R and 1010C, respectively.

Genetic characterization of woodrats

The species identities of 11 arenavirus-infected woodrats (Table 3) were confirmed by analysis of cytochrome *b* (*Cyrb*) gene sequences. The complete nucleotide sequences of the *Cyrb* genes of TK83707 and TK91001 were determined in previous studies (Edwards and Bradley 2002, Cajimat et al. 2007), the complete nucleotide sequences of the *Cyrb* genes of TK92349 and TK179749 were determined in this study from DNA isolated from liver, and the nucleotide sequences of a 1122- to 1125-bp fragment of the *Cyrb* genes of the seven other woodrats were determined in this study from DNA isolated from liver or skeletal muscle. The PCR assays used *GoTaq*[®] DNA Polymerase (Promega Corp., Madison, WI) in conjunction with oligonucleotides LGL 765 forward (Bickham et al. 1995) and LGL 766 reverse (Bickham et al. 2004).

Sequencing reactions and analysis

The nucleotide sequences of both strands of each purified amplicon were determined directly, using the Big Dye[®] Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). The products of the sequencing reactions were analyzed on ABI PRISM[®] 3100-Avant[™] or 3130-Avant[™] Genetic Analyzers (Applied Biosystems). The N protein gene sequences were deposited into the GenBank nucleotide sequence database under accession numbers KC807183–KC807195 (Table 3), and the *Cyrb* gene sequences were deposited into the GenBank nucleotide sequence database under accession numbers KF250456–KF250464.

Data analysis

The big-eared woodrats and California mice from six sites in western Riverside County (RIV-2 through RIV-7) were assigned to three age classes (juvenile, subadult, adult) on the basis of overall body size, dental wear, and pelage coloration. The Fisher exact test was used to assess differences between proportions and differences between rates.

The analyses of N protein gene sequences included BBTV strains AV D0390174 (GenBank acc. no. EF619035) and AV D0390324 (EF619036); CTNV, AV A0400135 (DQ865244) and AV A0400212 (DQ865245); MPRV, AV I0130006 (JX560798) and AV M0040017 (JX560799); OCEV, AV B1030026 (JN897398); RCTV, AV H0030026 (GQ903697); SKTV, AV D1000090 (EU123328); TAMV, W-10777 (AF512828) and AV 97140103 (EU486821); TTCV, AV D0150144 (EF619033) and AV D0390060 (EF6190034); WWAV strain AV 9310135 (AF228063) and seven other Whitewater Arroyo species complex viruses (Cajimat et al. 2008) AV 96010024 (EU123331), AV 96010025 (EU486820), AV 96010151 (EU123330), AV 98490013 (FJ032027), TVP-6038 (FJ719107), AV D1240007 (EU123329), and AV H0380005 (EU910959); and two South American Tacaribe serocomplex viruses, Guanarito virus (GTOV) strain INH-95551 (AY129247) and Pirital virus (PIRV) strain VAV-488 (AF485262). The analyses of N protein gene sequences were restricted to a 616-nucleotide fragment near the 3' end of the intergenic region of the small genomic segment. The predicted amino acid sequences were aligned by using the computer

program CLUSTAL W1.7 (Thompson et al. 1994). The nucleotide sequence alignment was constructed manually, guided by the computer-generated amino acid sequence alignment, and sequence nonidentities were equivalent to uncorrected distances.

The analysis of *Cyrb* gene sequences included white-throated woodrats (*N. albigula*) MVZ147667 (GenBank acc. no. AF108704), NK1330 (AF186814), NK17583 (AF186804), TK50148 (AF186808), TK77854 (AF186816), TK93637 (EU141961), and TK114533 (EU141960); Bryant's woodrats (*N. bryanti*) MVZ186296 (DQ781160), NK5372 (AF307832), TK26165 (AF376467), and TK77284 (AF307835); bushy-tailed woodrats (*Neotoma cinerea*) BYU17790 (DQ179859) and TK36287 (AF186799); Arizona woodrat (*Neotoma devia*) MVZ200714 (DQ781302); dusky-footed woodrats (*Neotoma fuscipes*) MVZ195212 (DQ781303), MVZ196356 (DQ179826), and MVZ196405 (DQ179822); Goldman's woodrat (*Neotoma goldmani*) TK28315 (AF186829); Angel Island woodrat (*Neotoma insularis*) UCLA19911 (DQ781161); desert woodrats (*N. lepida*) BYU18300 (DQ781254), MVZ195324 (DQ781162), NK2150 (AF307831), and NK54420 (AF307833); white-toothed woodrats (*Neotoma leucodon*) TK48594 (AF186809) and TK49716 (AF186806); big-eared woodrats (*N. macrotis*) MDM800 (DQ179841), MVZ198597 (DQ781304), TK77285 (AF307836), and TK77287 (AF376475); Mexican woodrats (*Neotoma mexicana*) NK36282 (AF298841), TK119202 (FJ716222), and TK123380 (FJ716223); Stephen's woodrats (*Neotoma stephensi*) MVZ197170 (DQ781305) and TK77928 (AF308867); and Allen's woodrat (*Hodomys alleni*) TK45043 (AF186802). The *Cyrb* gene sequences were aligned manually.

The analyses of the N protein gene and *Cyrb* gene sequences were done with MRBAYES v. 3.1.2 (Huelsenbeck and Ronquist 2001) and programs in the computer software package PAUP* (Swofford 2002). The Bayesian analyses used a GTR+I+G model of evolution with a site-specific gamma distribution and the following options in MRBAYES v3.1.2—two simultaneous runs of four Markov chains, 5 million generations (N protein gene sequence data) or 10 million generations (*Cyrb* gene sequence data), and sample frequency = every 1000th generation. The GTR+I+G model of evolution was selected on the basis of preliminary analyses of the nucleotide sequence data (Nylander 2004). The outgroup was GTOV strain INH-95551 or Allen's woodrat TK45043; the first 1000 trees were discarded after review of the likelihood scores, convergence statistics, and potential scale reduction factors. A majority-rule consensus tree was constructed from the remaining trees, and clade probability values were calculated *a posteriori*.

Results

Anti-arenavirus antibody (IgG) was found in 145 (3.6%) of 3977 neotomine rodents captured in six counties in southern California (Table 1). The majority (122 or 84.1%) of the 145 antibody-positive rodents were *N. macrotis* or *P. californicus*. By species, the median antibody titers in the antibody-positive rodents ranged from 320 (*P. maniculatus*) to ≥ 5120 (*N. albigula*, *N. lepida*, *N. macrotis*, *P. boylii*, *P. californicus*, and *P. eremicus*) (Table 4).

The antibody-positive rodents were from 33 sites—one in Kern County, three in Santa Barbara County, 11 in Los

TABLE 4. FREQUENCY OF TITERS OF ANTI-ARENAVIRUS ANTIBODY IN 3977 NEOTOMINE RODENTS CAPTURED IN SIX COUNTIES IN SOUTHERN CALIFORNIA IN 1998–2013, BY SPECIES

Species	Titer ^a				Total
	<320	320	1280	≥5120	
<i>Neotoma albigula</i>	2			1	3
<i>Neotoma bryanti</i>	548	5	4	4	561
<i>Neotoma cinerea</i>	1				1
<i>Neotoma lepida</i>				1	1
<i>Neotoma macrotis</i>	361	6	10	45	422
<i>Onychomys torridus</i>	2				2
<i>Peromyscus boylii</i>	513		1	2	516
<i>Peromyscus californicus</i>	766	8	8	45	827
<i>Peromyscus crinitus</i>	49				49
<i>Peromyscus eremicus</i>	628	1		2	631
<i>Peromyscus maniculatus</i>	814		2		816
<i>Peromyscus truei</i>	29				29
<i>Reithrodontomys megalotis</i>	119				119
Total	3832	20	25	100	3977

^aEndpoint antibody (IgG) titers to Whitewater Arroyo virus strain AV 9310135.

Angeles County, six in Orange County, six in western Riverside County, one in eastern Riverside County, five in San Diego County (Fig. 1). Antibody to WWAV was found in multiple species at 13 sites: SB-2, SB-3, LA-6, ORA-2, ORA-4, ORA-6, RIV-1, RIV-2, RIV-3, RIV-4, RIV-5, RIV-6, and SD-5 (Table 2).

Anti-arenavirus antibody was found in *N. macrotis* and/or *P. californicus* at five sites in the Santa Monica Mountains (LA-6, LA-7, LA-9, LA-10, LA-11), three sites in the San Jacinto Valley (RIV-2, RIV-3, RIV-4), and eight sites in the Santa Ana Mountains (ORA-2 through ORA-6, RIV-5, RIV-6, RIV-7). The antibody-positive rodents included nine (56.3%) of 16 big-eared woodrats and none of 21 California mice captured in the Santa Monica Mountains ($p < 0.0001$), five (26.3%) of 19 big-eared woodrats and 32 (23.5%) of 136 California mice captured in the San Jacinto Valley ($p > 0.4924$), and 26 (22.0%) of 118 big-eared woodrats and 26 (5.3%) of 490 California mice captured in the Santa Ana Mountains ($p < 0.0001$).

The 651 rodents from RIV-2, RIV-3, RIV-4, RIV-5, RIV-6, and RIV-7 were captured in January, 2000–September, 2002 (Table 5). Fifty Sherman live traps were set at each site on one night every 3 months, beginning in January, 2000 (RIV-2, RIV-3, RIV-4), February, 2000 (RIV-5, RIV-6), or March, 2000 (RIV-7). By site, trapping success ranged from 92 rodents/550 trap-nights (16.7%, RIV-5) to 133 rodents/550 trap-nights (24.2%, RIV-6) ($p = 0.0027$). The prevalence

FIG. 1. Map of southern California showing the locations of: (1) The 33 sites at which the 145 antibody-positive rodents in this study were captured, (2) ORA-HJC (Holy Jim Canyon, Santa Ana Mountains), and (3) ORA-SC (near the city of San Clemente). SLO, San Luis Obispo County; KRN, Kern County; SBD, San Bernardino County; SB, Santa Barbara County; VEN, Ventura County; LA, Los Angeles County; ORA, Orange County; RIV, Riverside County; SD, San Diego County; IMP, Imperial County. Arenaviral RNA was found in antibody-positive rodents captured at SB-1, SB-3, LA-6, LA-9, LA-11, ORA-6, RIV-1, RIV-4, and RIV-7 in this study; arenavirus was isolated from antibody-positive rodents captured at ORA-3, ORA-4, RIV-5, and RIV-6 in previous studies (Cajimat et al. 2007, Fulhorst et al. 2002); and the rodents from ORA-HJC and ORA-SC were positive for anti-arenavirus antibody in a previous study (Bennett et al. 2000). The distances among the nine sites in the Santa Ana Mountains (ORA-2 through ORA-6, ORA-HJC, RIV-5, RIV-6, and RIV-7) ranged from 0.7 km (ORA-3 and ORA-4) to 36.2 km (ORA-2 and RIV-7), and the distances among the three sites in the San Jacinto Valley (RIV-2, RIV-3, RIV-4) ranged from 0.6 km (RIV-3 and RIV-4) to 4.6 km (RIV-2 and RIV-4).

TABLE 5. PREVALENCE OF ANTI-ARENAVIRUS ANTIBODY IN 651 NEOTOMINE RODENTS CAPTURED AT SIX SITES IN RIVERSIDE COUNTY, CALIFORNIA IN JANUARY, 2000–SEPTEMBER, 2002, BY SITE AND QUARTER YEAR^{a,b}

Species ^c	2000				2001				2002			Total
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	
RIV-2												
Nmac	1/1	1/1		0/1								2/3
Pcal	2/8	0/3				0/2		0/4		0/3	0/1	2/21
Other	0/14	0/8	0/5	0/7	0/9	0/4	0/7	0/6	0/7	0/7	0/4	0/78
RIV-3												
Nmac		2/2	0/1	0/1	0/1	0/1	0/2	0/1		0/3		2/12
Pcal	1/1	1/4		0/1	0/1	1/3	1/1	0/2	1/4		0/2	5/19
Other	0/7	0/9	0/7	0/6	0/12	0/11	0/9	0/5	0/11	0/6	0/8	0/91
RIV-4												
Nmac					1/1	0/2		0/1				1/4
Pcal	10/10	3/4	1/3	2/6	7/20	0/10	0/8	1/9	0/7	1/7	0/3	25/87
Other		0/1			0/1		0/4	0/1	0/2			0/9
RIV-5												
Nmac	2/2			1/3	1/4	0/1	1/2					5/12
Pcal	0/5	1/1		0/2	0/7	0/4	0/1	0/3	0/2	0/7	0/3	1/35
Other	0/5	0/7	0/1	0/2	0/9	0/1	0/1	0/7	0/4	0/5	0/3	0/45
RIV-6												
Nmac		0/2	2/3	1/2	0/1			0/1	0/4	2/4	0/1	5/18
Pcal	1/8	2/7	0/5	1/8	1/21	0/8	0/4	0/6	0/12	0/15	0/8	5/102
Other							0/1	0/3	0/5	0/4		0/13
RIV-7												
Nmac		2/2		0/2		1/2	0/1	1/1	1/1	0/3	1/2	6/14
Pcal			0/6	0/4	0/3	0/1	0/5	0/1	0/9	0/8	0/2	0/39
Other	0/9	0/3		0/3	0/3	0/3	0/2	0/7	0/7	0/5	0/7	0/49
Total	17/70	12/54	3/31	5/48	10/93	2/53	2/48	2/58	2/75	3/77	1/44	59/651

^aRIV-2, RIV-3, and RIV-4 were in the San Jacinto Valley; RIV-5 and RIV-6 were in the Santa Ana Mountains; and RIV-7 was on the Santa Rosa Plateau, which is a southeastern extension of the Santa Ana Mountains in Riverside County.

^bQ1, first quarter (January 1–March 31); Q2, second quarter (April 1–June 30); Q3, third quarter (July 1–September 30); Q4, fourth quarter (October 1–December 31). Prevalence = number positive/number of rodents tested for IgG to Whitewater Arroyo virus strain AV 9310135.

^cNmac, *Neotoma macrotis*; Pcal, *Peromyscus californicus*; Other, *Neotoma bryanti* (Nbry), *Onychomys torridus* (Otor), *Peromyscus boylii* (Pboy), *Peromyscus eremicus* (Pere), *Peromyscus maniculatus* (Pman), *Peromyscus truei* (Ptru), *Reithrodontomys megalotis* (Rmeg). RIV-2, Nbry ($n=7$), Otor ($n=1$), Pboy ($n=45$), Pere ($n=13$), Pman ($n=12$); RIV-3, Nbry ($n=6$), Pboy ($n=60$), Pere ($n=9$), Pman ($n=15$), Ptru ($n=1$); RIV-4, Nbry ($n=3$), Pboy ($n=3$), Pere ($n=2$), Ptru ($n=1$); RIV-5, Nbry ($n=1$), Pboy ($n=35$), Pman ($n=7$), Rmeg ($n=2$); RIV-6, Pboy ($n=7$), Pere ($n=6$); RIV-7, Nbry ($n=3$), Pboy ($n=34$), Pere ($n=1$), Pman ($n=11$).

of big-eared woodrats among captured rodents ranged from 3/102 (2.9%, RIV-2) to 18/133 (13.5%, RIV-6) ($p=0.0049$); and the prevalence of California mice among captured rodents ranged from 19/122 (15.6%, RIV-3) to 102/133 (76.7%, RIV-6) ($p<0.0001$).

Twelve (20.3%) of the 59 antibody-positive animals from RIV-2, RIV-3, RIV-4, RIV-5, RIV-6, and RIV-7 were juvenile or subadult—one big-eared woodrat captured at RIV-2 in April, 2000, one California mouse captured at RIV-3 in January, 2000, one California mouse captured at RIV-3 in April, 2000, one California mouse captured at RIV-4 in January, 2000, two California mice captured at RIV-4 in November, 2000, five California mice captured at RIV-4 in January, 2001, one big-eared woodrat captured at RIV-6 in May, 2002. The antibody titers in the subadult woodrats were 320, the antibody titers in the juvenile California mice were 320 ($n=1$) and ≥ 5120 ($n=1$), and the antibody titers in the eight subadult California mice ranged from 320 ($n=4$) to ≥ 5120 ($n=2$). In contrast, the antibody titers in the 19 antibody-positive adult woodrats and 28 antibody-positive adult California mice from RIV-2, RIV-3, RIV-4, RIV-5, RIV-6, and RIV-7 ranged from 1280 ($n=2$) to ≥ 5120 ($n=17$)

and 1280 ($n=2$) to ≥ 5120 ($n=26$), respectively. Arguably, the anti-arenavirus antibody in some of the subadult woodrats, juvenile California mice, and subadult California mice was maternal in origin rather than a consequence of infection.

Anti-arenavirus antibody was found in four (25.0%) of the 16 adult big-eared woodrats and 22 (20.8%) of the 106 adult California mice from RIV-2, RIV-3, and RIV-4 ($p=0.7464$), and in 15 (39.5%) of the 38 adult big-eared woodrats and six (4.1%) of the 145 adult California mice from RIV-5, RIV-6, and RIV-7 ($p<0.0001$). By site, the prevalence of antibody in adult big-eared woodrats and adult California mice ranged from 2/10 (20.0%, RIV-3) to 6/11 (54.5%, RIV-7) and from 0/32 (RIV-7) to 17/74 (23.0%, RIV-4), respectively (Table 6). The prevalence of antibody in adult big-eared woodrats was not significantly different from the prevalence of antibody in adult California mice at any of the three sites in the San Jacinto Valley (RIV-2, RIV-3, RIV-4); however, the prevalence of antibody in adult big-eared woodrats was significantly greater than the prevalence of antibody in adult California mice at each of the three sites in the Santa Ana Mountains (RIV-5, RIV-6, RIV-7) (Table 6).

TABLE 6. PREVALENCE OF ANTI-ARENAVIRUS ANTIBODY IN 54 ADULT BIG-EARED WOODRATS (*NEOTOMA MACROTIS*) AND 251 ADULT CALIFORNIA MICE (*PEROMYSCUS CALIFORNICUS*) CAPTURED AT SIX SITES IN RIVERSIDE COUNTY IN JANUARY, 2000–SEPTEMBER, 2002, BY SITE AND SPECIES

Site ^a	Species ^b		Probability ^c
	<i>Neotoma macrotis</i>	<i>Peromyscus californicus</i>	
RIV-2	1/2 (50.0%)	2/18 (11.1%)	0.2842
RIV-3	2/10 (20.0%)	3/14 (21.4%)	> 0.6680
RIV-4	1/4 (25.0%)	17/74 (23.0%)	> 0.6581
RIV-5	5/11 (45.5%)	1/32 (3.1%)	0.0025
RIV-6	4/16 (25.0%)	5/81 (6.2%)	0.0382
RIV-7	6/11 (54.5%)	0/32 (0.0%)	< 0.0001
Total	19/54 (35.2%)	28/251 (11.2%)	< 0.0001

^aRIV-2, RIV-3, and RIV-4 were in the San Jacinto Valley; RIV-5 and RIV-6 were in the Santa Ana Mountains; and RIV-7 was on the Santa Rosa Plateau, which is a southeastern extension of the Santa Ana Mountains in Riverside County.

^bNumber positive/number of animals tested for immunoglobulin G (IgG) to *Whitewater Arroyo virus* strain AV 9310135.

^cThe statistical significance of differences between prevalences were assessed by using the Fisher exact test.

Arenavirus was isolated from woodrat TK91001, none of 108 other antibody-positive rodents, and none of 337 antibody-negative rodents captured alongside antibody-positive big-eared woodrats or antibody-positive California mice. Arenaviral N protein gene RNA was found in samples of kidney from 11 (26.2%) of 42 antibody-positive, culture-negative rodents—one white-throated woodrat, none of 13 Bryant's woodrats, five (26.3%) of 19 big-eared woodrats, one desert woodrat, one (50.0%) of two brush mice, three California mice, none of three cactus mice. The voucher numbers of the N protein gene RNA-positive rodents were TK90192, TK90226, TK90919, TK92349, TK93458, TK93461, TK113612, TK160419, TK160422, TK179749, and TK179750 (Table 3).

The alignment of N protein gene sequences was 634 characters in length, with an 18-nucleotide gap in the sequences of all the North American viruses except OCEV strain AV B1030026. The Bayesian analysis segregated the 18 California viruses (Table 3) into two groups, designated "A" and "B" (Fig. 2), with each group supported by results of analyses done *a posteriori* (i.e., clade probability values ≥ 0.95). Group A comprised the viruses from Santa Barbara County, Los Angeles County, Orange County, and western Riverside County. Group B included BBTv, SKTV, TTCV, and the arenaviruses associated with *N. albigula* and *N. lepida* in eastern Riverside County (RIV-1).

Nonidentities among the N protein gene sequences of the 18 viruses from California ranged from 0.0% (AV D1100015 and AV D1100036) to 29.2% (AV B0640008 and AV F0190012) (Table 7). Nonidentities among the 211-amino-acid sequences predicted from the N protein gene sequences ranged from 0.0% (AV A0060207 and AV 98470029, AV C0120030 and AV C0120039, AV D1100015 and AV D1100036, AV F0190005 and AV F0190012) to 20.5% (AV A0060209 and AV F0190005, AV A0060209 and AV F0190012) (Table 7). Together, the results of the Bayesian

analysis and pairwise comparisons of N protein gene sequences indicated that the 16 viruses in group A (Fig. 2) are strains of BCNV and the viruses associated with *N. albigula* and *N. lepida* in eastern Riverside County (i.e., AV F0190005 and AV F0190012) are strains of an arenavirus different from BCNV.

Discussion

Specific rodents (usually one or two closely related species) are the principal hosts of the arenaviruses for which natural host relationships have been well characterized. For example, the southern plains woodrat (*Neotoma micropus*) is the principal host of CTNV in southern Texas (Milazzo et al. 2013). The present-day principal host relationships of WWAV, CTNV, and some of the other North American Tacaribe serocomplex viruses may represent an ancient relationship between the Arenaviridae and genus *Neotoma* (Cajimat et al. 2011).

This study extends our knowledge of the geographical range of BCNV from Orange County, the Santa Ana Mountains in Riverside County, and western Los Angeles County to western Santa Barbara County and the San Jacinto Valley in Riverside County. The broad geographical distribution of BCNV in association with *N. macrotis* suggests that the BCNV–*N. macrotis* relationship was established long ago. Hypothetically, BCNV is the arenavirus associated with *N. macrotis* in Kern and San Diego counties.

The results of previous studies (Fulhorst et al. 2002, Cajimat et al. 2007, 2011) indicated that the big-eared woodrat (*N. macrotis*) in the Santa Monica Mountains (LA-11), big-eared woodrat in the Santa Ana Mountains (RIV-5), and California mouse (*P. californicus*) in the Santa Ana Mountains (ORA-3, ORA-4, and RIV-6) are natural hosts of BCNV. The results of this study indicate that the big-eared woodrat in Santa Barbara County, brush mouse (*P. boylii*) in Santa Barbara County, and big-eared woodrat in southern Orange County (ORA-SC) also are natural hosts of BCNV (Table 3).

In a previous study (Fulhorst et al. 2002), BCNV was isolated from 5 (55.6%) of nine antibody-positive California mice and none of 22 antibody-negative California mice captured at ORA-3 (El Cariso #1), ORA-4 (El Cariso #2), and RIV-6 (Bear Canyon Trailhead). In this study, arenavirus was isolated from none of four antibody-negative big-eared woodrats and none of 18 antibody-negative California mice captured alongside the BCNV-infected big-eared woodrats from LA-6, LA-9, and LA-11; arenavirus was isolated from none of 18 antibody-negative big-eared woodrats and none of 132 antibody-negative California mice captured alongside antibody-positive big-eared woodrats or antibody-positive California mice from ORA-3, ORA-4, ORA-6, RIV-4, RIV-5, and RIV-6; and BCNV N protein gene RNA was found in five (26.3%) of 19 antibody-positive, culture-negative big-eared woodrats and three (100%) of three antibody-positive, culture-negative California mice. Altogether, this information indicates that antibody (IgG) to WWAV strain AV 9310135 is a reasonably accurate measure of BCNV infection in *N. macrotis* and *P. californicus*. We acknowledge that the anti-arenavirus antibody in some rodents in this study, including rodents tested for N protein gene RNA, may have been maternal in origin.

The results of the analyses of N protein gene sequence data together with the results of the statistical analyses of antibody prevalence data indicate that *N. macrotis* is the principal host

FIG. 2. Phylogenetic relationships among North American Tacaribe serocomplex viruses based on Bayesian analysis of nucleotide sequences of a 616-nucleotide fragment of the nucleocapsid protein gene. The branch labels include (in the following order) virus, strain, host species, state, and county or municipality. BBTV, Big Brushy Tank virus; BCNV, Bear Canyon virus; CTNV, Catarina virus; MPRV, Middle Pease River virus; PVEV, Palo Verde virus; RCTV, Real de Catorce virus; SKTV, Skinner Tank virus; TAMV, Tamiami virus; TTCV, Tonto Creek virus; WWAV, Whitewater Arroyo virus. Nalb, *Neotoma albigula*; Ncin, *N. cinerea*; Nlep, *N. lepida*; Nleu, *N. leucodon*; Nmac, *N. macrotis*; Nmex, *N. mexicana*; Nmic, *N. micropus*; Nsp, *Neotoma* species (presumed to be *N. albigula*); Pboy, *Peromyscus boylii*; Pcal, *P. californicus*; OCEV, Ocozocoautla de Espinosa virus; PIRV, Pirital virus; GTOV, Guanarito virus. The length of the scale bar is equivalent to 0.05 substitution per site. A black dot at a node indicates that the probability values in support of the clade were ≥ 0.95 .

of BCNV in the Santa Monica Mountains and Santa Ana Mountains. The lack of a statistically significant association between rodent species (*N. macrotis* versus *P. californicus*) and antibody prevalence in the adult big-eared woodrats and adult California mice from the San Jacinto Valley may be related to habitat and/or the months in which rodents were trapped. As stated previously, traps were set at RIV-2, RIV-3,

and RIV-4 in January, April, July, and October; RIV-5 and RIV-6 in February, May, August, and November; and RIV-7 in March, June, September, and December.

Chronic infections in rodents appear to be critical to the long-term maintenance of arenaviruses in nature. Exposure to virus early in life, if not lethal, usually results in lifelong infection with persistent viruria (Childs and Peters 1993).

TABLE 7. NONIDENTITIES AMONG THE NUCLEOTIDE SEQUENCES OF A 616-NUCLEOTIDE FRAGMENT OF THE NUCLEOCAPSID PROTEIN GENES OF 23 TACARIBE SEROCOMPLEX VIRUSES NATURALLY ASSOCIATED WITH NEOTOMINE RODENTS IN CALIFORNIA OR ARIZONA, AND NONIDENTITIES AMONG THE 211-AMINO-ACID SEQUENCES PREDICTED FROM THE NUCLEOTIDE SEQUENCES

Virus(es) ^b	Nucleotide sequence nonidentities (%) ^a							
	BCNV (SB)	BCNV (LA)	BCNV (ORA)	BCNV (RIV)	PVEV	BBTV	SKTV	TTCV
BCNV (SB)	—	15.9–17.7	14.6–18.7	15.4–17.7	26.3–26.9	24.7–26.5	25.8–27.6	26.1–27.4
BCNV (LA)	8.3–9.8	—	10.6–14.8	10.6–13.1	26.9–27.8	25.2–27.8	26.6–28.1	26.9–28.9
BCNV (ORA)	5.9–9.3	2.9–5.9	—	2.8–13.3	26.9–28.7	25.2–27.1	24.7–26.3	25.3–28.6
BCNV (RIV)	6.3–10.7	2.9–6.3	0.0–4.4	—	24.8–29.2	24.8–27.1	25.5–27.3	25.3–28.9
PVEV	18.5–19.5	19.0–20.0	17.1–20.5	17.1–20.0	—	20.6–20.9	18.2–18.3	19.0–20.3
BBTV	15.6–18.0	16.6–18.5	15.6–18.5	16.6–18.0	10.2–10.7	—	20.6–21.3	19.3–20.8
SKTV	17.1–18.0	18.0–18.5	15.1–17.6	16.6–17.1	11.7	12.2–12.7	—	17.2–19.2
TTCV	18.0–20.0	18.5–21.0	17.1–21.5	18.5–21.0	12.2	11.7–13.2	8.8–9.3	—
Amino acid sequence nonidentities (%)								

^aNucleotide sequence nonidentities and amino acid sequence nonidentities are listed above and below the diagonal, respectively.

^bBCNV-SB, Bear Canyon virus (BCNV) strains from Santa Barbara County, AV C0900009, AV D1100015, AV D1100036; BCNV (LA), Los Angeles County, AV B0160054, AV B0300019, AV B0300052; BCNV-ORA, Orange County, AV A0060071, AV A0060083, AV A0060207, AV A0060209, AV C0120030, AV C0120039; BCNV-RIV, Riverside County, AV 98470029, AV A0070039, AV B0170044, AV B0640008; PVEV, Palo Verde virus strains AV F0190005 and AV F0190012; BBTV, Big Brushy Tank virus strains AV D0390174 and AV D0390324; SKTV, Skinner Tank virus strain AV D1000090; TTCV, Tonto Creek virus strains AV D0150144 and AV D0390060. Nonidentities among the nucleotide sequences (and amino acid sequences) of the BCNV strains from Santa Barbara, Los Angeles, Orange, and Riverside counties ranged from 0.0–14.6% (0.0–8.3%), 6.3–11.4% (2.9–6.8%), 1.5–11.5% (0.0–3.9%), and 1.6–13.0% (0.5–4.4%), respectively. The nucleotide sequence of PVEV strain AV F0190005 was 99.8% identical to the nucleotide sequence of PVEV strain AV F0190012.

Assumedly, BCNV infections in some big-eared woodrats are chronic and BCNV-infected big-eared woodrats can initiate intra- and interspecific (*N. macrotis*-to-*P. californicus*) virus transmission. Whether California mice can transmit BCNV to other California mice is the subject of an ongoing laboratory study.

Big-eared woodrats typically construct large houses (up to 1.8 meters in height) from twigs, small branches, bark, plant cuttings, and miscellaneous objects (Gander 1929). These houses are used by woodrats for resting, food storage, nesting, and protection from predators and temperature extremes. Studies done in the Santa Ana Mountains (Pequegnat 1951) and San Diego County (Gander 1929) revealed that California mice frequently seek refuge and nest in houses of big-eared woodrats, even when occupied by woodrats. The association of *P. californicus* with the houses of big-eared woodrats may be a strong determinant of the risk of BCNV infection in *P. californicus*, including newborn and juvenile animals. Indeed, the high prevalence of anti-arenavirus antibody in *P. californicus* at RIV-4 in January, 2000, and January, 2001, may be a consequence of nesting in woodrat houses occupied by BCNV-infected woodrats or contaminated with BCNV.

The results of the Bayesian analysis of N protein gene sequences indicated that the Tacaribe serocomplex virus associated with *N. albigula* and *N. lepida* in eastern Riverside County (RIV-1) is phylogenetically more closely related to BBTV, SKTV, and TTCV than to BCNV. Further work is needed to determine whether the arenavirus in eastern Riverside County is a variant of BBTV, SKTV, or TTCV, or a novel arenavirus, tentatively named Palo Verde virus (PVEV).

In this study, BCNV N protein gene RNA was found in five (26.3%) of 19 antibody-positive, culture-negative big-eared woodrats and none of 13 antibody-positive, culture-negative Bryant's woodrats ($p=0.0641$). The failure to detect N

protein gene RNA in the Bryant's woodrats may be due to small sample size; however, the median antibody titer in the 13 antibody-positive Bryant's woodrats was at least fourfold less than the median antibody titer in the 61 antibody-positive big-eared woodrats (Table 4). Perhaps the arenavirus associated with *N. bryanti* is antigenically different from BCNV in ELISA, and detection of RNA of the *N. bryanti*-associated arenavirus in tissues of infected rodents will require oligonucleotides different from those used to prime the PCR assays in this study.

Neotoma macrotis occurs in woodland, chaparral, and sage scrub communities from San Luis Obispo County, southward along the Pacific Coast, into western Imperial County and northwestern Baja California (Gander 1929, Chew et al. 1959, Horton and Wright 1944, Pequegnat 1951, Cameron 1971, M'Closkey 1972, Meserve 1974, Spevak 1983, Matocq 2002). The antibody-positive big-eared woodrats in this study were captured on a military base (SB-1) and in city or county parks (LA-6, SD-5), state parks (KRN-1, SD-1), national forests (SB-2, SB-3, ORA-2, ORA-4, ORA-6, RIV-5, RIV-6, SD-2), a national recreation area (LA-9, LA-10, LA-11), open space alongside public roads (RIV-2, RIV-3, RIV-4), an ecological preserve (RIV-7) and ecological reserve (SD-3), residential area (LA-7), and a public utility property (LA-3) and ecological preserve (SD-4) in the vicinity of residential areas. As such, persons who work or take recreation outdoors in southern California or northern Baja California may be exposed to BCNV. The human health significance of BCNV has not been investigated.

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Author Disclosure statement

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