

Ecology of Catarina Virus (family *Arenaviridae*) in Southern Texas, 2001–2004

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Abstract

A total of 3941 rodents were captured during a 46-month prospective (mark-recapture) study on the ecology of Catarina virus in southern Texas. Antibody reactive against Catarina virus was found in 73 (11.9%) of 611 southern plains woodrats (*Neotoma micropus*) and none of 3330 other rodents; strains of Catarina virus were isolated from 6 antibody-negative and 9 antibody-positive southern plains woodrats; and the infections in at least 3 southern plains woodrats were chronic. These results affirm the notion that the southern plains woodrat is the principal host of Catarina virus and suggest that Catarina virus infection is highly specific to *N. micropus*.

Key Words: Arenavirus—*Arenaviridae*—Catarina virus—*Neotoma micropus*—Southern plains woodrat—Tacaribe serocomplex.

Introduction

THE NORTH AMERICAN MEMBERS of the Tacaribe serocomplex (family *Arenaviridae*, genus *Arenavirus*) include Bear Canyon virus (BCNV), Catarina virus (CTNV), Tamiami virus (TAMV), and Whitewater Arroyo virus (WWAV) (Cajimat et al. 2011). Specific members of the rodent family Cricetidae (Musser and Carleton 2005) are the principal hosts of the Tacaribe serocomplex viruses for which natural host relationships have been well characterized. For example, the hispid cotton rat (*Sigmodon hispidus*) in southern Florida is the principal host of TAMV (Bigler et al. 1975, Calisher et al. 1970, Jennings et al. 1970).

The results of a previous study (Fulhorst et al. 2002a) suggested that the southern plains woodrat (*Neotoma micropus*) in southern Texas is the principal host of CTNV. The objective of this study was to extend our knowledge of the ecology of CTNV.

Materials and Methods

Study area

The Chaparral Wildlife Management Area (CWMA) comprises 6151 hectares (ha) of brush country in southern Texas (Ruthven and Synatzske 2002). The topography is level to

gently rolling (average elevation, 152.4 meters). The dominant vegetation includes blackbrush (*Acacia rigidula*), whitebrush (*Aloysia gratissima*), guayacan (*Guaiacum angustifolium*), prickly pear (*Opuntia* spp.) and other cacti, mesquite (*Prosopis glandulosa*), and a number of different forbs and grasses (McLendon 1991, Ruthven and Synatzske 2002). The terrestrial mammalian assemblage comprises coyote (*Canis latrans*), bobcat (*Lynx rufus*), common raccoon (*Procyon lotor*), white-tailed deer (*Odocoileus virginianus*), collared peccary (*Tayassu tajacu*), eastern cottontail (*Sylvilagus floridana*), striped skunk (*Mephitis mephitis*), northern pygmy mouse (*Baiomys taylori*), northern grasshopper mouse (*Onychomys leucogaster*), white-footed mouse (*Peromyscus leucopus*), fulvous harvest mouse (*Reithrodontomys fulvescens*), hispid cotton rat, southern plains woodrat, hispid pocket mouse (*Chaetodipus hispidus*), Ord's kangaroo rat (*Dipodomys ordii*), Merriam's pocket mouse (*Perognathus merriami*), Mexican ground squirrel (*Spermophilus mexicanus*), desert shrew (*Notiosorex crawfordi*), domestic cow (*Bos taurus*), and feral pig (*Sus scrofa*).

Trapping sites

Rodents were captured at 3 sites (Fig. 1) in January 4, 2001 to October 8, 2004 (Table 1). A trapping web (Anderson et al. 1983) was established on site I (Universal Transverse Mercator

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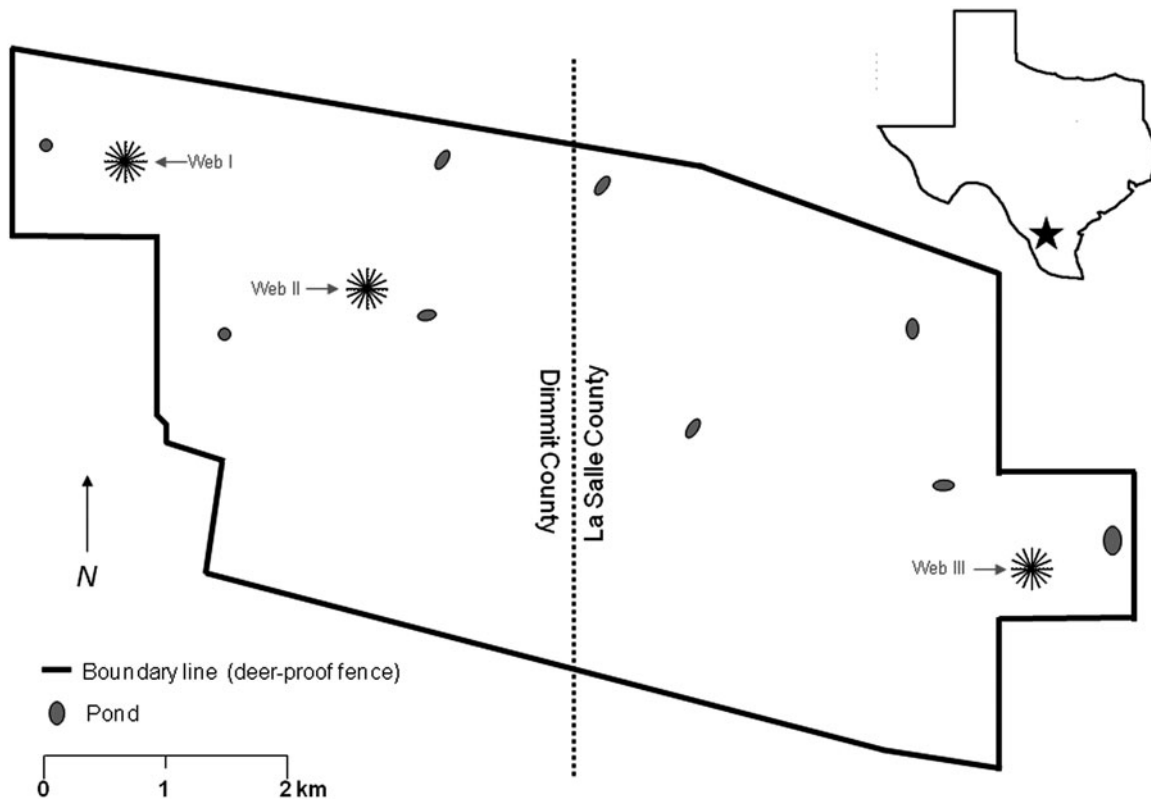


FIG. 1. Map of the Chaparral Wildlife Management Area showing the locations of Webs I, II, and III. The inset shows the location of the area in Texas.

coordinates, 14-465980E-3131609N) in January, 2001, site II (14-454285N-3136441E) in March, 2001, and site III (14-458569N-3134894E) in January, 2001. Each web (31,400 square meters) consisted of 16 trap lines and 320 trap stations, with 20 stations on each line at 5-meter intervals, beginning 5 meters from the center of the web.

Collection and processing of rodents

A Sherman live trap (H.B. Sherman Traps, Inc., Tallahassee, FL, model LFATDG) was set at each trap station on Web I (W-I) and Web III (W-III) on 3 consecutive nights in January, March, June, and October in January, 2001–October, 2004; a Sherman live trap was set at each trap station on Web II (W-II) on 3 consecutive nights in March, June, and October in 2001, and 3 consecutive nights in January, March, June, and October in January, 2002–October, 2004; a Tomahawk trap (Tomahawk Live Traps, Mfg., Hazelhurst, WI, model 201) was set at a trap station on the second and third nights of a trapping session if the Sherman trap was moved from the station, presumably by a small mammal, on the first night of the same trapping session; and a Tomahawk trap was set at a trap station on the third night of a trapping session if the Sherman trap was moved from the station on the second night of the same trapping session. Traps were set 1 h prior to sunset; baited with a mixture of rolled oats, cracked corn, and birdseed; and checked at dawn the following day. Animals captured in the traps or found dead on the webs were processed in a laboratory on the CWMA.

Individual animals were processed only once each trapping session. The live animals were restrained with ketamine hy-

drochloride (1 µg/g body weight, administered by intramuscular injection) to facilitate accurate measurement of the animals and the collection of samples. At first capture, each animal was assigned a unique identification (TK) number and “marked” with a unique toe-clip pattern (Gannon et al. 2007). Information collected on a standardized form included date of capture, web and trap station, species identity (based on external morphological features), gender, body weight, total length (nose to tip of tail), length of tail, and age class (juvenile, subadult, adult based on body size, and condition and coloration of the pelage). Blood samples, throat (oropharyngeal) swabs, and urine samples were collected from the woodrats. Only blood samples were collected from the other mammals. Blood from a bleeding (amputated) toe was collected on a Nobuto strip (Advantec MFS, Inc., Pleasanton, CA); blood from a retro-orbital venous plexus was collected with a sterile microhematocrit tube or sterile Pasteur pipette; cardiac blood from rodents that were euthanized or found dead was collected in an aseptic manner by trans-thoracic puncture with a sterile 25-gauge × 5/8” needle on a tuberculin syringe. Oropharyngeal secretions (OPsec) and other materials in the oropharynx were collected on a sterile cotton-tipped swab wetted with 0.01 M phosphate-buffered saline (PBS; pH 7.4) and then mixed with 0.3 mL of PBS containing 10% v/v heat-inactivated fetal bovine serum (FBS). Urine was collected by midstream catch or by antepubic cystocentesis, using a 25-gauge × 5/8” needle on a tuberculin syringe. The animals captured before the third day in the October, 2004, trapping session were processed, then provided small pieces of apple for hydration, and, when fully recovered from the effects of

TABLE 1. PREVALENCE OF CATARINA VIRUS INFECTION IN RODENTS CAPTURED AT 3 SITES ON THE CHAPARRAL WILDLIFE MANAGEMENT AREA, JANUARY 4, 2001–OCTOBER 8, 2004, BY TRAPPING SESSION^a

Web P ^b	Jan 01	Mar 01	Jun 01	Oct 01	Jan 02	Mar 02	Jun 02	Oct 02	Jan 03	Mar 03	Jun 03	Oct 03	Jan 04	Mar 04	Jun 04	Oct 04
Btay	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Nmic	0/4	2/11	9/29	5/20	0/4	1/11	1/4	0/11	2/11	0/8	2/22	0/27	0/2	0/10	0/28	0/4
Oleu	0/1	–	–	–	0/4	0/2	–	–	0/4	0/3	0/5	0/5	0/10	0/9	0/13	0/20
Pleu	0/19	0/20	0/13	0/16	0/30	0/21	0/4	–	0/53	0/34	0/25	0/36	0/32	0/21	0/11	0/22
Rful	0/7	0/1	0/3	0/3	0/12	0/3	–	–	0/7	0/1	0/3	0/4	0/20	0/8	–	0/1
Shis	0/3	0/5	0/30	0/27	0/6	0/6	0/1	–	0/25	0/20	0/23	0/57	0/64	0/49	0/120	0/146
Chis	–	0/6	0/12	0/10	0/2	0/5	0/15	0/24	0/11	0/20	0/24	0/49	0/5	0/3	0/3	0/6
Dord	0/1	0/2	0/1	–	0/4	0/4	0/3	–	0/9	0/11	0/3	0/9	0/6	0/3	0/3	0/2
Pmer	0/1	0/4	0/22	0/10	–	0/5	0/20	0/29	0/35	0/24	0/36	0/55	0/13	0/10	0/19	0/12
Smex	–	–	–	0/1	–	0/2	0/1	–	–	–	–	–	–	–	–	–
Total	0/36	2/49	9/110	5/87	0/62	1/59	1/48	0/64	2/155	0/121	2/141	0/245	1/173	0/129	0/215	0/217
Web II	Jan 01	Mar 01	Jun 01	Oct 01	Jan 02	Mar 02	Jun 02	Oct 02	Jan 03	Mar 03	Jun 03	Oct 03	Jan 04	Mar 04	Jun 04	Oct 04
Btay	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Nmic	3/11	3/11	5/15	6/19	5/21	3/11	0/4	3/22	1/16	0/8	0/39	1/36	0/17	0/9	0/21	0/36
Oleu	–	–	–	0/1	–	–	–	–	0/1	–	–	0/1	–	0/4	0/1	–
Pleu	0/17	0/17	0/17	0/18	0/24	0/11	0/6	0/2	0/32	0/27	0/43	0/37	0/23	0/18	0/3	0/2
Rful	–	–	–	–	0/4	0/2	–	–	0/3	0/2	0/1	–	0/10	0/2	–	–
Shis	0/2	0/2	0/2	0/8	0/3	0/1	0/1	0/1	0/14	0/17	0/46	0/104	0/68	0/97	0/128	0/110
Chis	0/9	0/5	0/5	0/11	0/1	0/1	0/6	–	0/2	0/4	0/12	0/18	0/1	–	0/3	0/5
Dord	0/2	0/2	0/2	–	–	–	–	–	–	–	–	0/4	0/1	0/2	0/2	0/1
Pmer	0/10	0/10	0/14	0/14	0/1	–	0/16	0/1	0/19	0/32	0/28	0/62	0/8	0/5	0/3	–
Smex	–	–	–	–	–	0/2	–	–	–	–	–	–	–	–	–	–
Total	3/51	3/51	5/55	6/71	5/54	3/28	0/33	3/26	1/87	0/90	0/170	1/264	0/132	0/140	0/161	0/158
Web III	Jan 01	Mar 01	Jun 01	Oct 01	Jan 02	Mar 02	Jun 02	Oct 02	Jan 03	Mar 03	Jun 03	Oct 03	Jan 04	Mar 04	Jun 04	Oct 04
Btay	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Nmic	1/5	3/11	4/32	5/27	1/8	1/4	0/12	3/19	0/8	0/11	0/14	0/18	0/8	0/13	0/20	0/39
Oleu	0/2	0/1	–	0/4	0/7	0/2	–	–	0/9	0/8	0/6	0/8	0/17	0/13	0/13	0/12
Pleu	0/25	0/27	0/28	0/20	0/47	0/34	0/16	0/2	0/86	0/45	0/52	0/45	0/31	0/32	0/18	0/10
Rful	0/8	0/3	–	0/1	0/3	0/3	–	–	0/12	0/6	0/8	0/2	0/18	0/10	0/7	0/3
Shis	0/8	0/9	0/11	0/17	0/5	0/1	0/2	0/1	0/11	0/12	0/87	0/103	0/93	0/92	0/132	0/135
Chis	–	0/9	0/8	0/7	0/2	0/1	0/19	0/13	0/12	0/9	0/20	0/19	0/3	0/1	0/3	0/1
Dord	–	–	–	–	0/2	0/3	0/1	–	–	–	–	0/1	–	–	–	0/1
Pmer	–	0/3	0/6	0/10	0/1	0/4	0/13	0/14	0/25	0/13	0/14	0/25	0/6	–	0/7	0/1
Smex	–	0/1	0/1	0/1	–	0/2	0/3	–	–	0/1	0/1	–	–	–	–	–
Total	1/48	3/64	4/86	5/87	1/75	1/54	0/66	3/49	0/163	0/105	0/206	0/228	0/197	0/174	0/204	0/202

^aTrapping on Webs I and III began in January, 2001; trapping on Web II began in March, 2001. A total of 1049 (26.6%) of the 3941 rodents captured during the 46-month study period were captured in 2 or more trapping sessions.

^bBtay, *Batomys taylori*; Nmic, *Neotoma micropus*; Oleu, *Onychomys leucogaster*; Pleu, *Peromyscus leucopus*; Rful, *Reithrodontomys fulvescens*; Shis, *Sigmodon hispidus*; Chis, *Chaetodipus hispidus*; Dord, *Dipodomys ordii*; Pmer, *Perognathus merriami*; Smex, *Spermophilus mexicanus*.

TABLE 2. ARENAVIRUSES ISOLATED FROM 6 ANTIBODY-NEGATIVE WOODRATS AND 9 ANTIBODY-POSITIVE WOODRATS CAPTURED ON THE CWMA, JANUARY, 2001–JANUARY, 2003^a

Animal identifier	Web	Sample date	Age class	Antibody titer ^b	Arenavirus (strain) isolated from		
					Blood	OPsec ^c	Urine
TK100150 ^d	W-I	Mar 2001	Subadult	0	AV C0410175	–	–
TK100313 ^d	W-I	Jun 2001	Juvenile	0	AV C0690111	–	–
TK100206 ^d	W-II	Mar 2001	Subadult	0	AV C0410194	AV C0410273	–
TK100126 ^d	W-III	Mar 2001	Adult	0	AV C0410166	AV C0410246	–
TK100453 ^d	W-III	Oct 2001	Juvenile	0	AV C1170025	AV C1170026	–
TK100529 ^d	W-III	Oct 2001	Adult	0	–	AV C1170108	–
TK100312	W-I	Jun 2001	Adult	7	–	AV C0690110	–
TK100312	W-I	Oct 2001	Adult	7	–	–	AV C1170179
TK100316 ^d	W-1	Jun 2001	Adult	6	–	–	AV C0690122
TK100380	W-I	Jun 2001	Adult	7	AV C0690203	AV C0690204	–
TK100380	W-I	Jan 2003	Adult	7	–	–	AV E0020168
TK100381 ^d	W-I	Jun 2001	Adult	3	–	AV C0690206	–
TK100385 ^d	W-I	Jun 2001	Subadult	4	–	–	AV C0690218
TK100445	W-I	Oct 2001	Adult	7	–	–	AV C1170006
TK100445	W-I	Jun 2002	Adult	6	–	–	AV D0660002
TK100190	W-II	Mar 2001	Adult	7	AV C0410190	–	–
TK100190	W-II	Jun 2001	Adult	6	–	–	–
TK102276 ^d	W-II	Oct 2002	Adult	7	–	–	AV D1030087
TK102318 ^d	W-III	Oct 2002	Adult	7	–	–	AV D1030150

^aNone of the woodrats captured in March, 2003–October, 2004 were positive for arenavirus.

^b0, < 320; 1 = 320; 2 = 1280; 3 = 5120; 4 = 20,480; 5 = 81,920; 6 = 327,680; 7, ≥ 1,310,720.

^cOropharyngeal secretions.

^dCaptured in only 1 trapping session.

the ketamine HCl and stress of processing, were released at the trap stations at which they were captured the previous night. The animals captured on the third day in the October, 2004, trapping session, animals found moribund, and animals with incapacitating injuries were euthanized by intraperitoneal injection of a lethal dose of a mixture of ketamine hydrochloride and xylazine hydrochloride (0.8 mg and 0.16 mg/10 grams body weight, respectively).

The samples of blood, OPsec, and urine were transported on dry ice to a biosafety level 3 (BSL-3) laboratory on the campus of The University of Texas Medical Branch, Galveston. Skins, skulls, and other voucher materials from the animals that died during processing and animals that were euthanized or found dead were deposited into the Museum of Texas Tech University.

Antibody assay

The blood samples were tested for anti-arenavirus immunoglobulin G (IgG), using an enzyme-linked immunosorbent assay (ELISA) (Bennett et al. 2000). The test antigen was a detergent lysate of Vero E6 cells infected with CTNV strain AV A0400135 (Cajimat et al. 2007), the negative control (comparison) antigen was a detergent lysate of uninfected Vero E6 cells. Serial fourfold dilutions (from 1:80 through 1:5,120) of each blood sample were tested against both antigens. Woodrat, grasshopper mouse, pygmy mouse, white-footed mouse, cotton rat, and harvest mouse IgG bound to antigen were detected by using a mixture of a goat anti-rat IgG peroxidase conjugate and goat anti-*Peromyscus leucopus* IgG peroxidase conjugate in conjunction with the ABTS Microwell Peroxidase Substrate System (Kirkegaard and Perry Laboratories, Gaithersburg, MD). Kangaroo rat, hispid pocket

mouse, Merriam’s pocket mouse, squirrel, and shrew IgG bound to antigen were detected by using Pierce Immuno Pure[®] Protein A/G, Peroxidase Conjugated (Pierce, Rockford, IL) in conjunction with the ABTS Microwell Peroxidase Substrate System. Optical densities (OD) were measured at 410 nm (reference, 490 nm). The adjusted OD (AOD) of a blood–antigen reaction was the OD of the well coated with the test antigen less the OD of the well coated with the negative control antigen. A blood sample was considered positive for anti-CTNV antibody if the AOD at 1:80 was ≥ 0.250, the AOD at 1:320 was ≥ 0.250, and the sum of the AOD for the series of fourfold dilutions (from 1:80 through 1:5,120) was ≥ 0.750 (Fulhorst et al. 2001). End point titers were measured in all samples from the antibody-positive rodents, using serial fourfold dilutions from 1:80 through 1:1,310,720. The antibody titer in an antibody-positive sample was the reciprocal of the highest dilution for which the AOD was ≥ 0.250.

Virus assay

The samples of cardiac blood, OPsec, and urine from the woodrats, and samples of kidney from the woodrats captured on the third day of the October, 2004, trapping session were tested for arenavirus by cultivation in Vero E6 cells (Fulhorst et al. 1996). Briefly, 0.2 mL of a 10% v/v suspension of blood or urine in sterile PBS, 0.2 mL of OPsec stored in 0.3 mL of PBS-FBS, or 0.2 mL of a crude 10% w/v homogenate of kidney in sterile PBS was inoculated onto a confluent monolayer of Vero E6 cells in a 12.5-cm² plastic culture flask. Cell spots were prepared from the monolayer on the 13th or 14th day after inoculation, and arenaviral antigen in the cell spots was detected by using an indirect fluorescent antibody test (IFAT) in

TABLE 3. PREVALENCE OF ARENAVIRUS INFECTION IN 611 SOUTHERN PLAINS WOODRATS AND ANTIBODY TO CATARINA VIRUS IN 3330 OTHER RODENTS CAPTURED AT 3 SITES ON THE CHAPARRAL WILDLIFE MANAGEMENT AREA, JANUARY 4, 2001–OCTOBER 8, 2004^a

Species	Web I	Web II	Web III	Total
<i>Baiomys taylori</i>	0/8	0/11	0/35	0/54
<i>Neotoma micropus</i>	17/183	24/232	16/196	57/611
<i>Onychomys leucogaster</i>	0/40	0/8	0/65	0/113
<i>Peromyscus leucopus</i>	0/212	0/177	0/335	0/724
<i>Reithrodontomys fulvescens</i>	0/57	0/21	0/69	0/147
<i>Sigmodon hispidus</i>	0/460	0/441	0/525	0/1426
<i>Chaetodipus hispidus</i>	0/174	0/65	0/97	0/336
<i>Dipodomys ordii</i>	0/32	0/9	0/5	0/46
<i>Perognathus merriami</i>	0/192	0/180	0/96	0/468
<i>Spermophilus mexicanus</i>	0/4	0/2	0/10	0/16
Total	17/1362	24/1146	16/1433	57/3941

^aTrapping on Webs I and III began in January, 2001; trapping on Web II began in March, 2001.

which the primary antibody was a hyperimmune mouse ascitic fluid raised against WWAV strain AV 9310135.

Genetic characterization of viruses

The nucleotide sequences of a 587-nucleotide fragment of the nucleocapsid (N) protein genes of isolates AV C0410166, AV C0410175, AV C0410194, AV D0660002, AV D1030087, and AV D1030150 (Table 2) were determined from RNA isolated from monolayers of infected Vero E6 cells. Reverse transcription of N protein gene RNA and amplification of first-strand cDNA were done by using the ABgene *Reverse iT™* One-Step RT-PCR Kit (ABgene House, Surrey, UK) in conjunction with oligonucleotides AVNP119 (5'-ACAGCC AATGATTCCACACTCTTC-3') and AVNP121 (5'-GTCAGG TCAAAGATGCATCACTCATGATG-3'). Both strands of each gel-purified PCR product were sequenced directly, using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, CA). The nucleotide sequences

of the 587-nucleotide fragment of the N protein genes of the isolates were deposited into the GenBank nucleotide sequence database under accession nos. JQ063083 through JQ063088.

The analyses of the N protein gene sequences included CTNV strain AV A0400135 (GenBank accession no. DQ865244), CTNV strain AV A0400212 (DQ865245), BCNV strain AV A0070039 (AY924390), WWAV strain AV 9310135 (AF228063), and TAMV strain W 10777 (AF512828). Strains AV A0400135 and AV A0400212 were originally isolated from southern plains woodrats captured on the CWMA in 1999 (Fulhorst et al. 2002a); AV A0070039 from a California mouse (*Peromyscus californicus*) captured in California in 1998 (Fulhorst et al. 2002b); W 10777 from a hispid cotton rat captured in Florida in 1965 (Calisher et al. 1970); and AV 9310135 from a white-throated woodrat (*N. albigula*) captured in New Mexico in 1993 (Fulhorst et al. 1996). The 11-taxa nucleotide sequence alignment was generated by using the computer program CLUSTAL W1.7 (Thompson et al. 1994); the neighbor-joining analysis of genetic (p) distances was done with *MEGA*, version 4.0 (Tamura et al. 2007); and bootstrap support (Felsenstein 1985) for the results of the neighbor-joining analysis was based on 1000 pseudoreplicate datasets generated from the original multiple nucleotide sequence alignment.

Results

A total of 3941 rodents, representing 10 species, were captured in 16 trapping sessions in January 4, 2001–October 8, 2004 (Table 3). A desert shrew was the only other mammal captured on the webs during the 46-month study period.

In all, 220 (5.6%) of the 3941 rodents were found dead in traps ($n=128$), died during processing ($n=91$), or were killed for humane reasons ($n=1$). The dead rodents included 6 northern grasshopper mice, 95 white-footed mice, 23 fulvous harvest mice, 40 hispid cotton rats, 16 southern plains woodrats, 11 hispid pocket mice, 2 Ord's kangaroo rats, and 27 Merriam's pocket mice. The deaths of a majority of these rodents were attributed to hypothermia and/or severe fatigue associated with multiple captures in a single trapping session.

Counting multiple captures of individual rodents in the same trapping session, the 3941 rodents were captured 8559

TABLE 4. SUMMARY OF 3941 RODENTS CAPTURED IN 45,120 TRAP STATION-NIGHTS ON THE CHAPARRAL WILDLIFE MANAGEMENT AREA, JANUARY 1, 2001–OCTOBER 8, 2004

Species	Number captured	Total number of captures ^a	Number captured in ≥ 2 trapping sessions ^b	Number recaptured ≥ 1 yr after initial capture
<i>Baiomys taylori</i>	54	87 (6, 1)	12 (22.2%)	2 (3.7%)
<i>Neotoma micropus</i>	611	950 (7, 1)	123 (20.1%)	47 (7.6%)
<i>Onychomys leucogaster</i>	113	257 (11, 2)	33 (29.2%)	8 (7.1%)
<i>Peromyscus leucopus</i>	724	2081 (18, 2)	261 (36.0%)	55 (7.6%)
<i>Reithrodontomys fulvescens</i>	147	240 (8, 1)	27 (18.4%)	4 (2.7%)
<i>Sigmodon hispidus</i>	1426	3248 (11, 2)	370 (25.9%)	33 (2.3%)
<i>Chaetodipus hispidus</i>	336	713 (9, 2)	75 (22.3%)	28 (8.3%)
<i>Dipodomys ordii</i>	46	114 (8, 2)	19 (41.3%)	11 (23.9%)
<i>Perognathus merriami</i>	468	852 (12, 1)	129 (27.6%)	44 (9.4%)
<i>Spermophilus mexicanus</i>	16	17 (2, 1)	0 (0.0%)	0 (0.0%)
Total	3941	8559	1049 (26.6%)	232 (5.9%)

^aTotal number of captures (maximum number of times an individual rodent was captured, median of the number of times that individual rodents were captured).

^bNumber (%) captured in 2 or more trapping sessions.

^cNumber (%) captured ≥ 12 months after initial capture.

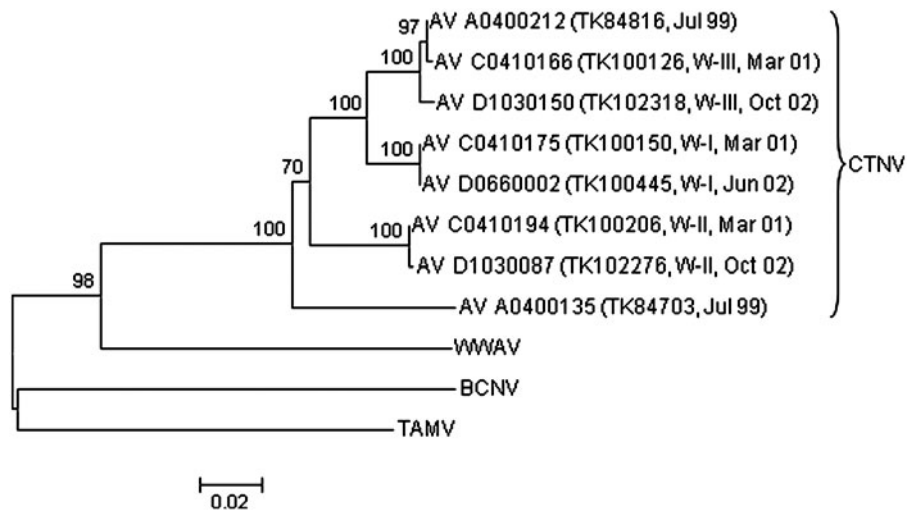


FIG. 2. Phylogenetic relationships among 8 strains of Catarina virus (CTNV) isolated from southern plains woodrats (*Neotoma micropus*) captured on the Chaparral Wildlife Management Area (CWMA), based on a neighbor-joining analysis of nucleocapsid protein gene sequence data. BCNV, Bear Canyon virus strain AV A0070039; TAMV, Tamiami virus strain W 10777; WWAV, Whitewater Arroyo virus strain AV 9310135. Branch lengths are proportional to genetic (p) distances; the numbers at the nodes indicate the percentage of 1000 bootstrap replicates that supported the interior branches; bootstrap support values less than 70% are not listed; and BCNV was the designated outgroup. The branch labels for the CTNV strains include (in the following order) virus strain, woodrat (TK) number, and month and year in which the virus-positive sample was collected.

times in 45,120 trap station-nights, with an overall trap success rate of 19.0%. Individual rodents were captured on as many as 18 nights (median, 2 nights); 1049 rodents each were captured in 2 or more trapping sessions; and 232 rodents each were captured in 2 or more trapping sessions separated by 12 months or longer (Table 4).

Antibody (IgG) reactive with CTNV strain AV A0400135 was found in 73 (11.9%) of 611 woodrats and none of the 3330 other rodents. The antibody titers in the antibody-positive blood samples ranged from 320 to $\geq 1,310,720$. One woodrat seroconverted between first capture (January, 2002) and second capture (January, 2003). The 72 other antibody-positive woodrats were positive for antibody to CTNV at first capture.

Arenavirus was isolated from samples of blood, OPsec, and/or urine from 6 antibody-negative woodrats and 9 antibody-positive woodrats (Table 2). The assays for arenavirus in the kidneys of the 51 woodrats captured on the last day in the October, 2004, trapping session were negative.

The results of the neighbor-joining analysis of genetic (p) distances (Fig. 2) indicated that arenaviruses AV C0410166, AV C0410175, AV C0410194, AV D0660002, AV D1030087, and AV D1030150 are strains of CTNV. Non-identities among the nucleotide sequences of these 6 strains, CTNV strain AV A0400135, and CTNV strain AV A0400212 ranged from 0/587 (AV C0410175 and AV D0660002) to 60/587 (AV A0400135 and AV D1030150).

The antibody titers in the first blood samples from 16 of the 19 antibody-positive, culture-negative juvenile woodrats and 5 of the 7 antibody-positive, culture-negative subadult woodrats were low (320 or 1280) relative to the antibody titers in the first blood samples from 44 of the 46 antibody-positive adult woodrats (Table 5). Hereafter, it was presumed that the anti-arenavirus antibody in the 21 young woodrats with antibody titers ≤ 1280 was maternal rather than a consequence of infection.

Overall, 57 (9.3%) of the 611 woodrats were infected; that is, positive for CTNV and/or positive for CTNV-reactive antibody in response to infection (Table 6). By study site, the prevalence of infection among woodrats ranged from 8.2% (W-III) to 10.3% (W-II) (Table 3).

Fifty-four (21.3%) of the 254 woodrats captured in January, 2001–October, 2002, and 3 (1.2%) of the 339 woodrats captured in January, 2003–October, 2004, were infected (Fig. 3). Only 1 of the 3 infected woodrats captured in January, 2003–October, 2004 (*i.e.*, TK100380) was positive for arenavirus (Table 2).

Forty-eight (89.5%) of the 54 infected woodrats captured in January, 2001–October, 2002, and the 3 infected woodrats

TABLE 5. ANTIBODY TITERS TO CATARINA VIRUS IN THE FIRST BLOOD SAMPLES FROM 73 ANTIBODY-POSITIVE WOODRATS CAPTURED ON THE CWMA, JANUARY, 2001–OCTOBER, 2004, BY AGE CLASS

Age class	Antibody titer ^{a,b}							Total	
	0	1	2	3	4	5	6		7
Juvenile	–	9	7	2	1	–	–	–	19
Subadult	–	2	3	–	1 ^c	1	1	–	8
Adult	1 ^b	–	1	7 ^c	11	13	5 ^c	8 ^c	46
Total	1	11	11	9	13	14	6	8	73

^a0, <320; 1=320; 2=1280; 3=5120; 4=20,480; 5=81,920; 6=327,680; 7, $\geq 1,310,720$.

^bOne adult woodrat seroconverted between first capture (January, 2002) and recapture (January, 2003).

^cArenavirus was isolated from samples collected at first capture from the subadult woodrat with a titer of 20,480; 1 of 7 adult woodrats with titers of 5120; 1 of 5 adult woodrats with titers of 327,680; and 6 of 8 adult woodrats with titers $\geq 1,310,720$.

TABLE 6. PREVALENCE OF CATARINA VIRUS INFECTIONS AMONG SOUTHERN PLAINS WOODRATS CAPTURED ON THE CHAPARRAL WILDLIFE MANAGEMENT AREA, 2001–2004, BY STUDY SITE

	Web I	Web II	Web III	Total
Prevalence of infection among all woodrats captured in				
January, 2001–October 2004	17/183 (9.3%)	24/232 (10.3%)	16/196 (8.2%)	57/611 (9.3%)
March–October, 2001–2004	17/162 (10.5%)	20/202 (9.9%)	14/182 (7.7%)	51/546 (9.3%)
March–October, 2001	15/55 (27.3%)	13/41 (31.7%)	11/65 (16.9%)	39/161 (24.2%)
March–October, 2002	2/25 (8.0%)	6/35 (17.1%)	4/33 (12.1%)	12/93 (12.9%)
March–October, 2003	3/54 (5.6%)	1/74 (1.4%)	0/37 (0.0%)	4/165 (2.4%)
March–October, 2004	0/48 (0.0%)	0/62 (0.0%)	0/64 (0.0%)	0/174 (0.0%)
Prevalence of infection among adult male woodrats captured in				
January 2001–October, 2004	5/49 (10.2%)	10/56 (17.9%)	5/49 (10.2%)	20/154 (13.0%)
March–October, 2001–2004	5/40 (12.5%)	7/41 (17.1%)	5/45 (11.1%)	17/126 (13.5%)
March–October, 2001	4/11 (36.4%)	4/6 (66.7%)	4/16 (25.0%)	12/33 (36.4%)
March–October, 2002	0/7 (0.0%)	3/7 (42.9%)	1/5 (20.0%)	4/19 (21.1%)
March–October, 2003	1/6 (16.7%)	1/16 (6.3%)	0/9 (0.0%)	2/31 (6.5%)
March–October, 2004	0/16 (0.0%)	0/12 (0.0%)	0/15 (0.0%)	0/43 (0.0%)
Prevalence of infection among adult female woodrats captured in				
January, 2001–October, 2004	8/61 (13.1%)	11/75 (14.7%)	9/55 (16.4%)	28/191 (14.7%)
March–October, 2001–2004	8/53 (15.1%)	10/68 (14.7%)	8/52 (15.4%)	26/173 (15.0%)
March–October, 2001	7/15 (46.7%)	6/16 (37.5%)	7/16 (43.8%)	20/47 (42.6%)
March–October, 2002	2/9 (22.2%)	4/10 (40.0%)	2/10 (20.0%)	8/29 (27.6%)
March–October, 2003	2/19 (10.5%)	0/18 (0.0%)	0/11 (0.0%)	2/48 (4.2%)
March–October, 2004	0/20 (0.0%)	0/28 (0.0%)	0/22 (0.0%)	0/70 (0.0%)

captured in January, 2003–October, 2004, were adult and infected at first capture. The prevalence of infection among male woodrats was 26/289 (9.0%), the prevalence of infection among female woodrats was 31/322 (9.6%), and the difference between 26/289 and 31/322 was not statistically significant (Fisher’s exact test, two-tailed, $p > 0.50$).

Counting multiple captures of individual rodents in the same trapping session, infected woodrats were captured 70 times at 55 trap stations in January, 2001–October, 2002; 801 rodents other than woodrats were captured 1606 times at 585 trap stations in January, 2001–October, 2002; and 52 rodents (35 white-footed mice, 1 fulvous harvest mouse, 5 hispid cotton rats, 3 hispid pocket mice, 2 northern grasshopper

mice, 1 Ord’s kangaroo rat, 3 Merriam’s pocket mice, and 2 Mexican ground squirrels) were captured 63 times at 26 of the 55 stations at which infected woodrats were captured in January, 2001–October, 2002. Collectively, these observations suggest that close physical interactions between rodents other than woodrats and infected woodrats in January, 2001–October, 2002, were rare.

A total of 297 woodrats in this study were juvenile or subadult at first capture (Table 7). In all, 123 (99.2%) of the 124 juvenile woodrats were captured in March, June, or October, indicating that breeding in *N. micropus* on the CWMA in 2001–2004 usually began in early spring and ended in fall.

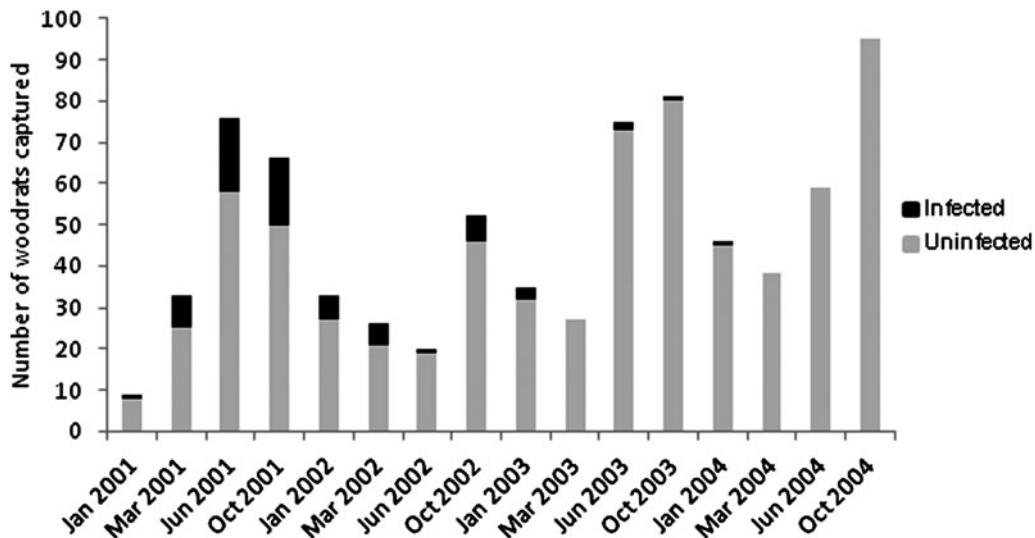


FIG. 3. Prevalence of Catarina virus infections among 611 southern plains woodrats captured on the Chaparral Wildlife Management Area, January, 2001–October, 2004, by trapping session.

TABLE 7. NUMBERS OF JUVENILE WOODRATS AND SUBADULT WOODRATS CAPTURED ON THE CHAPARRAL WILDLIFE MANAGEMENT AREA, 2000–2004, BY MONTH OF TRAPPING SESSION AND YEAR^a

Month	Juvenile woodrats ^b				Subadult woodrats ^b			
	2001	2002	2003	2004	2001	2002	2003	2004
January	–	–	–	0/1	0/3	0/7	0/11	0/7
March	0/14	–	0/4	0/11	2/6	0/4	0/6	0/4
June	1/17	0/1	0/16	0/4	2/19	0/7	0/25	0/16
October	2/12	2/23	0/13	0/8	1/15	0/12	0/16	0/15
Total	3/43	2/24	0/33	0/24	5/43	0/30	0/58	0/42

^aTraps were set on Webs I and III but not Web II in January, 2001.
^bNumber infected/number captured.

By year, the proportion of juvenile woodrats captured in March or June ranged from 1/24 (4.2%) in 2002 to 31/43 (72.1%) in 2001 (Table 7), and the amount of precipitation in January–April ranged from 1.5 cm in 2002 to 14.1 cm in 2003 (Fig. 4). There was a positive association between the number of pups captured in March or June and total precipitation in January–April ($r = 0.81$).

Discussion

In a previous study (Fulhorst et al. 2002a), strains of CTNV were isolated from 5 southern plains woodrats, anti-arenavirus was found in 4 (14.3%) of 28 other southern plains woodrats, and neither arenavirus nor anti-arenavirus antibody was found in 60 hispid cotton rats, 13 white-footed mice, 3 northern grasshopper mice, 1 northern pygmy mouse, 1 fulvous harvest mouse, 3 Ord’s kangaroo rats, 62 Merriam’s pocket mice, or 32 hispid pocket mice captured on the CWMA in a 1-week period in July 1999. Collectively, these findings suggested that the southern plains woodrat is the principal host of CTNV on the CWMA and that CTNV infection is highly specific to *N. micropus*. The infections in 57 (9.3%) of 611 woodrats and none of the 3330 other rodents in this study affirm the notion that CTNV infection is highly specific to *N. micropus*.

The analysis of capture data suggested that close physical interactions between infected woodrats and rodents other than woodrats in January, 2001–October, 2002, were rare. A low frequency of intimate physical interactions with infected woodrats may explain the failure to find evidence of arenavirus infections in rodents other than woodrats in this study. Alternatively, rodents other than woodrats are refractory to CTNV infection or CTNV-infected woodrats are rarely infectious to rodents other than woodrats.

A characteristic of arenaviruses is their ability to establish chronic infections in their respective principal hosts. The isolation of CTNV from samples collected from TK100312 in June, 2001, and October, 2001, TK100380 in June, 2001, and January, 2003, and TK100445 in October, 2001, and June, 2002 (Table 2) indicate that CTNV infections in some southern plains woodrats are chronic. Whether virus shedding in chronically infected woodrats is persistent remains to be determined.

The results of a previous study (Suchecky et al. 2004) suggested that breeding in *N. micropus* on the CWMA begins in early spring and ends some time in fall. As such, chronic infections in individual woodrats likely are critical to long-term maintenance, in particular—overwintering, of CTNV on the CWMA.

The results of a laboratory study (Milazzo and Fulhorst 2012) suggested that the duration of CTNV infection in southern plains woodrats is dependent upon age of woodrat at the onset of infection; for example, 6 (85.7%) of 7 southern plains woodrats inoculated at age 1 day with strain AV A0400135 were viruric through 5 months of age, whereas all 4 adult southern plains woodrats inoculated with the same strain, same dose, and by the same route sterilized their infections within 30 days of inoculation. Hypothetically, the majority of chronic infections in southern plains woodrats on the CWMA result from exposure to CTNV early in life; and, as such, vertical (dam-to-progeny or sire-to-progeny) virus transmission in *N. micropus* plays an important role in the long-term maintenance of CTNV on the CWMA.

The prevalence of infection among woodrats captured in March–October in this study decreased from 39/161 (24.2%) in 2001 to 0/174 (0.0%) in 2004 (Table 6). The majority (67.0%)

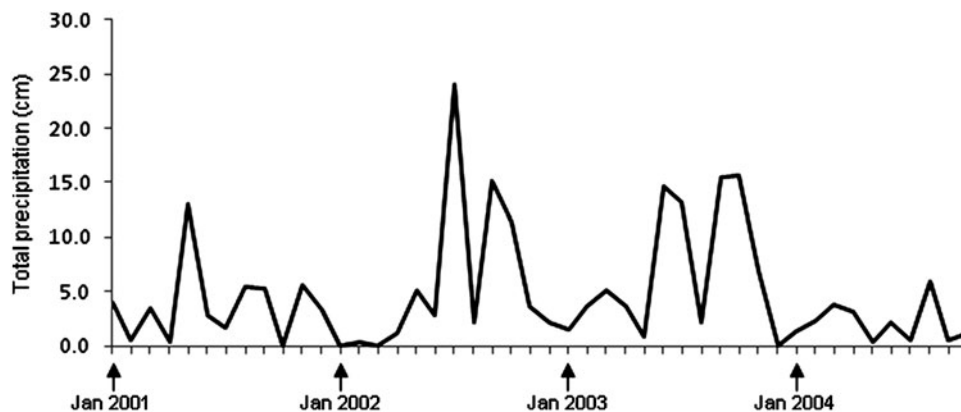


FIG. 4. Precipitation on the Chaparral Wildlife Management Area, January, 2001–October, 2004, by month and year. Precipitation was measured with a Vantage Pro2™ weather station (Davis Instruments Corp., Hayward, CA) located on the Area. Total precipitation in January–April in 2001, 2002, 2003, and 2004 was 8.2 cm, 1.5 cm, 14.1 cm, and 10.6 cm, respectively. The precipitation data were kindly provided by D. Synatzske and D. Walker (Texas Parks & Wildlife Department).

of the 100 juvenile woodrats captured in 2001, 2003, and 2004 were captured in January, March, or June (Table 7). In contrast, only 1 (4.2%) of the 24 juvenile woodrats captured in 2002 was captured before the October trapping session (Table 7). The onset of breeding in *N. micropus* in 2002 may have been delayed by conditions related to the drought in January–April in 2002; alternatively, the majority of pups whelped in spring of 2002 did not survive through early summer. Regardless, a high level of reproductive success in *N. micropus* early in spring may be essential to long-term maintenance of CTNV on the CWMA.

The analysis of N protein gene sequence data in this study revealed a significant level of genetic diversity among CTNV strains isolated from woodrats captured on the CWMA in July, 1999–October, 2002. Previous studies (Méndez-Harclerode et al. 2005, 2007) revealed a high level of genetic diversity among southern plains woodrats captured on the CWMA in January, 2001–October, 2004. Hypothetically, virus strain and host genetics, as well as age of woodrat at onset of infection, affect the duration of CTNV infection in the southern plains woodrat (Childs and Peters 1993).

The results of a previous study (Milazzo et al. 2011) suggested that arenaviruses naturally associated with woodrats (*Neotoma* spp.) are etiological agents of acute central nervous system disease or undifferentiated febrile illnesses in humans in the United States. It is generally accepted that humans usually become infected with arenaviruses by inhalation of virus in aerosolized droplets of saliva, respiratory secretions, urine, or blood from infected rodents. The isolation of CTNV from samples of OPsec or urine from woodrats in this study suggests that secretions and excretions from naturally infected southern plains woodrats may be infectious to humans. *Neotoma micropus* in Texas is principally associated with habitats dominated by cactus or thorny desert shrubs (Braun and Mares 1989). As such, persons who work, live, or enjoy outdoor activities in rural areas in southern Texas may be at risk of CTNV infection.

Acknowledgments

Joel Brant, Nevin D. Durish, Heath Garner, Kara Graham, Mariko Kageyama, Michelle Knapp, Peter Larson, Lisa K. Longhofer, L. Rex McAliley, Lisa Mitchell, Vicki Swier, and Amy Vestal (Texas Tech University); Jason E. Comer, Eleanor Dearthoff, and Sammie Hartmann (The University of Texas Medical Branch, Galveston); Emmanuel Rollin (University of Georgia, Athens, GA); and Stephen G. Bennett (Orange County Vector Control District, Santa Ana, CA) assisted with the capture and processing of rodents. Bal M. Mishra (The University of Texas Medical Branch, Galveston) assisted with the genetic characterization of the CTNV strains.

This study was supported by National Institutes of Health (NIH) grant AI-41435, “Ecology of emerging arenaviruses in the southwestern United States.” James Meegan and Patricia Repik (National Institute of Allergy and Infectious Diseases) facilitated the grant support for this study.

Author Disclosure Statement

No competing financial interests exist.

References

Anderson, DR, Burnham, KP, White, GC, Otis, DL. Density estimation of small-mammal populations using a trapping web and distance sampling methods. *Ecology* 1983; 64:674–680.

- Bennett, SG, Milazzo, ML, Webb, JP Jr, Fulhorst, CF. Arenavirus antibody in rodents indigenous to coastal southern California. *Am J Trop Med Hyg* 2000; 62:626–630.
- Bigler, WJ, Lassing, E, Buff, E, Lewis, AL, et al. Arbovirus surveillance in Florida: Wild vertebrate studies 1965–1974. *J Wildl Dis* 1975; 11:348–356.
- Braun, JK, Mares, MA. *Neotoma micropus*. In: *Mammalian Species*. The American Society of Mammalogists 1989; no. 330:1–9.
- Cajimat, MNB, Milazzo, ML, Bradley, RD, Fulhorst, CF. Catarina virus, an arenaviral species principally associated with *Neotoma micropus* (southern plains woodrat) in Texas. *Am J Trop Med Hyg* 2007; 77:732–726.
- Cajimat, MNB, Milazzo, ML, Haynie, ML, Hanson, JD, et al. Diversity and phylogenetic relationships among the North American Tacaribe serocomplex viruses (family *Arenaviridae*). *Virology* 2011; 421:87–95.
- Calisher, CH, Tzianabos, T, Lord, RD, Coleman, PH. Tamiami virus, a new member of the Tacaribe group. *Am J Trop Med Hyg* 1970; 19:520–526.
- Childs, JE, Peters, CJ. Ecology and epidemiology of arenaviruses and their hosts. In: *The Arenaviridae*. Salvato, MS, ed. New York: Plenum Press, 1993:331–384.
- Felsenstein, J. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 1985; 39:783–791.
- Fulhorst, CF, Bowen, MD, Ksiazek, TG, Rollin, PE, et al. Isolation and characterization of Whitewater Arroyo virus, a novel North American arenavirus. *Virology* 1996; 224:114–120.
- Fulhorst, CF, Charrel, RN, Weaver, SC, Ksiazek, TG, et al. Geographic distribution and genetic diversity of Whitewater Arroyo virus in the southwestern United States. *Emerg Infect Dis* 2001; 7:403–407.
- Fulhorst, CF, Milazzo, ML, Carroll, DS, Charrel, RN, et al. Natural host relationships and genetic diversity of Whitewater Arroyo virus in southern Texas. *Am J Trop Med Hyg* 2002a; 67:114–118.
- Fulhorst, CF, Bennett, SG, Milazzo, ML, Murray, HL, et al. Bear Canyon virus: An arenavirus naturally associated with *Peromyscus californicus* (California mouse). *Emerg Infect Dis* 2002b; 8:717–721.
- Gannon, WL, Sikes, RS, et al. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. *J Mammal* 2007; 83:809–823.
- Jennings, WL, Lewis, AL, Sather, GE, Pierce, LV, et al. Tamiami virus in the Tampa Bay area. *Am J Trop Med Hyg* 1970; 19:527–536.
- McLendon, T. Preliminary description of the vegetation of south Texas exclusive of coastal saline zones. *Texas J Sci* 1991; 43:13–32.
- Méndez-Harclerode, FM, Hanson, JD, Fulhorst, CF, Milazzo, ML, et al. Genetic diversity within the southern plains woodrat (*Neotoma micropus*) in southern Texas. *J Mammal* 2005; 86:180–190.
- Méndez-Harclerode, FM, Strauss, RE, Fulhorst, CF, Milazzo, ML, et al. Molecular evidence for high levels of intrapopulation genetic diversity in woodrats (*Neotoma micropus*). *J Mammal* 2007; 88:360–370.
- Milazzo, ML, Campbell, GL, Fulhorst, CF. Novel arenavirus infection in humans, United States. *Emerg Infect Dis* 2011; 17:1417–1420.
- Milazzo, ML, Fulhorst, CF. Duration of Catarina virus infection in the southern plains woodrat (*Neotoma micropus*). *Vector Borne Zoonotic Dis* 2012; 12:321–324.
- Musser GG, Carleton MD. Family Cricetidae. In: Wilson DE, Reeder DM, editors. *Mammal Species of the World: A Taxonomic*

- and Geographic Reference*, 3rd ed. Baltimore: The Johns Hopkins University Press; 2005:955–1189.
- Ruthven, DC III, Synatzske DR. Response of herbaceous vegetation to summer fire in the western south Texas plains. *Texas J Sci* 2002; 54:195–210.
- Suchecki, JR, Ruthven, DC III, Fulhorst, Bradley RD. Natural history of the southern plains woodrat *Neotoma micropus* (Rodentia: Muridae) from southern Texas. *Texas J Sci* 2004; 56:131–140.
- Tamura K, Dudley J, Nei M, Kumar S. *MEGA4*: molecular evolutionary genetics analysis (MEGA) software, version 4.0. *Mol Biol Evol* 2007; 24:1596–1599.
- Thompson, JD, Higgins, DG, Gibson, TJ. CLUSTAL W (1.7): Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choices. *Nucleic Acids Res* 1994; 22:4673–4680.

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