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Duration of Catarina Virus Infection in the Southern Plains Woodrat (*Neotoma micropus*)

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Abstract

Four adult male, 6 sub-adult, and 7 newborn southern plains woodrats (*Neotoma micropus*) each were inoculated subcutaneously with 3.1 $\log_{10}$ median cell culture infectious doses ($CCID_{50}$) of Catarina virus strain AV A0400135 (virus family *Arenaviridae*). The inoculated animals and the mothers of the newborn animals all became infected and remained asymptomatic. The infections in the adult male woodrats and in the mother woodrats were transient, the infections in 2 (33.3%) of the sub-adult woodrats persisted through month 4 post-inoculation, and 6 (85.7%) of the newborn woodrats were viruric through month 5 post-inoculation. Collectively these findings indicate that the duration of infection in the southern plains woodrat is dependent upon age at exposure to Catarina virus. The results of this study also indicate that chronically infected woodrats persistently shed Catarina virus into the environment.

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

Introduction

The North American members of the virus family *Arenaviridae* include Catarina virus (CTNV), Tamiami virus (TAMV), and Whitewater Arroyo virus (WWAV). Specific rodents are the principal hosts of the arenaviruses, for which natural host relationships have been well characterized. For example, the hispid cotton rat (*Sigmodon hispidus*) is the principal host of TAMV (Calisher et al. 1970; Jennings et al. 1970), and the short-tailed cane mouse (*Zygodontomys brevicauda*) is the principal host of Guanarito virus (GTOV) (Fulhorst et al. 1999). The hallmark of the arenaviruses is their ability to establish chronic infections in their respective principal hosts (Childs and Peters 1993).

Eight arenaviruses naturally cause severe febrile illnesses in humans: lymphocytic choriomeningitis virus (LCMV), Lassa virus (LASV) in western Africa, Lujo virus (LUJV) in southern Africa, GTOV in Venezuela, Junín virus (JUNV) in Argentina, Chaparé virus (CHPV) and Machupo virus (MACV) in Bolivia, and Sabiá virus (SABV) in Brazil (Peters 2002; Delgado et al. 2008; Briese et al. 2009). 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 results of a recently published study (Milazzo et al. 2011) indicated that WWAV or arenaviruses antigenically closely related to WWAV are etiological agents of acute central nervous system disease or undifferentiated febrile illnesses in humans in the United States. The arenaviruses that are antigenically closely related to WWAV include CTNV.

The southern plains woodrat (*Neotoma micropus*) is the putative principal host of CTNV (Fulhorst et al. 2002; Cajimat et al. 2007). The objective of this study was to determine the duration of infection and virus shedding in southern plains woodrats experimentally infected with the CTNV prototype strain AV A0400135 (Cajimat et al. 2007).

Materials and Methods

Safety

All work with live animals was done in an animal biosafety level 3 (ABSL-3) laboratory located on the campus of The University of Texas Medical Branch, Galveston. Personal protective equipment worn while working in this laboratory included a face mask (3M N95 Health Care Particulate Respirator and Surgical Mask, model 1860), disposable gown, disposable booties, and latex rubber gloves.

Virus

The inocula were prepared from a single stock of CTNV strain AV A0400135. This strain was originally isolated from a

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southern plains woodrat captured in 1999 on the Chaparral Wildlife Management Area (CWMA) in southern Texas (Fulhorst et al. 2002). The passage history and titer of the stock virus in Vero E6 cells were Vero E6 + 3 and 5.8 $\log_{10}$ median cell culture infectious doses ($CCID_{50}$)/0.1 mL, respectively.

Experimental animals

The experimental animals included 4 adult male woodrats, 6 sub-adult woodrats (aged 10–11 weeks) from 3 mother woodrats, and 7 newborn pups (aged < 24 h) from 3 other mother woodrats. The adult male woodrats and the mother woodrats were captured on the CWMA in 2001–2002 (Méndez-Harclerode et al. 2005), and the mother woodrats were pregnant when they were captured. Tests on blood samples, throat swabs, and urine samples collected from the adult male and mother woodrats within 2 days of capture were negative for evidence of arenavirus infection. Tests on blood samples and urine samples collected from the sub-adult woodrats prior to inoculation with CTNV were also negative for evidence of arenavirus infection.

Husbandry, inoculation, and sampling of animals

The woodrats were housed in polyester filter-bonneted cages in laminar flow bioisolators. The adult male woodrats and sub-adult woodrats were caged individually, and the woodrats inoculated at birth were separated from their mothers at 35–40 days of age.

The adult male, sub-adult, and newborn woodrats each were inoculated subcutaneously at one site over the scapular region with 0.1 mL of a -2.8 $\log_{10}$ dilution of the stock virus. The inocula were prepared in cold sterile 0.01 M phosphate-buffered saline (PBS), pH 7.40.

Samples of blood, oropharyngeal (OP) secretions, and urine were collected from each inoculated animal at approximately 1-month intervals. The woodrats were anesthetized with methoxyflurane (Metofane®; Mallinckrodt Veterinary, Inc., Mundelein, IL) to facilitate collection of these samples. Blood was collected by puncture of a retro-orbital venous plexus, using a sterile microhematocrit tube or sterile Pasteur pipette. OP secretions were collected on a sterile, cotton-tipped swab, and then stored in 0.3 mL of PBS containing 10% v/v heat-inactivated fetal bovine serum. Urine was collected by mid-stream catch. The adult male animals were killed 3 months post-inoculation (PI), the animals inoculated at age 10–11 weeks were killed 4 months PI, the animals inoculated at birth were killed 5 months PI, and the mothers of the animals inoculated at birth were killed 25–30 days after they were separated from their pups. The inoculated woodrats and mother woodrats were killed by asphyxiation in an atmosphere of CO_2, and samples of cardiac blood, OP secretions, urine, and kidney were collected from each animal at necropsy.

Virus assays

The samples of blood (diluted 1:10 v/v in PBS), OP secretions, urine (diluted 1:10 v/v in PBS), and kidney (prepared as 1:10 w/v suspensions in PBS) were assayed for arenavirus by cultivation in monolayers of Vero E6 cells in 12.5-cm^2 culture flasks (Fulhorst et al. 2001). The inoculum volume was 0.2 mL, cell spots were prepared from the Vero E6 cell monolayers on day 13 PI, and arenavirus antigen in the cell spots was detected using an indirect fluorescent antibody test (IFAT), in which the primary antibody was a hyperimmune mouse ascitic fluid (HMAF) raised against WWAV strain AV 9310135 (Fulhorst et al. 1996).

The titers of arenavirus in the inocula, 8 culture-positive urine samples from 3 woodrats inoculated at age 10–11 weeks, and 15 culture-positive urine samples from 3 woodrats inoculated at birth, were measured in monolayers of Vero E6 cells in 24-well plastic plates (each well was 1.78 cm^2). Serial 10-fold dilutions of each sample were prepared in PBS, the infectivity of each dilution was tested in 4 monolayers, the inoculum volume was 0.05 mL, cell spots were prepared from the monolayers on day 13 PI, and arenavirus antigen in the cell spots was detected using the IFAT described above. The titers of virus in the inocula and urine samples were calculated as described previously (Reed and Muench 1938).

Antibody assay

Blood samples were tested for immunoglobulin G (IgG) against CTNV strain AV A0400135, using an enzyme-linked immunosorbent assay (ELISA) (Bennett et al. 2000). The test antigen was a lysate of Vero E6 cells infected with AV A0400135, the control (comparison) antigen was a lysate of uninfected Vero E6 cells, and serial fourfold dilutions (from 1:80 through 1:5120) of each blood sample were tested against both antigens. Woodrat IgG bound to antigen was detected by using a mixture of a goat anti-rat IgG peroxidase conjugate and goat anti-*Peromyscus leucopus* IgG peroxidase conjugate (Kirkegaard and Perry Laboratories, Gaithersburg, MD), in conjunction with the ABTS Microwell Peroxidase Substrate System (Kirkegaard and Perry Laboratories). Optical densities (OD) were measured at 405 nm (reference, 490 nm); the adjusted OD (AOD) was the OD of the well coated with the test antigen minus the OD of the well coated with the control antigen; a sample was considered positive if the AOD at 1:80 was ≥ 0.250, the AOD at 1:320 was ≥ 0.250, and the sum of the AOD at 1:80 and AOD at 1:320 was ≥ 0.750; and the antibody titer in a positive sample was considered to be the highest dilution for which the AOD was ≥ 0.250.

Results

The titers of CTNV in the inocula ranged from 2.9 $\log_{10}$ to 3.6 $\log_{10}$ $CCID_{50}$/0.1 mL (geometric mean, 3.1 $\log_{10}$ $CCID_{50}$/0.1 mL). The inoculated animals and the mothers of the woodrats inoculated at birth all became infected with CTNV, no overt sign of illness was observed in any of the inoculated woodrats or mother woodrats, and the carcasses of all the woodrats were grossly unremarkable at necropsy.

The results of the tests for arenavirus in the samples of blood, OP secretions, urine, and kidney from the animals inoculated with virus, and the results of the ELISA for anti-CTNV IgG in the samples of blood from these animals are summarized in Table 1. None of the 4 adult male woodrats, 2 (33.3%) of the 6 woodrats inoculated at age 10–11 weeks, and 6 (85.7%) of the 7 woodrats inoculated at birth were positive for arenavirus at necropsy.

The titers of arenavirus in the urine samples from 3 animals inoculated at age 10–11 weeks, and 3 animals inoculated at birth, ranged from 0.8 to 2.3 $\log_{10}$ $CCID_{50}$/0.1 mL, and from 1.1 to 4.3 $\log_{10}$ $CCID_{50}$/0.1 mL, respectively (Fig. 1). The geometric mean of the titers in the samples collected 1 month

Table 1. Results of Assays for Arenavirus and Antibody to Catarina Virus in Samples from 17 Southern Plains Woodrats Inoculated with Catarina Virus, by Age at Inoculation

	Months post-inoculation[a]				
	1	*2*	*3*	*4*	*5*
Adult (male, wild-caught)					
Virus isolated from[b]					
Blood	0/4	0/4	0/4	—	—
Throat swab	0/4	0/4	0/4	—	—
Urine	3/4	1/4	0/4	—	—
Kidney	—	—	0/4	—	—
Culture positive	3/4	1/4	0/4	—	—
Antibody positive	4/4	4/4	4/4	—	—
Antibody titers[c]	2 (2–3)	3	3	—	—
10–11 weeks (captive-borne)					
Virus isolated from[b]					
Blood	2/6	0/6	0/6	0/6	—
Throat swab	3/6	1/6	0/6	0/6	—
Urine	5/6	4/6	2/6	2/6	—
Kidney	—	—	—	2/6	—
Culture positive	5/6	4/6	2/6	2/6	—
Antibody positive	5/6	6/6	6/6	6/6	—
Antibody titers[c]	2 (1–3)	3 (2–3)	3	3	—
1 day (captive-borne)					
Virus isolated from[b]					
Blood	5/7	1/7	0/7	0/7	0/7
Throat swab	4/7	2/7	1/7	0/7	0/7
Urine	6/7	7/7	6/7	6/7	5/7
Kidney	—	—	—	—	6/7
Culture positive	7/7	7/7	6/7	6/7	6/7
Antibody positive	4/7	7/7	7/7	7/7	7/7
Antibody titers[c]	2 (0–3)	3 (2–3)	3	3	3

[a]1 month, 30 days; 2 months, 58–60 days; 3 months, 91–95 days; 4 months, 118–124 days; 5 months, 150 or 152 days.
[b]Number positive/number tested.
[c]Median (range): 0, <320; 1, 320; 2, 1280; 3, ≥5120.

PI from the 3 animals inoculated at age 10–11 weeks was statistically significantly different from the geometric mean of the titers in the samples collected at age 1 month from the 3 animals inoculated at birth ($p < 0.01$ by two-tailed Student's *t*-test). Lastly, the titers of arenavirus in the samples collected at age 1 month from the 3 animals inoculated at birth were significantly greater than the titers of arenavirus in the samples collected from the same animals at age 5 months ($p < 0.05$ by two-tailed Student's *t*-test).

The tests for arenavirus in the samples of blood, urine, and kidney collected from the 4 mother woodrats at necropsy were negative. The titers of anti-CTNV IgG in the blood samples collected from these 4 woodrats at necropsy were ≥5120.

Discussion

The results of this study suggest that the duration of CTNV infection in the naturally infected southern plains woodrat is dependent upon age at onset of infection. Other factors that may significantly affect the duration of infection (and virus shedding) include inoculum dose, route of exposure, virus genetics, and host genetics (Childs and Peters 1993). We note that previous studies revealed a high level of genetic diversity among CTNV strains isolated from southern plains woodrats captured on the CWMA in 1999 (Fulhorst et al. 2002), and a high level of genetic diversity among southern plains woodrats captured on the CWMA in 2001–2002 (Méndez-Harclerode et al. 2005, 2007).

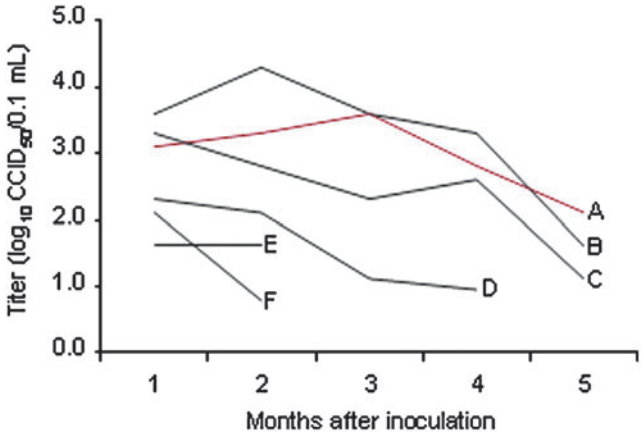

FIG. 1. Titers of arenavirus in the culture-positive urine samples from 3 woodrats inoculated at age 1 day (A, B, and C), and 3 woodrats inoculated at age 10–11 weeks (D, E, and F). These 6 woodrats were from different mothers. The assays for arenavirus in the urine samples collected from woodrats E and F at 3 and 4 months post-inoculation were negative ($CCID_{50}$, cell culture infectious dose). Color images available online at www.liebertonline.com/vbz

Breeding in *N. micropus* on the CWMA occurs primarily in late winter and early spring (Suchecki et al. 2004). As such, chronic infections in southern plains woodrats likely are critical to long-term maintenance of CTNV in *N. micropus* in southern Texas. Collectively, the results of this study suggest that the chronic carrier state usually results from exposure to CTNV early in life.

The house mouse (*Mus musculus*) is the principal host of LCMV (Childs and Peters 1993), the prototypical arenavirus. In the laboratory, female house mice inoculated with LCMV prior to or shortly after mating transmitted their infections to a high proportion of their pups (Skinner and Knight 1974). Hypothetically, transmission of CTNV from an infected male to a female southern plains woodrat during mating can result in infection of a litter, and may thereby initiate the carrier state in subsequent generations of *N. micropus*.

The 7 woodrats inoculated at age 1 day in this study all developed a humoral antibody response to CTNV by day 60 PI, yet the infections in 6 (85.7%) of these woodrats persisted through age 5 months. Together, these results suggest that sterilization of infection in the southern plains woodrats is dependent upon cellular immunity.

The gestation period of the southern plains woodrat is about 33 days (Schmidly 2004). In this study, 3 of the 4 adult woodrats inoculated with CTNV were positive for arenavirus and antibody to CTNV on day 30 PI, suggesting that female woodrats infected during or soon after mating are antibody-positive to CTNV before they whelp their next litters. Whether maternal antibody affects the efficiency of vertical CTNV transmission may depend upon whether this mode of virus transmission occurs pre- or postnatally. In the latter scenario, the efficiency of transmission may be affected by the capacity

of maternal antibody to neutralize the infectivity of CTNV in newborn southern plains woodrats.

Lastly, the results of this study suggest that the titer of CTNV in urine from an infected southern plains woodrat is dependent upon age of infection as well as age at onset of infection. As such, young woodrats infected at birth likely pose a greater risk of infection to humans than older infected woodrats.

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Author Disclosure Statement

No competing financial interests exist.

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