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Tacaribe Virus Causes Fatal Infection of An Ostensible Reservoir Host, the Jamaican Fruit Bat

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Tacaribe virus (TCRV) was first isolated from 11 *Artibeus* species bats captured in Trinidad in the 1950s during a rabies virus surveillance program. Despite significant effort, no evidence of infection of other mammals, mostly rodents, was found, suggesting that no other vertebrates harbored TCRV. For this reason, it was hypothesized that TCRV was naturally hosted by artibeus bats. This is in stark contrast to other arenaviruses with known hosts, all of which are rodents. To examine this hypothesis, we conducted experimental infections of Jamaican fruit bats (*Artibeus jamaicensis*) to determine whether they could be persistently infected without substantial pathology. We subcutaneously or intranasally infected bats with TCRV strain TRVL-11573, the only remaining strain of TCRV, and found that low-dose (10^4 50% tissue culture infective dose [$TCID_{50}$]) inoculations resulted in asymptomatic and apathogenic infection and virus clearance, while high-dose (10^6 $TCID_{50}$) inoculations caused substantial morbidity and mortality as early as 10 days postinfection. Uninoculated cage mates failed to seroconvert, and viral RNA was not detected in their tissues, suggesting that transmission did not occur. Together, these data suggest that *A. jamaicensis* bats may not be a reservoir host for TCRV.

Bats (order Chiroptera) represent approximately one-fifth of the 5,676 recognized species of mammals (4). In recent years, bats of several species have been identified as hosts for viruses of human and veterinary importance, and they are known reservoirs of other important pathogens. Examples of viruses for which bats are reservoirs include rabies virus and related lyssaviruses, henipaviruses, filoviruses, and coronaviruses causing severe acute respiratory syndrome (4, 8, 15, 25, 28). Little is known about virus-host interactions in bats because bats have not been extensively studied in regard to their physiology, including immunology and responses to infections. Bats of at least some species may become persistently infected with viruses without apparent ill effect (4, 7), and because of a general lack of knowledge of bat biology, the mechanism by which this occurs is unknown.

Tacaribe virus (TCRV) is an arenavirus (family *Arenaviridae*, genus *Arenavirus*) having a segmented, ambisense RNA genome (3, 22). Although isolates were obtained from 11 artibeus bats, only one, TRLV-11573, remains, and it has been passaged 24 times in suckling mice and twice in Vero cells. While TCRV is not known to naturally cause human disease, at least one instance of nonfatal laboratory infection with flu-like symptoms has occurred (G. Gard, personal communication). The Tacaribe virus complex (New World) arenaviruses include Junín, Sabiá, Guanarito, Machupo, and Chapare viruses, which cause Argentine hemorrhagic fever (HF), Brazilian HF, Venezuelan HF, Bolivian HF, and an as-yet-unnamed hemorrhagic fever (3, 5, 24).

TCRV was first isolated from Jamaican fruit bats (*Artibeus jamaicensis*) and great fruit-eating bats (*Artibeus lituratus*) in the 1950s near Port-of-Spain, Trinidad, during a rabies virus surveillance program (11, 20). Examination of more than 2,000 mammals, principally small rodents, in Trinidad at that time failed to provide serological evidence of infection with TCRV, leading to the suggestion that artibeus bats were reservoirs of TCRV (11). This is unusual in that all other arenaviruses with known hosts are rodent borne (12, 16). When TCRV was isolated from artibeus fruit bats, it was observed that some appeared healthy while others were mistaken for rabid bats (11). Previous experimental infections of rodents in the 1970s and 1980s found that TCRV was lethal to newborn mice, causing paralysis and necrosis in the central nervous system (1, 5). In adult mice, limited replication was observed, and infection was without signs of disease. Experimental infections of Jamaican fruit bats with TCRV were attempted shortly after the virus was first isolated; however, the bats used in those experiments were wild caught and not prebled to determine their serologic status prior to the experiment. The results from those studies were inconclusive and may have been influenced by previous natural infections (11). TCRV can cause persistent infection of Vero E6 cells; however, it was unknown whether it can cause persistent infections in Jamaican fruit bats (9, 10).

Considering these ambiguous results, the suggestion that artibeus bats might serve as reservoirs of TCRV, and the fact that all other recognized arenaviruses have rodent reservoirs, we reexamined the question with one of the species under controlled laboratory conditions. We conducted experimental infections of Jamaican fruit bats with TCRV to determine pathology, virus tropism, viral shedding, and whether they could become persistently infected.

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MATERIALS AND METHODS

Bats. All procedures were approved by the University of Northern Colorado (UNC) and Colorado State University Institutional Animal Care and Use Committees and were in compliance with the U.S. Animal Welfare Act. The University of Northern Colorado bat colony has neotropical fruit bats of two species, *Carollia perspicillata* and *A. jamaicensis*. They are maintained together in a 36-m^3 room that allows unrestrained free flight by all individuals. The colony has been closed for 16 years and is rabies virus free; PCR screening of rectal swab samples from 20 of these bats has failed to detect coronaviruses (K. Holmes, personal communication), and serum samples for TCRV infections have been negative by neutralization testing. The bats had access to roosting areas in the form of ceiling-hung baskets and various cloth drapes and were maintained under a light cycle of 12 h of light/12 h of darkness. Ambient temperature was maintained between 20°C and 25°C and humidity between 50% and 70% by a computer-controlled heating, ventilating, and air conditioning (HVAC) system. The bats were fed daily at midday with a mixture of grueled apples, monkey chow (Harlan Teklad, Denver, CO), molasses, nonfat dry milk, cherry gelatin with raisins, and fresh bananas. In addition, a variety of other fruits, including mangos, papayas, cantaloupes, bananas, grapes, and watermelons, were provided in food trays and also arrayed on skewers throughout the room to stimulate foraging behavior. For additional enrichment, artificial trees and vines were provided.

During each infection experiment, bats were placed three to a cage in a total of four cages. Water was made available in bowls inside each cage, and individual bats were identified using numbered wing tags.

Experimental infections. We performed two sets of experimental infections over the course of 2 years. Tacaribe virus strain TRVL-11573 was used for these studies. The virus had 24 passages in suckling mice and 2 passages in Vero E6 cells (R. Tesh, personal. communication). The titer of virus stock was 10^7 50% tissue culture infective doses ($TCID_{50}$) per ml of BA1 medium (MEM, 1% bovine serum albumin, 350 mg/liter sodium bicarbonate, 50 ml/liter 1 M Tris [pH 7.6], 5 mg/liter phenol red) containing 5% heat-inactivated fetal bovine serum (FBS).

For the initial experiment, each of 12 male bats were inoculated with 100 μl containing 10^6 $TCID_{50}$ subcutaneously (s.c.) in their abdominal walls, and 25 μl containing 2.5×10^5 $TCID_{50}$ intranasally (i.n.). The bats were monitored for 18 days for signs of illness. As part of our protocol, some bats were euthanized to determine disease progression. Two bats each were euthanized on day 4 postinfection (p.i.) and two others on day 8 p.i. The remaining eight bats were euthanized as they began to show signs of illness. In this experiment, one bat that was not housed with the 12 infected bats was included as a negative control.

The second experiment included three male and nine female Jamaican fruit bats randomly divided into four experimental groups receiving different doses by different routes of virus inoculation. Group 1 consisted of two bats that received 10^6 $TCID_{50}$ i.n. and one bat that received sterile phosphate-buffered saline (PBS) i.n. Group 2 consisted of two bats that received 10^4 $TCID_{50}$ i.n. and one bat that received sterile PBS i.n. Group 3 consisted of two bats that received 10^6 $TCID_{50}$ s.c. in the abdomen and one bat that received sterile PBS s.c. in the abdominal wall. The fourth group consisted of two bats that received 10^4 $TCID_{50}$ s.c. in the abdominal walls and one bat that received sterile PBS s.c. in its abdominal wall. The bats were monitored for development of signs of illness over the course of 45 days. Any moribund bats were immediately euthanized.

In both experiments, intranasal inoculations were done using a micropipettor and a sterile tip to administer the correct dose. Subcutaneous injections were done using a sterile hypodermic syringe and needle. Inoculations were performed in a biosafety cabinet without anesthetizing the bats.

Weights. Bat weights were recorded only on the day of inoculation for the initial experiment and on the day of inoculation and every 2 days thereafter during the second experiment. During the second experiment, weights were recorded to examine the effect of viral infection on feeding behavior and weight gain or loss. The bats were carefully rolled in a piece of cloth and placed on a balance to obtain their weight in grams.

Oral and rectal swab collection. Oral and rectal swabs were collected from each bat, using sterile cotton-tipped swabs, every 2 days to test for virus shedding. The oral and rectal swabs were collected in 500 μl of BA1 medium containing 5% FBS and were then frozen at −80°C until they were processed.

Serum collection. Serum samples were collected from each bat every 2 days. The bats were bled from the front left wing vein using a sterile 22-gauge needle and capillary collection tubes. Approximately 100 μl of blood was collected from each bat each time and placed into 200 μl of BA1 medium containing 5% FBS. Following euthanasia, serum samples were collected by cardiac puncture. The serum samples were used for virus isolation attempts and for neutralizing-antibody tests.

Organ collection. All bats were euthanized by an intracardiac injection of sodium pentobarbital. Necropsies were performed directly following euthanasia. Organ samples were collected for testing using conventional PCR and virus isolation in Vero E6 cells followed by immunofluorescent-antibody tests. Organ samples were frozen at −80°C until they were processed for RNA or fixed in buffered formalin for histopathology. In the first experiment, testes, kidney, heart, lung, liver, spleen, large intestine, small intestine, and brain samples were collected. In the second experiment, organ sampling was the same, except for the collection of the uteri from female bats and salivary glands from all bats.

Oral and rectal swab RNA extraction. Using QIAamp (Qiagen, Valencia, CA) viral RNA minikits according to the manufacturer's instructions, TCRV RNA was extracted from BA1 medium containing 5% FBS that had been used to collect oral and rectal swabs. All RNA samples were frozen at −80°C until they were used for reverse transcription.

Organ sample RNA extraction. RNA was extracted from bat organ samples collected in RNAlater stabilization reagent (Qiagen, Valencia, CA). The organ samples were placed in 1.5-ml screw-cap tubes, and approximately 12 2.3-mm zirconia/silica beads (BioSpec Products Inc., Bartlesville, OK) were added to each tube. A mini-bead-beater (BioSpec Products Inc.) was used to homogenize the organ samples. RNA extraction was done using QiaShredder columns (Qiagen) and RNeasy minikits (Qiagen) according to the manufacturer's instructions. All RNA samples were frozen at −80°C until they were used for reverse transcription.

Reverse transcription and conventional PCR. RNA from oral swabs, rectal swabs, and organ samples was reverse transcribed using qScript cDNA synthesis kits (Quanta Biosciences, Gaithersburg, MD) according to the manufacturer's instructions. PCR was performed using a Promega (Madison, WI) 2× PCR master mix kit and appropriate TCRV primers (forward, 5′-AATTTGCGATCGAGAGCCTA-3′; reverse, 5′-AGCTCAT CCCAAACCATGAG-3′). Amplification was performed using 35 cycles of 94°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 1 min. After 35 cycles, the reaction mixtures were held at 72°C for 10 min and then held at 4°C until they were used. Amplified PCR samples were then resolved and visualized using a 1% agarose gel. The assay has a sensitivity of as few as 5 copies using a quantified plasmid (NanoDrop 1000; Thermo Scientific, Wilmington, DE) template.

Cloning and sequence analysis of PCR products. Several PCR products from organ samples were cloned and sequenced to verify amplification of TCRV sequences. PCR products were cloned into the TOPO-TA (Invitrogen, Carlsbad, CA) cloning vector according to the manufacturer's directions. The clones were then used to transform One Shot chemically competent *Escherichia coli* cells (Invitrogen) that were subsequently plated and grown on LB-ampicillin plates. Bacterial-colony screening was done using PCR to verify plasmid inserts. Colonies with the TCRV insert were then incubated at 37°C overnight in 4 ml of LB broth with 50 μg/ml ampicillin. The plasmids were purified using a QIAprep spin Miniprep kit (Qiagen) according to the manufacturer's directions. Plasmid recovery was confirmed using a 1% agarose gel. Sequencing reactions were performed using T7 and T3 primers with BigDye Terminator (Applied Bio-

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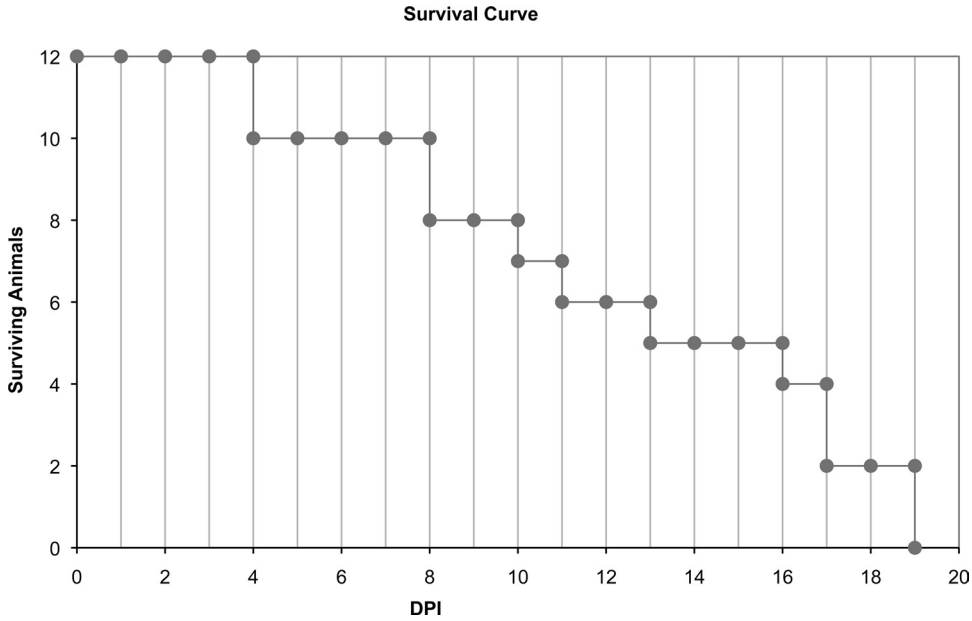

FIG 1 Tacaribe virus is lethal to Jamaican fruit bats. Two bats were euthanized on days 4 and 8 p.i. (DPI) and were asymptomatic. However, on day 10, one bat was found dead, and during the next 8 days, the remaining bats died or were euthanized for humane reasons.

systems, Foster City, CA). Sequencing reactions were done by CSU Macromolecular Services. Sequencing files were edited using Sequencher (GeneCodes, Ann Arbor, MI), and BLAST was used for gene identification.

Virus isolation. Organs that had previously been frozen at −80°C were thawed in 500 μl of BA1 medium containing 5% FBS. Approximately 12 2.3-mm zirconia/silica beads were added to each tube, and tissues were homogenized using a mini-bead-beater. The tubes were centrifuged at 13,000 rpm for 5 min to separate virus-containing supernatant from tissue debris. The supernatants were diluted in 96-well tissue culture plates in a $\log_{10}$ dilution series beginning with neat supernatant and leaving the last row uninoculated as negative controls. The supernatants were added to a corresponding plate containing confluent Vero E6 cells. The cells were incubated with 100 μl of virus dilutions for 24 h, at which point 200 μl of fresh medium was added to replace the old medium. The cells were kept at 37°C and 5% CO_2 for 7 days.

Cells were evaluated for the presence of virus using immunofluorescent-antibody staining. The medium was removed, and the cells were fixed by incubation with cold acetone for 2 h. They were then incubated with mouse anti-TCRV ascites fluid (kindly provided by R. Tesh) diluted 1:200 in PBS for 30 min at 37°C. The cells then were washed with PBS and incubated with a 1:1,600 dilution of DyLight goat anti-mouse IgG conjugated to fluorescein isothiocyanate (Jackson ImmunoResearch, West Grove, PA) and incubated for 30 min at 37°C. The cells were again washed, stored in PBS, and protected from light until they were evaluated with a fluorescence microscope. The same protocol for virus isolation was used on terminal serum samples from each experiment. Wells were considered positive for virus isolation if a large proportion of Vero E6 cells were fluorescing.

Neutralizing antibody. Terminal serum samples from each bat were screened for anti-TCRV neutralizing antibody and by immunofluorescence. Fifty microliters of each serum sample was diluted with 75 μl of BA1 cell culture medium and heat inactivated in a water bath for 10 min. The serum samples were further diluted 1:2 with BA1 cell culture medium and placed in the first well of a 96-well tissue culture plate. A $\log_2$ dilution series was prepared for each sample. A dilution series with mouse anti-TCRV ascites fluid in BA1 cell culture medium was done without the addition of serum to serve as an anti-TCRV positive control. A negative-control sample was included by using a dilution series with TCRV in BA1 cell culture medium without the addition of serum. Fifty microliters containing 10 $TCID_{50}$ TCRV was added (1:2 dilution). The plates were incubated at 37°C for 60 min, and 50 μl containing 4×10^5 Vero E6 cells was then added to each well. The plates were incubated at 37°C and 5% CO_2 for 7 days, and the cells were then screened for the presence of TCRV using immunofluorescence, as described above for organ sample virus isolations.

Histopathology. Formalin-fixed specimens were prepared and sectioned by Colorado Histprep, Inc. (Fort Collins, CO), for evaluation by one of the authors, a veterinary pathologist (D.G.).

RESULTS

Experimental infections. In the first experiment, 12 bats were inoculated with 10^6 $TCID_{50}$ (100 μl s.c.) and 2.5×10^5 $TCID_{50}$ (25 μl i.n.) to determine susceptibility to TCRV. For the initial experiment, we had planned to euthanize two bats each on days 4, 8, 12, 16, and 28 p.i. Two bats were euthanized on day 4 and day 8 p.i., but on day 10, some bats began to show signs of illness and others exhibited tremors and were unable to fly. One bat was found dead on day 10 p.i., and another was moribund on day 11 p.i. and was euthanized (Fig. 1); one bat was found dead on day 13 p.i. and another on day 16 p.i.; Two other bats became moribund on day 17 p.i. and were euthanized. On day 18 p.i., a bat was found dead, and the last bat was moribund and was euthanized. Signs in moribund bats included poor responses to mechanical stimuli; wing, ear, and head tremors; incoordination; and inability to fly when released.

In the second experiment, two bats each were inoculated with a high dose i.n. (10^6 $TCID_{50}$), a low dose i.n. (10^4 $TCID_{50}$), a high dose s.c. (10^6 $TCID_{50}$), or a low dose s.c. (10^4 $TCID_{50}$) and were euthanized only if they showed signs of illness. As sentinels for the determination of aerosol or other routes of transmission, we included in each of these four cages one uninfected bat. On days 21 and 25 p.i., bats that had received a 10^6 $TCID_{50}$ i.n. inoculation

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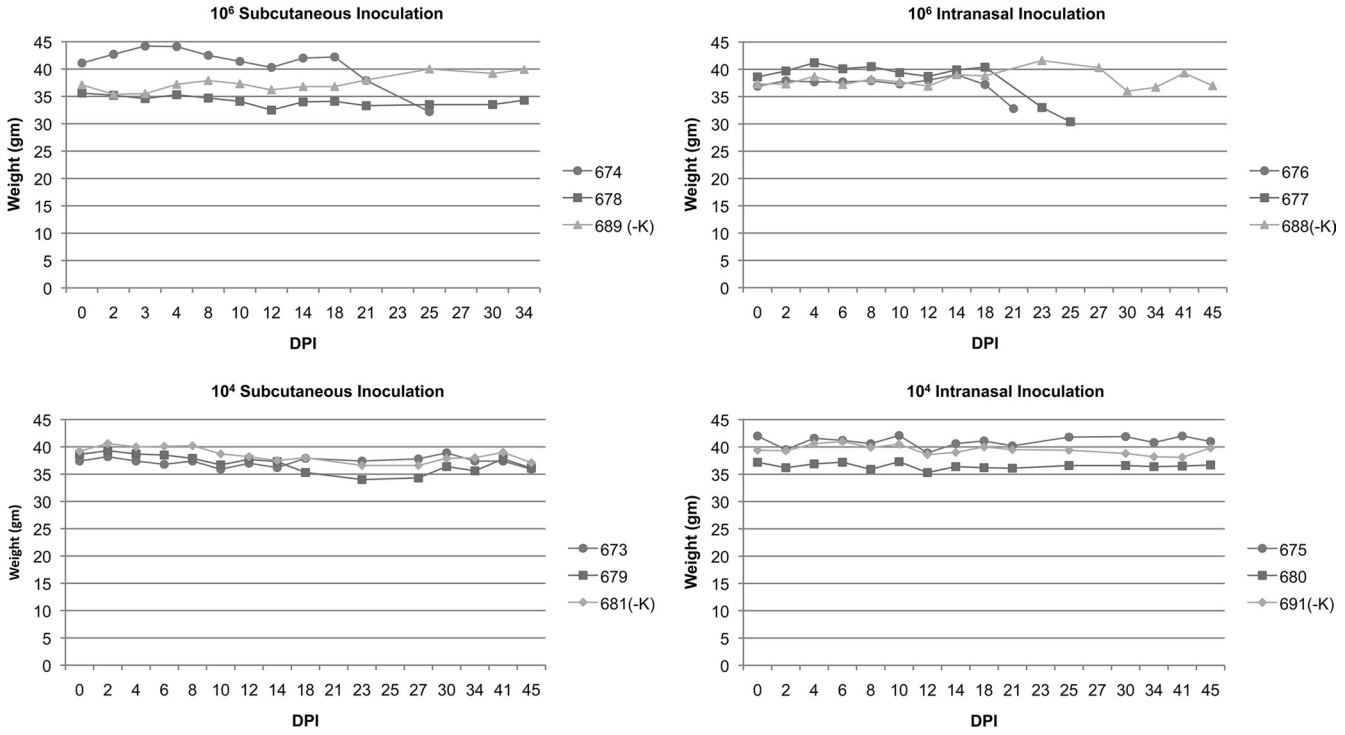

FIG 2 Weight loss with lethal Tacaribe virus infection. Weight loss was recorded for the 10^6 s.c., 10^6 i.n., 10^4 s.c., and 10^4 i.n. groups. Bats that survived had minimal weight fluctuations during the 45-day experiment. However, bats that became moribund (674, 676, and 677) each had weight decline prior to euthanasia.

became moribund and were euthanized, and on day 23 p.i., a bat that had received a 10^6 $TCID_{50}$ s.c. inoculation became moribund and was euthanized. The remaining nine bats did not exhibit signs of illness during the 45-day experiment. The three bats that became moribund experienced the same signs described for the first experiment.

Bat weights. In the second experiment, bat weights were recorded every 2 days, as well as on days when bats became moribund or were found dead. Bat weights fluctuated minimally throughout the experiment, with a notable decrease in weight as bats became increasingly ill (Fig. 2).

Oral swab PCR. In the first experiment, oral swabs collected on days 0, 2, and 4 p.i. were negative for TCRV RNA by PCR (Table 1). On day 6 p.i., oral swabs from several bats had detectable TCRV RNA and remained PCR positive for the duration of the experiment.

In the second experiment, TCRV RNA was undetectable by PCR in oral swabs collected on day 0 (Table 2). On day 2 p.i., the oral swab collected from one bat had detectable TCRV RNA. None of the day 4 p.i. oral swabs had detectable TCRV RNA, but by day 6 p.i., oral swabs from five of the bats had detectable TCRV RNA. Most of the oral swabs had TCRV RNA for the duration of the experiment.

Rectal swab PCR. In the first experiment, rectal swabs collected on days 0, 2, and 4 p.i. were negative for viral RNA by PCR. By day 6 p.i., and continuing through the entire experiment, rectal swabs from the remaining eight bats had detectable TCRV RNA (Table 1).

In the second experiment, rectal swabs collected on days 0, 2, 4, and 6 p.i. were negative for TCRV RNA. From day 8 p.i. through day 45 p.i., rectal swabs for all bats were positive for TCRV RNA by PCR on several collection dates (Table 2).

Organ sample PCR. Organ samples collected during the first experiment showed that 11 of the 12 bats were positive for TCRV RNA by PCR in several organ samples. One bat had no detectable TCRV RNA in any organ sample, and two other bats were positive in one organ each. Multiple organs of the remaining nine bats were PCR positive (Table 3).

Organ samples collected during the second experiment showed that only the three bats that died had detectable TCRV RNA in various organ samples. One bat was positive in the brain only, while the other two bats were positive in multiple organ samples (Table 4).

Virus isolation. Organ homogenates from the bats that died or were euthanized in the first experiment were tested for virus in Vero E6 cells using an immunofluorescence assay (IFA) (Table 3) and were congruent with the PCR results. Virus isolation from the second experiment also supported the findings from the organ PCR results, with only two bats (674 and 677) positive for virus in organs (Table 4). Virus was not isolated from terminal serum samples collected in either of the infection experiments.

Neutralizing antibody. Neutralizing-antibody tests on terminal serum samples from the first experiment, in which bats died or were euthanized between 10 and 18 days p.i., were negative ($<10^2$). Three bats (673, 678, and 679) from the second experiment had neutralizing-antibody titers of 10. These three bats were in groups of bats inoculated subcutaneously and were negative for virus by PCR (Table 4).

Histopathology. Eleven bats from the first experiment had neutrophilic interstitial pneumonia that ranged from mild to moderate in severity and with multifocal to diffuse distribution, while bat 738 exhibited no remarkable lesions. Six of the bats (bats 718, 747, 714, 720, 729, and 738) exhibited myocardial lesions

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TABLE 1 Oral and rectal swab PCR results from the first experiment

Bat	Day p.i. euthanized/ died	Swab	Results on day p.i.[a]: 0	2	4	6	8	10	12	16
645	4	Oral								
		Rectal								
647	4	Oral								
		Rectal								
747	8	Oral				×	×			
		Rectal								
705	8	Oral								
		Rectal				×	×			
714	11	Oral								
		Rectal						×		
703	12	Oral								
		Rectal					×			
718	13	Oral				×			×	
		Rectal						×	×	
748	16	Oral							×	
		Rectal						×	×	
712	17	Oral								
		Rectal						×		
720	17	Oral								×
		Rectal				×		×	×	×
729	18	Oral								×
		Rectal				×			×	
738	18	Oral						×		×
		Rectal						×		

[a] × indicates a sample that had detectable TCRV RNA; for all other samples, no viral RNA was detected.

without inflammation, fibrosis, or degenerate myofiber loss. Three bats (747, 714, and 748) exhibited mild to moderate multifocal adrenocortical necrosis and mild to moderate multifocal hepatocellular necrosis. Vacuolar hepatocellular degeneration was observed in five bats (645, 647, 705, 747, and 712). Most bats exhibited mild to moderate neutrophilic enteritis with crypt necrosis (necrosis of intestinal crypt cells) and epithelial degeneration.

Spleens from bats were grouped in two categories based upon histopathology: acute neutrophilic splenitis and white pulp hyperplasia/plasmacytic and histiocytic splenitis. Neutrophilic infiltration of the white pulp with fibrin tagging of the capsule occurred in six bats (645, 647, 705, 747, 714, and 712). This may have been related to changes in the peritoneum; however, no conspicuous peritonitis was observed. The white pulp changes appear to be the result of proliferating lymphocytes instead of inflammation, as plasma cells often were abundant.

Two bats (712 and 729) had lymphocytic leptomeningitis and mild to moderate multifocal gliosis in the brainstem and prosencephalon. Bat 712 also had mild neutrophilic encephalitis of the prosencephalon (Fig. 3). Both of these bats, as well as bat 738, were PCR positive for TCRV RNA and exhibited tremors prior to euthanasia. Bat 714, which had died on day 11, also was PCR positive, but no tremors had been observed the previous day.

No remarkable lesions were observed in the kidney, ureter, bladder, testicle, thymus, esophagus, salivary gland, pancreas, or stomach.

DISCUSSION

Based on previous research, TCRV is the one known arenavirus thought to be hosted by bats and not rodents. On the other hand, the results of our experiments suggest that Jamaican fruit bats may not be reservoirs of TCRV.

Conducting experimental infections with bat models is challenging because of the unique attributes of bats. To establish breeding colonies, it is usually necessary to have free-flight enclosures; appropriate foods; enrichment, including stimulation of foraging behavior; and appropriate climate control, all to facilitate the highly social characteristics of the colony. In addition, reproductive female bats typically produce one to three offspring per year and invest heavily in maternal care (19). For artibeus bats, reproductive females typically produce two pups per year in the wild, which is also what females in the UNC colony produce. This presents difficulty in conducting experiments with large sample sizes and thus can be a limiting factor in research with bats. Despite these limitations, we succeeded not only in maintaining the colony, but in infecting Jamaican fruit bats with TCRV.

Different doses and routes of TCRV inoculation were used in these experiments. Initial experiments were done with coinoculation of high doses by two routes (i.n. and s.c.) to determine whether Jamaican fruit bats could be infected with TCRV strain TRVL-11573. This virus was isolated by Downs et al. in 1956 from the brain of a great fruit-eating bat (11).

We found that s.c. and i.n. inoculation resulted in infection in Jamaican fruit bats and, to our surprise, coincided with signs of illness, death, and organ pathology. With the exception of the first four bats, which were euthanized as previously scheduled (two each on days 4 and 8), all remaining bats either died or became moribund and were euthanized. None of the bats survived past day 18 p.i.

In the second experiment, dose and route were partitioned between four groups (10^4 $TCID_{50}$ s.c. or i.n. or 10^6 $TCID_{50}$ s.c. or i.n.). Three of the eight inoculated bats became moribund and were from high-dose groups.

The results of testing oral and rectal swabs by PCR for TCRV RNA suggest that some infected bats excreted virus, which typically occurs in rodent reservoirs of other arenaviruses (13). In the first experiment, not all bats shed virus. However, the two bats with negative oral or rectal swabs were euthanized on day 4 p.i., and shedding may occur later in the course of infection. The remaining 10 bats had positive oral or rectal swabs at some point during the experiment (Table 1). Many bats appeared to be intermittently shedding virus (detected as viral RNA) either orally or rectally during the experiment. It is likely they were shedding virus or viral RNA throughout the study but not at levels sufficient for detection by PCR. This pattern was observed with many of the oral and rectal swabs collected from each of the bats in both experiments (Tables 1 and 2). During the first experiment, all bats appeared to be shedding TCRV RNA by day 10 p.i. A similar pattern was observed in the second experiment, as all bats appeared to be shedding virus either orally or rectally by day 18 p.i. While three

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TABLE 2 Oral and rectal swab PCR results from the second experiment

Bat	Dose ($TCID_{50}$) and route	Day p.i.	Swab	Results on day p.i.[a]: 0	2	4	6	8	10	12	14	18	23	27	30	34	41	45
691	UI[b] 10^4 i.n.	45	Oral									×	×		×	×		
			Rectal						×					×		×		
680	10^4 i.n.	45	Oral				×					×	×		×	×		
			Rectal					×										
675	10^4 i.n.	45	Oral				×	×							×	×		
			Rectal											×			×	×
688	UI 10^6 i.n.	45	Oral				×					×		×		×		
			Rectal									×		×		×		
677	10^6 i.n.	25	Oral						×	×		×						
			Rectal							×	×	×	×					
676	10^6 i.n.	21	Oral							×		×						
			Rectal							×								
681	UI 10^4 s.c.	45	Oral									×				×		×
			Rectal											×		×		
679	10^4 s.c.	45	Oral					×				×			×	×		×
			Rectal					×				×		×	×	×		×
673	10^4 s.c.	45	Oral				×					×			×	×	×	
			Rectal					×	×			×				×		×
689	UI 10^6 s.c.	45	Oral					×						×		×		
			Rectal						×			×		×			×	
678	10^6 s.c.	45	Oral				×					×		×	×	×		
			Rectal							×		×	×	×	×	×	×	
674	10^6 s.c.	23	Oral		×				×			×						
			Rectal					×	×			×	×					

[a] × indicates a sample that had detectable TCRV RNA; for all other samples, no viral RNA was detected.
[b] UI, uninfected.

bats became moribund and were euthanized during the second experiment, the other nine remained healthy but appeared to still be shedding viral RNA, which we assume is a surrogate for infectious virus, on day 45 p.i. without signs of illness. However, it is unknown whether infectious virus was still present in the intestines of the surviving bats despite attempts to isolate virus on Vero E6 cells.

Organ sample PCR results from the first experiment showed

TABLE 3 Organ sample PCR and virus isolation results from the first experiment

Parameter	Value											
Bat	645	647	747	705	714	703	718	748	712	720	729	738
Day p.i. euthanized/died	4	4	8	8	11	12	13	16	17	17	18	18
Organ[a]												
Testes					×		×	×		×	×	
Kidney			×	×	×		×	×		×	×	
Heart					×		×				×	
Lung			×	×	○	×	○	×		×	×	
Liver			×	×	×		○	×		×	×	
Spleen	×		○	○	×	×	×	×		×	×	×
Large intestine			×	×	×	×	○	○		×	×	×
Small intestine				×	×		×	○		○	○	×
Brain					×				×		○	×

[a] × indicates the sample had detectable TCRV RNA; ○ indicates that viral RNA was detected and virus was isolated on Vero E6 cells; blank indicates that no viral RNA was detected.

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TABLE 4 Organ sample PCR, virus isolation, and neutralizing-antibody test results from the second experiment

Parameter	Value											
Bat	691	680	657	688	677	676	681	679	673	689	678	674
Dose ($TCID_{50}$) and route	UI[b]	10^4 i.n.	10^4 i.n.	UI 10^6 i.n.	10^6 i.n.	10^6 i.n.	UI 10^4 s.c.	10^4 s.c.	10^4 s.c.	UI 10^6 s.c.	10^6 s.c.	10^6 s.c.
Day p.i. euthanized/died	45	45	45	45	25	21	45	45	45	45	45	23
Organ[a]												
Reproductive												
Kidney					×							
Heart												
Lung					×							×
Liver												
Spleen					○							×
Large intestine					×							
Small intestine					○							×
Brain					×	×						○
Salivary												
NAb titer[c]	<5	<5	<5	<5	<5	<5	<5	10	10	<5	10	<5

[a] × indicates the sample had detectable TCRV RNA; ○ indicates that viral RNA was detected and virus was isolated on Vero E6 cells; blank indicates that no viral RNA was detected.
[b] UI, uninfected.
[c] NAb, neutralizing antibody.

that all but one bat had TCRV RNA in various organs. The one negative bat was euthanized on day 4, likely before virus could be detected in the organs. There appeared to be no discernible pattern of virus distribution in the organs, except that it was first detected in the spleen and last detected in the heart and brain (Tables 3 and 4). It is possible that once virus reaches the brain death ensues rapidly; however, not all bats that became moribund or died in the first experiment had detectable virus in the brain. In the second experiment, only three bats (which died) had TCRV in the organs, and there was no pattern of virus progression through the organs over time. For each experiment, virus isolation results were congruent with PCR results (Tables 3 and 4).

Neutralizing antibodies were detected in the terminal serum samples of three bats from the second experiment. These results, along with lack of detection of virus, suggest that the bats cleared the virus.

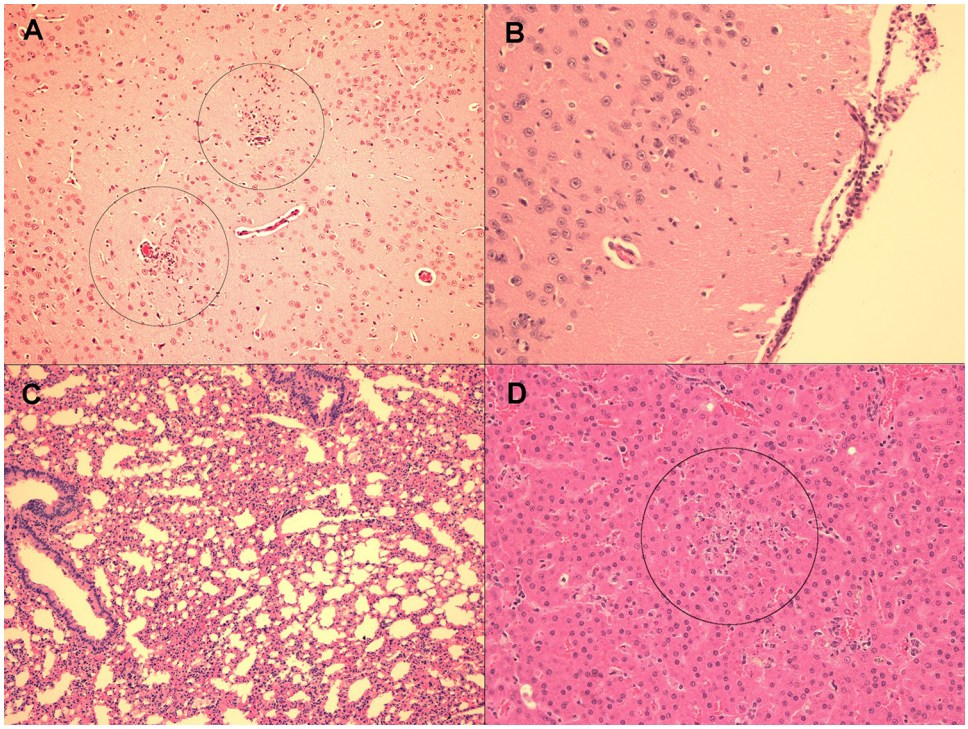

FIG 3 Histologic studies of tissues from TCRV-infected bats in the first experiment. (A) Bat 712 with encephalitis and multifocal gliosis. (B) Bat 712 with lymphocytic meningitis. (C) Bat 712 with interstitial pneumonia. (D) Bat 718 with focal hepatic necrosis.

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Virus transmission was assessed during the second experiment by including one uninfected bat in each experimental group. All uninoculated bats were negative for virus by PCR of collected organ samples; however, all of these bats had detectable viral RNA in oral or rectal samples by PCR at some point in the experiment. None of the bats seroconverted, and none had virus in organs, as determined by PCR or virus isolation, making it likely that the PCR results represent contaminating virus from the infected bats, since these highly social bats huddle close to one another. This experiment provided no support for bat-to-bat transmission by biological means.

Histopathologic findings from the first experiment revealed multiple-organ involvement in TCRV disease. Pneumonia was noted in all but one bat, and the livers and spleens had pathological changes, as well. Three bats had brain lesions, and each exhibited tremors and had TCRV RNA in the brain, while a fourth brain, from a bat that died on day 11, had TCRV RNA, and also exhibited tremors, had no remarkable brain lesions. Tremors are a feature of some cases of human South American hemorrhagic fevers (14), suggesting the possibility that bats may serve as potential models for these diseases.

These experimental infections show that Jamaican fruit bats are susceptible to TCRV and that a high dose results in significant and fatal disease. Low-dose infections resulted in no disease, seroconversion in some, and no detection of virus in organs. We were unable to demonstrate contact transmission of virus between bats despite the close proximity of infected and uninfected bats for 45 days. Together, these data do not support the hypothesis that Jamaican fruit bats are reservoirs of Tacaribe virus.

The distributional range of Jamaican fruit bats is throughout tropical Central and South America; the West Indies, including the Greater and Lesser Antilles; Trinidad and Tobago; the Yucatan peninsula of Mexico; and the Florida Keys (19). Jamaican fruit bats are capable of flying long distances to forage (17–19, 21), and genetic studies indicate bidirectional movement of Jamaican fruit bats between the Antilles and mainland Mexico and South American countries (6, 21). In Mexico, Jamaican fruit bats commonly fly between Cozumel Island and the Yucatan Peninsula, a distance of 20 km (J. Ortega-Reyes, personal communication). Trinidad, where TCRV TRVL-11573 was isolated, is 10 km from Venezuela and easily within the flight range of artibeus bats (21), although there are no reports of TCRV circulation in Venezuela. Antibodies to TCRV were also detected in later studies of Trinidadian bats in the 1970s (20), including *A. jamaicensis*, *A. lituratus*, the little yellow-shouldered bat (*Sturnira lilium*), and Heller's broad-nosed bat (*Platyrrhinus* [*Vampyrops*] *helleri*), and in the vampire bat (*Desmodus rotundus*). One great fruit-eating bat captured in Guatemala in the 1990s had neutralizing antibodies to TCRV (26). It is possible that the artibeus bats from which TCRV was first isolated had been infected with the virus in Venezuela or elsewhere and subsequently introduced the virus to Trinidad before experiencing signs of illness. This could account for the lack of serological evidence in other mammals surveyed in Trinidad, as reported by Downs et al. (11).

With the exception of strain TRVL-11573, the 19 isolates from 11 bats reported by Downs et al. (from 6 Jamaican fruit bats and from 5 great fruit-eating bats) have been lost, although they were shown to be indistinguishable by complement fixation testing (11). It is also possible that passage of TCRV TRVL-11573 in newborn mice (24 passages) and Vero cell cultures (2 passages) has led to the selection of mutations that have altered the genotypic characteristics of the virus, so that wild-type viruses may indeed be hosted by artibeus bats but with different infectious outcomes. Perhaps the routes of infection we used (s.c. or i.n. with uninfected cage mates) do not mirror natural transmission, such as the transovarial transmission observed in some instances of Machupo virus in its reservoir host, the large vesper mouse (*Calomys callosus*), or neonatal or juvenile transmission in experimental infections of short-tailed cane mice (*Zygodontomys brevicauda*) with Guanarito virus (13, 27); other routes of infection may alter the infectious outcome. It is also possible that some small proportion of bats may be "superspreaders" (23), but because of the small sample sizes we used, they were not detected in our studies. Given the unavailability of the other 18 original isolates, it is impossible to determine whether strain TRVL-11573 is representative of Trinidadian TCRV isolates. Additional field work will be required to reisolate TCRV from Trinidadian bats or other mammals. Finally, it is important to note that strain TRVL-11573 was isolated from a great fruit-eating bat, and thus, its phenotypic characteristics may differ in Jamaican fruit bats.

With arenaviruses, such as Guanarito virus, infection of its natural reservoir, the short-tailed cane mouse (13), and hantaviruses in their rodent hosts (2), persistent infection occurs without pathology, which is contrary to our results with TCRV and Jamaican fruit bats; thus, the work presented here does not support the hypothesis that these bats are natural reservoirs. It is possible that other bat species, including other members of the genus *Artibeus*, may be reservoirs. Considering the hosts of other arenaviruses, it is also possible that TCRV is hosted by a rodent species. Several are indigenous to Trinidad, including the reservoir of Guanarito virus (the short-tailed cane mouse), and some of these species are arboreal, which may not have been sampled in the early work on TCRV. Additional field work in Trinidad will be required to identify possible hosts and to reisolate TCRV.

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