A Common-Source Outbreak of Callitrichid Hepatitis in Captive Tamarins and Marmosets

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Callitrichid hepatitis (CH) is a highly fatal, emerging arenavirus disease of captive South American marmosets and tamarins (Callitrichidae), including the endangered golden lion tamarin. A common-source outbreak of CH in golden lion tamarins and pygmy marmosets at a US zoo resulted from a single feeding of the primates with newborn mice inapparently infected with lymphocytic choriomeningitis virus (LCMV). Isolates from livers of mice and primates were related to isolates from previous CH outbreaks and to laboratory strains of LCMV by serology and nucleic acid hybridization, and 2 surviving animals developed antibody to other $LCMV_{CH}$ isolates and to laboratory strains of LCMV. Thus, LCMV, an arenavirus prevalent in wild mice in the US, can cause sporadic fatal hepatic disease in primates. Exposure of humans to wild or laboratory mice or to marmosets and tamarins that are infected with wild-type strains of LCMV poses the danger of serious disease.

In 1981 and 1982, outbreaks of acute fatal hepatitis of unknown etiology were reported in several species of marmosets and tamarins (Callitrichidae) in zoos in the United States [1] and Great Britain [2]. A 9-year retrospective pathologic study showed that 57 marmosets and tamarins died in 12 outbreaks at 10 North American zoos, with a high incidence in the endangered golden lion tamarin (*Leontopithecus rosalia*) [3]. Because of the threatened status of this primate and the zoonotic potential of viral diseases of nonhuman primates, we explored the etiology and epidemiology of this emerging virus disease. We showed that callitrichid hepatitis (CH) was caused by a virus [4] and that this virus, originally designated callitrichid hepatitis virus, was an arenavirus, lymphocytic choriomeningitis virus (LCMV) [5]. Because LCMV is enzootic in *Mus musculus* worldwide [6], we suspected that mice were a source of the virus initiating CH outbreaks [5]. The modes of introduction and transmission of LCMV in zoos and animal parks, however, had not been determined in any of the previously reported outbreaks.

This paper reports a new, highly fatal common-source outbreak of CH that occurred in golden lion tamarins and pygmy marmosets (*Callithrix pygmaea*) at a US zoo.

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Materials and Methods

Cells, viruses, and antibodies. Vero E6 cells were used for virus isolation, immunofluorescence, and virus growth (American Type Culture Collection, Rockville, MD). Fetal human intestine (FHI) cell line 349 (obtained from M. Vincent, Uniformed Services University of the Health Sciences), also was used for virus isolation. Viruses used were an isolate from a CH outbreak at the Oklahoma City Zoo, $LCMV_{CH}$(OKCZ1), and the Armstrong strain of LCMV ($LCMV_{ARM}$; ATCC 134-VR). Anti-LCMV polyclonal guinea pig serum and monoclonal antibodies were provided by M. Buchmeier (Scripps Research Institute, La Jolla, CA). Anti-$LCMV_{CH}$ serum was obtained from a tamarin at the Oklahoma City Zoo that was exposed to $LCMV_{CH}$(OKCZ1).

Monkey colony history. The epizootic of CH occurred at the Fort Worth Zoo in a colony of 7 golden lion tamarins housed in two separate exhibit buildings on the zoo grounds and in a breeding group of 7 pygmy marmosets housed in a building ~4 km from the zoo. In addition to the standard diets for callitrichids held in zoos, once weekly the animals were hand-fed with live neonatal mice for protein supplementation. These feed mice previously were obtained from a commercial supplier (Mouse House, Houston), but the week before the outbreak, the vendor was changed to a local mouse breeder.

CH outbreak and clinical course. The epizootic began in April 1991, when 4 of the 7 golden lion tamarins became ill 8–9 days after being hand-fed neonatal mice from a single batch from the new supplier. Details of this feeding relative to the ensuing morbidity and mortality are summarized in figure 1. Clinical signs in the tamarins included weakness, anorexia, and ataxia, with grand mal seizures in 3 of the 4 ill tamarins (nos. 5–7), and mucus-covered feces in all 4. Each of the animals with seizures died 24–48 h after onset of clinical signs (figure 1), despite treatment with acyclovir, sulfamethazine, and diazepam. Clinicopathologic findings in these 3 animals were reminiscent of previous CH outbreaks. Golden lion tamarin 4 recovered after 3 days of illness, although inguinal petechiae appeared 4 days after other clinical signs disappeared. On the seventh day after the last onset of clinical signs, ribavirin therapy (150 mg/kg, intramuscularly, once daily for 6 days) was instituted in all surviving tamarins. One hour after the first ribavirin injection, tamarin no. 3 aborted a late-stage fetus. This tamarin had appeared well for ~6 weeks after being fed the neonatal mice but then developed mild ataxia that lasted several days. Two other tamarins (nos. 1, 2) never showed signs of illness.

The outbreak in the 7 pygmy marmosets occurred after they were fed neonatal mice on the same day and from the same batch as the tamarins (figure 1). Five marmosets developed weakness, incoordination, and (variably) jaundice 11–20 days after eating the mice. One marmoset that refused any mouse material remained clinically normal despite continued close contact with the sick animals; a pregnant female died after surgery for dystocia, and no material was available for further study. The clinical course was more protracted in the marmosets than in the tamarins. The 5 affected marmosets all died 16–23 days after eating mice and 5–7 days after the onset of illness.

Serum and tissue collection. Blood samples were obtained by cardiac puncture from the 3 necropsied tamarins and by venipuncture from the 4 surviving tamarins. Specimens of liver and, in some cases, brain were obtained from the 3 tamarins, abortus from tamarin no. 3, and most of the marmosets at necropsy. Portions of the tissues were frozen at −70°C. Neonatal mice and several adult breeding mice obtained from the new feed-mice vendor on 30 April and 3 May 1991 were euthanized with CO_2 and frozen at −70°C.

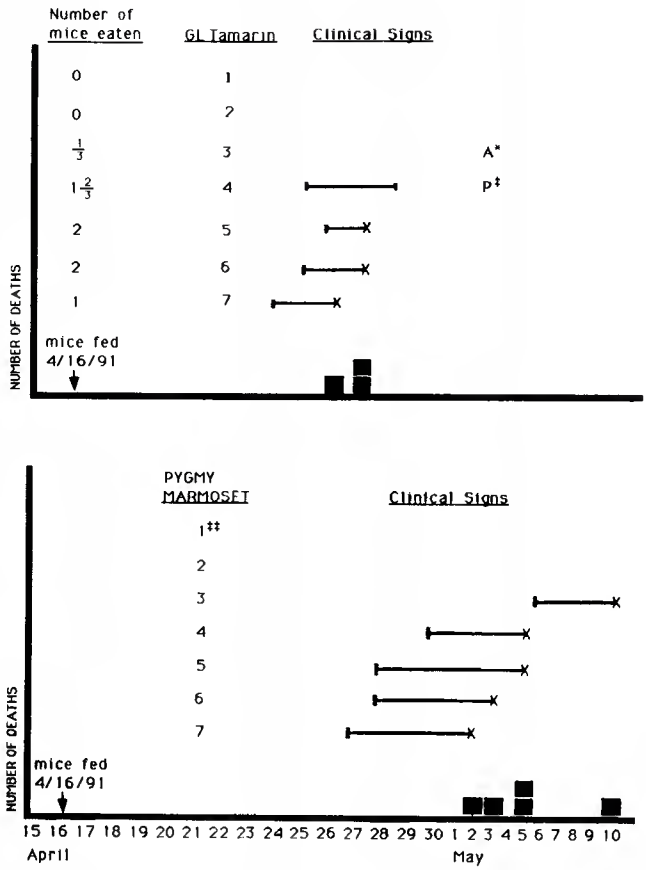

Figure 1. Course of callitrichid hepatitis outbreak at Fort Worth Zoo in golden lion tamarins and pygmy marmosets. *, Spontaneous abortion; ‡, petechiae; ‡‡, died of dystocia.

Solid-phase and immunoblot immunoassays. Frozen liver samples from the affected tamarins and marmosets, mice, a control common marmoset, and a common marmoset experimentally inoculated with $LCMV_{CH}$ [4] were homogenized in TEN (10 m*M* TRIS, 1 m*M* EDTA, 150 m*M* NaCl, pH 7.4) and centrifuged at 1000 *g;* the supernatants were respun at 12,000 *g* and the resultant pellets resuspended in TEN with 0.5% (vol/vol) NP-40. Dot blots were done [4] with convalescent serum from a tamarin surviving a previous outbreak and polyclonal anti-$LCMV_{ARM}$ guinea pig serum. SDS-PAGE and immunoblots were done [8] using the above sera and monoclonal antibody 33.3, specific for LCMV gp2.

RNA hybridization. Total cellular RNA was isolated from livers and control or virus-infected Vero cell cultures using RNAzol B (Tel-Test, Friendswood, TX). RNA was blotted onto nitrocellulose (Schleicher & Schuell, Keene, NH) and hybridized with a labeled cDNA probe corresponding to the 5′ 500

nucleotides of the S RNA genome segment of either $LCMV_{CH}$(OKCZ1) or $LCMV_{ARM}$. The cDNA was radiolabeled with [^{32}P]dCTP using the Prime-It random primer labeling kit (Stratagene, La Jolla, CA).

Results

Histopathology. The 3 golden lion tamarins that died had extensive hepatitis with formation of acidophilic bodies as seen in previous CH outbreaks [3]. Liver lesions in the pygmy marmosets were less necrotizing, with more intense portal inflammatory cell infiltration and portal vein vasculitis, while brains of the pygmy marmosets showed encephalitis and more prominant gliosis than did those of the tamarins that had only minimal meningitis and vasculitis.

Virus isolation. Virus was isolated in Vero cells from the livers of affected tamarins, marmosets, and neonatal mice obtained from the same source that supplied the implicated mice to the zoo. Immunofluorescence assays done with anti-$LCMV_{ARM}$ and anti-$LCMV_{CH}$ sera confirmed that these virus isolates were LCMV-related (data not shown).

Viral antigens in tissues. By solid-phase immunoassay using guinea pig polyclonal $LCMV_{ARM}$-specific serum, viral antigens were detected in the livers of all tamarins and marmosets that died in this outbreak, as well as in the livers of neonatal mice obtained ~1 week after the outbreak from the vendor that had supplied the zoo with the implicated feed mice (figure 2A). Viral antigen was also detected in the liver of the aborted tamarin fetus. Anti-$LCMV_{CH}$ serum also detected viral antigens in these livers. The level of antigen in the neonatal mice livers as detected by anti-$LCMV_{ARM}$ was low and with anti-$LCMV_{CH}$ was barely perceptible. Several $LCMV_{CH}$-infected but not control livers gave faint positive signals either with the control sera or without added serum (data not shown) because of binding of the radioiodinated staphylococcal protein A to antibodies found in livers of the infected animals. Immunoblot analysis demonstrated the 60-kDa LCMV nucleocapsid protein in livers from infected marmosets and tamarins and in the experimentally inoculated common marmoset but not in the liver of an uninoculated common marmoset (figure 2B). This protein also was seen in the livers of previous CH cases [7].

RNA hybridization. The cDNA probe generated from $LCMV_{CH}$(OKCZ1) hybridized strongly with RNA extracted from the livers of tamarins that died in this outbreak. This probe also hybridized with RNA isolated from the liver of a common marmoset experimentally inoculated with $LCMV_{CH}$(OKCZ1) and from Vero cells inoculated with $LCMV_{CH}$(OKCZ1) or $LCMV_{ARM}$. The cDNA probe generated from $LCMV_{ARM}$ hybridized weakly with RNA from $LCMV_{CH}$-infected livers and $LCMV_{CH}$(OKCZ1)-inoculated Vero cells (figure 2C).

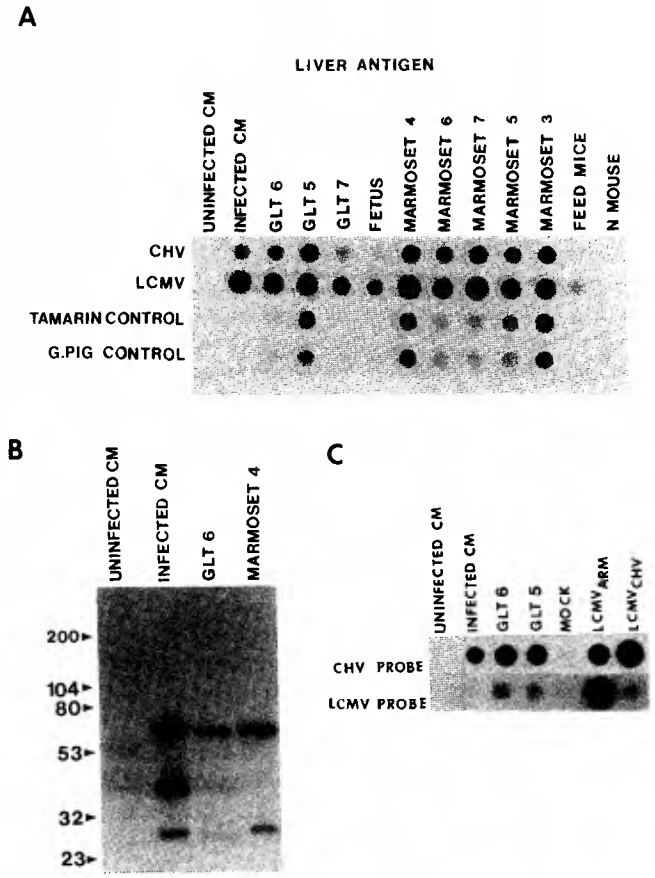

Figure 2. Detection of lymphocytic choriomeningitis virus (LCMV) antigens and nucleic acid in livers of tamarins and marmosets that died in Fort Worth Zoo outbreak, aborted fetus of 1 tamarin, feed mice from new vendor, and, as controls, mouse from LCMV-free colony, common marmosets (uninoculated and experimentally inoculated with $LCMV_{CH}$[OKCZ1]), and Vero cells infected with $LCMV_{CH}$(OKCZ1) or $LCMV_{ARM}$ or mock infected. **A**, Solid-phase immunoassay of viral antigens; **B**, immunoblot of viral antigens (molecular masses are at left); **C**, nucleic acid hybridization.

Discussion

This highly fatal outbreak of callitrichid hepatitis provides an important example of the potential danger of sporadic epizootics of infection with wild-type strains of LCMV. We report that a common-source outbreak of CH in a highly susceptible New World primate family (Callitrichidae) was caused by a single feeding of neonatal mice inapparently infected with LCMV. The clinical signs and pathologic changes observed in the affected tamarins and marmosets in this outbreak were consistent with previous occurrences of CH in other zoos and animal parks [3]. Immunofluorescence, immunoblot, and nucleic acid hybridization analysis of tissues and viruses isolated from the affected golden lion tamarins and pygmy marmosets provided evidence that they were infected by LCMV, as shown for earlier CH outbreaks.

Neonatal mice obtained from the vendor that supplied feed mice to the zoo also were infected with this virus.

In this outbreak, the occurrence and severity of disease among the primates were generally related to the amount of murine tissue ingested by the animals (figure 1). Animals that did not eat mice neither became ill nor seroconverted to LCMV even after close contact with sick primates. Thus, direct primate-to-primate transmission of LCMV was not observed in this outbreak, although such a mode of transmission remains a possibility. Vertical transmission of LCMV to an aborted tamarin fetus, however, was demonstrated. While this abortion coincided with an injection of ribavirin, a known abortifacient, spontaneous abortion in humans is recognized as a complication of Lassa fever, which is caused by an Old World arenavirus related to LCMV [6].

The establishment of arenavirus-contaminated feed mice as the source of LCMV in this epizootic probably explains the sporadic nature of the previous outbreaks of CH in zoos in the US and the absence of CH in primate research centers in which mice are not fed to the primates. Outbreaks of CH have occurred in zoos that did not feed rodent material to callitrichids (personal communication, L. J. Gage, Marine World, Vallejo, CA), presumably from contact of callitrichids with wild mice infected with LCMV. Thus, these studies underscore the importance of stringent rodent control programs in zoos and primate centers, particularly in areas housing callitrichids.

Arenaviruses generally cause a mild or inapparent persistent infection in one reservoir species, usually a rodent, but more serious disease can occur when the virus infects a different species. Both New World and Old World arenaviruses can be naturally transmitted from rodents to humans, causing either inapparent infections or highly lethal diseases such as Lassa fever, Argentinean and Bolivian hemorrhagic fever, and Venezuelan hemorrhagic fever, a newly described arenavirus syndrome [8]. Before the emergence of CH, however, natural arenavirus infections never have been reported in nonhuman primates, although serious disease can result from experimental infection of rhesus monkeys and New World primates with arenaviruses such as Lassa fever virus, Tacaribe virus, and the WE strain of LCMV [9–11].

LCMV has long been recognized as the cause of serious zoonotic disease in humans [6]. The potential exposure of many caretakers and zoo visitors to LCMV-infected tamarins and marmosets makes it particularly important to determine the transmissibility of LCMV from callitrichids to humans and to identify the strains of LCMV associated with this disease. There is extensive strain variation in LCMV isolates, with differences in antigenicity, nucleotide sequence, virulence, host range, and tissue tropism [12, 13]. Nucleotide sequencing of cDNA from different isolates of $LCMV_{CH}$ is in progress and will provide more definitive information for classifying LCMV strains that cause CH.

In the 13 zoo outbreaks identified to date, human infections have been identified by seroconversion in 2 veterinarians, 1 who had necropsied an animal with CH and 1 who had been bitten by an infected animal. Neither person experienced any illness at the time of exposure [4]. Nevertheless, considerable caution is strongly recommended for veterinary staff exposed to infected primates or their tissues and excreta. It has long been known that LCMV infections of humans can cause flu-like illness and central nervous system disease [14]. This report suggests that some strains of LCMV also may play a role in sporadic liver disease in humans.

Further detailed studies on CH and the LCMV strains that cause it will help to control a devastating emerging virus disease that may threaten the conservation of the endangered golden lion tamarin [15]. Currently, surveillance programs are being done to monitor the LCMV antibody status of golden lion tamarins in breeding and renaturalization programs to avoid reintroduction of animals that might serve as a reservoir for the virus. In addition to helping protect populations of tamarins and marmosets, studies on the molecular pathogenesis of $LCMV_{CH}$ will provide important information for comparison of the pathogenesis of LCMV with other serious or fatal arenavirus infections, such as Lassa fever.

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