

CALLITRICHID HEPATITIS: EPIZOOTIOLOGY OF A FATAL HEPATITIS IN ZOO TAMARINS AND MARMOSETS

Edward C. Ramsay, D.V.M., Richard J. Montali, D.V.M., Michael Worley, D.V.M., Ph.D., Charles B. Stephensen, Ph.D., and Kathryn V. Holmes, Ph.D.

Abstract: Epizootics of an acute, fatal hepatitis affecting tamarins and marmosets (family Callitrichidae) have been reported in two zoos in the U.S. and Europe. Two new epizootics of hepatitis affecting seven golden lion tamarins (*Leontopithecus rosalia*) and three emperor tamarins (*Saguinus imperator*) were identified at the Oklahoma City Zoo in 1984 and 1986. Livers from these animals showed hepatocellular swelling and necrosis accompanied by inflammatory cells and acidophilic bodies. Virus-like particles 85–105 nm in diameter were seen in hepatocytes. Using pathologic and clinical criteria, a case definition of the disease now called callitrichid hepatitis (CH) was developed. Health records of tamarins in zoos in the U.S. were surveyed for the occurrence of CH, and 12 outbreaks of CH were retrospectively identified at 10 zoos or animal parks. These studies show that CH is a highly infectious disease with a high mortality rate. This disease is apparently much more prevalent in zoos than previously recognized, and may therefore pose a serious threat to the survival of captive tamarins, particularly endangered species.

Key words: Hepatitis, virus, callitrichid, marmoset, tamarin, *Leontopithecus* sp., *Saguinus* sp., endangered.

INTRODUCTION

In 1981 and 1982, acute fatal hepatitis was reported in tamarins and marmosets at two zoos. The etiology of the hepatitis was not determined but a new viral pathogen was suspected.^{4,8} This paper describes the clinical and pathological characteristics of two additional hepatitis epizootics (henceforth called callitrichid hepatitis, CH) at the Oklahoma City (OKC) Zoo and reports results of a survey identifying 10 additional outbreaks of CH since 1982 at nine other U.S. zoos or animal parks.

From the Department of Pathology, National Zoological Park, Smithsonian Institution, Washington, D.C. 20008, USA (Ramsay, Montali); Center for Reproduction of Endangered Species, The Zoological Society of San Diego, California 92112, USA (Worley); and the Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814, USA (Stephensen, Holmes). Present addresses: Department of Medicine, School of Veterinary Medicine, University of California, Davis, California 95616, USA (Ramsay); and the Department of Public Health Sciences, University of Alabama, Birmingham, Alabama 35299, USA (Stephensen). Reprint requests should be addressed to Dr. Richard J. Montali, Department of Pathology, National Zoological Park, Washington, D.C. 20008, USA.

CASE REPORT

Investigation of OKC Zoo epizootics

In 1984 and 1986, two epizootics of CH occurred at the OKC Zoo. Two family groups of captive-born golden lion tamarins (GLT) (*Leontopithecus rosalia*) were involved in the outbreak in 1984 and a family group of emperor tamarins (EMT) (*Saguinus imperator*) was involved in 1986. The three family groups were exhibited in widely separate locations in the zoo and cared for by different personnel. The groups of tamarins had no direct contact with each other and no known recent exposure to other non-human primates.

The first group of GLT's to become ill in the 1984 outbreak included an adult female and her two female offspring born in February 1982. Between January and March 1984, the three tamarins were found dead about a month apart from each other without any premonitory signs. The adult female had been kept with an adult male GLT in the exhibit from October 1980 until the male's death from a condition similar to CH in March 1982.

The second group affected by CH in 1984 was a pair of adult GLT's, their juvenile

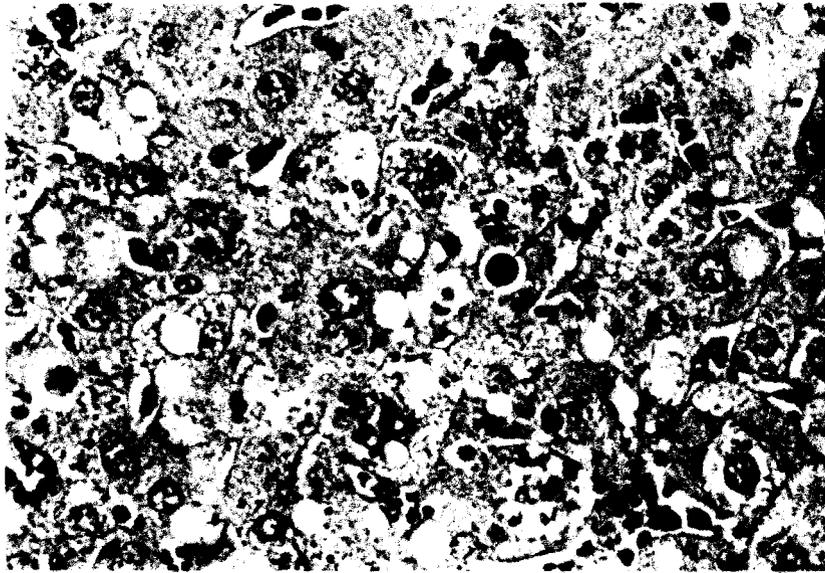


Figure 1. Liver from emperor tamarin with callitrichid hepatitis (CH) from the Oklahoma City (OKC) Zoo outbreak in 1986 shows swollen and necrotic hepatocytes, scattered inflammatory cells, and an acidophilic body (arrow). H&E, $\times 205$.

male offspring, and an unrelated juvenile male. The adults had been exhibited together since June 1980. The family group was initially kept in the zoo office building but was moved to the reptile house in February 1984. The unrelated juvenile male was from another group of GLT's at the zoo that was unaffected by CH. He had been hand reared from birth in the animal nursery and was introduced to the family group in April 1984. At the end of May, one juvenile GLT was found dead, and the adult male and the other juvenile were moribund. Despite parenteral antibiotic and fluid therapy, the male died the next day and the other juvenile died 8 days later. The female GLT (OKC 717337) survived and has remained healthy.

The 1986 epizootic occurred in a wild-caught pair of adult EMT's and their yearling male offspring. In August 1985, this group had been moved into the exhibit formerly occupied by the first affected GLT group. The exhibit had been disinfected twice following the deaths of the GLT's and was used as an aviary during the interim. Late in March, both the female and the ju-

venile male EMT became weak and anorectic and received parenteral antibiotics and glucocorticoid therapy, but they died within 10 days after the onset of illness. The adult male was also ill and, despite 4 days of antibiotic therapy, became moribund and was euthanized at the time the others died.

Pathologic findings

Necropsies of the GLT's and EMT's that died in these outbreaks showed some or all of the following gross changes: jaundice, subcutaneous and intramuscular hemorrhages, hepatosplenomegaly, and pleuropericardial effusions, which were occasionally sanguinous. Liver and heart blood and lung or peritoneal fluid cultivated on sheep blood agar and in thioglycollate broth were negative for aerobic and anaerobic bacterial pathogens.

The major histologic changes were in the liver and consisted of hepatocellular swelling and necrosis associated with a few lymphocytes and neutrophils distributed throughout hepatic lobules. These were accompanied by acidophilic bodies (remnants

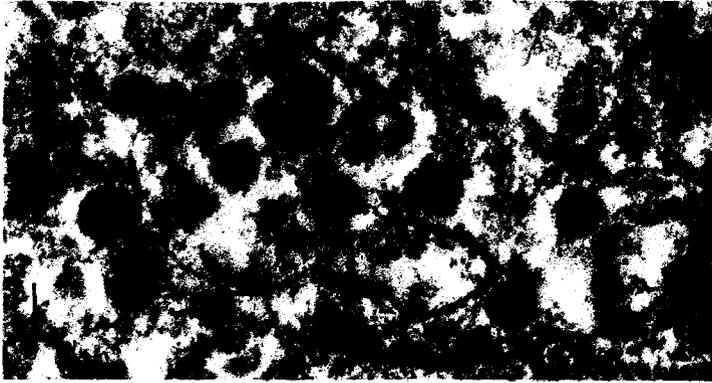


Figure 2. Electronmicrograph of liver from golden lion tamarin (OKC 129-84) with callitrichid hepatitis (CH) shows virus-like particles 85–105 nm in diameter in a hepatocyte with extensive cytoplasmic degeneration. Bar = 100 nm.

of degenerated hepatocytes) (Fig. 1) and often by portal phlebitis. The liver appeared to be the target organ, but necrosis and inflammation were also consistently observed in lymph nodes and spleen and to a much lesser degree in other parenchymal organs.

Liver specimens from affected tamarins preserved in a mixture of 1% glutaraldehyde and 1% paraformaldehyde showed enveloped virus-like particles that ranged in size from 85 to 105 nm within degenerated hepatocytes (Fig. 2).

RETROSPECTIVE SURVEY FOR CH AT OTHER ZOOS

A retrospective study of mortality data in the *International Golden Lion Tamarin Studbook* was performed to determine the prevalence of hepatitis in the captive population of GLT's. This studbook is maintained at the National Zoological Park (NZN) which has GLT's on breeding loans to many zoos in the U.S. and Europe.¹ Necropsy reports were reviewed for all animals listed as having hepatitis or whose cause of death was presumed to be virus induced. From 1981 to 1987, cause of death was recorded for 291 juvenile and adult GLT's. Of these (excluding tamarins from the OKC CH epizootics), 15 GLT's died of hepatitis of unknown or presumed viral etiology and an

additional 17 died of generalized illness with presumed viral etiology.

These observations led us to eight zoos in the U.S. (in addition to the OKC Zoo) that had experienced outbreaks of presumed viral hepatitis in tamarins, marmosets, and Goeldi's monkeys (*Callimico goeldii*, recently reclassified from the Callitrichidae to Callimiconidae). The dates, locations, species, and numbers of animals that died are shown in Table 1. An outbreak was defined as death of more than one animal within a 2-mo period, with the major lesion noted as hepatitis and without identification of an etiologic agent. Histologic criteria used to identify CH included diffuse hepatocellular swelling and necrosis with acidophilic bodies (Fig. 1). We reviewed pathology reports and a limited number of histological slides and paraffin blocks from four zoos, and complete sets of slides, blocks, and pathology reports from four other zoos. Histologic lesions in the livers from animals in these eight zoos were compatible with CH in some or all of the animals examined (Table 1). While this manuscript was in preparation, an additional outbreak of CH occurred in a California animal park in which two of five saddleback tamarins (*S. fuscicollis*) died of a disease histologically compatible with CH (Table 1). In summary of

Table 1. Epizootics of callitrichid hepatitis (CH) at U.S. zoos and animal parks.

Location of facility	Year	Species ^a	No. dead	Cases available for histopathology	Cases histologically compatible with CH
Nebraska (Henry Doorly Zoo, Omaha)	1980/1981 ^{b,c}	GLT	9	6	6
	1985	GLT	2	2	2
Tennessee (Memphis Zoo)	1983	GLT	7	3	2
Colorado (Denver Zoo)	1984	GM	4	4	4
Oklahoma (Oklahoma City Zoo) ^c	1984	GLT	7	7	7
	1986	EMT	3	3	3
Illinois (Brookfield Zoo, Chicago)	1986/1987	GLT	8	2	2
Indiana (Mesker Park Zoo, Evansville)	1986	GLT	2	2	2
Kansas (Sedgwick County Zoo, Wichita)	1986/1987	GLT, GM	5	5	5
New York (Buffalo Zoo)	1987	BHT, CM, CTT	4	4	3
Illinois (Lincoln Park Zoo, Chicago)	1987	EMT, WFT	3	2	2
California Marine World, Vallejo)	1988	ST	3	3	2

^a GLT, golden lion tamarin (*Leontopithecus rosalia*); GM, Goeldi's monkey (*Callimico goeldii*); EMT, emperor tamarin (*Saguinus imperator*); BHT, black-headed tamarin (*L. r. chrysomelas*); CM, common marmoset (*Callithrix jacchus*); CTT, cotton-top tamarin (*S. oedipus*); WFT, white-fronted tamarin (*S. nigricollis*); ST, saddleback tamarin (*S. fuscicollis*).

^b / indicates continuing outbreak.

^c The Oklahoma City Zoo outbreaks are used as index cases in the present study.

the data in Table 1, 40 of 44 (91%) animals that died in all known U.S. outbreaks had histopathologic lesions compatible with CH.

DISCUSSION

The clinical features of CH were often nonspecific because of the acute onset and rapid course of the disease, which usually resulted in the death of the animal. In animals which had a longer clinical course, icterus and elevated liver enzymes were reported. Two saddleback tamarins which survived the Marine World outbreak (Table 1) developed elevated aspartate aminotransferase (AST or SGOT) of up to 2,695 U/L, which returned to normal 6 wk later (60–200 U/L)³ (L. Gage, pers. comm.).

Pathologic criteria for the diagnosis of CH include diffuse hepatocellular swelling and necrosis with acidophilic body formation and a mild infiltration of inflammatory cells throughout the liver. Based on these criteria, 12 CH outbreaks were identified in 10 zoos or animal parks in the U.S. Thus, callitrichid hepatitis is a much more prevalent disease among zoo tamarins and marmosets

than had been considered previously. Table 1 shows that at least 40 animals died of CH in U.S. zoos since 1981. More than half of these animals were GLT's, an endangered species. Many of the animals that died were juveniles; therefore, the development of effective treatment and/or prevention of CH would be a major contribution to these rare and endangered species.

Little information was uncovered in the survey about the source of these outbreaks. Two zoos (Henry Doorly and OKC) each had two outbreaks separated by several years (Table 1). In the case of the OKC Zoo, the second outbreak was in the same enclosure as the first, although the area had been decontaminated several times. Interzoo exchanges of marmosets and tamarins are rather common and are usually traceable, but we only identified one possible connection between two of these epizootics in which CH occurred in a zoo after the arrival of a previously exposed GLT. Thus, the primary source for the introduction of CH into zoo tamarins remains to be determined. There are three possible sources of infection: (1)

the CH agent may sometimes produce an inapparent infection in tamarins, which can be activated in some as yet unrecognized manner; (2) the CH agent may cause a minor or inapparent infection in other zoo species or by carrier hosts such as wild rodents, which then transmit it to tamarins; or (3) the CH agent may be transmitted to callitrichids by keepers or visitors. However, no illness that could be attributed to CH occurred in zoo personnel involved in the CH epizootics that we investigated.

Based on epizootiologic, pathologic, and ultrastructural findings, CH appears to be of viral etiology. To further support this premise we recently transmitted CH to three common marmosets using a bacteria-free filtrate prepared from frozen liver tissue from a naturally infected emperor tamarin that died in the 1986 outbreak at the OKC Zoo.⁵ In that study, sera was used from several tamarins naturally exposed to CH to develop an immunoassay which demonstrated a CH-associated antigen in the livers of infected marmosets but not in uninfected animals' livers.

The virus which induces CH has not yet been characterized, but it does not appear to be human hepatitis A (HAV), B (HBV), or D (HDV or delta).⁵ An intriguing candidate for the agent of CH is the large (85–105 nm in diameter) enveloped virus-like particle observed in the cytoplasm of hepatocytes of tamarins with natural CH infections. Similar particles were observed in the livers of marmosets experimentally infected with CH.⁵ Based on size, structure in thin sections, and cytoplasmic location,² it appears to be an RNA virus compatible with a classification of either bunyavirus or coronavirus. Bunyaviruses cause febrile illnesses in humans and hepatitis in mice with Councilman bodies and some are rodent borne;⁹ coronaviruses cause hepatitis in mice and cats,^{6,7,11} and coronavirus-like particles have been observed in feces of marmosets.¹⁰ Efforts are now underway to isolate and characterize the CH virus.

We note that, to date, CH seems to have been limited to zoos or animal parks and has not been observed in colonies of marmosets or tamarins reared for laboratory use. The reason for this difference in occurrence of CH is not clear, but the epizootiology of the disease could be dependent upon dissimilar management practices between the two types of institutions. In view of the frequent outbreaks and high mortality rate of CH, it seems likely that this disease poses a threat to all captive colonies of callitrichids and callimicos.

Acknowledgments: This study was supported by a grant from the Friends of the National Zoo (FONZ-10C), by grant no. RO7403 from the Uniformed Services University of the Health Sciences, and by funds provided by the Zoological Society of San Diego. We thank B. Baumeister and J. Wenzel of the Oklahoma City Zoo and the staff of Oklahoma Animal Disease Diagnostic Laboratory for assistance with the original pathology studies and the veterinary staffs from all of the institutions represented in Table 1 for contributing information and material for this study. The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting views of the Department of Defense or the Uniformed Services University of the Health Sciences.

LITERATURE CITED

1. Ballou, J. 1986. The International Golden Lion Tamarin Studbook. National Zoological Park, Washington, D.C.
2. Dubois-Dalcq, M., K. V. Holmes, and B. Rentier. 1984. The Budding Enveloped RNA Viruses. Springer-Verlag, Vienna.
3. Holmes, A. W., M. Passavoy, and R. B. Capps III. 1967. Blood chemistry of laboratory-kept marmosets. *Lab. Anim. Care* 27: 11–29.
4. Lucke, V. M., and A. M. Bennett. 1982. An outbreak of hepatitis in marmosets in a zoological collection. *Lab. Anim.* 16: 73–77.
5. Montali, R. J., E. C. Ramsay, C. B. Stephensen, M. Worley, J. A. Davis, and K. V. Holmes. In press. A new transmissible viral hepatitis of marmosets and tamarins. *J. Infect. Dis.*
6. Montali, R. J., and J. D. Strandberg. 1972. Extraperitoneal lesions in feline infectious peritonitis. *Vet. Pathol.* 9: 109–121.

7. Pederson, N. C. 1987. Coronavirus diseases (coronavirus enteritis, feline infectious peritonitis). In: Holzworth, J. (ed.). *Diseases of the Cat*. W. B. Saunders Co., Philadelphia, Pennsylvania. Pp. 193–213.

8. Phillips, L. G. 1981. Suspected viral hepatitis in golden lion tamarins—case report. *Annu. Proc. Am. Assoc. Zoo Vet.* Pp. 34–35.

9. Shope, R. E. 1985. Bunyaviruses. In: Fields, B. N. (ed.). *Virology*. Raven Press, New York, New York. Pp. 1052–1082.

10. Smith, G. C., T. L. Lester, R. L. Heberling, and S. S. Kalter. 1982. Coronavirus-like particles in non-human primate feces. *Arch. Virol.* 72: 105–111.

11. Wege, H., S. Siddell, and V. Ter Muelen. 1982. The biology and pathogenesis of coronaviruses. *Curr. Top. Microbiol. Immunol.* 99: 165–200.

Received for publication 29 June 1988.

Journal of Zoo and Wildlife Medicine 20(2): 183, 1989
Copyright 1989 by American Association of Zoo Veterinarians

BOOK REVIEW

Techniques in Large Animal Surgery, 2nd ed., A. Simon Turner and C. Wayne McIlwraith. Lea and Febiger, Philadelphia, Pennsylvania, USA. 1989. 381 pages, 121 illustrations. Price: \$54.00.

The scope of general zoo practice necessitates surgical intervention on the larger mammalian species. Although this text is written for the traditional large animal practitioner, it has many applications in the nondomestic realm. The authors' goal is to provide a reference book demonstrating the fundamental techniques of the most commonly encountered large animal surgical procedures. This is ably done using a wealth of high quality illustrations supported by easy to understand text.

Chapters 1–6 review the basics of presurgical considerations, anesthesia, fluid therapy, surgical instrumentation, and suture materials as well as suture patterns. Although these chapters are geared more to the student surgeon, there are excellent descriptions of regional anesthesia and suture patterns. These discussions with the corresponding illustrations would serve the zoo practitioner well in review.

Chapters 7 and 8 detail the principles of wound management, the use of drains, and reconstructive surgery of wounds. The techniques discussed have great application for the nondomestic large animal. The sliding H-flap and the Z-plasty illustrations were especially well done.

Chapters 9–12 concentrate on particular equine surgeries, many of which would have limited applicability to the zoo veterinary surgeon. This is especially true

for the 40 pages that deal with orthopedic procedures. There are, however, excellent descriptions of certain urogenital, dental, and gastrointestinal procedures along with postoperative management suggestions that are of great value for our specialty.

The bovine surgery section (Chapters 13–15) is filled with procedures that zoo practitioners may find themselves performing. In-depth descriptions of the rumenotomy, rumenostomy, abomasopexy, urethrostomy, caesarean section, cosmetic dehorning, teat laceration repair, enucleation, and digit amputation are among the 23 procedures illustrated.

The last two chapters describe a number of surgeries for swine, dehorning of mature goats, and canine tooth removal in the llama. An appendix with the names of materials mentioned in the text as well as manufacturers' addresses may serve as a helpful resource for certain difficult to acquire items.

The intent of *Techniques in Large Animal Surgery* is to provide the student and practicing veterinary surgeon with an easy to use resource for assistance in performing the more common large animal procedures. The book performs this task admirably. Although some of the procedures would have no applicability to zoo practice, and the beginning chapters seem more geared to student concerns, I still feel this is an excellent reference text for the zoo practitioner. With the intermittent nature of zoo surgery, a fast, complete, and well-illustrated text such as this will serve a useful function in the zoo library.—Reviewed by **Robert A. Cook, V.M.D.**, Animal Health Center, New York Zoological Society, Bronx, New York 10460, USA.