A canine distemper virus epidemic in Serengeti lions (Panthera leo)

Melody E. Roeke-Parker*†, Linda Munson‡, Craig Packer*‡, Richard Kock†, Sarah Cleaveland†‡, Margaret Carpenter*, Stephen J. O'Brien**, Andrea Pospischil††, Regina Hofmann-Lehmann†‖, Hans Lutz‡‖, George L. M. Wmawengelle‡, M. N. Mgasasa‡, Brian A. Summers‡‡ & Max J. G. Appel††

* Serengeti Wildlife Research Institute, Tanzania National Parks, Arusha, Tanzania
† Messerli Foundation, Zürich, Switzerland
‡ Department of Pathology, College of Veterinary Medicine, University of Tennessee, Knoxville, Tennessee 37901, USA
§ Department of Ecology, Evolution, and Behavior, University of Minnesota, St Paul, Minnesota 55105, USA
∥ Kenya Wildlife Services, Nairobi, Kenya
‖ Institute of Zooology, London NW1 4RY, UK
# London School of Hygiene and Tropical Medicine, London WC1E 7HT, UK
* Laboratory of Viral Carcinogenesis, National Cancer Institute, Frederick, Maryland 21702, USA
** Institute of Veterinary Pathology, and † Department of Veterinary Internal Medicine, University of Zurich, 8057 Zürich, Switzerland
†† Department of Veterinary Pathology, Sokono University of Agriculture, Morogoro, Tanzania
‡‡ Veterinary Investigation Center, Arusha, Tanzania
‡§ Department of Pathology, and †† James Baker Institute of Animal Health, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853, USA

**To whom correspondence should be addressed.

Canine distemper virus (CDV) is thought to have caused several fatal epidemics in canids within the Serengeti-Mara ecosystem of East Africa, affecting silver-backed jackals (Canis mesomelas) and bat-eared foxes (Otocyon megalotis) in 1978 (ref. 1), and African wild dogs (Lycaon pictus) in 1991 (refs 2, 3). The large, closely monitored Serengeti lion population15 was not affected in these epidemics. However, an epidemic caused by a morbillivirus closely related to CDV emerged abruptly in the lion population of the Serengeti National Park, Tanzania, in early 1994, resulting in fatal neurological disease characterized by grand mal seizures and myoclonus; the lions that died had encephalitis and pneumonia. Here we report the identification of CDV from these lions, and the close phylogenetic relationship between CDV isolates from lions and domestic dogs. By August 1994, 85% of the Serengeti lion population had anti-CDV antibodies, and the epidemic spread north to lions in the Maasai Mara National reserve, Kenya, and uncounted hyenas, bat-eared foxes, and leopards were also affected.

In early 1994, six lions in the Serengeti National Park, Tanzania, were observed with grand mal seizures, and three other lions developed facial and forelimb myoclonus (recurrent twitching). Additional lions were noted to be disoriented, ataxic and profoundly depressed. Between January and March 1994, 11 lion carcasses were found, representing a dramatic increase in mortality over previous years and indicating that a serious epidemic was emerging.

To investigate the epidemic, tissue and serum samples were obtained from 23 lions that died or were killed in a moribund state, 13 live lions with obvious signs of disease, and 72 apparently healthy, anaesthetized lions. Sera from 111 healthy lions sampled previously exposure of this population to viruses. Tissues from 19
dead lions, examined by histopathology, had either encephalitis, interstitial pneumonia, and/or lymphocytic depletion in lymph nodes and spleen (Fig. 1; Table 1). Rare multinucleated syncytia and intranuclear and/or intracytoplasmic viral inclusions characteristic of morbilliviral infection were also found in these lions (Table 1). Because these lesions were seen in zoo cats in the United States in the 1991 and 1992 canine distemper epidemics, we used monoclonal and polyclonal CDV antibodies to confirm that CDV nucleocapsid antigens were present in affected tissues. We then tested all available lion sera for neutralizing antibody titres to CDV, and found that 63 of the 72 apparently healthy lions and 8 of the 11 sick lions sampled in 1994 had CDV titres (Fig. 2a). We isolated CDV from the cerebrospinal fluid of one lion cub with grand mal seizures that subsequently died with CDV encephalitis. The monoclonal antibody-binding pattern of this virus was compared to binding patterns of viruses isolated from: a bat-eared fox (Otocyon megalotis), a spotted hyaena (Crocuta crocuta), and a domestic dog that died during the 1994 Serengeti epidemic; and with a virulent CDV (A75-17, 1975) and two attenuated CDV from domestic dogs (Rockborn 1958 and Onderstepoort 1948). Monoclonal antibodies were donated by C. Orvell (Huddinge, Sweden), and were directed against viral nucleoprotein (N), polymerase (P), fusion glycoprotein (F), and haemagglutinin glycoprotein (H). Lymphocytes infected with the Serengeti lion virus bound the same group of CDV monoclonal antibodies as cells infected with these other viruses, suggesting that the lion morbillivirus was CDV. To characterize further the lion morbillivirus, the genomic sequences of the CDV P gene, a conserved region of the virus, were amplified from buffy-coat lymphocytes of two lions with neurological signs. Phylogenetic relationships between the P-gene sequences of the two RT-PCR-derived lion CDV and the P-gene sequences of other morbilliviruses were then examined. These analyses (Fig. 3) indicated that the Serengeti CDV was closely related to the Onderstepoort strain of canine distemper virus isolated from a domestic dog in South Africa. The sequences of the epidemic are given in Table 1.

Later in 1994, CDV-infected lions were identified in the northern and western areas of the Serengeti National Park and in the Maasai Mara National Reserve in Kenya (Fig. 2b). Of 54 apparently healthy lions sampled from October 1994 to March 1995 in the Mara and neighbouring areas of Kenya, 23 had high serum titres of CDV antibodies, suggesting recent exposure. During 1994, 39 CDV-associated lion deaths were documented, but this is probably an underestimation of true mortality statistics because most of the Serengeti lion population outside the Seronera study area is not under close observation. The overall lion population in the Serengeti ecosystem, estimated at 3,000 before the outbreak, is now estimated at 2,000. In 1994 and 1995, CDV-related deaths were also confirmed in seven spotted hyaenas (Crocuta crocuta) by pathology and virology. Because CDV is historically not pathogenic in lions or hyaenas, the emerging biotype of CDV has apparently extended its host range. During the epidemic, CDV-induced disease also was confirmed in two bat-eared foxes, and CDV-like neurological disease was observed in a common jackal (Canis aureus) and two silver-backed jackals, indicating that the Serengeti CDV biotype conserves its pathogenicity for canids.

The magnitude of this epidemic may be explained in part by the lion population being immunologically naive to CDV when it was introduced in 1994. All but one of the 34 lions sampled between 1990 and 1993 were seronegative, and the seropositive lion was
Fig. 2a, Temporal patterns of mortality and seroconversion in the long-term study population of lions in the Serengeti National Park. Top, number of lions resident in the long-term study area, measured monthly from August 1974 to February 1995 (refs 4, 5). Middle, prevalence of canine distemper virus (CDV) antibodies in Serengeti lions from 1990 to 1994. The single seropositive lion in 1993 was sampled in December. Bottom, age-specific mortality (proportion dying per year) in female lions in the study population. The solid line represents the combined mortality rate from 1984 to 1993; the broken line represents mortality in 1994. Male mortality was comparable to female mortality, but was not included here because male disappearances could also be caused by normal, non-disease-related dispersals.

To determine if the high lion mortality during the 1994 CDV epidemic was due to co-infection with another viral pathogen, we compared the prevalences of serum antibody titres to feline immunodeficiency virus (FeiV), feline parvovirus (FePV), feline herpesvirus 1 (FeHV1), feline coronavirus (FeCoV), and feline calici virus (FeCV) between lions with CDV disease (6/10 FeiV+, 3/5 FePV+, 6/6 FeHV+, 2/6 FeCoV+, 1/6 FeCV+) and healthy CDV-seropositive lions (15/16 FeiV+, 11/12 FePV+, 13/13 FeHV+, 8/13 FeCoV+, 8/13 FeCV+). The discordance between CDV disease and antibodies to other viruses (Fisher's exact test: FeiV, P > 0.055; FePV, P > 0.19; FeCoV, P > 0.35; FeCV, P > 0.14) fails to support a role for these viruses as necessary cofactors in CDV morbidity.

CDV infections in equatorial African wildlife usually occur as periodic epidemics because environmental factors limit viral persistence outside susceptible carnivore hosts, and these hosts usually succumb or rid themselves of virus. CDV persists in dense populations of domestic dogs because pups provide a constant reserve of susceptible hosts. The source of CDV in the Serengeti epidemic was probably the domestic dogs of the local villages, acting as a reserve of susceptible hosts. The source of CDV in the Serengeti ecosystem had previously been confirmed in the endemic infection was confirmed in the Serengeti district where CDV seropositivity was detected in pups (<12 months old) in each year of the study (1992-1994). This district also has one of the highest dog population densities. In the Llolido and Ngorongoro areas, seropositivity was detected in pups in 1994 (3/7), but not in 1992 (0/6) or 1993 (0/17).

METHODS. Sera were tested against the Onderstepoort strain of CDV adapted to Vero cells as described previously. Sera were also tested against CDV isolated from a lion during the California epidemic (A92-27/20). Log titres of 1.0 or greater were considered positive.
ecular analyses were derived from 20 ml blood taken from anaesthetized
lions. Viral RNA fragments of 429 bp were amplified by RT-PCR from RNA of thymus and white blood cells isolated from two lions with neurological signs. Oligonucleotide primers were synthesized based on conserved regions of the CDV phosphoprotein (P) gene as described previously,55 PCR product fragments were cloned and sequenced, and the sequences aligned with P-gene sequences from other morbilliviruses. Derived P-gene sequences from two lions were 99% identical. A phylogenetic analysis using cladistic, phenetic and maximum-likelihood methods revealed a close relationship between the lion morbillivirus and the Onderstepoort strain of CDV, the sequences having 95% nucleotide identity.

Derivation of CDV P-gene sequences from two lions was 99% identical. A phylogenetic analysis using cladistic, phenetic and maximum-likelihood methods revealed a close relationship between the lion morbillivirus and the Onderstepoort strain of CDV, the sequences having 95% nucleotide identity.

**TABLE 1. Summary of CDV disease in Serengeti lions**

| Criteria indicating CDV infection | Number of affected lions |
|-----------------------------------|-------------------------|
| Seizures                          | 12                      |
| Myoclonus                         | 15                      |
| Other neurological signs          | 27                      |
| Cancruses recovered               | 23                      |
| Disappearance from observed       | 31                      |
| population of 250 lions           |                         |
| CDV seroprevalence†               | 71 of 83                |
| Lions with histopathological lesions of CDV | 18 of 19            |
| Lions with viral inclusions in tissues | 14 of 19            |
| Lions with CDV nucleocapsid proteins in tissue§ | 14 of 19            |
| Lions with CDV isolated‡          | 1 of 7                  |
| Lions with CDV RNA obtained by RT-PCR‡ | 2                   |

† Number designates lions observed during 1994. Other neurological signs included ataxia, disorientation, profound depression or stupor, hyperaesthesia, and inappropriate behavioural responses.

‡ Immunohistochemistry and viral isolation were performed by methods published previously.2,12 Buffy-coat isolation for viral cultures and molecular analyses were derived from 20 ml blood taken from anaesthetized lions.

§ Methods are described in Fig. 3 legend.

Most of the lion deaths in the Serengeti National Park occurred between January and September 1994, and mortality rates have subsequently returned to previous levels (Fig. 2b). Although this CDV epidemic claimed approximately 30% of the Serengeti and Mara lions, the impact on other carnivore species is unknown. Less dense populations of endangered species, such as cheetahs or wild dogs, are a clear cause for concern if exposed to a virulent pathogen such as this putative new biotype of CDV. The Serengeti is surrounded by approximately 30,000 domestic dogs, most of which are not vaccinated against canine pathogens (including CDV), representing a large reservoir for carnivore diseases. The CDV epidemic clearly emphasizes the need for continued disease surveillance to monitor infectious diseases in valuable wildlife resources, and for initiating vaccination programs for domestic animals in contact with wildlife.
A common molecular basis for three inherited kidney stone diseases

Sarah E. Lloyd*, Simon H. S. Pearce*, Simon E. Fisher*, Klaus Steinmeyer*, Sarah E. Lloyd*, Simon H. S. Pearce*, Brian Harding*, Alessandra Bolino*, Marcello Devoto*, Paul Goodyer*, Susan P. A. Rigden#, Oliver Wrong*, Thomas J. Jentsch*, Ian W. Craig† & Rajesh V. Thakker††

* MRC Molecular Endocrinology Group, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0NN, UK
† Genetics Laboratory, University of Oxford, South Parks Road, Oxford OX1 3QU, UK
‡ Centre for Molecular Neurobiology Hamburg (ZMNH), Hamburg University, Martinistrasse 52, D-20246 Hamburg, Germany
§ Department of Medicine, State University of New York Health Science Center, Syracuse, New York 13210, USA
¶ Laboratorio di Genetica Molecolare, Istituto G. Gaslini, Largo Gaslini 5, 16148 Genova, Italy
# Centre hospitalier de Montreal pour Enfants, 2300 rue Tupper, A717 Montreal, Quebec H3H1P3, Canada
$ Department of Paediatric Nephrology, Guy’s Hospital, St Thomas’ Street, London SE1 9RT, UK
& Department of Nephrology, The Middlesex Hospital, Mortimer Street, London W1N 8AA, UK

Kidney stones (nephrolithiasis), which affect 12% of males and 5% of females in the western world, are familial in 45% of patients and are most commonly associated with hypercalciuria. Three disorders of hypercalciuric nephrolithiasis (Dent’s disease), X-linked recessive nephrolithiasis (XRN), and X-linked recessive hypophosphatemic rickets (XLHR) have been mapped to Xp11.22 (refs 5–7) have been mapped to Xp11.22 (refs 5–7). A microdeletion in one Dent’s disease kindred allowed the identification of a candidate gene, CLCN5 (refs 8,9). A putative renal chloride channel. Here we report the investigation of 11 kindreds with these renal tubular disorders for CLCN5 abnormalities; these identified three nonsense, four missense and two donor splice site mutations, together with one intragenic deletion and one microdeletion encompassing the entire gene. Heterologous expression of wild-type CLCN5 in Xenopus oocytes yielded outwardly rectifying chloride currents, which were either abolished or markedly reduced by the mutations. The common synonym for Dent’s disease, XRN and XLHR indicates that CLCN5 may be involved in the renal tubular dysfunction associated with kidney stones.

Analysis of CLCN5 reverse transcriptase–polymerase chain reaction (RT–PCR) products encompassing the entire 2238-bp coding sequence from probands of 11 kindreds with Dent’s disease, XRN and XLHR, revealed different CLCN5 mutations (Table 1). Each mutation was confirmed and demonstrated to cosegregate with the disease by using genomic DNA together with the appropriate PCR primers and restriction enzymes, or by sequence-specific oligonucleotide (SSO) probe analysis (Table 1 and Fig. 1). In addition, the absence of these CLCN5 abnormalities in 110 alleles from 69 (28 males and 41 females) unrelated normal individuals established that they were not common polymorphisms. CLCN5 belongs to a family of voltage-gated chloride-channel genes (CLCN1 to CLCN5 and CLCN-Ka and -Kb), which encode proteins (CLC-1 to CLC-5, CLC-Ka and CLC-Kb) that have about 12 transmembrane domains. These chloride channels are important for the control of membrane excitability, transepithelial transport, and possibly regulation of cell volume. However, mutations have been identified previously in only CLCN1, which is expressed in muscle and is associated with myotonia congenita. Thus, to assess further the functions of CLC-5 and its mutations, we performed heterologous expression studies in Xenopus oocytes. Expression of the human wild-type (WT) CLC-5 reproducibly yielded strongly outwardly rectifying, essentially time-independent currents (Fig. 3). Ion substitution experiments indicated that these were carried by anions, with a chloride-to-lactate conductance sequence (Fig. 3b,e), as is the case with other chloride channels (CLC-4, CLC-1 and CLC-2) of this family (12,13). However, these human CLC-5 currents, which were indistinguishable from those of rat CLC-5 (ref. 17), differed from the others in being strongly outwardly rectifying and in being observed only at potentials more positive than 110 mV. Although positive potentials of even +40 mV have been observed in apical membranes of some actively transporting epithelia (14), we are not aware of renal cells where these voltages would be reached in vivo. CLC channels are known to function as multimeric complexes, which are most likely to be tetramers (15), and it seems possible that CLC-5 forms homotetramers with as yet unknown subunits in situ that may render the channels open at a more physiological voltage. Our functional expression of CLC-5 provides a valuable means of investigating this further and in assessing the functional effects of the CLC-5 mutations.

Expression of the four missense and three nonsense mutations (Table 1), and the in-frame deletion of the predicted transmembrane domain D2 (Fig. 1), abolished the CLC-5 currents or reduced them (S244L and S520P) to levels where they were difficult to distinguish from endogenous chloride currents (Fig. 3d,f). As a control, we also expressed more conservative changes at codons 244 and 520; S244T, S520T, S244A and S520A all elicited chloride currents that were similar to the WT (data not shown). Thus, the mutations found in the hypercalciuric nephrolithiasis pedigrees grossly and specifically affect CLC-5 function, thereby strongly suggesting a causal role in the disease. Dent’s disease, which is characterized by low-molecular-weight proteinuria (LMWP), hypercalciuria, nephrocalcinosis, nephrolithiasis, rickets and eventual renal failure (16), has phenotypic similarities to XRN and XLHR (Fig. 2). However, there are important differences as rickets is absent in XRN, and nephrocalcinosis and moderate renal failure are more notable in XLHR. A correlation between these phenotypic differences, the different mutations (Table 1 and Fig. 1) and the resulting abnormal chloride currents (Fig. 3c) could not be established. Thus, Dent’s disease was found to be associated with the mutations W279X,
ERRATUM

doi:10.1038/nature08888

A canine distemper virus epidemic in Serengeti lions (*Panthera leo*)

Melody E. Roelke-Parker, Linda Munson, Craig Packer, Richard Kock, Sarah Cleaveland, Margaret Carpenter, Stephen J. O'Brien, Andreas Pospischil, Regina Hofmann-Lehmann, Hans Lutz, George L. M. Mwamengele, M. N. Mgasa, G. A. Machange, Brian A. Summers & Max J. G. Appel

*Nature* 379, 441–445 (1996)

In this Letter, the received and accepted dates for the manuscript were incorrectly listed as being in 1994, instead of 1995. The correct dates are: received 2 October; accepted 24 November 1995.