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


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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 population 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 from previous years and indicating that a serious epidemic was emerging.

To investigate the epidemic, tissue and serum samples were obtained from 13 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 between 1984 and 1994 (refs 6–8) were also analysed to track previous 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 giant cells 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 (10), and found that 53 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 (10). The monoclonal antibody-binding pattern of this virus was compared to binding patterns of viruses isolated from: a bat-eared fox (O. megalotis), a spotted hyena (Crocuta crocuta), and a domestic dog that died during the 1994 Serengeti epidemic; with CDV isolated from a raccoon (Procyon lotor) and fox (Vulpes vulpes); and with a virulent CDV (AT5-17, 1975) and two strains of 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 morbilli-viruses were then examined. These analyses (Fig. 3) indicated that Serengeti lion CDV virus is closely related to the Onderstepoort strain of canine distemper virus isolated from a domestic dog in South Africa. The statistics 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 neighboring 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 Serengeti 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 hyenas (C. crocuta) by pathology and virology. Because CDV is historically not pathogenic in lions or hyenas (10), the emerging biotype of CDV has apparently extended its host range. During the epidemic, CDV-induced disease was also 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 by the fact that 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. 2 a. 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 anti-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 1966 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. *b*, Chronology and geographic distribution of CDV disease in the Serengeti ecosystem. The months in boxes are dates in 1994 when lions were first observed with neurological disease. The proportions of CDV-seropositive domestic dogs in regions surrounding the Serengeti National Park are represented by pie charts for 1992 to 1994 (n, number of dogs tested). The domestic dog that was confirmed to die from canine distemper disease in September 1994 was located in the Ngorongoro Conservation Area. 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 Loliondo and Ngorongoro areas, seropositivity was detected in pups in 1994 (3/7), but not in 1992 (0/6) or 1993 (0/7).

METHODS. Sera were tested against the Onderstepoort strain of CDV adapted to vero cells as described previously. Sera were also tested against CDV isolates from a lion during the California epidemic (A92-2785). Log titres of 1.0 or greater were considered positive.

Sampled in December of 1993 (Fig. 2a). Although this abrupt seroconversion indicates a recent exposure of these lions to CDV, analysis of sera from 77 lions in the region from 1984 to 1989 disclosed that 22 lions had antibodies to CDV (none of these previously tested lions was alive during the 1994 epidemic), indicating that lions in the Serengeti ecosystem had previously encountered CDV without an increase in disease-related mortality. 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 corona virus (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 adjacent to the National Park. CDV seroprevalence increased in
FIG. 3 Sequences and phylogenetic analysis of 389 bp from 14 novel morbillivirus P genes. a, Total 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. PCR product fragments were cloned and sequenced, and two sequences aligned with P-gene sequences from other morbilliviruses. Derived P-gene sequences from two lions were 99% identical. A phylogenetic analysis using cladespecific, 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. PAUP maximum parsimony tree (left), with branch lengths equal to the number of nucleotide substitutions. Numbers shown on branches are branch lengths and number of homoplasies, in that order. Tree length, 543; consistency index, 0.790. PHYLIP neighbour-joining tree (centre) generated using a transition/transversion ratio of 0.84, with branch lengths equal to the percent of nucleotide differences. The number of bootstrap iterations (out of 100) which support the nodes are shown in parentheses. PHYLIP maximum-likelihood tree (right) generated with a transition/transversion ratio of 0.84 (in likelihood, -2561.52297; 155 trees examined). Branch lengths are the expected number of substitutions per site x 100. 94-52-10 and 94-74 are sequences from Serengeti lions, CDV, canine distemper (Genbank X51869); PIV, pochona distemper virus (Genbank X75960); MV, measles virus (Genbank X16659); PPR, rinderpest virus (Genbank X86531); DMV, dog morbillivirus; PMV, porcine morbillivirus; and PPR, peste des petits ruminants. A, Translated amino-acid sequence of lion morbillivirus compared to CDV and PIV.

TABLE 1 Summary of CDV disease in Serengeti lions

<table>
<thead>
<tr>
<th>Criteria indicating CDV infection</th>
<th>Number of affected lions</th>
</tr>
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<tbody>
<tr>
<td>Seizures</td>
<td>12</td>
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<tr>
<td>Myoclonus</td>
<td>15</td>
</tr>
<tr>
<td>Other neurological signs</td>
<td>27</td>
</tr>
<tr>
<td>Lion mortalities</td>
<td>23</td>
</tr>
<tr>
<td>Carcasses recovered</td>
<td>32</td>
</tr>
<tr>
<td>Disappearance from observed</td>
<td>71 of 83</td>
</tr>
<tr>
<td>population of 250 lions</td>
<td></td>
</tr>
<tr>
<td>CDV seroprevalence†</td>
<td>71 of 83</td>
</tr>
<tr>
<td>Lions with histopathological lesions of CDV</td>
<td>18 of 19</td>
</tr>
<tr>
<td>Lions with viral inclusions in tissues</td>
<td>14 of 19</td>
</tr>
<tr>
<td>Lions with CDV nucleocapsid proteins in tissue</td>
<td>14 of 19</td>
</tr>
<tr>
<td>Lions with CDV isolated†</td>
<td>1 of 7</td>
</tr>
<tr>
<td>Lions with CDV RNA obtained by RT–PCR</td>
<td>2</td>
</tr>
</tbody>
</table>

†Numbers designate 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. Spleen extract for viral cultures and molecular analysis were derived from 20 ml blood taken from anaesthetized lions.

I Methods are described in Fig. 3 legend.

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