Canine Distemper Epizootic in Lions, Tigers, and Leopards in North America

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What is This?
Canine distemper epizootic in lions, tigers, and leopards in North America


Abstract. Canine distemper virus (CDV) infection occurred in captive leopards (Panthera pardus), tigers (Panthera tigris), lions (Panthera leo), and a jaguar (Panthera onca) in 1991 and 1992. An epizootic affected all 4 types of cats at the Wildlife Waystation, San Fernando, California, with 17 mortalities. CDV-infected raccoons were thought to be the source of infection in these cats. Two black leopards died at the Naibi Zoo, Coal Valley, Illinois, and 2 tigers died at the Shambala Preserve, Acton, California. Initial clinical signs were anorexia with gastrointestinal and/or respiratory disease followed by seizures. Canine distemper virus was isolated from 3 leopards, 3 tigers, and 3 lions that died or were euthanized when moribund. Monoclonal antibody testing identified the virus isolates as CDV. Gross and histopathologic findings were similar to those found in canids with distemper with a few exceptions. There were fewer lesions in the brain, and there was a pronounced type 2 cell proliferation in the lung, with inclusion bodies and CDV antigen demonstrated by immunohistology. Neutralizing antibody to CDV was found in high titers in serum from most animals but was absent or was found only in low titers in some cats that succumbed after CDV infection. There was a marked difference in neutralizing antibody titers when tests were done with different strains of CDV.

Canine distemper virus (CDV) is a common pathogen in dogs and is well known in canids, mustelids, procyonids, and viverrids. Encephalitis in javelinas (collared peccaries) after natural infection with CDV occurred in Arizona. Experimental infection with CDV in domestic cats and pigs resulted in seroconversion but no clinical disease. Morbilliviruses closely related to CDV have been isolated from epizootics in seals, dolphins, and porpoises.

A major outbreak of canine distemper (CD) in large cats occurred in the fall of 1992 at the Wildlife Waystation, San Fernando, California. Seventeen large old world cats, including lions (Panthera leo), tigers (Panthera tigris), and leopards (Panthera pardus), plus a jaguar (Panthera onca) succumbed to the disease with respiratory, enteric, and central nervous system (CNS) signs. This is the first time that CD has been incriminated in a disease outbreak of such proportion in large cats. Two tiger cubs died from CD in the Shambala Preserve, Acton, California. CDV was isolated from 1 of 2 black leopards that died in the Rock Island Forest Preserve, Naibi Zoo, Coal Valley, Illinois, in December 1991. There are few previous reports of CD in large cats. Canine distemper was diagnosed by fluorescent antibody demonstration of CDV antigen in 2 snow leopards (Panthera uncia) that died following feline panleukopenia infection in the Blank Park Zoo in Des Moines, Iowa, in 1988. CD was also diagnosed in a Bengal tiger by histopathology and serology and in 2 lion cubs by blood transfer into a dog and was suggested in a Siberian tiger.

There are other reports of encephalitis in large cats. A fatal nonsuppurative encephalitis in lions and tigers was observed in a safari park in Germany. It was believed to be caused by an infectious agent that has not been isolated but was found to be unrelated to CDV by serology and immunohistology. A similar encephalitis of unknown origin is seen in domestic cats in the United States and Europe. A paramyxovirus-like agent was associated with demyelinating lesions in the CNS and optic neuritis in cats. This agent has.
not been isolated and a relation to CDV has not been substantiated (Cosby, personal communication).

In this report, we describe an epizootic of CD in large old and new world cats, including clinical signs, pathology, serology, virus isolation, and identification.

Case report

In December 1991, two black leopards (ages 14 and 19 years) died in the Rock Island Forest Preserve, Nai-bi Zoo (Coal Valley, IL). A chronic illness of approximately 1 month duration with anorexia, respiratory distress, seizures, and paraparesis in the final stages were observed in both animals. A jaguar died in the same location following a respiratory illness with terminal CNS signs.

In October 1992, a 4-month-old tiger in the Shambala Preserve (Acton, CA) died after showing clinical signs as described above. This animal was ill before it was transported to the Preserve from an animal shelter. A littermate recovered from respiratory disease. In May 1993, another 6-month-old Bengal tiger developed intestinal, respiratory, and CNS signs, including staggering gait and seizures. The animal was euthanized. At the same time, 4 additional tigers and 1 lion showed intestinal and respiratory signs without CNS involvement. These animals recovered.

Between June and December 1992, a disease outbreak occurred among old world large cats and a jaguar at the Wildlife Waystation, Angeles National Forest (San Fernando, CA). A total of 35 of 74 cats showed respiratory, gastrointestinal, and/or the CNS clinical signs. Seventeen cats succumbed after developing CNS signs, including seizures, ataxia, myoclonus, and coma. The animals included 7 African lions, 1 Siberian tiger, 1 Bengal tiger, and 2 Siberian/Bengal tiger hybrids, 4 leopards, 1 Chinese leopard (Panthera pardus japonica), and 1 jaguar. The age of the animals ranged from 2 to 19 years and both male and female cats were equally involved. Other indigenous North American cats were unaffected.

The animals were housed in outdoor enclosures either singly or in groups of 2-7 cats per enclosure. A mixture of raw meat was fed 5 days/week, and water was available ad libitum. Vaccinations for feline panleukopenia, calicivirus, rhinotracheitis, and rabies\(^a\) had been administered annually since their arrival at the facility. All animals had received booster vaccinations in March 1992. Routine screening for Toxocara canis and \(T.\ catti\) and Toxascaris leonina and treatment if indicated (ivermectin) was performed 2 or 3 times per year.

Clinical disease in the 35 large exotic felines at the Wildlife Waystation displayed two patterns. One group of animals developed an acute onset of CNS abnormalities (6/35), and the second group experienced an insidious onset and progression of clinical signs (29/35). Cats in this group became anorexic and depressed with intermittent diarrhea for 1-2 weeks. Coughing, dyspnea, and ocular or nasal discharge were infrequently observed during this period. Fourteen of these animals recovered with supportive care, including broad spectrum antibiotic treatment. Animals not responding to conservative therapy developed neurologic signs following gastrointestinal or respiratory disease (15/35). Twelve of these animals died or were euthanized when they became moribund. Of the 6 cats with acute onset of CNS disease, 4 cats were found acutely moribund, and 2 underwent a behavioral change characterized as escape behavior and fear. These animals were observed racing around their enclosures and colliding into exhibit fencing. Pronounced mydriasis was also observed during these episodes. Additional CNS abnormalities developed within 1 day or less of the observed behavioral change without the development of any other additional clinical signs. One animal survived.

Generalized seizure activity was the most common CNS disorder regardless of the nature of disease onset. During seizure episodes, animals collapsed into lateral recumbency and displayed chewing motions of the jaw with paddling of all 4 feet. Seizures lasted 1-2 minutes, and 13 of 17 cats had multiple episodes of seizures. After the onset of seizures, most animals became moribund and died within 1-2 days. A few cats survived for up to 2 weeks with longer intervals between seizures before they became comatose and died. Over the 2 weeks preceding death, cats frequently developed mucopurulent ocular and nasal discharge. Partial motor seizures of the limbs were seen in 2 Siberian/Bengal tigers. One animal developed the focal seizures 2 days prior to generalized seizure activity, and the second animal never developed generalized seizures.

During the same time period, an unusually large number of raccoons and skunks died with CD in the area (Hughes, personal communication).

Testing for rabies and for organochlorine and other poisons (arsenic, lead, mercury) was performed on a few animals with negative results. Cerebrospinal fluid analysis and electroencephalogram were negative in the first lion with CNS signs at the Wildlife Waystation. These investigations were terminated when the diagnosis of CD was made. Similar tests were also made on the Illinois leopards. A variety of nonpathogenic bacteria were isolated that were not considered to be involved in the pathogenesis of the disease complex.

Material and methods

Clinical examination

After the onset of seizures, cats at the Wildlife Waystation were immobilized with 2 mg/kg xylazine\(^b\) and 2 mg/kg ke-
tamine for physical examination and treatment. The dosage was decreased for depressed or moribund animals. A total of 25 blood samples were obtained from 11 animals for complete blood cell counts and biochemical profiles. Serology was performed on 22 animals. Heparinized whole blood was obtained from 5 cats for virus isolation from buffy coat cells. Multiple fecal samples were taken for parasitologic examinations.

**Necropsies**

Necropsies were performed on cats that either died or were euthanized when moribund. Two black leopards were necropsied in Illinois and at Iowa State University in December 1991. Five African lions, 3 tigers, 4 leopards, and 1 jaguar were necropsied between September 1992 and January 1993 in California. Gross pathologic findings were recorded, and tissues were fixed in 10% buffered formalin for histopathology and immunohistochemistry. Tissues from 1 Illinois leopard were fixed in gluteraldehyde and processed for electron microscopy. Fresh tissues were submitted for bacterial culture, and additional tissues were frozen at -70°C for virus isolation. Buffy coat culture from heparinized blood was also used for virus isolation. Serum samples were collected for serology.

In addition to large cats, necropsy material from 2 raccoons with clinical signs of CD trapped near the Wildlife Waystation was available for virus isolation and histopathology.

**Immunocytochemistry**

Sections from tissues that showed CDV lesions and inclusion bodies by hematoxylin and eosin (HE) staining were deparaffinized, treated to remove endogenous peroxidase, and incubated with a mouse monoclonal antibody to a CDV-N protein (MAb N3.991). To localize MAb binding to CDV antigen, a commercial avidin-biotin preparation was used. Sections were counterstained within Gill’s hematoxylin, dehydrated, and mounted in Permount. Sections of lung from tissues were fixed in 10% buffered formalin for histopathology and immunohistochemistry. Tissues from 1 Illinois leopard and 1 African lion were used for virus isolation. Buffy coat culture from heparinized blood was also used for virus isolation. Serum samples were collected for serology.

**Virus isolation**

CDV. Isolation of CDV was made in canine blood lymphocytes (CBL) as previously described. CBL were separated on ficoll/sodium diatrizoate from heparinized blood from specific-pathogen-free (SPF) beagles and resuspended in RPMI 1640 medium with l-glutamine plus 20% fetal calf serum at a density of 5 x 10⁶ cells/ml. Cells were stimulated with 15 µg/ml phytohemagglutinin (PHA) and incubated at 37°C in 95% air, 5% CO₂. One or 2 days later, these CBL were cocultivated with an equal number of blood lymphocytes (BL) from diseased cats or they were inoculated with tissue suspensions in RPMI 1640 at a final dilution of 1:50. (Lower dilutions may be toxic for CBL.) Infected CBL were then incubated for an additional 6 days. Three and 6 days after infection, cells were pelleted by cytopsin and examined for the presence of CDV antigen by direct immunofluorescence (IF).

Supernatants from CDV-positive cultures and original tissue suspensions were inoculated into secondary dog kidney (DK) cells and into dog lung macrophage (DLM) cultures. The cultures were examined for cytopathic effects and by IF for CDV antigen 5-8 days later in DK cells and 3-5 days later in DLM cultures.

**Feline viruses.** Blood lymphocytes as prepared above and tissue suspensions were inoculated into cloned Crandell feline kidney (NLFK) cells in an attempt to isolate feline herpesvirus, calcivirus, or parvovirus. Cells were examined daily for cytopathic effects for 1 wk. Cells were then passaged and examined again.

**Retrovirus.** Whole blood samples from a feline immunodeficiency virus (FIV) antibody-positive lion, an FIV antibody-negative lion, and an FIV antibody-negative tiger (all CDV positive) were collected into tubes containing preservative-free heparin. Virus isolation was attempted using techniques similar to those previously described. The BL were separated from other cellular blood components by centrifugation over ficoll/sodium diatrizoate. To localize MAb binding to CDV antigen, a commercial avidin-biotin preparation was used. Sections were counterstained within Gill’s hematoxylin, dehydrated, and mounted in Permount. Sections of lung from tissues were fixed in 10% buffered formalin for histopathology and immunohistochemistry. Tissues from 1 Illinois leopard and 1 African lion were used for virus isolation. Buffy coat culture from heparinized blood was also used for virus isolation. Serum samples were collected for serology.

In replicate negative control sections, MAb for influenza virus replaced the MAb for CDV. Positive control sections were from a confirmed case of CDV encephalitis in a dog.

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were tested and compared with earlier isolates of virulent CDV from a fox (1989) and from dogs (1956 and 1975). Comparison was also made with the attenuated Onderstepoort and Rockborn CDV strains.

Serology

Serum samples from diseased cats that succumbed or recovered were tested for CDV neutralizing antibody against the Onderstepoort strain of CDV adapted to Vero cells as previously described. This strain is routinely used for titration of CDV neutralizing antibody. Using the same microtiter techniques, the serum samples were also tested against the Rockborn strain of CDV and against a Vero cell-adapted CDV isolate from an African lion that died (A92-27/20) (Table 1). The isolated CDV was adapted to Vero cells through 8 passages. Final results with the Onderstepoort strain were recorded after 3 days. In contrast, results with the Rockborn strain and the adapted lion isolate were recorded after 8 days, with fluid changes on days 3 and 6.

Further serum samples were obtained from healthy large cats from the Los Angeles Zoo, from the Ringling Brothers Circus, and from the National Zoo (Washington, DC). Serum samples were received from the Hawthorn Corp. (Chicago, IL) from 5 tigers that had survived an outbreak of severe respiratory and enteric disease 2 yr earlier. One additional sample was taken from a tiger that was added to the colony after the outbreak had occurred.

Sera were also tested by hemagglutination inhibition (HI) for the presence of feline panleukopenia antibody as previously described. Antibody tests for feline leukemia virus (FeLV) and FIV were made by Professional Animals Laboratories (Tustin, CA) and by the Veterinary Diagnostic Laboratory (Urbana, IL).

Serum samples from the Wildlife Waystation were tested for antibody against lymphocytic choriomeningitis (LCM) virus. Sera from the 2 Illinois leopards were tested for antibody to encephalomyocarditis (EMC) virus.

Results

Clinical pathology. Initial blood samples taken after onset of clinical disease revealed a lymphopenia (≤2,000 cells/µl with the lowest count of 155 cells/µl) and a neutrophilia (> 10,000 cells/µl with the highest count at 28,340 cells/µl in all 11 animals sampled. Fecal floating revealed occasional Toxocara. Elevation of muscle and liver enzymes (creatine phosphokinase, aspartate aminotransferase, alanine aminotransferase), blood urea nitrogen, and creatinine were the more consistent clinical pathologic findings.

Necropsies (gross pathology). Necropsies were performed on 13 of 17 large cats in California and on 2 leopards in Illinois. The majority of animals were in excellent body condition. Three animals were underweight because of extended periods of coma or anorexia. The dominant gross postmortem lesions were congestion or consolidated lungs (11 / 13) and congestion of meningeal vessels (7/13). Ocular and nasal mucopurulent discharge was seen in most animals.

Histopathology. On histopathologic examination, significant differences were not seen among different species or among cats from different locations.

Lesions in the brain were focal and only occasionally severe. In most animals, there was a mild nonsuppurative polioencephalitis, lymphocytic meningitis, and mild microgliosis in white matter. Astrocytosis and perivascular cuffing was an exceptional finding. Loss of neurons, slight satellitosis, and neuronal and astrocytic CDV inclusion bodies were seen occasionally, predominantly in the hippocampus and cerebellum (Fig. 1A). One leopard had pronounced ischemic neurons in the pyramidal layer of the hippocampus. One tiger had severe focal cerebellar necrosis with mineralization of the molecular layer. Protozoal cysts were seen in the midbrain of I leopard and I lion.

A diffuse interstitial pneumonia with intra-alveolar giant cells and numerous eosinophilic inclusion bodies
in bronchiolar epithelium was seen in most animals. In a single animal, there was extensive squamous metaplasia of the bronchiolar epithelium. Proliferation of this epithelium produced nodules and tissue within the adjacent lung parenchyma (Fig. 2). In bronchioles showing this metaplastic change, viral inclusion bodies were much more numerous in the squamous cells. In 6/10 animals, prominent alveolar type 2 cell proliferation with intracytoplasmic and intranuclear inclusions was observed (Fig. 3A). Alveolar edema with macrophages in alveoli was frequently seen. PMN infiltration in alveoli and bronchioles and fibrin exudation or hemorrhage were frequent findings. In 2 lions and 1 leopard, the pneumonia was necrotizing and multifocal in association with many protozoal cysts.

Severe lymphoid depletion with viral inclusions in macrophages was noticed in the spleen and lymph nodes from all cats. Syncytial cell formation in the cortex of lymph nodes was seen in some animals; necrosis, edema, and hemorrhage were seen in others. Two cats (1 leopard, 1 lion) had protozoal cysts with eosinophil infiltration in lymph nodes and spleen.

Lesions in other tissues were not consistent. Autolysis of the stomach, intestine, liver, pancreas, and kid-

Figure 1. Brain; leopard. A. Area of the hippocampus contains three syncytial astrocytes and cells with intranuclear viral inclusions (arrows). HE, 350x. B. Brain; lion. Viral antigen in a Purkinje cell and stellate cells of the molecular layer of the cerebellar cortex. Immunohistology, 350 x.
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Figure 2. Lung; tiger. A bronchiole (B) shows diffuse squamous metaplasia and contains many neutrophils. Nodules of epithelium bud from the bronchial wall (arrows) and form islands of squamous epithelium within the parenchyma (asterisks). HE, 180 x.

In all large cats, inclusion bodies were not found in any of these tissues nor in the urinary bladder. Ulceration and gastritis was seen in 1 leopard. Necrosis of the lamina propria of the small intestine occurred in 1 tiger. Sinusoidal and hepatocellular pigmentation was present in the liver of 2 leopards and a lion, and focal areas of necrosis were observed in a tiger, a lion, and a leopard. Protozoal cysts and hepatocellular degeneration were seen in the livers of a leopard (Fig. 4) and 2 leopards. In the kidneys, a mild mesangial thickening of glomeruli and Bowman’s capsule was noticed in some cats; a few mononuclear cell aggregates were seen in the interstitium of the cortex in others. One lion and 1 leopard had acute necrotizing pancreatitis. Multiple foci of nodular hyperplasia were seen in the pancreas of another leopard.

In 2 raccoons, multifocal interstitial pneumonia with areas of suppurative inflammation was most prominent. Intranuclear and intracytoplasmic inclusion bodies were seen predominantly in bronchiolar epithelium and occasional syncytia in alveolar spaces. There were numerous intracytoplasmic inclusions in the urinary bladders of both animals. Brain and lymphatic tissues were not available.

Electron microscopy. Paramyxovirus-like nucleocapsids were seen in bronchiolar epithelial cells from a leopard in Illinois (Fig. 5).

Immunocytochemistry. Immunocytochemical studies of CDV antigen were performed on specimens of 3 brains, 3 lungs, and 2 spleens from large cats. Staining was most consistent in pulmonary tissue, where a common finding was diffuse cytoplasmic staining of hypertrophic alveolar type 2 cells (Fig. 3B). Most staining was cytoplasmic, but occasional intranuclear inclusions were also seen. Scattered staining was also found in bronchiolar epithelium. Staining in the brain was focal. In 1 animal, infection of neurons in the pyramidal cell layer of the hippocampus was conspicuous and was associated with antigen-positive syncytial astrocytes. In another animal, focal infection of the cerebellar cortex affected Purkinje cells and stellate neurons of the molecular layer (Fig. 1B). Unlike the dog, in which neuronal or glial infection is typically widespread in the brain, infection in these cats was limited to such populations. Staining of cells in the red and white pulp of the spleen was seen. Immunocytochemical studies of protozoal cysts with *Toxoplasma gondii* antibody were positive.

In lung sections from 2 raccoons, positive staining was mostly seen in bronchiolar epithelium and occasionally in alveolar cells and spaces.

Virus isolation. Canine distemper virus was isolated in CBL from tissues or BL from 3 leopards, 3 tigers, 3 lions, and 2 raccoons. Tissues from which virus was isolated included lung (3 leopards, 1 lion, 1 raccoon), brain (1 lion, 1 leopard, 1 raccoon), and spleen (1 leopard). Buffy coat cells from heparinized blood were the source of virus from 3 tigers and 1 lion. Uninoculated control cultures remained free of virus after 1 passage in CBL.

The isolated viruses produced syncytia in DLM cultures and did not replicate in secondary DK cells or Vero cells without adaptation. These criteria were previously associated with virulent CDV. By indirect IF testing with MAbs to CDV and PDV, the isolated viruses from large cats were identified as CDV (Fig. 6).

Attempts to isolate feline viruses in NLFK cells by inoculating tissue suspensions or buffy coat cells were unsuccessful. Likewise, attempts to isolate retroviruses from large cat buffy coat cells by cocultivation with domestic cat BL were negative.

The FIV antibody-positive lion BL culture was discarded when no Mg2+-dependent RT activity was detected after 4 weeks of cultivation. The BL from the
other 2 cats were discarded after 2-3 weeks because of extensive cell death in the cultures. No Mg\(^{2+}\)-dependent RT activity was detected in these cultures. A second attempt to culture BL from these cats ended with similar results.

By PCR, positive signals were detected in the FIV antibody-positive lion. Results for tissues from other cats remained inconclusive.

Serology. Four large cats that died or were acutely ill did not have CDV neutralizing antibody. Neutralization of CDV by sera from cats that died or were euthanized was seen in 13/16 animals. However, marked differences in neutralizing antibody titers were found when tests were done with different strains of CDV (Table 1). In 6 large cats, antibody titers were < 1:10 when tested with the egg- and later Vero cell-adapted Onderstepoort strain of CDV, but titers were considerably higher when tested with either a Vero cell-adapted strain of Rockborn CDV or a Vero cell-adapted CDV isolate from a lion (A92-27/20). Titer differences were less marked in sera from other diseased large cats and in cats that had recovered or did not show clinical signs after infection.

Serum samples obtained from healthy large cats in

Figure 3. Lung; tiger. A. Alveolus is lined by hypertrophic type 2 pneumocytes, which contain many intracytoplasmic viral inclusions (arrows). A central multinucleated giant cell is prominent. HE, 560 x. Lung; leopard. B. Hypertrophic type 2 pneumocytes are positive in the cytoplasm for CDV antigen. Immunohistology 560 x.
different locations were also tested by the standard CDV neutralization test. Serum taken from a healthy mountain lion (*Felis concolor*) at the Wildlife Waystation was negative when taken in February 1992 but had a titer of 1:300 when taken in October 1992. Serum from a healthy Asian leopard had a titer of ≥ 1:300 in October 1992. A tiger cub that died at the Shambala Preserve had no neutralizing antibody to CDV, but a littermate that recovered had a titer of 1:800. An additional tiger died with a serum titer of 1:300. Four tigers with mild respiratory and gastrointestinal signs had CDV serum neutralization titers between 1:800 and 1:1,600. CDV was isolated from the blood of an acutely ill lion in the same location, but the serum was CDV antibody negative, and this lion recovered. A tiger, a lion, and a leopard without clinical signs were CDV antibody negative, but 1 tiger with a mammary carcinoma and otherwise no clinical signs had a titer of 1:100. Sera from 2 jaguars, 1 snow leopard, and 1 mountain lion from the Los Angeles Zoo were negative. One mountain lion and 1 tiger from the same location both had CDV antibody titers of 1:5,000. Sera from 7 tigers were obtained from the Ringling Brothers Circus. Five of the 7 tigers had titers between 1:100 and 1:300. One tiger had a titer of 1:30, and 1 tiger was seronegative.

Sera from 5 tigers at the Hawthorn Corp. that had survived respiratory and enteric disease 2 years earlier had CDV antibody titers between 1:1,000 and > 1:10,000. Serum from 1 additional tiger that had been added to the group after the disease outbreak had no CDV antibody.

**Figure 4.** Liver; lion. Focus of hepatocellular necrosis (n). A cluster of *Toxoplasma* organisms can be seen (arrow). HE 350 x.

**Figure 5.** Electron micrograph. Lung; black leopard. Intracytoplasmic viral nucleocapsids, consistent with a paramyxovirus, are located within the bronchiolar epithelial cells Bar = 0.5 µm.
Canine distemper in exotic felids

Anti CDV Monoclonal Antibodies

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Figure 6. Canine distemper virus (CDV) in acetone-fixed canine blood lymphocytes was tested by indirect immunofluorescence using a panel of mouse anti-CDV monoclonal antibodies (MAb) (Anti CDV Monoclonal Antibodies) and mouse anti-phocine distemper virus (PDV) MAb (Anti PDV Monoclonal Antibodies). MAbs were directed against viral nucleoprotein (N), polymerase (P), fusion glycoprotein (F), and hemagglutinin glycoprotein (H). Five CDV isolates from lions, tigers, and leopards from San Fernando and Acton.

Sera from 65 cheetahs (Acinonyx jubatus), 55 leopards, 20 lions, 17 tigers, 16 leopard cats (Felis bengalensis), 13 bobcats (Felis rufus), 12 servals (Felis serval), 8 Geoffreys cats (Felis geoffroyi), and 12 domestic cats from the National Zoo (Washington, DC) collected between 1985 and 1993 all were free of CDV antibody. However, sera from 2 jaguars in the same location had CDV antibody titers of 1:100 and 1:200. Sera of all animals tested were free of antibody to FeLV, and only 1 lion that died had antibody to FIV. Sera from all animals had positive HI antibody titers against feline parvovirus. Sera from large cats from the Wildlife Waystation were free of antibody to LCM virus. Sera from the 2 leopards from Illinois were free of antibody to EMC.

Discussion

Canine distemper virus is a known pathogen of an extremely wide range of animal species. The populations of susceptible animals seem to be expanding. In recent years, CDV and closely related morbilliviruses have been found unexpectedly in fatal disease outbreaks in seals, dolphins, and porpoises and in javelinas (collared peccaries). We are now reporting the first major outbreak of disease caused by CDV in large Old and New World cats.

Large cats have lived in the wild and have been kept in zoological parks for centuries in fairly close contact with canids, mustelids, and procyonids, which have experienced frequent outbreaks of distemper before modified live vaccines became available. Yet, CD in large cats has not been diagnosed. There are only 4 reports in the literature describing possible CD in single tigers, in 2 snow leopards, and in 2 lion cubs. Virus isolation to confirm the diagnosis were not made.

There are several possibilities that might explain the CDV outbreaks that occurred in 1991 and 1992.

First, it could be argued that the holding facilities for large cats in wildlife parks today are different from the typical isolation quarters in zoological gardens. Closer contact with CDV-infected raccoons and other wildlife may allow greater risks of exposure.

Second, a mutation of CDV could have occurred that made a new variant of CDV virulent for large cats, similar to the occurrence of PDV that was found in seals or to the feline parvovirus that mutated to be...
come virulent for dogs. In our MAb studies, we found no evidence for that assumption. Six CDV isolates from large cats and another from a raccoon that died from distemper in the same area had MAb profiles of classical CDV (Fig. 6). The viral isolates were identical to earlier isolates of CDV from dogs and from wildlife. However, remarkable biological differences can occur among CDV strains that have the same MAb pattern as, for example, the Snyder Hill strain, a highly virulent virus, and the Onderstepoort strain, an attenuated virus (Fig. 6). Therefore, the possibility of a new “bio-
type” cannot be ruled out. Comparison of the viral RNA sequences from the different CDV isolates has not yet been made.

The characteristics of the CDV isolates were those of virulent virus. They replicated in dog lymphocyte and macrophage culture (with syncytial formation) but they did not replicate in first passage in secondary dog kidney cells.\(^{1,15}\)

Because of the increased number of fatal cases of CDV infection in raccoons and skunks in the area around the California wildlife park (Hughes, personal communication), we hypothesize that virulent CDV was transmitted from infected raccoons to large cats. There may have been a similar source of infection in the Shambala Preserve and in the Illinois outbreak.

Transmission of virulent CDV to domestic cats did not produce disease in the past. Experimental infection of domestic cats with CDV that was virulent for dogs produced subclinical infection that was restricted to lymphatic tissues. The virus was not shed to uninfected cats in close contact.\(^{6}\) In contrast, cat-to-cat transmission probably has occurred in the recent outbreak that produced fatal disease. Why now and not in the past? There may be differences in CDV susceptibility between domestic cats and large cats. However, we have not ruled out the possibility of a virus mutation.

A third possible explanation would be dual infections, where one agent (as for example FIV or FeLV) would induce immunosuppression allowing the second infection with CDV to become virulent. We have searched for antibody or agents in addition to CDV that might support this hypothesis but were not successful. Antibody to FIV was found in only 1 lion, and virus isolation attempts from blood lymphocytes from that animal were negative, although PCR results were positive. The difficulty in maintaining some of the large cat lymphocyte cultures may have been the result of CDV infection, in spite of the presence of anti-CDV antibody. Isolation of FIV from large cats appears to be difficult, and the agent(s) differs somewhat from FIV of domestic cats.\(^{7,8,22}\) Antibody to FeLV was not found in any of the cats. There was antibody to parvovirus in all cats as the result of vaccination. Dual infection with parvovirus and CDV was reported in 2 snow leopards in 1989.\(^{16}\) However, virus isolation attempts of feline parvovirus or other known feline agents were negative in our study.

It is possible that a retrovirus or another unknown virus is present in these cats but cannot be detected by conventional methods. A lentivirus isolate from a mountain lion was found to be different from FIV,\(^{24}\) and considerable differences among FIV isolates have been reported.\(^{20}\) Inconclusive results have been obtained by PCR in large cats in our study.

The most remarkable lesion seen in most animals on histopathologic examination was a diffuse alveolar type 2 cell hyperplasia with intracytoplasmic and intranuclear viral inclusions. Immunohistochemical evaluation revealed a strong response to CDV antigens in the type 2 cells. The diffuse and pronounced type 2 cell hyperplasia has not been seen in distemper in canids or other species known to be susceptible to CDV. This lesion could reflect an unusual host response to a standard virus or it could be the result of mutant CDV. Because this lesion was not found in raccoons with distemper in the same area, the former explanation appears to be more likely. There were also metaplastic and proliferative changes in the bronchial epithelium very similar to the changes reported for measles pneumonitis in humans.\(^{10}\)

As always seen in canine distemper, there was a severe lymphoid depletion with viral inclusions and sometimes giant cells in the spleen and lymph nodes of all cats examined.

The lesions seen in brain tissue of most cats were mild and patchy in comparison to the lesions seen in canids. Mild polioencephalitis with microgliosis and lymphocytic meningitis were most frequently seen. Neuronal degeneration with viral inclusions were seen in a few cases. Vascular cuffing and white matter astrocitosis with demyelination, as frequently seen in distemper in canids, was missing in the cats. There were no consistent significant lesions in other tissues of the affected cats.

Toxoplasma gondii cysts were found in the lungs of 3 cats, lymph nodes of 2 cats and brains of 2 cats in association with focal necrosis. Activation of persistent Toxoplasma infection secondary to CDV infection is well recognized in the dog.

Failure of some moribund cats to develop neutralizing antibody to CDV was not surprising. Similar observations have been made in diseased dogs and mus-telids.\(^{2}\) High CDV neutralizing antibody titers in both moribund or dead and surviving cats support the diagnosis of distemper.

When serum neutralizing antibody titers to an attenuated strain of CDV (Onderstepoort) were compared with titers to CDV isolated from a lion in California, it became apparent that “early” antibody titers...
from animals with acute disease varied considerably, whereas antibody titers from animals later in the disease course or from recovered animals were similar. These test results indicate that the cat CDV isolates may be different from conventional CDV. However, when the same comparison was made with another attenuated CDV strain (Rockborn) that had the same MAb pattern as the lion isolate (Fig. 6), little difference in antibody titers was found.

The findings for early and late antibody are not uncommon in other virus infections. For example, when dogs are inoculated with canine adenovirus type 1 or 2, serum collected 5 days after inoculation neutralizes only the inoculated virus. When serum is collected after 8 days, it neutralizes both viruses (M. J. G. Appel, unpublished data).

Some serum samples from clinically normal exotic felids had CDV antibody, which suggests that not all large cats that become infected with CDV develop disease. High CDV antibody titers in tigers from the Hawthorn Corp. that had recovered from a disease outbreak suggest that CDV was involved in the disease outbreak. CDV neutralizing antibody was found in 6 of 7 tigers from the Ringling Brothers Circus. None of these animals had been vaccinated for CDV. The seventh tiger was born after an episode of gastrointestinal disease about 6 years ago in the other 6 tigers. He was the only seronegative animal. Because all 7 tigers were kept in close contact for the past 6 years, it is unlikely that the 6 tigers became infected after the seventh tiger was born. It can be assumed that serum antibody titers persisted in the 6 tigers over that time period but that chronic shedding of CDV did not occur.

The high percentage of CDV antibody positive large cats in the Hawthorn and Ringling Brothers collection prompted testing of a large collection of sera from large cats in North American zoos. Only sera from 2 jaguars in a collection of more than 200 sera taken from exotic felids between 1985 and 1993 were CDV antibody positive, suggesting that zoo animals are better protected from contact with CDV-infected carnivores than are circus or wildlife park animals.

Animals at the Wildlife Waystation included lions, tigers, leopards, jaguars, mountain lions, bobcats, a serval cat, and a margay (Felis wiedii). Seizures and death after CDV infection was seen only in Old World cats and in 1 jaguar, not in indigenous large cats or the smaller exotic cats. However, several mountain lions had mild signs (diarrhea, anorexia, coughing), which may have been from CDV infection. Unfortunately, only a limited number of serum samples prior to the outbreak were available. It would have been of interest to test whether the indigenous cats had been exposed to CDV previously and were immune before the disease outbreak or whether they were subclinically infected with the same strain of virus. Indigenous cats may be intrinsically more resistant to CDV than are the Old World cats. Evidence for CDV resistance was found in 1 mountain lion, which was seronegative in February 1992 and seropositive in October 1992. Another mountain lion was still seronegative in October 1992.

This is the first report of multiple mortalities in exotic felids in which CDV infection has been confirmed. This infection must now be considered in large cats with respiratory, gastrointestinal, and CNS disease. Serologic studies indicate that some animals have subclinical infections. CDV may be the basis for some unexplained mortalities in zoo cats. The epidemiology of these infections remains unexplained. Vaccination may be advised, but killed virus preparations may be safer until modified live products have been tested in these animals. Because chick embryo-adapted CDV vaccines are in general safer than dog tissue culture-adapted CDV vaccines, the egg vaccines should be used for testing safety and efficacy in large cats.

Addendum. At the time that this article was going to press, we confirmed an epizootic of canine distemper virus infection in lions in the Serengeti, Tanzania.

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Sources and manufacturers

a. Fel-O-Vax PCT or PCT-R, Fort Dodge Laboratories, Fort Dodge, IA.
b. AnaSed, Lloyd Laboratories, Shenandoah, IA.
c. Ketaset, Aveco Co., Fort Dodge, IA.
d. ZYMED KPL Kit, Zymed Labs, San Francisco, CA.
e. Histopaque 1077, Sigma Chemical Co., St. Louis, MO.
f. Sigma Chemical Co., St. Louis, MO.
g. Shandon, Pittsburgh, PA.
h. GeneAmp, Perkin Elmer Cetus, Norwalk, CT.
i. Thermal Reactor, Hybaid, Middlesex, U.K.
k. Donated by Claes Orvell, Karolinska Institute, Stockholm, Sweden.

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