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Viral Distemper Virus in Domesticated Cats and Pigs

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SUMMARY

Twenty-two cats and 17 pigs, ranging in age from 2 days to 2 years, were found to be susceptible to intranasal inoculation of virulent canine distemper (CD) virus. Virus replicated in lymphatic tissues and in macrophages, but not in surface epithelium or brain. Infection with CD virus in cats and pigs was inapparent. Pathologic changes were mild and were restricted to lymphatic tissues and lungs. Intracerebral inoculation did not result in clinical signs or pathologic changes in the brain. Virus spread from infected dogs to cats, but not from infected cats to dogs, cats to cats, or pigs to pigs. Serum samples from 14 of 150 cats had neutralizing antibody to CD virus.

Canine distemper virus has a wide host range under natural and experimental conditions. The natural host range includes animals of the order Carnivora, suborder Fissipeda or land carnivores, with variations in animals among the families. Until recently, animals in the family Felidae were thought to be resistant to CD virus. Piat, in 1950, observed CD in two 4-month-old lion cubs which came in contact with a dog affected with CD. Injection of blood of the lion cubs into susceptible dogs caused signs of CD. Chicken embryos, mice, hamsters, monkeys, and man also have been reported to be susceptible to CD virus after artificial exposure. Benner inoculated 2 pigs with a virulent strain of CD virus. Both pigs developed swine influenza-like signs 5 days after exposure. The purpose in the present report is to confirm the susceptibility of cats and pigs to CD virus.

Materials and Methods

Virus—Canine distemper virus, Snyder Hill strain, was passaged in specific-pathogen-free Beagle dogs. Thymus and spleen were harvested from a dog 5 days after intravenous inoculation of log₅ median tissue culture infective doses (TCID₅₀). A 20% suspension of this thymus and spleen in medium 199 was clarified by centrifugation, and the supernatant was frozen at -70°C in 1-ml amounts. This viral stock suspension contained CD virus at concentration of log₅ TCID₅₀/ml.

Viral Isolation and Titration—Viral isolations and titrations were made in canine lung macrophage cultures as previously described, with minor modifications. Bovine fetal serum was used, and culture fluids were changed 1 day after seeding of cells before they were inoculated with viral suspensions.

Lung macrophage cultures also were prepared from lungs of noninfected and from CD virus-infected cats and pigs. Cultures from noninfected 1- to 2-week-old animals were inoculated with 10³ TCID₅₀ of stock virus for viral replication tests in these cells.

Serologic Tests—Serum samples from experimental cats and pigs were tested for CD virus-neutralizing antibody in microtitration plates as previously described. In addition, serum samples from 150 cats originating in New York and California were tested by the same technique.

Serum samples from experimental cats were tested for the presence of precipitating antibody against feline syncytia-forming virus as previously described.

Inoculation of Cats—Mixed-breed cats were obtained from the Ithaca, NY, area and from the cat colony at the New York State Veterinary College. Nine 2-day-old kittens, ten 6- to 8-week-old kittens, and three 1- to 2-year-old cats were intranasally inoculated with 1 ml each of CD virus stock suspension and studied under isolation conditions. Necropsies were done on postinoculation (PI) days 5, 7, 9, and 21, as indicated (Table 1). Two additional 6-week-old cats were anesthetized, and each was intracerebrally inoculated with 0.2 ml of CD viral stock suspension. These 2 cats were killed for necropsy examination on PI day 21 (Table 1).

Three 6-week-old cats and two 8-week-old Beagle dogs were placed in contact with four 7-week-old cats that had been exposed to CD virus 2 days earlier. Four 8-week-old cats were placed in contact with two 6-week-old Beagle dogs that had been exposed to CD virus 2 days earlier (Table 2).

All cats and kittens used in experimental inoculation studies were free of CD virus-neutralizing antibody before study.

Inoculation of Pigs—Yorkshire pigs and African minipigs from the specific-pathogen-free colony at the Veterinary Virus Research Institute were used. Seven 2-day-old Yorkshire pigs, six 6- to 8-week-old Yorkshire pigs, and four 2-year-old African minipigs were each intranasally inoculated with 1 ml of CD viral stock suspension and studied under isolation conditions. Necropsies were done on PI days 5, 6, 7, 10, 14, and 21, as indicated (Table 3). Two 6-week-old Yorkshire pigs were anesthetized, and each was intracerebrally inoculated with 0.2 ml of CD viral stock suspension; they were necropsied on PI day 21 (Table 3).

Three 3-day-old pigs were placed in contact with three 1-day-old pigs that had been inoculated with CD virus on the previous day. One of these 3 pigs was necropsied 6 days after contact exposure; the other 2 pigs were necropsied 21 days after contact exposure.

Viral Passages—Viral passages in 6- to 8-week-old cats and Yorkshire pigs were made by intranasal inoculation of 1 ml of 20% spleen suspension in medium 199 from each
cat or pig on PI day 5. Three serial passages were made in each species, and viral titrations of tissues harvested at PI day 5 were compared.

**Fluorescent Antibody Staining of Viral Antigen**—Frozen samples of spleen, thymus, mesenteric lymph node, lung, cerebellum, and urinary bladder from cats and pigs collected at PI days 5 and 6 were sectioned and stained with CD virus fluorescent antibody (FA) as described. Canine distemper virus hyperimmune serum was prepared in dogs. Conjugation of hyperimmune serum with fluorescein isothiocyanate was made according to the method of The and Feltkamp.

Cultured lung macrophages from CD virus-infected cats and pigs prepared at PI day 5 were stained with FA 2 or 3 days after preparation. Lung macrophage cultures from noninfected cats and pigs were infected with $10^8$ TCID$_{50}$ of stock virus and stained with FA 2 or 3 days later.

**Histopathologic Studies**—Pieces of spleen, thymus, lymph node, lung, kidney, and intestine were fixed in 10% buffered formalin and stained with hematoxylin and eosin.

**Results**

**Clinical Signs**—The only clinical sign observed in cats experimentally exposed to CD virus was slight increase in body temperature (maximally 1 degree C) in all young and mature cats between PI days 4 and 7. Temperatures of 2-day-old kittens were not tested. A similar temperature increase without other clinical signs was also seen in 6- to 8-week-old and 2-year-old pigs. Two of the three 1-day-old pigs were more severely affected. All CD virus-exposed 2-day-old pigs developed clinical signs of pneumonia, signs not seen in noninfected or in littermates placed in contact with infected pigs. One inoculated pig died of bronchopneumonia 10 days after exposure.

**Viral Isolation and Titration**—Canine distemper viral isolations and titrations from experimentally infected cats and pigs are summarized (Tables 1 and 3). Virus was isolated only from lymphatic tissue and lung, but not from brain or urinary bladder epithelium. Highest viral titers were found in spleen, thymus, and mesenteric lymph nodes from newborn and weaned cats and pigs at PI days 5 and 6. The titers ranged from $\log_{10}$1.7 to $\log_{10}$4.8 TCID$_{50}$. The highest lung titer was $\log_{10}$4.5 TCID$_{50}$. At PI day 7, viral titers were greatly reduced or absent in all cats and in weaned pigs. Higher viral titers persisted in two 1-day-old pigs at PI day 7, but virus was not detected at PI day 10 or 14. One 2-year-old cat and pigs were only tested at PI days 5 and 6. Distribution of viral in these cats and pigs was the same as that in younger groups, but viral titers were lower.

In 3 serial passages in weaned cats and pigs, viral titers of spleen remained between $\log_{10}$3.5 and $\log_{10}$4.5 TCID$_{50}$ at PI day 5.
Virus was not isolated from cats and pigs placed in contact with infected animals.

**Distribution of Viral Antigen by FA Method**—Canine distemper viral antigen was seen in all spleen, lymph nodes, and mesenteric lymph nodes from experimental cats and pigs at day 5 or 6. Viral antigen was located in scattered groups of cells, predominantly located in and around spleen corpuscles and lymph follicles and in the medulla of the thymus. In lungs, only a few cells with viral antigen were found in peribronchial lymph follicles and around alveoli, but not in bronchial epithelium. At day 7 or later, viral antigen was found only in the same locations in tissues from newborn pigs. Viral antigen was not seen at any time in brain or urinary bladder.

**Susceptibility to CD Virus of Feline and Porcine Lung Macrophages**—When examined with FA, feline and porcine lung macrophage cultures from cats and pigs at day 5 contained scattered single cells and very few fused cells with CD viral antigen. Similarly, when lung macrophage cultures were prepared from noninfected cats and pigs and these cultures were inoculated with CD virus, single cells and a few small syncytia were seen with viral antigen 2 or 3 days later. However, when supernatant fluids from these cultures were passaged to freshly prepared cultures from noninfected cats and pigs, syncytia or viral antigens were not found.

**Macroscopic Lesions**—The thymus was markedly small in newborn cats and pigs 5 to 9 days after inoculation of CD virus. A gelatinous appearance of the thymus was noticed in some animals. The smallness of the thymus was also noticed in weaned cats and pigs, but was not as pronounced. Newborn pigs developed bronchopneumonia with secondary bacterial infections which were not seen in noninfected littermates and littermates placed in contact with infected pigs. Gross lesions were not observed in lung or other tissues of weaned or adult cats or pigs after CD viral infection.

**Microscopic Lesions**—Microscopic lesions after CD viral infection in cats and pigs were restricted to lymphatic tissues and lung. Changes varied in severity among animals, but were more pronounced in newborn cats and pigs than in older animals. In newborn cats and pigs examined at day 5, there was interlobular edema in the thymus, with proteinaceous exudation. In lobules, there were degeneration and moderate depletion, of lymphocytes, with an irregular network of primitive reticulum-type cells and histiocytic cells remaining. Lesions were minimal to absent in weaned adult cats and pigs. In spleen and lymph nodes of newborn and weaned cats examined histologically at day 5 or 6, there were focal necrotic areas in lymphoid follicles. These changes were minimal in adult animals. Pulmonary lesions were evident in all animals examined at day 5 or 6. In newborn pigs, there was pneumonitis characterized by proliferation of pneumocytes and leukocytic infiltration. Bronchitis and bronchiolitis was a prominent feature in these pigs. In older pigs and in cats of all ages examined, there was patchy thickening of alveolar septums with a few pneumocytes scattered in some alveoli. Inclusion bodies were not demonstrated in tissues examined.

**Immune Response**—The immune response of cats and pigs to CD virus was tested by serum-neutralization procedures (Tables 1–3). In weaned cats and pigs, low serum-neutralizing antibody titers were found at day 7, and high titers, at day 21. In newborn animals, only traces of antibody were seen at day 7, with increasing titers at day 9 and later.

### Table 3—Canine Distemper Viral Isolation and Titration of Tissues from Pigs Inoculated with Canine Distemper Virus

<table>
<thead>
<tr>
<th>Pig identification number (post-inoculation day)</th>
<th>Necropsy day</th>
<th>Spleen</th>
<th>Thymus</th>
<th>Mesenteric lymph nodes</th>
<th>Lung</th>
<th>Brain</th>
<th>Urinary bladder</th>
<th>Neutralizing antibody in logs</th>
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<td>2.5</td>
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<td>P70-5/1</td>
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<td>1.8</td>
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<td>2.3</td>
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<td>NT</td>
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</tbody>
</table>

Pigs were intranasally inoculated, except for pigs 7/1 and 7/2 which were intracerebrally inoculated and pigs P70-2/2, 5/6, and 3/6 which were placed in contact with CD virus-infected pigs.

* Died.
Serum-neutralizing antibody to CD virus was found in 14 of 150 cats tested. Neutralizing antibody titers ranged from $-\log_{10} 2.1$ to $-\log_{10} 3.4$, with an average of 2.2.

The presence of precipitating antibody to feline syncytia-forming virus was independent of neutralizing antibody to CD virus. Only 2 of the adult cats (2-year-old cats C70-2/0 and 4/0) and the newborn litter of 1 of these cats (C70-2/1, 2/2, and 2/3) had precipitating antibody to feline syncytial virus, but lacked neutralizing antibody to CD virus. None of the cats which developed neutralizing antibody to CD virus developed precipitating antibody to feline syncytia-forming virus.

Discussion

Viral CD virus produced an infection in pigs and cats that resembled infection of dogs with attenuated CD virus. In both instances, macrophages and lymphatic tissues became infected and virus replicated, but in contrast to virulent CD virus infection in dogs, surface epithelium and brain did not become infected. Infected cells in the lungs seemed to be lymphocytes and macrophages. Virus was not shed, therefore precluding transmission. Virulent CD virus did not spread from infected cats to dogs, cats or pigs to pigs, since CD virus could not be recovered from contact animals, nor did neutralizing antibody develop. Cats and pigs, therefore, could not have any role in the transmission of CD virus. Susceptible cats placed in contact with infected dogs, however, did become infected. Canine distemper viral transmission from dogs to cats appears naturally. Of 150 cats tested in the present study, 10% had neutralizing antibody to CD virus.

Virus produced in lymphatic tissues of cats and pigs was infective, as demonstrated by 3 serial passages in each species. Viral titers in spleen tissue did not decrease after 3 serial passages.

Cultured lung macrophages of cats and pigs were susceptible to virulent CD virus; however, only a few cells became infected in contrast to multiple infections in canine lung macrophage cultures. Serial passages in feline and porcine macrophage cultures were not accomplished. A difference in macrophage susceptibility to virulent and attenuated virus has been observed earlier. However, the limited viral replication in feline and porcine lung macrophages cannot be explained by differences in viral strains, since only one virulent strain was used in the present studies. It may be explained by a limited susceptibility of macrophages from the different species. A similar limitation in vivo may contribute to restricted infection in cats and pigs. Infection of surface epithelium in dogs has been found to be preceded by the presence of viral antigen in histiocyte-type cells in adjacent connective tissue. Similarly, brain infection was preceded by the presence of viral antigen in meningeal macrophages.

Viral CD viral infection in cats and pigs produced apparent infections. The slight temperature increase in cats and pigs after inoculation of virulent CD virus was similar to the temperature reaction of dogs after inoculation with certain strains of attenuated CD virus. Thymic depletion of lymphocytes and necrotic foci in spleen and lymph nodes were mild in most experimental cats and pigs after CD viral exposure. The bronchopneumonia which developed in newborn pigs after CD viral infection has been diagnosed as a bacterial infection, but bacterial isolation was not attempted. Since this bronchopneumonia was found only in CD virus-inoculated pigs, but not in noninoculated littermates, it can be assumed that the CD viral infection contributed to the pathogenesis of bronchopneumonia. Canine distemper virus apparently did not affect the respiratory epithelium, but it may have affected the alveolar macrophages.

Canine distemper virus-neutralizing antibody in cats and pigs appeared earlier than that in dogs. Antibody was first detected at 37 days in intranasally inoculated dogs and at 37 days 7 in cats and pigs.

A possible relationship between CD virus and feline syncytia-forming virus was tested in order to rule out the possible influence of feline syncytia-forming virus on CD viral syncytia formation in canine lung macrophages and on the development of CD virus-neutralizing antibody. Since antibodies to CD virus and to feline syncytia-forming virus seemed independent of each other, the CD viral infection of cats and pigs apparently was not influenced by feline syncytia-forming virus.

Naturally occurring and experimentally induced CD viral infections, resulting in clinical signs and pathologic conditions of infection, can occur in a wide variety of species. Apparently, CD viral infections without clinical signs can occur in additional species, indicating an even wider host range exists.

References