Seroprevalence of Canine Distemper Virus in Cats

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A seroepidemiological survey of canine distemper virus (CDV) infection in Asian felids revealed that the prevalence of antibodies varied depending on region and, in some cases, exposure to dogs. The serologic pattern in cats with antibodies indicated that they had likely been exposed to field strains rather than typical CDV vaccine strains.

Canine distemper virus (CDV) is a member of the genus Morbillivirus of the family Paramyxoviridae. Affected dogs show severe immunosuppression and gastrointestinal and respiratory signs and frequently develop disorders in the central nervous system (2, 13). Increasing numbers of cases of typical canine distemper even in vaccinated animals suggest the emergence of CDVs with different antigenic properties from the vaccine strains (4, 7, 11, 17, 19). Recently, we have reported the genetic and antigenic diversities between vaccine strains and field isolates from dogs in Japan (10).

CDV has a wide host range under natural and experimental conditions (2). However, although Appel et al. (1) reported the susceptibility of domestic cats to CDV infection by experimental infection, the animals in the family Felidae, including domestic cats, have long been thought to be resistant to natural CDV infection until some researchers reported the prevalence of CDV infection in large felids (8). Blythe et al. (5) reported chronic encephalomyelitis caused by CDV in a tame Bengal tiger. Subsequently, apparently unrelated enzootic outbreaks of CDV infections among large African felids have been noted in different zoos in the United States (3). Recently, an epidemic of fatal neurological diseases caused by CDV emerged in the lion population of the Tanzanian Serengeti National Park (16, 18). On the other hand, the prevalence of CDV in Asian wild felids remains obscure, and there are limited reports about naturally occurring morbillivirus infections in domestic cats. In the present study, we investigated the seroprevalence of CDV in leopard cats (Felis bengalensis) and domestic cats in Asian countries.

B95a cells, an adherent derivative of B95-8 cells (12), were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum and antibiotics. A recent field isolate of CDV from Japan, Yanaka strain (10), was used in this study. The Ondersteopoort strain was also used as a standard vaccine strain.

Plasma samples were taken from nine leopard cats in Vietnam and two leopard cats and four gem-faced civets (Paguma larvata) in Taiwan as reported previously (9). All 15 animals were apparently healthy at the time of sampling. Plasma samples from Taiwanese and Vietnamese domestic cats have been reported elsewhere (14, 15, 20). Plasma samples taken from Japanese domestic cats in 1970 to 1990 were reported previously (6). Plasma samples from Japanese domestic cats in 1998 were collected at a veterinary hospital in Tokyo.

To detect the virus-neutralizing (VN) antibodies in plasma samples, one-point VN assays were performed. Plasma samples were heat inactivated at 56°C for 30 min, and 50 μl of 10-fold-diluted samples were mixed with 25 50% tissue culture infective doses (TCID50) of Yanaka strain (50 μl) in quadruplicate. These mixtures were incubated at 37°C for 30 min, and 50 μl of uninfected B95a cells (2 × 104 cells/ml) was added to the mixtures. Seven days after cultivation, VN antibody activities were detected as protection of the cells from typical cytopathic effects (CPEs). For further characterization of the plasma samples, cross-VN assays using a typical vaccine strain and a recent field isolate were performed. Fifty microliters of serially diluted plasma samples (1:10 to 1:1,280) were mixed with 25 TCID50 of Yanaka or Ondersteopoort strain (50 μl) in duplicate. These mixtures were incubated at 37°C for 30 min, and 50 μl of uninfected B95a cells (5 × 104 cells/ml) was added to the mixtures. Seven days after cultivation, VN antibody titers were determined and defined as the reciprocal of the highest plasma dilution that protected the cells from showing CPEs.

Detection of antibodies against CDV in leopard cats and civets. Plasma samples from 11 leopard cats and 4 gem-faced civets were tested. By the one-point VN assay, one leopard cat in Taiwan was shown to have VN antibodies, and no VN activity was detected in the other 14 animals (Table 1).

Detection of antibodies against CDV in domestic cats in Taiwan and Vietnam. Plasma samples from domestic cats were examined by the one-point VN assay. The CDV-seropositive rates in certain areas in Taiwan in 1993 to 1994 and in 1998 ranged from 0 to 33.3% and 0 to 88.8%, respectively, and the
overall positive rates were 14.7% (11 of 75) in 1993 to 1994 and 28.1% (9 of 32) in 1998 (Table 1). As shown in Table 1, no relationship was observed between the CDV-seropositive rate and the physical condition of the cat populations. The seroprevalence, however, varied greatly depending on geographical location in Taiwan. When the 69 samples from Vietnam in 1997 were examined, all samples were shown to be CDV seronegative (Table 1).

**Retrospective survey of CDV seroprevalence in domestic cats in Japan.** To examine CDV infection in Japanese domestic cats retrospectively, 100 samples collected from 1970 to 1990 were tested by the one-point VN assay. As shown in Table 2, one plasma in 1982 in Sapporo was shown to have antibodies against CDV. On the other hand, no CDV-positive sample was detected among 84 plasma samples taken in Tokyo from 1970 to 1990 (Table 2). In contrast, when the 38 samples from a veterinary hospital in Tokyo in 1998 were examined, four animals were registered as positive and the positive rate was 10.5% (4 of 38) (Table 2).

**Comparison of clinical findings between seropositive and seronegative cats.** To examine possible CDV-induced diseases in domestic cats, clinical findings were compared between the four CDV-seropositive and the 24 CDV-seronegative cats in the veterinary hospital in 1998. Although the number of samples is small, the number of anemic cats was much higher in the CDV-seropositive cat group (3 of 4) than in the seronegative cat group (6 of 24) (data not shown). However, it was revealed that all three CDV-seropositive and anemic cats had been blood-transfused several times from healthy cats kept as blood donors in the hospital. In contrast, only one of the six CDV-seronegative and anemic cats had a history of blood transfusion in the hospital. These facts suggested the possibility that the seroprevalence of CDV observed in the hospital could be due in part to the blood transfusion. For further analysis of the relationship between CDV infection and blood transfusion, the CDV-seropositive rates among cats kept in that hospital were examined, and four of the nine cats, which were kept separately in cages as blood donors, were shown to be CDV seropositive. When the other three cats, which were infected with *Haemobartonella felis* and kept separately in cages with caged healthy dogs in the same room, were examined, all three cats were shown to have antibodies against CDV.

**Cross-VN assays using Onderstepoort and Yanaka strains.** To examine the titers of VN antibodies against Onderstepoort and Yanaka strains in the plasma samples, cross-VN assays were performed. Cross-VN assays using the 12 Taiwanese samples including the plasma from a leopard cat revealed that the VN activities against Yanaka strain were generally higher than those against Onderstepoort strain (Table 3). Similar results were also observed in most plasma samples from cats in Japan.

| TABLE 1. Detection of VN antibodies against CDV in civets, leopard cats, and domestic cats in Taiwan and Vietnam |
|-------------------------------------------------|---------|--------|-----------------------|-----------------|-----------------|-----------------|
| Animal   | Yr     | Area   | Source   | No. of animals tested | Physical condition | No. (%) positive animals |
|----------|--------|--------|----------|---------------------|--------------------|-------------------------|
| Civet    | 1994   | Taichung | BC | 4 | Healthy | 0 (0.0) |
| Leopard cat | 1989 | Nantou | Captive | 1 | Healthy | 1 (100.0) |
|          | 1994   | Nantou | Captive | 1 | Healthy | 0 (0.0) |
|          | 1997   | HCMC   | Captive | 1 | Healthy | 0 (0.0) |
|          | 1997   | Hanoi  | Captive | 3 | Healthy | 0 (0.0) |
|          | 1997   | Hanoi  | Free-ranging | 3 | Healthy | 0 (0.0) |
| Domestic cat | 1993 | Taichung | Hospital A | 32 | Sick | 2 (6.3) |
|          | 1993   | Taichung | Hospital B | 13 | Sick | 0 (0.0) |
|          | 1993   | Chan-Hua | BC | 6 | Sick | 1 (16.7) |
|          | 1993–94 | Tam-Sui | Shelter | 24 | Healthy | 8 (33.3) |
|          | 1998   | Taipei | Shelter | 6 | Healthy | 0 (0.0) |
|          | 1998   | Tam-Sui | Shelter | 11 | Healthy | 9 (88.8) |
|          | 1997   | Hanoi  | RS | 69 | Healthy | 0 (0.0) |

*a* Taichung, Nantou, Chang-Hua, Tam-Sui and Taipei are in Taiwan, and Hanoi and Ho Chi Minh City (HCMC) are in Vietnam.

*b* BC, breeding cattery; RS, random sources (mainly collected from animal markets).

| TABLE 2. Detection of VN antibodies against CDV in domestic cats in Japan |
|-------------------------------------------------|--------|--------|----------|-----------------|-----------------|
| Group   | Yr     | Area   | Source   | No. of animals tested | Physical condition | No. (%) positive animals |
|---------|--------|--------|----------|---------------------|--------------------|-------------------------|
| 1       | 1971–75 | Tokyo | Institute A | 8 | Healthy | 0 (0.0) |
| 2       | 1978   | Tokyo | Institute B | 8 | Healthy | 0 (0.0) |
| 3       | 1979   | Tokyo | Institute C | 16 | Healthy | 0 (0.0) |
| 4       | 1985   | Tokyo | Institute D | 8 | Healthy | 0 (0.0) |
| 5       | 1988–90 | Tokyo | Hospital A | 44 | Sick | 0 (0.0) |
| 6       | 1982–83 | Sapporo | Hospital B | 16 | Sick | 1 (6.3) |
| 7       | 1998   | Tokyo | Hospital A | 38 | Sick | 4 (10.5) |
TABLE 3. Cross-VN assay using representative plasma samples

| Sample (source) | VN titer against strain: |  |
|-----------------|--------------------------|---|
|                 | Yanaka Onderstepoort     |   |
| FB-1 (a)        | 80 60                    |   |
| TC-1 (b)        | 160 80                   |   |
| TC-2 (b)        | 80 40                    |   |
| TS-1 (c)        | 40 30                    |   |
| TS-2 (c)        | 120 40                   |   |
| TS-3 (c)        | 320 240                  |   |
| TS-4 (c)        | 240 240                  |   |
| TS-5 (d)        | 400 320                  |   |
| TS-6 (d)        | 160 240                  |   |
| TS-7 (d)        | 160 80                   |   |
| TS-8 (d)        | 120 40                   |   |
| TS-9 (d)        | 80 40                    |   |
| SP-1 (e)        | 160 320                  |   |
| TK-1 (f)        | 1,280 320                |   |
| TK-2 (f)        | 80 40                    |   |
| TK-3 (f)        | 40 20                    |   |
| TK-4 (f)        | 20 <20                   |   |
| D-1 (g)         | 320 160                  |   |
| D-2 (g)         | 480 160                  |   |
| D-3 (g)         | 160 80                   |   |
| D-4 (g)         | 240 120                  |   |
| H-1 (h)         | 80 20                    |   |
| H-2 (h)         | 30 <20                   |   |
| H-3 (h)         | 160 20                   |   |

*CDV-seropositive samples in Taiwan were obtained from a leopard cat (a) and domestic cats (b) in Taichung in 1993 and in Tam-Sui in 1993 (c) and 1998 (d). CDV-seropositive samples in Japan were collected from domestic cats in Sapporo in 1982 (e) and in Tokyo in 1998 (f, g, and h). D-1 to D-4 were cats kept as blood donors in veterinary hospital A. H-1 to H-3 were *Haemobartonella*-infected cats kept in the same hospital.

while the oldest CDV-seropositive sample, obtained in 1982 (SP-1), showed higher VN activities against Onderstepoort than Yanaka strain. The samples from the three blood-transfused cats (TK-2, TK-3, and TK-4) showed relatively low VN activities against CDV strains, but the sample from cat TK-1 was shown to have rather high VN antibodies.

In the present article, we report the seroprevalence of CDV in feral and domestic cats in Japan and Taiwan. Additionally, our findings indicate strong circumstantial evidence of common CDV transmission from infected dogs to unaffected cats.

Several researchers have reported the prevalence of CDV infection in large felids, such as lions, leopards, and tigers, in Africa and in different zoos in the United States (3, 16, 18). As shown in Table 1, one leopard cat in Taiwan was shown to have antibodies against CDV, indicating that Asian small wild felids as well as African or American large felids are susceptible to CDV infection. As for seroprevalence of CDV in domestic cats, Appel et al. (1) reported that about 10% of serum samples originating from cats in the United States had VN antibodies. As shown in Tables 1 and 2, several cats in Taiwan and Japan had VN antibodies, indicating naturally occurring CDV infection in domestic cats. The evidence of CDV infection was obtained from cat sera taken as early as 1982 in Japan (Table 2) and 1974 in the United States (1), suggesting that CDV might have accompanied cats for a considerably longer time worldwide. Cross-VN tests revealed that the recent plasma samples from Taiwanese and Japanese domestic cats had higher VN activities against Yanaka strain than Onderstepoort strain (Table 3). Thus, it is probable that the recently prevalent viruses in Asian domestic cats are similar to the recent field isolates of CDV. On the other hand, the cross-VN tests using the oldest CDV-seropositive sample from Sapporo in 1982 suggested the possibility that the virus infecting the cat in 1982 might be more similar antigenically to vaccine strains.

Although the cats examined in the shelter in Tam-Sui, Taiwan, were isolated in a house not in contact with dogs, more than 1,500 dogs were kept outside the house in the same shelter. As shown in Table 1, it was clearly demonstrated that CDV-seropositive rates were quite high in the cats in the shelter in Tam-Sui (33.3 and 88.8% in 1993 to 1994 and in 1998, respectively). Although the nine cats used as blood donors in the veterinary hospital in Tokyo were caged separately, about 20 dogs used for blood donors were kept in cages in the next room. As in Tam-Sui, four of the nine cats had antibodies against CDV. Significantly, the three cats infected with *H. felis* in the hospital were kept with dogs in the same room, and all three cats were shown to be CDV seropositive. Since these cats seem to be in a location of comparatively high exposure to dogs, it is suggested that the major mode of CDV spread among domestic cats in the natural setting is transmission from infected dogs to susceptible cats by direct or indirect contact. The cats in the shelter in Tam-Sui or in the hospital have not had direct contact with dogs, and thus it is possible that droplet infection occurs from infected dogs to unaffected cats.

Experimental infection of cats with the virulent Snyder-Hill strain of CDV resulted in a slight increase in body temperature (1). Similar results were also observed in specific-pathogen-free cats infected with CDV from large felids (8). As shown in Table 1, we could not find any relationship between CDV seropositivity and the physical conditions of the cats examined in Taiwan. These observations indicate that CDV has low pathogenicity in domestic cats. Although we found four CDV-seropositive cats among the 38 sick cats brought to the veterinary hospital in Tokyo in 1998 (Table 2), three of the four seropositive cats (TK-2, TK-3, and TK-4) had histories of blood transfusion from healthy cats in the hospital. Since most of the blood donors in the hospital were shown to be CDV seropositive (Table 3) and the titers of VN antibodies against CDV were relatively low in the three sick cats (Table 3), VN activities in the three cats might result from passive immunity due to the blood transfusion. In contrast, the remaining CDV-seropositive cat, TK-1, had not been blood transfused, and the titer of VN antibodies was relatively high (Table 3), suggesting that the cat was naturally affected with CDV. Although this cat was brought to the hospital because of a fever, low appetite, and low activity lasting for a month, no significant clinical finding but slight rhinotracheitis and hepatopathy was observed. Therefore, it remains possible that CDV infection can contribute to respiratory illness or pathogenesis of the liver in domestic cats. Further in vivo experiments with domestic cats using a recent field CDV isolate will be necessary to assess CDV pathogenesis in cats.

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