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Serosurvey for Selected Virus Infections of Wild Carnivores in Taiwan and Vietnam

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ABSTRACT: Serum samples from two leopard cats (*Felis bengalensis*) and four Formosan gem-faced civets (*Paguma larvata taivana*) in Taiwan, September 1995, and nine leopard cats in Vietnam, August and December 1997, were examined for the prevalence of antibodies against feline parvovirus, feline herpesvirus type 1, feline calicivirus and feline immunodeficiency virus. All civets and nine of 11 leopard cats were shown to have antibodies against feline parvovirus (FPV), and FPV's were isolated from mononuclear cells in the peripheral blood of the six leopard cats.

Key words: Feline calicivirus, feline herpesvirus type 1, feline immunodeficiency virus, feline parvovirus, *Felis bengalensis*, gem-faced civet, leopard cat, *Paguma larvata*, serosurvey.

Significant disease syndromes in domestic cats are associated with viral infections, and several viruses of these are known to infect wild felines (Artois and Remond, 1994). In domestic cats, feline herpesvirus type 1 (FHV-1) causes rhinotracheitis (Gaskell and Povey, 1979), feline calicivirus (FCV) is responsible for stomatitis, gingivitis, and circumscript lesions of the tongue (Wardly and Povey, 1977), feline leukemia virus (FeLV) causes several neoplastic and non-neoplastic diseases (Neil et al., 1991), feline immunodeficiency virus (FIV) induces immunosuppression (Pedersen et al., 1987; Carpenter and O'Brien, 1995) and feline parvovirus (FPV) is associated with clinical signs of gastroenteritis and panleukopenia (Parrish, 1995).

Formosan gem-faced civets (*Paguma larvata taivana*) in Taiwan and leopard cats (*Felis bengalensis*) in Asia are threatened species of the Viverridae and Felidae, respectively. Although parasites of wild felidae in Thailand have been reported (Patton and Rabinowitz, 1994), little is known about the prevalence of viral diseases in Asian wild Felidae or Viverridae. In the present study, we investigated the presence of certain feline viruses in a community of leopard cats and gem-faced civets in Taiwan and Vietnam and attempted the isolation of FPV from these animals.

A list of animals examined and their assigned numbers are shown in Table 1. All 15 animals examined were apparently healthy. Blood samples of eight leopard cats, LVT1 to LVT8, were collected in Hanoi district (Vietnam; 21°05′N, 105°55′E) in 1997. LVT1 was kept in the Hanoi Zoological Garden. LVT2 and LVT3 were captured and protected in cages in a wildlife conservation center. Five free-ranging cats, LVT4 to LVT8, were captured in mountain areas near Hanoi City (Vietnam). LVT9 was a captive leopard cat, which was sold at an animal market in Ho Chi Minh City (Vietnam; 10°58′N, 106°40′E) in 1997. Two leopard cats, LTW1 and LTW2, had been captured in Nantou County (Taiwan; 24°02′N, 121°20′E) in 1994 and 1989, respectively and kept in cages as pets. Four gem-faced civets were born in a breeding cattery at Taichung County (Taiwan; 24°15′N, 121°02′E) in 1994, and kept in cages as pets. Blood samples of these six animals from Taiwan were collected in 1995.

Peripheral blood mononuclear cells (PBMCs) of the animals were isolated by Ficoll-Paque (Pharmacia LKB Biotechnology AB, Upsalla, Sweden) and stimulated by 10 μg/ml of concanavalin-A (con-A) for

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3 days as reported previously (Miyazawa et al., 1989). Con-A stimulated PBMCs were maintained in RPMI1640 growth medium supplemented with 10% fetal calf serum, antibiotics and 100 units/ml of recombinant human interleukin-2.

Serum antibodies against FHV-1, FCV, and FPV were detected by indirect immunofluorescence (IF) assays using glass slides coated with the Crandell feline kidney (CRFK) cells (Crandell et al., 1973) infected with the respective viruses. The cells were fixed in cold acetone for 30 min, then incubated at 37 C for 30 min with the 100-fold diluted serum samples. After incubation, the cover slips were washed twice with phosphate-buffered saline (PBS), and incubated with 200-fold diluted goat anti-cat immunoglobulin G (whole molecule) conjugated with fluorescein isothiocyanate (Cappel, Aurora, Ohio, USA). After incubation at 37 C for 30 min, the glass slides were washed twice with PBS, mounted in buffered glycerol and examined by fluorescence microscopy. Sera from FHV-1-, FCV-, FPV- or mock-infected specific pathogen-free cats were used as positive and negative controls. Additionally, the titers of virus neutralizing (VN) antibodies against FPV in the plasma samples were determined as reported previously (Ikeda et al., 1998a). For detection of FPV antigens in the cultured PBMCs, an indirect IF assay using anti-FPV VP2 monoclonal antibody (mAb) 2D9 (Mochizuki et al., 1989) was performed as reported previously (Ikeda et al., 1998b). FeLV antigen and antibodies against FIV were checked using an ELISA method using commercial kits (Cite-Combo: IDEXX Co., Portland, Maine, USA).

Recently, we reported that the eight leopard cats in Vietnam were not infected with FIV or FeLV (Miyazawa et al., 1998). By using a commercial kit, it also was shown that the cat in Ho Chi Minh City and two cats and four civets in Taiwan did not have FeLV-specific antigens or antibodies against FIV. By using the indirect IF assay, it was demonstrated that the two leopard cats and four civets in Taiwan had antibodies against FPV but not FHV-1 or FCV. Alternatively, the leopard cats in Vietnam were shown to have antibodies against FHV-1 (1/9), FCV (3/9) and FPV (7/9). In addition, the titers of VN antibodies against FPV were demonstrated to range from 160 to >5,120 in the four civets and 640 to >5,120 in the nine leopard cats (Table 1).

The isolation of FPV from the PBMCs of the infected domestic cats with high VN antibodies has been reported (Miyazawa, et al., 1999). For isolation of FPV from the civets or the feral cats, the Con-A-stimulated PBMCs of the all civets and 10 cats were maintained for more than 2 wk in culture. At 3 to 9 days after cultivation, the PBMCs of the six leopard cats showed cytopathic effects (CPEs) such as cell rounding and nuclear disintegration. The observed CPE's were similar to those observed in FPV-infected feline PBMCs (Ikeda et al., 1998). By the indirect IF assay using mAb 2D9, PBMCs showing CPEs from six leopard cats were shown to contain FPV-specific antigens (Table 1). In contrast, no FPV-specific antigen was detected in the other eight cultured samples.

In the present study, we found the seroprevalence of FCV, FHV-1 and FPV in leopard cats and the susceptibility of gem-faced civets to FPV. Additionally, we isolated FPV from the PBMCs of six leopard cats with high VN antibodies.

Since the number of samples is unfortunately low, it is difficult to conclude that the observed prevalence of the viruses is representative of the local population of feral cats or civets in Taiwan or Vietnam. However, these results indicate that leopard cats are susceptible to infection with FHV-1, FCV and FPV and that the civets also are susceptible to FPV infection. Infection of FHV-1 or FCV in domestic cats is known to occur by close contact between the animals. As shown in Table 1, most of the cats examined had antibodies against FPV but few of the cats had antibodies against FHV-1 or FCV, suggesting

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TABLE 1. Detection of antibodies against feline calicivirus, feline herpesvirus type 1, and feline parvovirus in Formosan gem-faced civets and leopard cats in Taiwan and Vietnam.

Animal	Number	Sex	FCV[a] IFA	FHV-1[b] IFA	FPV[c] IFA	FPV titer	FPV isolation
Civet	CTW1	M	−	−	+	>5,120	−
	CTW2	F	−	−	+	×2,560	−
	CTW3	F	−	−	+	×160	−
	CTW4	F	−	−	+	×5,120	−
Leopard cat	LTW1	F	−	−	+	×640	−
	LTW2	F	−	−	+	×640	+
	LVT1	M	+	+	+	>5,120	−
	LVT2	M	−	−	+	×2,560	−
	LVT3	F	−	−	+	>5,120	+
	LVT4	F	−	−	+	×2,560	+
	LVT5	?	−	−	−	<10	ND[d]
	LVT6	M	+	−	+	×640	+
	LVT7	M	+	−	+	×1,280	+
	LVT8	M	−	−	+	×2,560	+
	LVT9	M	−	−	−	<10	−

[a] Feline calicivirus.
[b] Feline herpesvirus type 1.
[c] Feline parvovirus.
[d] Not done.

that the leopard cats seldom come into contact with free-roaming domestic cats in these areas. Since FPV is generally shed only for a short period of time by domestic cats but survives in the environment for up to several months (Scott, 1980), the infection of FPV may be due to the environmental exposure. The absence of FeLV antigens and relative lack of antibodies against FHV-1 or FIV in the animals suggests that these viruses may not actively circulate in the population.

In the present study, we reported the isolation of FPV from the PBMCs of the leopard cats with high VN antibodies. This observation indicates that FPV can persistently infect the lymphocytes even in the presence of high VN antibodies. Although animals that recover from FPV infection are thought to be protected for life from re-infection, it is possible that the persistent virus stimulates the host immune system for life even after the recovery. At present, however, it remains to be determined whether shedding of the virus following periods of stress occurs after clinical recovery.

Since there are few reports on isolation of FPV from feral cats, the six FPV isolates reported here may provide insight into the FPV infection in Asian feral cats. Further studies will be necessary on biological and genetical differences between the six isolates and FPVs from domestic cats and dogs. Moreover, the method presented here may be applicable to isolate FPV from wild animals.

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