NOTE *Virology*

Recent Epidemiological Status of Feline Upper Respiratory Infections in Japan

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ABSTRACT. Epidemiology of upper respiratory infections of cats was studied. Nasal, ocular, and oral swabs collected from 111 cats presented at animal hospitals during the past 2.5 years were examined. Twenty-four (21.6%) and 4 (3.6%) cats were diagnosed as feline calicivirus (FCV) infection and feline viral rhinotracheitis, respectively, indicating FCV is more prevalent than feline herpesvirus-1, which revealed a considerable shift from data obtained in 1970s. Cat sera immunized by using vaccines containing either FCV F9 or 255 strains neutralized 42.9% and 66.7% of the FCV isolates, respectively. *Chlamydia psittaci,* examined by a PCR assay amplifying the *ompA* gene, was found in 26.9% of 26 diseased cats that typically showed conjunctivitis and rhinitis.

KEY WORDS: *Chlamydia psittaci*, feline calicivirus, feline herpesvirus.

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Feline herpesvirus-1 (FHV) and feline calicivirus (FCV) are well known viral pathogens involved in upper respiratory infections (URI) of cats, and other non-viral agents such as *Chlamydia psittaci (C. psittaci)* and *Bordetella bronchiseptica* have been increasingly recognized as significant pathogens in the URI [4]. Both live attenuated and inactivated FHV and FCV vaccines have been introduced, and monovalent bacterin or multivalent products incorporating such bacterins and inactivated feline leukemia virus are also commercially available in the Western countries recently. Although combined FHV and FCV systemic vaccines have been in wide use since 1986 in Japan, many URI cases are still observed clinically. Thus, in this communication, we described etiology of recent feline URI cases in Japan for considering more efficacious preventive measures matched for an existing epidemiological status.

During the past 2.5 years from March 1997 to September 1999, 111 cats with URI presented at private animal hospitals in Tokyo area were studied. Oral, nasal and ocular swabs were taken from 71, 10 and 20 cats, respectively. Furthermore, all three, both oral and nasal, and both oral and ocular swabs were taken from five, four and one cats, respectively. In all, 81 oral, 19 nasal and 26 ocular swabs were examined for viruses and *C. psittaci* as shown in Table 1. In addition, 92 oral swabs of apparently healthy cats were examined during the same period as the control group. Crandell feline kidney cell (ATCC CCL94) was used for isolation and identification of virus, and other methods applied here were essentially the same as the previous report [17]. Additionally, the reverse-transcriptase polymerase chain reaction (RT-PCR) assay which amplifies a gene segment of the amino acid motifs GLPSG and YGDD [10] was applied for identification of FCV in the virus-positive culture.

As shown in Table 1, FCV was isolated from 22, two and one of the oral, nasal and ocular samples, respectively. On the other hand, FHV was isolated from four and one of oral and nasal samples, respectively. In a cat for which three kinds of the swab sample were examined, FHV was isolated from both oral and nasal swabs. However, in the remaining all cases, either FCV or FHV was isolated from the one site of a cat, excepting a case in which both FCV and FHV were recovered from the oral swab. In consequence, 24 (21.6%), four (3.6%) and one (0.9%) cats were diagnosed as FCV infection, feline viral rhinotracheitis and a mixed infection of both viruses, respectively. In contrast, only one FCV was recovered from the oral site of 92 normal cats. Clinical signs in order of incidence of FCV-infected cats were ulcers in the oral cavity (47.4%), ocular and nasal discharges (36.8–47.4%), lacrimation (36.8%), sneezing (31.6%), hypersalivation (26.3%), coughing (10.5%), and vomiting (5.3%).

The ratio of FCV-infected to FHV-infected cats in the present study was 6:1, contrasting with the result reported more than a couple of decades ago in Japan where FCV and FHV were isolated from approximately equal numbers of cats [17]. This peculiar phenomenon was already pointed out 10 years ago by Harbour *et al.* [9]: the average ratio of total FCV

Table 1. Detection of feline calicivirus (FCV), feline herpesvirus-1 (FHV), and *Chlamydia psittaci* from 111 cats with upper respiratory disease signs during a period from March, 1997 to September, 1999

Sampling site	Number of swabs tested	Isolation of		DNA detection of
		FCV	FHV	*C. psittaci*
Oral	81	22 (27.2%)	4 (4.9%)	0[a)]
Nasal	19	2 (10.5%)	1 (5.3%)	0[a)]
Ocular	26	1 (3.9%)	0	7 (26.9%)

a) Twenty-one oral and two nasal swabs were examined, but no positive result was obtained.

isolates to FHV isolates during a decade from 1980 in England was 4.8:1.

Next, a neutralization profile of 21 FCV isolates was determined by using a micro-neutralization test (MNT) and cat sera immunized by vaccination. Two types of commercially available vaccines were used; the multivalent vaccines A and B containing live F9 and inactivated 255 strains of FCV, respectively. According to the recommended vaccination protocols, each vaccine was inoculated into five naive kittens and serum was obtained 2 weeks after the final administration. The sera were pooled and titrated for neutralizing activity against the homologous FCV strain used for immunization, and a working antibody solution containing 1:16 of MNT titer was prepared. The method of MNT was essentially the same as the previous report [18]. Uncloned FCV isolate was cultured a total of three times before use. In brief, after making fivefold serial virus dilutions in duplicate, one dilution was mixed with an equal volume of the antibody solution, and the other, as a control virus dilution, was mixed with a solution lacking antibody. They were incubated at 37°C for 1 hr, and then the cell suspension was added into all wells. The plate was incubated for 7 days and a reduction of virus infectivity was determined comparing the control virus dilution. No titer reduction was recorded as '–', while 5 to 5^2 and 5^3 or more times reduction were recorded as '+' and '++', respectively. When '++' was recorded in the homologous strain in each test, a titer of reduction was evaluated for a test isolate.

As shown in Table 2, four isolates were neutralized by both, two were neutralized by neither, and others were neutralized by either of antisera. Two (9.5%) and 7 (33.3%) were neutralized as '++' and '+', respectively, and 12 (57.1%) were not neutralized by the anti-F9 strain serum. In contrast, 8 (38.1%) and 6 (28.6%) were neutralized as '++' and '+', respectively, and 7 (33.3%) were not neutralized by anti-255 strain serum.

In Japan the vaccine containing F9 strain has been used predominantly since 1986 and no vaccine product incorporating 255 strain was applied before October 1999. Recently Hohdatsu *et al.* [11] also reported antigenic disagreement between commercially available vaccine strains and field FCV strains in Japan. They analyzed FCV isolates in early 1990s and showed a vaccine incorporating bivalent FCV strains had produced more cross-reactive antibodies in cats than the monovalent vaccine products. Although the neutralization profile of fresh FCV isolates in 1970s to 1980s when the vaccine was not yet introduced is uncertain, the present results suggest a vaccine adopting a novel antigenic FCV such as 255 strain may give animals more efficient immune protection than the existing F9 type vaccine for instance. In all cases, the epidemiological feature of URI revealed here is similar to those previously described in the Western countries [2, 3, 9, 13, 15, 20], especially in Britain where the majority of the vaccines incorporate F9 or F9-like strain of FCV have been used [2, 3, 9, 13]. It has been possibly concluded based on these observations that a change in the antigenicity of FCV over the past two decades resulted from either a natural selection or the widespread use of vaccines derived predominantly from a single strain, thus leading to low efficacy of the habitual vaccine [1, 4, 27]. For supporting such a hypothetical scenario, the following phenomena are suggestive: an evolution of FCV quasispecies leading to antigenic change in vitro as well as *in vivo* [23], and the antigenic variants generated from cats persistently infected with FCV [12].

Although there is probably no doubt that an introduction of novel antigenic vaccine strains into existing circumstances is immediately potent [3,11,13], they do not appear to protect against all FCV strains as also revealed here (Table 2) and another vicious circle may develop in time. As commented in one review article [1], a fundamental improvement of vaccine designation, such as a peptide vaccine designed from the conserved antigenic region [24], is desired.

Feline *C. psittaci* has been well known as a pathogen which causes clinical abnormalities that resemble *C. trachomatis* infections of humans, and the mucosal epithelial cells mainly of eyes are affected and typically resulted in a manifestation of chronic conjunctivitis. For detection of *C. psittaci* the PCR assay described by Sykes *et al.* [28] was followed in the present study. The assay amplifies 1,019 bp of the *omp*A gene-coding region. A protein encoded by this gene exists in the outer membrane of bacteria and is considered to be a major protective antigen targeted by the host immune system [25].

Table 2. Neutralization of FCV isolates by antibody of cats immunized by commercially available vaccines

Reference FCVs and isolates	Antibody of cats immunized by Vaccine A[a)]	Vaccine B
F9	++[b)]	+
255	+	++
97–003	–	–
97–011	–	++
97–014	–	+
97–018	++	+
97–019	–	++
97–027	+	–
97–087	+	–
98–003	–	++
98–005	+	++
98–008	–	++
98–009	–	+
98–010	–	–
98–012	+	+
98–027	+	–
98–038	+	–
98–039	++	–
98–040	–	++
98–047	–	++
98–054	–	+
98–058	–	++
98–060	+	+

a) The multivalent vaccines A and B contained live (F9 strain) and inactivated FCV (255 strain), respectively.
b) A reduction of virus infectivity by adding the antibody was determined comparing with the duplicated virus dilution with no antibody. No titer reduction was recorded as '–', while 5 to 5^2 and 5^3 or more times reduction were recorded as '+' and '++', respectively.

As shown in Table 1, 7 swabs only from the ocular site of the diseased cats were positive. No viral agent was recovered from them. They all typically suffered from conjunctivitis, and the second most frequent clinical sign was rhinitis found in 6 cats. The present prevalence rate (26.9%) was as high as those reported in Australia [28], Britain [16, 29], Canada [14], Japan [6, 21], New Zealand [7], and the U.S. [19] where cats with the URI or ocular disease were subjects of investigation. In contrast, the prevalence in healthy cat population was relatively low [8].

The PCR products were subjected to restriction fragment length polymorphism (RFLP) analysis with restriction enzymes *Alu* I and *Mse* I. In addition, they were sequenced and the predicted amino acid sequences were compared with the same region of known *C. psittaci* strains Cello and Pn-1 (ATCC VR120) in the GenBank database by the methods described previously [10]. A homogeneous RFLP pattern of the PCR products was observed between the field and the Cello strains (data not shown) as shown in literatures previously [26, 28]. Nucleotide and amino acid sequence homologies between them were found to be 99.5% and 99.1%, respectively (data not shown), again suggesting high homogeneity of the *omp*A gene of feline *C. psittaci*. It has been described that there are two genotypes in feline *C. psittaci*, for instance, based on the RFLP analysis of genomic DNA; types Fe1a and Fe1b represented by Pn-1 and Cello strains, respectively [5, 22]. In addition, it was pointed out that cats might have been infected with some antigenically different *Chlamydia* species on the basis of a serological study [21]. Although some genetic as well as antigenic heterogeneity of feline *C. psittaci* may exist in the field, the present data together with the previous one [6] indicate some control measure such as vaccination practice may be indicated in cat colonies where chlamydial conjunctivitis is considered to be a problem.

In conclusion, FCV has become a major viral pathogen of the URI of cats and more than half of recent FCV isolates were not neutralized by the antibody produced by an F9 type of FCV vaccine. Chlamydial conjunctivitis has also become a clinical concern.

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