ネコの呼吸器感染症からのウイルス分離

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A Survey of Feline Respiratory Infections

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ABSTRACT. A survey of viruses associated with feline respiratory diseases was conducted by virus isolation from swabs collected from cats visiting the University Veterinary Hospital and from apparently healthy laboratory cats in a medical school animal center. Twenty-nine caliciviruses and 2 herpesviruses were isolated from 29 of 65 cats with respiratory disease and 2 caliciviruses were from 2 of 49 cats with other diseases. From 50 laboratory cats 2 caliciviruses were also isolated.—KEY WORDS: feline calicivirus, feline herpesvirus, feline respiratory disease.

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Viral upper respiratory disease is a major clinical problem in feline medicine and is mainly caused by one of two viruses: feline herpesvirus (FHV) and feline calicivirus (FCV). Both viruses show a world-wide distribution [3, 7] and also have been isolated in Japan from cats exhibiting clinical signs of upper respiratory disease [2, 6, 8–10]. The FHV is responsible for feline viral rhinotracheitis (FVR), whose syndrome has been reviewed by Crandell [1]. The clinical syndrome associated with FCV infection may vary from an inapparent, or mild upper respiratory tract involvement to a severe, and frequently fatal respiratory disease [4]. Although FCV infection is far more frequently associated with ulcerative glossitis [4], virulent FCV infection is very difficult to differentiate from FVR by FHV.

The present study was carried out to determine the prevalence of FHV and FCV in cats showing clinical signs of upper respiratory infections and other diseases in the University Veterinary Hospital, and in apparently healthy laboratory cats in Animal Facilities for Experimental Medicine, School of Medicine, Akita University. This survey was made from January 1983 to September 1984.

CatS+L− cell line [11] was kindly supplied by Dr. H. Yoshikura, Institute of Medical Science, University of Tokyo, and grown at 37°C in Eagle's minimum essential medium (MEM) containing 10% newborn calf serum, 10% tryptose phosphate broth and kanamycin (0.06 mg/m*l*). For virus isolation and maintenance of cells, the serum concentration was reduced to 5%. The cell monolayers were prepared in 13×100 mm test tubes by seeding $1.5-2.0\times10^5$ cells suspended in 1 m*l* of growth medium and incubated at 37°C for 2 days. Prior to virus inoculation, the cell cultures were washed once with MEM and inoculated with 0.2 m*l* of virus material. After virus adsorption at 37°C for 90 min, the inoculated cultures were fed with 1.5 m*l* of maintenance medium and incubated for 5 days.

FHV strain 7301 [6] and FCV strain F4 [8], kindly supplied by Dr. S. Konishi, Department of Veterinary Microbiology, University of Tokyo, were propagated in CatS+L− cells and stored at −80°C as seed virus when extensive cytopathic effect (CPE) was detected in the inoculated cultures. The isolates

were also stored at −80°C.

For defining the physicochemical properties of the isolates, the isolates were treated with ether and chloroform (20°C for 10 min), tested in pH 3.0 stability (37°C for 60 min) and filtered through the membrane filters (Sartorius, West Germany) for estimating virion size. The infected cultures were treated with 5-iodo-2-deoxyuridine (IUDR) for typing the nucleic acid. These experiments were performed by the same methods described previously on MHV-2 strain of mouse hepatitis virus [5].

Oral or nasal materials were collected from the cats and inoculated into the cell cultures as soon as possible. Table 1 shows that 31 isolates were obtained from 29 of 65 cats with respiratory disease and 2 isolates were from 2 of 49 cats with other diseases. In the cases of 2 diseased cats (No 401 and 419), viruses were isolated from the nasal sample and ulcerative lesion. Table 2 shows sources of the isolates, and clinical signs (diagnosis) of diseased cats.

All the isolates grew rapidly in the cell cultures and showed marked CPE with cell-rounding. Except isolates 406 and 407, all the isolates were resistant to ether and chloroform, but sensitive to pH 3.0 treatment. These isolates passed through 50 nm filter. The replication of these isolates as well as the control FCV strain F4 in the cells was not affected with IUDR. These findings indicate that they are non-enveloped RNA viruses, and are smaller than 50 nm in diameter.

On the other hand, the growth of isolates 406 and 407, and control FHV strain 7301 in the cells was inhibited by IUDR treatment. In addition, two isolates were sensitive to both organic solvents and pH 3.0 treatment, but failed to pass through 100 nm filter. In the cell cultures inoculated with both isolates,

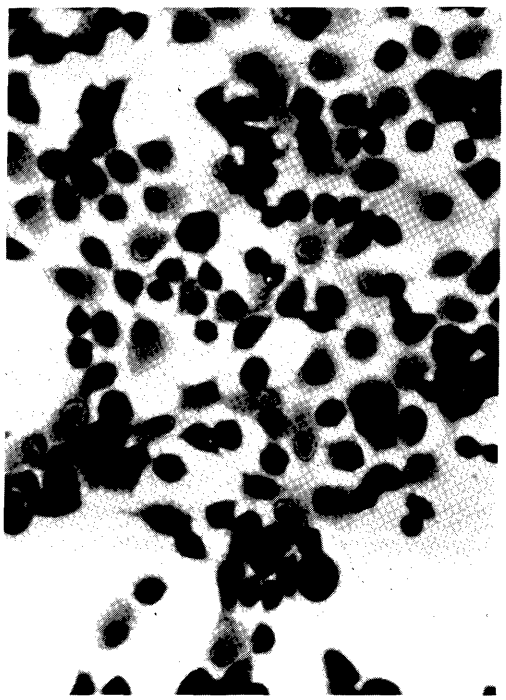

Fig. 1. Intranuclear inclusion bodies in the infected cells with isolate 406 at 12 hr postinoculation (hematoxylin-eosin staining).

Table 1. Virus isolation from cats with or without respiratory diseases

Cats with clinical signs	Number of isolates	Number of cats
		Virus positive/tested
Diseased cats (total)[a]	33	31/114 (27.2%)
with respiratory disease	31	29/ 65 (44.6%)
with other disease	2	2/ 49 (4.1%)
Apparently healthy[b] laboratory cats	2	2/ 50 (4.0%)

a) Cats visiting the University Veterinary Hospital.
b) Cats kept in Animal Facilities for Experimental Medicine, Akita University School of Medicine.

Table 2. Sources and idenfitication of the isolates

Virus isolate	Age of cat[a]	Clinical signs[b] (diagnosis)	Isolation site	Identified[c] as
8	3 Y	SN+CO (FVR)	Throat	FCV
9	6 M	SN+CO (FVR)	Throat	FCV
11	3.5 M	SN	Throat	FCV
12	7 Y	SN+CO (FVR)	Throat	FCV
35	4 Y	SN	Nose	FCV
38	1.5 Y	SN+CO	Throat	FCV
40	6 M	SN	Throat	FCV
43	8 Y	SN	Throat	FCV
49	5 Y	SN+CO (FVR)	Throat	FCV
53	10 M	SN	Throat	FCV
58	2 M	SN (FVR)	Throat	FCV
62	3 Y	SN (FVR)	Throat	FCV
64	2 Y	SN (FVR)	Throat	FCV
68	9 Y	SN (FVR)	Throat	FCV
74	8 Y	Fracture	Throat	FCV
79	?	SN (FVR)	Throat	FCV
82	1.5 Y	Diarrhea	Throat	FCV
83	8 Y	SN (FVR)	Throat	FCV
401N	3 Y	SN	Nose	FCV
401U			Oral ulcer	FCV
406	2 M	CO (FVR)	Throat	FHV
407	2 M	CO (FVR)	Throat	FHV
408	10 Y	SN+CO (FVR)	Throat	FCV
410	3 Y	SN+CO	Throat	FCV
412	4.5 Y	SN+CO	Oral ulcer	FCV
413	2 M	SN+CO	Throat	FCV
417	?	SN	Oral ulcer	FCV
419N	4 M	SN+CO	Nose	FCV
419U			Oral ulcer	FCV
T1	5 M	SN	Nose	FCV
T2	6 M	SN	Nose	FCV
T3	4 M	SN	Nose	FCV
T4	5 M	SN	Nose	FCV
A391	?	App. Healthy	Nose	FCV
A423	?	App. Healthy	Nose	FCV

a) Y: Year-old, M: Month-old.
b) SN: Snieveling or snuffles, CO: Coughing, FVR: Feline viral rhinotrachitis, App. Healthy: Apparently healthy, A391 and A423 from laboratory cats, 74 and 82 cats having no respiratory disease.
c) FCV: Calicivirus, FHV: Herpesvirus.

typical acidophilic intranuclear inclusion bodies were seen as presented in Fig. 1. The properties of 406 and 407 isolates were shown to be the same as those of FHV strain 7301.

The isolates 406 and 407 were neutralized in the same manner as FHV strain 7301 with anti-FHV rabbit serum supplied by Dr. K. Yagami, School of Medicine, Kumamoto University. The rest of the isolates were also neutralized with hyperimmune rabbit serum against FCV strain F4 although antigenic discrepancies were found beween FCV strain F4 and the isolates.

Electron microscopy was carried out on the isolates 38, 58, A391, A423 and strain F4 after negative staining with 2% phosphotungstic acid. As shown in Fig. 2, particles

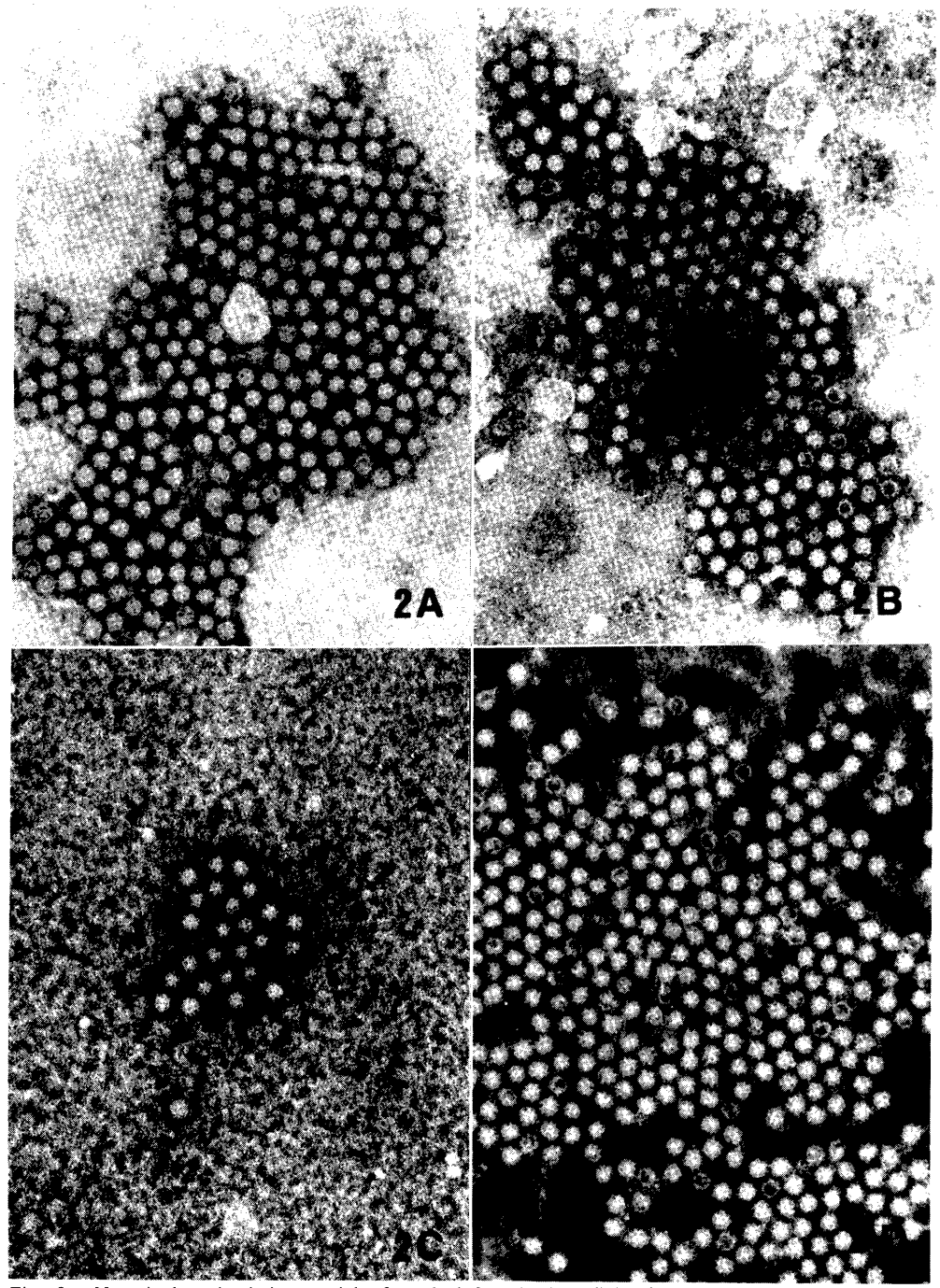

Fig. 2. Negatively stained virus particles from the infected culture fluid of the cells with isolate 38 (2A), 58 (2B), A391 (2C) and A423 (2D) (×90000).

of these isolates, 35 to 40 nm in diameter, had a characteristic nucleocapsid as observed with F4 strain.

The viruses were isolated from about 45% of the cats (29/65) with respiratory disease and mostly identified as FCV. In contrast with our observations, Mochizuki *et al.* [6] reported the isolation of FHV (37.5%), FCV (25%) and both viruses (37.5%) from 8 domestic cats with respiratory disease.

As reported by Povey and Johnson [7], it was very difficult to distinguish the virulent FCV infection from FVR due to FHV on the basis of clinical signs bacause FCV was isolated in high incidence from the cats diagnosed as having FVR in the University Veterinary Hospital. It is very important for diagnosis and medical treatment of the feline viral respiratory infection to define the causative agent. In the present study, FCV produced characteristic CPE with cell-rounding in the cell cultures and was isolated within 12 hr after inoculation. As soon as CPE was observed, all the isolates were examined for sensitivity to ether and chloroform to predefine them as FHV or FCV.

Recently, Yagami *et al.* [10] reported that FCV was isolated more frequently than FHV from clinically healthy cats for laboratory use. In our observations, the FCV was isolated from about 4% of the cats with manifestations other than respiratory disorders, and also from 4% of the apparently healthy laboratory cats. These findings suggest the possibility that some inapparently infected carrier cats as well as diseased cats shed the viruses to cause the new infection in healthy cats.

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要　約

ネコの呼吸器感染症からのウイルス分離（短報）：平野紀夫・佐藤れえ子[1)]・松田幸久[2)]（岩手大学農学部家畜微生物学教室，[1)]家畜内科学教室，[2)]秋田大学医学部動物実験施設）――岩手大学家畜病院に来院したネコと，健康と思われる研究用ネコからウイルス分離を行なった。呼吸器病のネコ65例中29例からヘルペスウイルス２株とカリシウイルス29株が分離され，他の疾患のネコ49例と研究用ネコ50例からは，各２例からカリシウイルス株が分離された。