

## An Etiological Investigation of Domestic Cats with Conjunctivitis and Upper Respiratory Tract Disease in Japan

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**ABSTRACT.** *Chlamydomphila felis* (*C. felis*), feline herpesvirus-1 (FHV-1) and feline calicivirus (FCV) were detected in 39 (59.1%), 11 (16.7%) and 14 (21.2%) cats respectively, from 66 domestic cats presented with conjunctivitis and upper respiratory tract disease (URTD) in 9 prefectures of Japan. Dual and multiple infections were found in 7 (10.6%) cats with both *C. felis* and FHV-1, 10 (15.2%) cats with both *C. felis* and FCV, and 1 (1.5%) cat with all three agents. *C. felis* was isolated from 11 (28.2%) of 39 PCR positive cats. Antigenic difference was found in a 96 kDa protein of our isolates and Fe/145 strain isolated in USA. In conclusion, *C. felis* is the most common agent of feline conjunctivitis and URTD, and the coinfection with *C. felis*, FHV-1 and FCV are also common in cats in Japan.

**KEY WORDS:** *Chlamydomphila felis*, conjunctivitis, FCV, FHV-1, upper respiratory tract disease (URTD).

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Conjunctivitis and upper respiratory tract disease (URTD) are common in domestic cats. The most common agents for these diseases include chlamydia and viruses such as feline herpesvirus-1 (FHV-1) and feline calicivirus (FCV) [11]. *Chlamydomphila felis* (*C. felis*), known as feline *Chlamydia psittaci* (*C. psittaci*) previously, was first isolated from feline pneumonia [1], and now it is considered as causing a conjunctival infection in cats [13]. Transmission of *C. felis* to human was reported with conjunctivitis and serious systemic infection in an immunocompromised person [16, 17]. On the basis of our previous study, feline chlamydial infection was thought to be transmitted by close contact with infected cats and possibly from infected birds [20]. Since the first isolation of FHV-1 and FCV [2, 4] and the subsequent recognition of these viruses as significant causes of respiratory disease in cats [11], the relative importance ascribed to *C. felis* as a respiratory tract pathogen in cats has been diminished. Some researchers in other countries reported dual infections with both FHV-1/*C. felis* and both FHV-1/FCV in cats [8, 18]. Our previous seroepidemiological studies indicated that feline chlamydial infection is widely spread in cats in Japan [5, 15, 20]. Mochizuki *et al.* [14] reported the epidemiological status of feline URTD and Iwamoto *et al.* [10] reported the isolation of *C. felis* in Japan. None of them, however, reported the coinfection of *C. felis* with FHV-1 and/or FCV. This study was conducted to determine the relative frequency of *C. felis*, FHV-1 and FCV in conjunctivitis and URTD of domestic cats in Japan. In addition, some characteristics of the Japanese isolates of *C. felis* were studied by genetic and antigenic analysis.

### MATERIALS AND METHODS

**Clinical samples:** Samples were collected from 66

domestic cats with clinical signs from November 1998 to April 2000 at private veterinary hospitals in Osaka, Aichi, Fukuoka, Ibaraki and another 5 prefectures in Japan. Among them, 18 cats presented conjunctivitis, 14 cats presented URTD and 34 cats presented conjunctivitis and URTD (Table 2). Conjunctival swabs were obtained from 65 cats, and nasal swabs from 8 cats. Specifically, both conjunctival and nasal swabs were obtained from 7 cats, conjunctival swabs only from 57 cats and nasal swabs only from 1 cat.

**Preparations of the strains:** *C. felis* Fe/145, FHV-1 C7301 and FCV-F4 strains were propagated in L cell suspension cultures (SL cells) and Crandell feline kidney (CRFK) cells, and used as positive controls in PCR detection.

**PCR detection:** Extraction of DNA of *C. felis* and FHV-1 from the swabs and PCR amplification were performed as described previously [3, 7]. Detection of FCV was performed by RT-PCR [9] using One Step RNA PCR Kit (Takara, Kyoto, Japan) according to the manufacturer's instruction.

**Isolation:** Isolation of *C. felis* was performed by yolk sac inoculation using embryonated eggs as described previously [19] and it was identified by PCR. Viral isolation was performed as described previously [8].

**Sequencing analysis:** The PCR products were purified using GeneClean II Kit (BIO 101, INC., U.S.A.). DNA sequencing was performed with a Thermo Sequenase Cy5.5 dye terminator cycle sequencing kit and SEQ 4 × 4 personal sequencing system (Amersham Pharmacia Biotech, U.S.A.) according to the manufacturer's instructions. Sequencing analysis was examined with the GENETYX-MAC Version 10 (Software Development Co., Ltd., Japan). The published sequences of FEPN (accession No. M73037), FPN/pring (accession No. X61096) and FP Cello (accession No. AF269258) from the DNA Data Bank of Japan (DDBJ, National Institute of Genetics, Mishima, Japan) were used in

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alignments and comparative analyses.

**Antigenic analysis:** *C. felis* elementary bodies (EBs) were purified from a monolayer of McCoy cells that were infected with yolk sac homogenate [6]. Polypeptide profiles of EBs were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) [6]. Western blot analysis was performed using anti-Fe/145 antiserum [6].

## RESULTS

**PCR detection and isolation:** The detection and isolation of *C. felis*, FHV-1 and FCV are shown in Table 1. Of 66 cats, *C. felis* was detected in 39 (59.1%) cats, and the agent was isolated from 11 (16.7%) cats. FHV-1 and FCV were detected and isolated from 11 (16.7%) and 14 (21.2%) cats, respectively. From 65 conjunctival swabs, *C. felis*, FHV-1 and FCV were detected in 38, 11 and 14 swabs, and from 8 nasal swabs, these agents were detected from 4, 2 and 2 swabs, respectively. In 7 cats for which two kinds of the swab sample were examined, *C. felis*, FHV-1 and FCV were detected in 3, 2 and 2 of both conjunctival and nasal swabs. The rates of distribution in various prefectures were from 50 to 77.8% for *C. felis*, 7.8 to 42.9% for FHV-1 and 12.5 to 33.3% for FCV, respectively (Table 1). Dual infection with *C. felis* and FHV-1 or *C. felis* and FCV was found in 7

(10.6%) and 10 (15.2%) cats, respectively (Table 2). Multiple infection with the three agents was found in 1 (1.5%) of 66 cats.

**Sequencing MOMP gene of *C. felis* isolates:** The MOMP gene fragments corresponding to the sequence from 302 (codon 69) to 1190 (codon 352) bp were compared between our isolates and several foreign strains. The nucleotide and amino acid sequences of our isolates were found to be identical to the Fe/145 strain but different from FEPN, FPN/pring and FP Cello strains at 710 and 711 bp (codon 198).

**Antigenic diversity of *C. felis* isolates:** When the protein profiles were compared to the Fe/145 strain, polypeptides of 38 (MOMP), 44, 61 and 120 kDa, as well as several other polypeptides were similar, but differed in a 96 kDa polypeptide (Fig. 1). Proteins of the Japanese isolates as well as the Fe/145 strain showed strong immunological reactivity to anti-Fe/145 antiserum in the 132, 75, 61, 52, 44, 38 (MOMP) and 30 kDa proteins. In contrast with the weak reaction of Fe/145 strain, our isolates presented a very strong reaction with the 96 kDa protein (Fig. 2).

## DISCUSSION

In the present study, *C. felis* was detected at a higher rate (59.1%) than FHV-1 (16.7%) and FCV (21.2%). The data

Table 1. Detection and isolation of *C. felis*, FHV-1 and FCV from 66 domestic cats in 9 prefectures

Place	No. of cats	<i>C. felis</i> (%) <sup>a)</sup>		FHV-1 <sup>b)</sup>	FCV <sup>c)</sup>
		PCR detection (%)	Isolation (%)	Isolation (%)	Isolation (%)
Osaka	29	17 (58.6)	4 (13.8)	4 (13.8)	7 (24.1)
Aichi	13	17 (53.8)	2 (15.4)	1 ( 7.8)	2 (15.4)
Fukuoka	9	7 (77.8)	3 (33.3)	2 (22.2)	3 (33.3)
Ibaraki	7	5 (71.4)	2 (28.6)	3 (42.9)	1 (14.3)
Others <sup>d)</sup>	8	4 (50.0)	0 0	1 (12.5)	1 (12.5)
Total	66	39 (59.1)	11 (16.7)	11 (16.7)	14 (21.2)

a) No. of positive/no. of cats. b) and c) The result of PCR detection was identical to the viral isolation. d) Chiba, Kanagawa, Kyoto, Hokkaido and Tokyo.

Table 2. The prevalence of infections by *C. felis*, FHV-1 and/or FCV in 66 domestic cats with conjunctivitis and URTD<sup>a)</sup>

Agents detected by PCR	Clinical symptoms (%) <sup>b)</sup>			Total
	Conjunctivitis	URT <sup>c)</sup>	Conjunctivitis and URTD	
<i>C. felis</i>	8 (44.4)	4 (28.6)	9 (26.5)	21 (31.8)
FHV-1	1 (5.6)	0 0	2 (5.9)	3 (4.5)
FCV	0 0	1 (7.1)	2 (5.9)	3 (4.5)
<i>C. felis</i> and FHV-1	0 0	1 (7.1)	6 (17.6)	7 (10.6)
<i>C. felis</i> and FCV	3 (16.7)	3 (28.6)	4 (8.8)	10 (15.2)
<i>C. felis</i> , FHV-1 and FCV	0 0	1 (7.1)	0 0	1 (1.5)
None <sup>c)</sup>	6 (33.3)	4 (28.6)	11 (32.3)	21 (31.8)
Total	18	14	34	66

a) Upper respiratory tract disease. b) The number of positive cats/cats with clinical signs. c) None of these agents was detected.

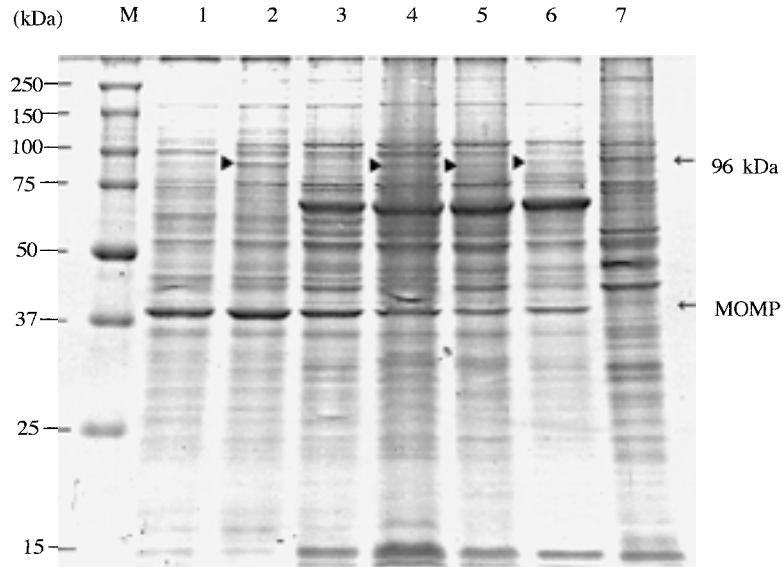


Fig 1. Comparison of protein profiles of the isolates and Fe/145 strain. Lane M, molecular weight standards; lanes 1 and 2 are purified EBs of Fe/145 and isolate Fe/C38; lanes 3 to 6 are McCoy cells infected with Fe/145, isolates of Fe/C60, Fe/C56 and Fe/C38, respectively; lane 7 is normal McCoy cells. Variation in the molecular weights of the 96 kDa protein was observed.

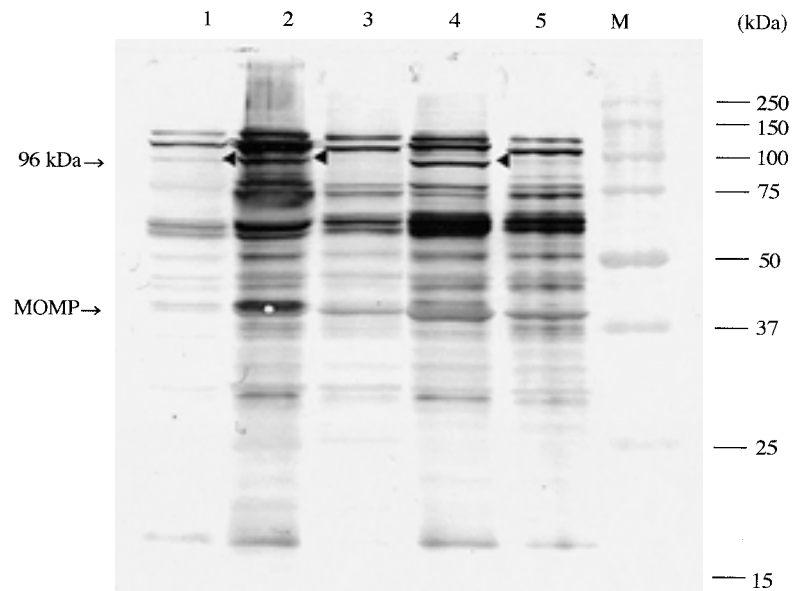


Fig 2. Immunoblots of the isolates and Fe/145 strain with antisera to Fe/145. Lanes 1 to 3 are cells infected with isolates of Fe/C56, Fe/C60 and Fe/145, respectively; lanes 4 and 5 are purified EBs of isolate Fe/C60 and Fe/145; lane M is molecular weight standard. The MOMP (38 kDa) of each isolate and Fe/145 strain showed the same reactivity. The 132, 75, 61, 52, 44 and 30 kDa bands were common to each isolate and Fe/145 strain. The 96 kDa band of the isolates is different from Fe/145 strain.

indicate that *C. felis* is the most common agent of feline conjunctivitis and URTD in 9 prefectures of Japan. Some papers reported that FHV-1 and FCV were common agents in feline conjunctivitis and URTD [11, 14]. Although only 66 cats from 9 prefectures were tested in the present study, combined with our previous seroepidemiological study, we suggest that *C. felis* causing feline conjunctivitis and URTD is more common than FHV-1 and FCV in Japan. It is necessary to study the etiological situation of these agents from other regions of Japan.

Dual and multiple infections of *C. felis* with FHV-1, FCV and both viruses were detected in 10.6, 15.2 and 1.5%, respectively. The results indicate that dual and multiple infections of *C. felis*, FHV-1 and FCV are common in the cat population in the prefectures investigated. Dual infection with both *C. felis* and FHV-1 or FCV has rarely been reported. Mochizuki *et al.* reported that FHV-1 and FCV were detected at 21.6 and 3.6% respectively, and that dual infection of both viruses was detected at 0.9% in feline URTD in Japan [14]. They, however, did not report coinfection of *C. felis* with FHV-1 and/or FCV. The rate of dual infection of both *C. felis* and FHV-1 was reported at 0.6% in Australia and 1.6% in U.S.A. [12, 18].

In the present study, though the PCR positive rate for the feline samples was high (59.1%), the isolation rate was lower (16.7%). Iwamoto *et al.* reported the isolation of *C. felis* from 6 of 52 cats with clinical signs of conjunctivitis and sneezing [10]. These results indicate that the isolation of *C. felis* might relate to other factors such as the development stage of the infection, contents of the agent in samples, preservation and transportation of samples, and so on.

We reported that *C. felis* could be differentiated from other *C. psittaci* based on restriction fragment length polymorphism (RFLP) analysis of polymorphic DNA [7], antigenicity of MOMP [6] and sequence analysis of the MOMP gene [7]. Polypeptide profiles and immunological specificities of MOMP were compared with *C. psittaci* strains isolated from various animals [6]. To our knowledge, no reports have been published on the antigenic variation among different *C. felis* strains. In the present study, we found some antigenic differences in proteins other than MOMP between our isolates and some foreign strains. Further studies will be necessary to analyze the immunological and genetic diversity of these isolates with the aim of developing an effective vaccine against *C. felis*.

In conclusion, although bordetella, mycoplasma and feline reovirus were not studied in the present study, *C. felis* is the most common agent of feline conjunctivitis and URTD, and coinfection with *C. felis*, FHV-1 and FCV are also common in cats in Japan. Therefore, chlamydial infection has also become a clinical concern and some control measures such as vaccination practice, and clinical treatment should be given importance.

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