Isolation of *Chlamydia psittaci* from Domestic Cats with Oculonasal Discharge in Japan

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ABSTRACT. Eight strains of *Chlamydia psittaci* were isolated in Japan from the nasal and conjunctival swabs of six household cats using the L929 cell line of mouse fibroblast origin. The isolates were identified as *C. psittaci* on the basis of the formation of characteristic inclusion bodies in the cell culture detected by Giemsa stain and immunofluorescence. Comparison of nucleotide sequences of the *ompA* gene amplified from the three isolates with the published sequence of feline FEPN strain of *C. psittaci* showed almost 100% homology.

KEY WORDS: *Chlamydia psittaci*, feline, isolation.

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When *Chlamydia psittaci* was first isolated from cats [1], the organism was considered to be the major cause of feline respiratory disease and was called feline pneumonitis agent. However, since the subsequent recognition of feline herpesvirus and feline calicivirus (FCV) as significant causes of feline upper respiratory tract disease, *C. psittaci* is now primarily considered as a conjunctival pathogen with or without rhinitis in cats [5, 12].

The isolation and identification of *C. psittaci* in cat populations with conjunctivitis have been reported in the U.S.A. [2], Canada [8], Australia [10], and the UK [4, 5, 12]. In Japan, positive antibody to chlamydia has been detected in as many as 20% of healthy and diseased cats [7]. Despite this high prevalence of seropositivity, the isolation of *C. psittaci* from domestic cats in Japan has not been reported yet.

A total of 156 samples were collected from 52 household cats kept in Osaka and Hiroshima Prefectures, Japan. The cats were taken to veterinary clinics for examinations from Sep. 1999 to Aug. 2000. All of the cats showed conjunctivitis or discharge or eye mucus. Of them, 48 cats showed either or both upper respiratory tract disease or stomatitis. All of the conjunctival, nasal and oral swabs were submitted for chlamydia isolation. The tip of the swab was placed into 2 ml chlamydia transport medium in a tube [9], and transported at 4°C and stored at –80°C until use. An attempt was made to isolate *C. psittaci* using the L929 cell subline of Mouse L cells. One milliliter of growth medium (Eagle MEM with 10% fetal calf serum (FCS)) containing 2.5 x 105 mouse L cells was added to each well of flat bottomed plastic dishes with a diameter of 10 mm, which were incubated at 37°C for 24 hr in the presence of 5% carbon dioxide. Before inoculation of clinical specimens, the growth medium was removed and monolayers were treated with 30 μg/ml DEAE-dextrane solution for 5 min at room temperature; the solution was removed and 0.1 ml of specimen was then added to each of four wells. Cultures were incubated at 37°C for 1 hr with agitation 2 to 3 times. The inoculum was replaced with 1 ml of maintenance medium (Eagle MEM with 5% FCS) containing 1 μg/ml cycloheximide. After incubation at 37°C for 3 to 5 days in 5% CO2, monolayer cells of two of the four wells were fixed in methanol. Cells in two other wells were harvested for subsequent passage after freeze/thaw. The rest of the cells fixed in methanol were stained with Giemsa and examined under a microscope for intracellular inclusion bodies. Another one monolayer was stained by indirect immunofluorescence with mouse serum containing antibodies against feline *C. psittaci* Fe/B166 strain and with fluorescein-conjugated anti-mouse IgG. The Fe/B166 strain was isolated from a cat with conjunctivitis in the UK in 1984, and obtained from the Department of Veterinary Microbiology, Faculty of Agriculture, Gifu University, Japan.

CRFK cell cultures were used for detection of feline viral agents. Chlamydiae were isolated from conjunctival swabs of 6 of 52 cats with clinical signs of marked bilateral conjunctivitis and sneezing. The clinical histories of the 6 cats from which 8 strains of *C. psittaci* were isolated are shown in Table 1. Among the 6 positive cats, chlamydiae were isolated from 2 nasal swabs of 2 cats at the same time. Feline caliciviruses were isolated from the nasal and oral swabs of cat No.1 from which the Fe/N-P7 and Fe/C-P8 strain were isolated, and from conjunctival, nasal and oral swabs of cat No.2 from which Fe/C-N24 strain was isolated in CRFK cell culture. All of the isolates formed typical intracytoplasmic chlamydial inclusion bodies in L929 cell monolayers. They were identified as *C. psittaci* by means of indirect immunofluorescence assay with antisera against Fe/B166 strain of *C. psittaci*.

For detection of *C. psittaci*, the polymerase chain reaction (PCR) assay was conducted as described by McDonald et al. [5]. A total of 156 conjunctival, nasal and oral swabs used for chlamydia isolation in this study were employed. Not
only all the positive samples but one sample negative for chlamydia isolation were positive by the PCR assay.

Oligonucleotide primers (5' - TCTT TAGG GTAG TATGA - 3' and 5' - GAA TCAGA ATGGACCAAT - 3') for PCR were selected from the feline FePn strain (Genbank accession no. M73037) to amplify a 1,191-bp fragment of the ompA gene spanning from position 22 to position 1,212. The nucleotide sequence of the 1,191 bp amplified from the three strains, Fe/C-P8, Fe/C-N24 and Fe/C-B2822, isolated in this study were analyzed. Comparison of the ompA gene of the FePn strain with that of the strains, Fe/C-P8, Fe/C-N24 and Fe/C-B2822, isolated in this study showed notably high percentages of 100, 100 and 99.9% in their homology, respectively.

The seroepidemiological surveillance of C. psittaci in cats was performed from 1980 to 1999 in Japan [3, 7, 13]. These reports showed the chlamydial infection in cats spread gradually and widely in Japan. Pudjiatmoko et al. [7] described that 10 out of 11 prefectures in Japan had C. psittaci antibody positive samples with prevalence rates of 5–50%. Recently, Mochizuki et al. [6] reported the prevalence rate of C. psittaci in Japan was found to be 26.9% in cats with signs of conjunctivitis and rhinitis by PCR assay amplifying the ompA gene. However, the isolation of C. psittaci from domestic cats in Japan has not been previously reported.

There is a controversy as to whether C. psittaci plays the leading role in upper respiratory tract disease in cats [5]. Most of the chlamydial strains of feline origin were isolated from cases of conjunctivitis [2, 5, 8, 10], and conjunctivitis has been recognized as one of the most documented clinical signs in cats. However, experimental C. psittaci infection to kittens produces fever, lethargy, lameness, and reduction in weight gain besides ocular signs [11]. In the present study, 2 of 8 strains were isolated from nasal swabs and 5 of 6 strains were isolated from conjunctival swabs with upper respiratory signs. The pathogenicity of C. psittaci strains isolated from cats in the present study remains to be investigated.

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REFERENCES