A Study on Indoor Air Contaminants Related to Pets in Japanese Dwellings

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Abstract
The behavior of indoor particulate matter as it relates to pets was studied from the viewpoint of architectural hygiene. The viable particles from pets can be classified into two types, pathogenic microorganisms and pet allergens. This study investigated Pasteurella, which is a pathogenic organ of Pasteurellosis, dog allergen Can f1 and cat allergen Fel d1 in eight dwellings in Tokyo. In six of these dwellings (the case dwellings), the dogs and/or cats were allowed free access to indoor areas. Pasteurella and the pet allergens were quantified by biochemical and immunological methods, respectively. The relationship between suspended particles and airborne Fel d1 was also examined.

Three species, P. canis, P. dagmatis and P. multocida, were isolated from the oral cavity of the dogs and cats. In the six case dwellings, dust allergen Can f1 and Fel d1 ranged from 1 to 1000 µg allergen/g dust and 100 to 5000 µg, respectively. The indoor air of the case dwellings contained concentrations of airborne Fel d1 ranging from 10 to 100 ng of allergen/m³ air. In addition, there was a significant relationship between the distributed concentration of suspended particles larger than 5 µm and airborne Fel d1 (P< 0.01). These findings suggest that the traditional reduction method for coarse particles, for example the use of a domestic air filtration cleaner, may be effective in removing airborne pet allergens.

Keywords: particulate; Pasteurella; pet allergen; dwelling; indoor air environment

1. Introduction
The World Health Organization (WHO) has warned of P. multocida infection as a form of zoonosis (WHO 1959), and the Japanese Ministry of Health and Welfare officially identified this infection as a zoonosis in 1989. In Japan, the number of cases of Pasteurellosis has increased by about 10 times over the last decade. Pasteurellosis tends to be an opportunistic infection, and is caused by the pathogen Pasteurella, which is a gram-negative short rod-shaped bacterium that usually exists in the oral cavity of many mammals, including cats and dogs. It is well known that P. multocida is a frequent cause of infection following animal bites or scratches. In Japan, the reported cases of P. multocida infection have predominantly involved local infections, followed by respiratory infection (Arashima et al., 1990, Arashima et al., 1993). On the other hand, about 30% of Japanese suffer from allergies such as asthma, hay fever, mite allergy and chemical sensitivity, to name a few. Pet allergens have been considered to be an important cause of the increase in allergies in Japan.

There are two main reasons for the increase in Pasteurellosis and dog/cat allergies. One is a change in lifestyle that now sees pets accepted as members of the family, which has in turn resulted in close contact between pets (and their allergens) and humans. The other is an increase in the air tightness of dwellings in recent years in Japan, which has resulted in a rise in the indoor contaminant concentration caused by pets.

The objectives of the present study were to clarify the pollution status caused by the various Pasteurella pathogens and pet allergens in Japanese dwellings, and to devise a control method by which to reduce the indoor air pollution related to pets, such as airborne pet allergens.

2. Methods
Outline of the dwellings in the study.
Investigations on indoor air environments were conducted during the 2004/2005 winter in Tokyo. Table 1. shows the details of the eight selected dwellings.

Dwellings B, C, E, F, G, and H were selected as the "case dwellings" and had more than one dog or cat that was allowed free access to indoor areas. Dwellings A and D were the control dwellings and did not have pets. The following measurements were taken in the living room of each of the eight dwellings.
Airborne microbes.

Air samplers (Model: BIOSAMP MBS-1000, Midori Anzen Co., Japan) were placed in the center of each living room. According to the Japanese Industrial Standards K3836, the "collection performance test method of an airborne bacillus measuring instrument", MBS-1000, with the impaction method, has a collection efficiency of 99% and can detect particles smaller than 1 μm in diameter. Three different culture media, soybean casein digest agar (SCDA), potato dextrose agar (PDA), and chocolate agar (CA) were used to detect airborne bacteria, fungi and Pasteurella, respectively. PDA were supplemented with 100 mg/L chloramphenicol.

Particles were collected by impaction onto SCDA, PDA and CA plates. The air was sampled for two minutes for each measurement. Sampling was repeated twice for every plate in each living room. The airborne microbe concentrations were calculated in colony forming units per cubic meter (cfu/m$^3$). The samples were incubated at 25°C for 72 hours for PDA, at 32°C for 48 hours for SCDA and at 37°C for 90 hours for CA. Fig.1 shows the process for identifying Pasteurella.

Airborne allergens.

Airborne Can f1 and Fel d1 in dwellings E, F, G, and H were measured at the center of the room at a height of 1.2 meters above floor level using a low-volume air sampler (Model: LV-15, Shibata Co. Japan), which consists of three units, an air pump, a filter box and a vinyl tube. Two 30-minute samples from each dwelling were collected using the LV-15 air sampler with an airflow rate of 15 L/min or 0.9 m$^3$/h.

The Fel d1 and Can f1 allergens were quantified by a fluorometric sandwich enzyme-linked immunosorbent assay (ELISA). An Immulon 2 ELISA plate (Dynatech, Alexandria, VA) was coated with 2 μg/ml of either monoclonal anti-Fel d1 or rabbit anti-Can f1 for 3 hours at 37°C. The plate was then emptied and coated with 1% BSA-PBS at 37°C for 1 hour. After washing, standard allergens or diluted samples were added to the wells and the plate was incubated overnight at 4°C.

The plate was then washed and 0.2 μg of biotinylated corresponding IgG antibodies were added as detector antibodies. The plate was incubated for 1 hour at room temperature. After washing, β-D-galactosidase conjugated streptavidin (Zymed Laboratories, San Francisco, CA) was added and the plate further incubated for 1 hour at room temperature. After a final washing, 0.1 mM 4-methylumbelliferyl-β-D-galactoside (Sigma, St. Louis, MO) was added to each well and the plate was incubated for 2 hours at 37°C. The enzyme reaction was stopped with 0.1 M glycine-NaCl (pH 10.2) and the fluorescence intensity was read as fluorescence units on a microplate fluorescence reader (Fluoroskan, Flow Laboratories, McLean, VA) (Sakaguchi, et al., 1993).

Settled microbes and allergen.

After measuring airborne microbes, the concentration of suspended particles and the air change rate per hour (ACH), house dust was collected from the surfaces of the living room floors using a paper filter equipped with a hand cleaner (Matsushita Co., Japan). These
samples were used for the analysis of Pasteurella, Can f1 and Fel d1. Pasteurella in the oral cavities of pets. Pasteurella in the mucus of the oral cavities of the dogs, cats and occupants was collected using oral swabs (EIKEN Co., Japan). These were then cultured in the laboratory. Suspended particles.

Suspended particles were measured using an optical particle counter KR-12A (Rion Co., Japan) simultaneously with measurements of bacteria, fungi, and Pasteurella species. The KR-12A counter measures the scattering of light to determine the concentration of suspended particles of different sizes. The size ranges of the KR-12A channels are 0.3–0.5, 0.5–0.7, 0.7–1, 1–2, 2–5 and >5 µm.

Air change rate.

The air change rate per hour (ACH) of each case room was measured using the tracer gas decay technique. SF6 was used as the tracer gas. Air temperature and relative humidity.

The air temperature and relative humidity of each room were measured at intervals of 5 minutes for one week (dwellings A, B, C and D) or for the duration of the measurements mentioned above (dwellings E, F, G and H).

Questionnaire.

A questionnaire survey mainly focusing on lifestyle and contact with pets was also carried out in this study.

3. Results

Air change rate.

The ACHs of dwellings B, C and D ranged from 0.50 to 1.31 h\(^{-1}\), while the others were less than 0.11 h\(^{-1}\). These results illustrated the increase in air tightness of dwellings in Japan. Fig. 2 shows the decay of the concentration of tracer gas in dwelling A. This result clearly demonstrated that opening windows is an effective method for ventilation. Fig. 3 shows the indoor air temperature and relative humidity of dwelling A, before, during and after opening windows. Although the air temperature decreased sharply after opening the windows, it returned quickly to its original value after closing the windows.

Air temperature and relative humidity.

The mean indoor air temperature of each dwelling was about 20°C, except for dwelling D where the lowest value of air temperature was about 16°C. The cause of the lower air temperature in dwelling D was due to poor insulation performance. On the other hand, the mean indoor relative humidity of each dwelling was about 40–60% and no significant differences were demonstrated. In winter, the indoor relative humidity was maintained at a higher level although the relative humidity of outdoor air was approximately 20%.

Airborne microbes.

Indoor bacterium and fungal spore concentration ranged from 50 to 700 cfu/m\(^3\) and 30 to 250 cfu/m\(^3\).
m³, respectively. There were no differences in the concentration of bacterium and fungal spores between the case dwellings and the control dwellings.

**Pasteurella.**

*P. canis, P. dagmatis* and *P. multocida* were isolated from the oral cavities of the dogs and cats (Table 2.).

However, no *Pasteurella* was isolated from settled particles, indoor air or the oral cavities of occupants. Suspended particles.

**Fig. 4. shows the mean concentrations of indoor suspended particles in each dwelling.** The concentrations of suspended particles larger than 5 µm in the case dwellings (B, C, E, F, G, H and I) were higher than those in the control dwellings (A and D).

**Dog and cat allergens.**

As expected, there were significantly higher levels of Can f1 and Fel d1 per gram of dust in the case dwellings than in the control dwellings (Fig.5.). Fig. 6. shows the airborne allergen concentrations of Can f1 and Fel d1. Airborne Fel d1 in dwelling F, with seven cats, was 10 times higher than in the other dwelling with 1 or 2 cats.

Although data collection was insufficient, there were obvious tendencies toward a greater amount of dust allergen leading to a higher concentration of airborne allergen (Fig.7.), and a higher concentration of suspended particles larger than 5 um leading to a higher airborne Fel d1 concentration (Fig.8.).

**Questionnaire.**

The results of the questionnaire surveys showed that a pet was often considered to be a family member (all cases). In all cases, the pets helped people reduce loneliness and were given free access to indoor areas.

On the other hand, regarding contact with pets, owners of dwellings B and C reported kissing their pets frequently, feeding their pets by mouth and sleeping with their pets. In these dwellings, contact between the owner and the pet was frequent.

**4. Discussion**

The indoor air quality, including air temperature, humidity, ACH and airborne microbes in the case dwellings did not differ significantly from that in the control dwellings. According to the results of measurements of ACH and the questionnaire surveys on lifestyle, two changes, namely the air tightness of
dwellings and close contact to a pet, were confirmed. Until now, only four species, *P. multocida*, *P. canis*, *P. dagmatis*, and *P. stomatis*, have been identified as Pasteurella. In the present study, 3 (60%) of 5 dogs and 9 (80%) of 11 cats harbored Pasteurella (*P. canis*, *P. dagmatis*, or *P. multocida*), confirming the high ratio of Pasteurella species in the oral cavities of dogs and cats. Regarding respiratory tract infections resulting from *P. multocida*, since 20% of cases have no history of contact with an animal, aerial infection is considered to be the infection route. However, no Pasteurella species were isolated from the indoor air or dust in the present study. This suggests that the capture of Pasteurella from an indoor environment is difficult, although generating Pasteurella from the sneeze of a cat, for example, is probably easy. Further investigations are needed to improve the method of sampling of Pasteurella in dwellings.

The relationships between settled dust and airborne allergens have been closely studied (Blay et al., 1991; Wickman et al., 1999; Munir et al., 2003). In the present study, a significant correlation was found between the Fel d1 in dust and that in air (P<0.05). Our measurements clearly indicated that airborne pet allergens are strongly influenced by the amount of dust allergen, which can be re-dispersed into indoor air by working and other activities. The dust Fel d1 and airborne Fel d1 values in dwelling F, with seven cats, are the highest ever-reported in Japan (Sakaguchi, 1993). From the viewpoint of architectural hygiene, ventilation and purification are the two most effective methods for reducing indoor air pollution concentrations, including particulate matter related to pets. Regarding the reduction of contaminant concentrations by ventilation, Wickman et al. (1999) reported a correlation between increased ACH and decreased levels of Fel d1 in the air. Our measurements verified the reduction of trace gas and the increase of ACH by opening the windows in dwelling A. Although the results were influenced by the outdoor climatic conditions, according to the measurement of ACH, the indoor air mixed with the fresh air in a very short period of time (2 minutes). These results suggest that opening windows (e.g., once per hour) is effective in reducing the indoor concentration of pollutants related to pets.

On the other hand, for the filtration of allergens by an air filter, Green et al. (1999) reported that HEPA (high efficiency particulate air filter) air cleaners reduced airborne Can f1 in homes with dogs. Gore et al. (2003) reported that the use of domestic air filtration units appeared to result in a reduction in inhaled cat allergen. As we clarified that airborne pet allergens are mostly particles larger than 5 µm, which are known as coarse particles, it is possible to reduce the indoor airborne pet allergen concentration using a domestic air filtration cleaner equipped with a medium efficiency particulate air filter.

5. Conclusions

[1] We confirmed a high ratio of *Pasteurella* species, *P. canis*, *P. dagmatis* and *P. multocida*, in the oral cavities of dogs (60%) and cats (80%) in Japan.

[2] Dust allergen Can f1 and Fel d1 ranged from 1 to 1000 µg/g and 100 to 5000 µg/g, respectively, in the six case dwellings. The concentration of airborne Fel d1 in the case dwellings ranged from 10 to 100 ng/m³. Both the 5000 µg/g (dust Fel d1) and 100 ng/m³ (airborne Fel d1) measurements are the highest ever-reported in Japan.

[3] The concentration of suspended particulate matter larger than 5 µm shows a significant relationship with airborne Fel d1 (P<0.01).

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