

## Short communication

# Pigeon allergens in indoor environments: a preliminary study

**Background:** Few studies have measured pigeon allergens in non pigeon coop environments. This study was conducted to determine approximate pigeon dropping allergen concentrations in indoor environments.

**Methods:** Polyclonal antibody serum was prepared by injecting a rabbit three times with crude wild pigeon dropping extract in 50 mM Tris buffer with Freund's adjuvant. One hundred and fifteen dust samples were collected in a pigeon-infested school, pigeon coops, homes and hospitals and analyzed by a direct competitive pigeon enzyme-linked immunosorbent assay (ELISA).

**Results:** The highest level of pigeon allergen inhibitory activity were recorded in four samples from pigeon coop bedding samples with a median activity of 11.2% relative to pigeon droppings. The second highest level of pigeon allergens was in a pigeon-infested high school with a median or 7.4% activity relative to pigeon droppings. At an entrance underneath pigeon roosts, one sample had a relative inhibitory activity of 62.3%. Pigeon allergen inhibitory levels were generally low in the home and hospital samples, but nevertheless 46 out of 89 of these samples were still above detection limit.

**Conclusions:** This study suggests that large concentrations of pigeon allergens can be found in buildings without domestic pigeons such as the pigeon-infested high school.

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It has long been known that pigeon keepers heavily exposed to domestic pigeons can develop asthma, rhinitis and an allergic alveolitis known as pigeon breeders lung (1–4). In addition, many infectious microbes can be spread by pigeon droppings, including *Chlamydia*, *Mycoplasma*, *Aspergillus fumigatus*, *Histoplasma capsulatum*, *Cryptococcus neoformans* and *Influenza* (3, 5). Only recently has it been recognized that less intensive exposure to wild pigeons can cause hypersensitivity reactions. A previously healthy 34-year old Dutch woman exposed to wild pigeons on the outdoor ledge of her house died following flu-like illness, shortness of breath and alveolitis (6). Her five children, aged 8–14 years, developed shortness of breath and high antibody titres to pigeon allergens (6). A 61-year-old Japanese woman exposed to wild pigeons at work developed dyspnea, non productive cough, lung opacities and high levels of antibodies to pigeon droppings (7). A recent Mexican study found that pigeon breeders disease was associated with certain histocompatibility alleles (8).

Pigeon-sensitive patients can develop immunoglobulin (Ig)G, IgA, IgE and IgM to a wide range of proteins in pigeon serum, feather and feces (9). Several studies have noted that pigeon intestinal mucin and pigeon IgA are highly allergenic (10–12). Pigeon droppings are a

highly complex mixture of many different proteins with at least 10 different allergenic fractions described (13–17).

Pigeons are very common in large cities and many buildings undergo extensive cleanup to remove pigeon droppings, feathers and dead birds (5). Relatively few studies have measured pigeon allergen in dust and air (3). Edwards and coworkers (18) utilized pigeon breeder lung serum antibodies to construct an ELISA for measuring pigeon allergens in pigeon loft litter.

This preliminary study was conducted to determine if significant pigeon allergens were present in schools, hospitals and homes in the greater Chicago area. Several pigeon-sensitive patients and an occupational physician had contacted the authors to inquire about possible measurement of indoor pigeon allergens.

## Material and methods

### Collection of house dust and bird dropping samples

House and school dust samples were taken from: (1) a pigeon infested three-story public high School in Chicago; (2) pigeon bedding and house dust from two Illinois pigeon fanciers; (3) two homes with indoor parakeets/cockateels or canaries; (4) samples from two Illinois hospitals and (5) samples from 15 other Illinois homes without indoor birds. Dust samples were collected with vacuum cleaners. Fresh dried pigeon (*Columbia livia*) dropping

samples were collected from a wild pigeon colony in Chicago. Fresh dried bird droppings from parrot (*Psittaciformes* species), parakeet (*Melopsittacus undulatus*), cockateel (*Nymphicus hollandicus*) and finch (order *Passeriformes*) were obtained from an Illinois pet shop. These droppings from other birds were collected to test for the relative specificity of the pigeon dropping antibody serum. Ten percent solutions of these bird droppings were dissolved in pH 7.4 phosphate-buffered saline (PBS), well homogenized and the supernatant collected for assays.

Airborne dust samples were also collected in five areas of the school including the cafeteria, south hallway near the cafeteria, rooms 203 and 302, and the plenum in the air-handling unit. About 7–8 m<sup>3</sup> of air was collected on 25 mm cellulose filters at an air flow of 0.028 m<sup>3</sup>/min. All dust samples, airborne dust samples and bird droppings were frozen at –20°C until analysis.

The school in which the dust samples were collected had long-standing problems with pigeons. One 55-year-old female teacher was physician diagnosed with allergic alveolitis.

#### Development of polyclonal antibodies to crude pigeon extract and conjugation

Polyclonal antibody serum was prepared by the method of Coligan and coworkers (19). Crude pigeon droppings were dissolved in 50 mM Tris Buffer, containing 0.3 M NaCl and 0.5% Triton X-100 at a pH of 7.6. This mixture was stirred and sonicated for 6 h at room temperature. At day 0, 2 ml of serum was removed from the rabbit to use as a control preimmune fraction. On day 1, the rabbit was then injected with a 1 : 1 mixture of the crude pigeon dropping Tris extract and Freund's Complete Adjuvant (Item #F5881, Sigma Chemicals, PO Box 14508, St. Louis, MO, USA). At 29 days, and 58 days, the rabbit was given additional injections of the pigeon Tris extract with Freund's incomplete adjuvant (Item #F5506, Sigma Chemicals). The animal was then bled at day 68 and the polyclonal antiserum was stored frozen until used.

The antibody was then conjugated with horseradish peroxidase using conjugation kit #1-829-696 (Roche Chemicals, Mannheim, Germany).

#### ELISA assays for pigeon allergens

A direct competitive ELISA for pigeon allergens was constructed by the method of Ausbel and coworkers (20). Sodium carbonate buffer solutions (100 µl at pH 9.6) containing 240 µg of pigeon dropping proteins were coated on ELISA plates overnight at 4°C. The plates used were Dynex Immolon 2HB High Binding Plastic Plates (Dynex Products, Helsinki, Finland). The plates were then washed three times with a PBS solution with 0.05% Tween and blocked for 1 h with 5% milk solution, and then washed again three times with PBS. Then 25 µl of sample solutions and pigeon allergen standards were mixed with 25 µl of a 1/2500 dilution of the antipigeon dropping antibody horseradish peroxidase conjugate and allowed to incubate 1 h at room temperature. Then 50 µl of the sample solution antibody conjugate solutions were added to the pigeon dropping coated plates

and allowed to incubate 2 h at room temperature. The plates were then washed again three times with PBS and a Sigma OPD solution was added (Sigma Chemicals). The color was then read at 492 nm with an automatic plate reader (Biotek Instruments, Vermont, USA).

The dried pigeon droppings were found to contain about 18% protein as measured by the Pierce 23227 BCA protein assay kit (Pierce Chemicals, Rockford, IL, USA).

As a control serum sample, a total unrelated antibody, horseradish peroxidase conjugated rabbit antileptin serum was randomly selected. This antileptin serum was used on samples of pigeon and five other bird droppings instead of the antipigeon-conjugated serum on several of the ELISA plates.

#### Data handling and statistics

A curve of ELISA inhibition by standard solutions of various pigeon dropping concentrations (containing 18% protein by dry weight) was prepared to calculate sample inhibition levels. Pigeon allergen activity was calculated on the basis of ELISA inhibition activity on a dry weight basis relative to the inhibition of pigeon droppings. Most of the above detection limit samples had a least one dilution in the 25–75% inhibition range.

Duplicate split sample assays for 15 of the samples from the high school and pigeon fanciers house/coops were run to estimate precision and reproducibility of the ELISA samples. The detection limit was calculated as two standard deviations above the blank. This corresponds to about 0.2% blocking activity of pigeon droppings. Because of variation in ELISA readings, some samples may have a slightly negative value for pigeon dropping activity.

Because the pigeon allergen inhibitory activities were not normally distributed, statistical differences between dust collected at various indoor sites were calculated with a two-sample comparison Mann–Whitney U nonparametric rank test using an SPSS program (21). Differences between groups with a *P*-value < 0.05 were considered statistically significant.

#### Results

Table 1 below gives summary statistics of pigeon allergen concentrations collected in various locations. Table 2 lists 11 samples taken in a pigeon infested high school. Table 3 below notes ELISA inhibitory activity of the rabbit antiserum to crude pigeon dropping extract to droppings from birds other than pigeons.

Of the five airborne samples, only the sample from the hallway near the cafeteria had any measurable pigeon inhibitory activity. This activity corresponded to about 3.9% of the pigeon droppings on a dry weight basis (the hall dust sample has about 6.2% of the pigeon dropping activity) or 0.97 µg pigeon dropping protein per cubic meter. Table 2 notes that the highest

Table 1. Summary statistics from pigeon dust allergen ELISA measurements

| Location                                 | Total number of buildings | Total number of samples | Percentage median inhibitory activity relative to pigeon droppings | Range of values | Number of samples over detection limit | Percentage of samples over detection limit |
|------------------------------------------|---------------------------|-------------------------|--------------------------------------------------------------------|-----------------|----------------------------------------|--------------------------------------------|
| Pigeon Loft Bedding Material             | 2                         | 4                       | 11.2%                                                              | 6.9% to 15.8%   | 4                                      | 100%                                       |
| High School*                             | 1                         | 11                      | 7.4%                                                               | 0.2% to 62.3%   | 11                                     | 100%                                       |
| Homes of pigeon breeders                 | 2                         | 4                       | 3.1%                                                               | 2.3% to 4.5%    | 4                                      | 100%                                       |
| Homes with parakeets/cockateels/canaries | 2                         | 7                       | 2.0%                                                               | –0.1% to 7.7%   | 5                                      | 71%                                        |
| Homes with no indoor birds               | 15                        | 62                      | 2.0%                                                               | –0.2% to 10.6%  | 36                                     | 58%                                        |
| Hospitals                                | 2                         | 27                      | 0.5%                                                               | –0.2% to 1.3%   | 10                                     | 37%                                        |

\*Only the high school dust samples included on this table, no airborne samples included.

Table 2. Pigeon ELISA measurements in a pigeon infested Chicago High School (all samples in dust unless noted as airborne)

| Location                                                           | Inhibitory activity relative to fresh pigeon dropping |
|--------------------------------------------------------------------|-------------------------------------------------------|
| Cafeteria                                                          | 1.2%                                                  |
| Hall next to cafeteria                                             | 6.3%                                                  |
| Airborne sample from hall next to cafeteria- airborne              | 3.9%                                                  |
| Classroom 203                                                      | 0.2%                                                  |
| Classroom 203- airborne                                            | Below detection limit                                 |
| Classroom 302                                                      | 0.3%                                                  |
| Classroom 302- airborne                                            | Below detection limit                                 |
| Plenum air-handling unit                                           | 2.3%                                                  |
| Plenum airborne                                                    | Below detection limit                                 |
| West entrance underneath many pigeon roosts                        | 62.3%                                                 |
| 1st floor hallway about 10 meters from west entrance               | 7.4%                                                  |
| Classroom 355 floor room near pigeon roosts (windows often open)   | 26.2%                                                 |
| Classroom 355 indoor window sill- near pigeon roosts               | 27.4%                                                 |
| South-east entrance underneath many pigeon roosts                  | 32.7%                                                 |
| First floor hallway about 10 meters from south-east entrance       |                                                       |
| Outdoor airborne sample taken about 10 meters north of high school | 30.1%                                                 |
|                                                                    | Below detection limit                                 |

inhibitory activities occurred in the pigeon coops and pigeon-infested high school. Mann-Whitney two-way comparison tests indicated that pigeon dust allergen activities in the pigeon coops were significantly higher than in the home with other birds ( $P=0.014$ ), homes without birds ( $P=0.0016$ ), and hospitals ( $P=0.0015$ ). Pigeon dust allergens activities in the high school were also significantly higher than in the homes without birds ( $P=0.011$ ) and hospitals ( $P=0.004$ ). Pigeon dust levels were also significantly lower in the hospitals as compared to pigeon breeders homes ( $P=0.0015$ ), homes with other birds ( $P=0.022$ ) and in homes with no birds ( $P=0.0001$ ). All other two-way comparison were insignificant at  $P = 0.05$ .

Table 3 notes that highest inhibitory activities were found at the west entrance (62.3% activity of pigeon dropping) and south-east entrance (32.7%). These entrances were directly beneath many pigeon roosts located on the window sill and 3rd floor roof. Many pigeon droppings fell over these entrances and were tracked in by students and teachers. High levels of pigeon allergen inhibition were also detected on the floor (26.2%) and the indoor windowsills (27.4%) of room 355, which is near many pigeon roosts and often has the windows opened. This room has the 55-year-old female teacher with hypersensitivity pneumonitis.

The 15 split-paired samples had a mean coefficient of variation of 16%.

Table 3. Activity of bird droppings to rabbit anti pigeon dropping serum

| Bird dropping       | Percentage activity $\pm$ SD relative to pigeon droppings* |
|---------------------|------------------------------------------------------------|
| Parrot droppings    | 40.9% $\pm$ 10.6%                                          |
| Cockateel droppings | 33.6% $\pm$ 5.0%                                           |
| Finch droppings     | 30.0% $\pm$ 2.4%                                           |
| Parakeet droppings  | 19.6% $\pm$ 2.5%                                           |

\*4 samples of each bird dropping were analyzed.

When the rabbit preimmune serum was used on 10 pigeon dropping samples, no binding occurred. When the rabbit leptin antibody serum was used instead of the rabbit antipigeon dropping antibody serum, no samples were above the detection limit for the 12 pigeon dropping samples and four samples each of parrot, parakeet, cockateil and finch droppings (the total was 28 samples). This indicates that neither the rabbit preimmune serum or the rabbit antileptin serum had any significant binding to the bird dropping antigens.

## Discussion

This study suggests that considerable quantities of pigeon allergens can be present in wild pigeon-infested schools and other environments in which pigeons are not kept indoors. Some of the school samples had higher percentages of pigeon allergens in the dust as compared to pigeon coop bedding material. Pigeon allergen concentrations in the 89 samples from hospitals and homes without indoor birds were generally low. Nevertheless, 46 of these samples (52%) still had pigeon allergen concentrations above the detection limit.

The rabbit antipigeon dropping serum reacted to all four of the bird-dropping samples tested with activities ranging from 19.3% in the fresh parakeet droppings to 40.9% in the fresh parrot droppings.

It has long been known that exposure to common indoor allergens such as dust mites, cats, dogs and fungi can worsen asthma and allergies (22, 23). Recent studies have demonstrated that high exposures to cockroach (24, 25) and mouse allergens (26, 27) often occur in inner-city dwellings, and that exposure to cockroach or mouse allergens are associated with higher asthma rates. Pigeon allergens may also play an important role in worsening asthma in certain urban environments which contain many pigeons. This study analyzed 115

dust samples from 24 buildings. Additional larger environmental studies and/or studies linking pigeon allergen exposure to adverse health effects would be very useful.

This study measured pigeon allergens primarily in indoor dusts, with only five airborne samples which collected only small amounts of air (7–8 m<sup>3</sup>). Further studies to measure pigeon allergens in larger air samples would be useful but would require many collection hours per sample with a high volume air sampler. Airborne measurements of allergens are probably a better estimate of human exposures than dust-borne exposures. Table 2 notes that the concentration of allergens in many of the school dust samples were higher than from dust from pigeon coops. However, schools are generally much less dusty than pigeon coops so that total amount of pigeon airborne pigeon allergens might be greater in the dusty pigeon coops than in the schools.

This study has shown that considerable amounts of pigeon allergen are present in some urban environ-

ments. Further use of pigeon allergen polyclonal assays may be useful in providing a quick and inexpensive estimate of exposure to pigeon allergens in indoor situations. Such exposure estimates should be very useful in conducting remediation work and/or health studies in buildings contaminated with pigeon droppings.

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This paper was prepared using the guidelines for nomenclature as described by Johansson et al. (28).

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