

Bird antigen persistence in the home environment after removal of the bird

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Timothy J. Craig, DO; Joyce Hershey; Renata J.M. Engler, MD; William Davis, MD; Gary B. Carpenter, MD; and Kalman Salata, PhD

Hypersensitivity pneumonitis (HP) secondary to bird exposure is treated with glucocorticosteroids and avoidance. Despite therapy, symptoms may persist for a prolonged time. Just as cat antigen, Fel d 1, may persist for greater than 20 weeks after cat removal, there may be persistent bird antigen to explain prolonged symptoms in bird HP. It was the intent of this study to determine household distribution and persistence of bird antigen after removal of the bird from the patient's home.

The homes of patients with birds were followed serially after bird removal with multiple samples collected using a hand-held vacuum cleaner. Bird antigen levels were determined by an inhibition enzyme-linked immunoassay. In five homes the antigen declined gradually despite extensive environmental control measures, with high levels still detectable at 18 months in one home.

This data suggests that high levels of bird antigen can be detected for prolonged periods of time after bird removal and environmental cleanup. The antigen may account for the persistence of disease in some patients with HP. In severe HP, the preferred therapy may be temporary relocation of the patient away from the room in which the bird was housed, in addition to corticosteroids, until the patient's environment is demonstrated to be relatively bird antigen-free.

INTRODUCTION

Hypersensitivity pneumonitis (HP) secondary to pigeon exposure has been well described.^{1,2} Unlike pigeon breeders who are exposed to large amounts of bird antigen on an intermittent basis, usually outside the home, patients with HP from pet birds are continuously exposed to the antigen from birds housed in the home. It appears that very low levels of antigen may be enough to induce HP.³ In addition, serology and sometimes symptoms and signs of HP persist despite removal of the pet bird from the home.⁴ A possible explanation for this is that bird antigen may persist, for long periods of time, as does cat antigen.⁵

From the Walter Reed Army Medical Center, Allergy-Immunology Service, Washington, DC.

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In this study, our goal was to assess bird antigen levels in the homes of patients with birds, and to follow the decrease of the bird antigen over time.

METHODS

Dust samples were collected using a portable hand vacuum (Douglas Rx 365 Hand Vacuum, model 6735 Douglas Products, Walnut Ridge, AR). A 1-square meter area was vacuumed for three minutes. At approximately monthly intervals, samples were collected from six homes, from the bird's room and (when possible) at least one additional room located at a distance from the bird. Three of the houses sampled on a monthly basis were houses of patients with HP (homes 1, 2, and 3). Controls included samples from homes with birds (positive - four homes) and samples collected in homes with no history of pet birds (negative - two homes). The dust was stored at -70°C until processed. All but two birds were cockatiels. The others included a parakeet and an African grey. Both

had good cross-reactivity with cockatiel.

Cockatiel droppings (CD) were collected from the bottom of bird cages, extracted and used as capture antigen. Dust samples and CD were extracted in similar fashion. The extract was prepared by using a 1:20 wt/vol dilution of CD or dust in phosphate buffered saline (PBS) pH 7.2. After overnight extraction, the samples were centrifuged at 1500 RPM for 20 minutes, followed by filtration with a 0.22-millipore filter; they were then frozen at -70°C until used. Serum was obtained from a patient with HP who had high titers of IgG against cockatiel antigen and was used at 1:2500 dilution. This serum contained high titers of anti-cockatiel IgG of subclasses 1, 2, and 3, as well as IgA. Goat anti-human IgG with an alkaline phosphatase label was obtained from Tago (Burlingame, CA) and used at a 1:1000 dilution. Optimal dilutions were determined in preliminary studies. Buffers used were running buffer [PBS with 0.1% bovine serum albumin (BSA) and 0.05% Tween], blocking buffer (PBS with

0.2% BSA) and wash buffer (PBS with 0.05% Tween). Substrate (*p*-nitrophenyl phosphate) was obtained from Sigma Chemical Company (St. Louis, MO).

Bird antigen in dust samples was assessed by a competitive ELISA inhibition assay in 96-well Immulon plate (2-U, Dynatech, Chantilly, VA). All samples were run in duplicates. Plates were coated with 1:2500 dilution of extracted CD in carbonate buffer (pH 9.6), and insulated overnight at 4 °C. Dust samples were diluted 1:2 over a range of 1:40 to 1:5120. Standards were prepared from the same extract used to coat the plate, and diluted 1:2 over a range of 7.8 units/mL to 1,000 units/mL. One unit is equal to 1 µg of extracted standard CD. Anti-cockatiel serum (50 µL) was mixed with dilutions of bird dropping standards (50 µL) and dust sample extracts (50 µL). After 90 minutes' incubation at 37 °C, and wash, 100 µL of 1:2000 goat anti-human IgG alkaline phosphatase was added to each well; after another 90 minutes incubation and wash, *p*-nitrophenyl phosphate in diethanolamine buffer (pH 9.8) was added for eight minutes at room temperature. Optical density was read at 405 nm on a Dynatech MR5000 microplate reader (Chantilly, VA). Optical density was measured in each well, and results were plotted against units of concentration. Optical density from dust extracts was compared to the standard curve to obtain the concentration of bird antigen in the dust samples. All data are expressed in units.

RESULTS

Intra-assay variability was 6.8% and interassay variability was 8.5%. The curve demonstrates good inhibition (Fig. 1). The lowest level of bird antigen was detected in homes without birds and the optical densities measured did not differ significantly from wells without dust added (Fig. 2). The one exception was a sample collected from an enclosed porch

with a deck around it, which was frequented by wild birds attracted by bird feeders. On the deck and around the door connecting the room to the deck there was a large

amount of bird droppings. Since this area was frequented by occupants of the house it is suspected that bird antigen was tracked in on their shoes. Measurements of this sample showed substantial amounts of bird antigen. Other samples collected from different rooms of this same home were free of bird antigen.

The highest levels of bird antigen were detected in samples from rooms in which a bird was living. Rooms distant from the one in which the bird was housed had significantly less antigen (Fig. 2). Only one (sample L) had antigen levels that were high in distant rooms and this was in a home where the bird was allowed free access to all rooms in the house.

Serial assessment of homes after the removal of the bird revealed a slow degradation of antigen over months (Figs. 3 and 4). At present, antigen has been detected at 4, 6, and 18 months (most recent date at which samples were collected). Five out of six homes followed serially had significant levels of antigen. The exception was home #6 where the bird was housed in a room with a wooden floor and separated from the nearest carpeted room by a solid

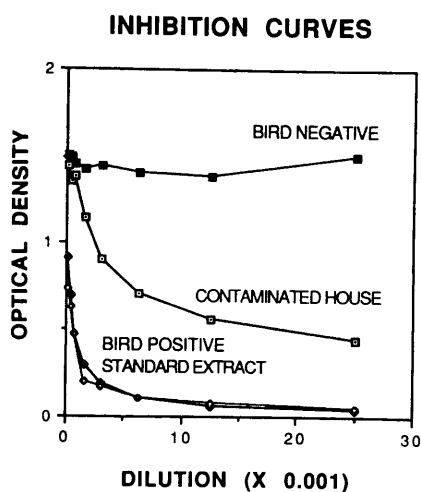


Figure 1. Inhibition curves for samples collected from homes without birds (bird negative), homes with birds (bird positive), standard extract, and from a partially contaminated home. The horizontal axis is the dilution of house dust extract and the standard bird dropping extract. The vertical axis is the mean optical density at each dilution. The lower the optical density, the more inhibition of anti-cockatiel IgG binding by bird antigen in the dust.

BIRD ANTIGEN LEVELS IN ROOMS

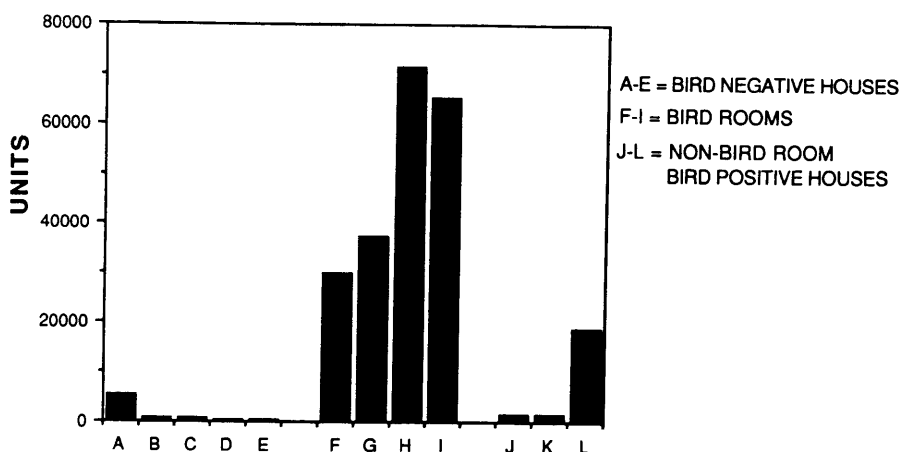


Figure 2. Comparison of extracts of dust collected in rooms from bird negative homes (A-E), rooms with birds (F-I) and rooms distant from where the bird is kept (J-L). Horizontal axis is the identity of the samples and the vertical axis is the units of cockatiel antigen in dust samples. One unit is equal to 1 µg of extracted cockatiel droppings. House A was contaminated by outside bird droppings.

barrier. Cleaning of the cage was carried out weekly and the wooden floor was cleaned 2 to 3 times per week.

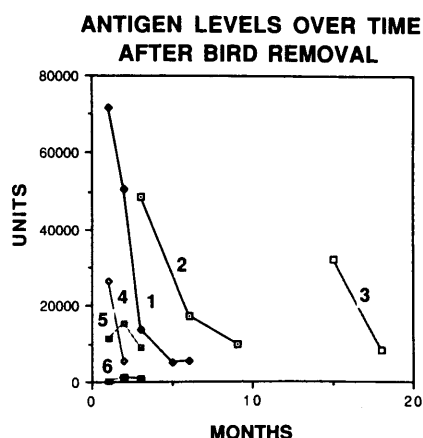


Figure 3. Horizontal axis is time over which serial samples were collected after the bird was removed from the house. Vertical axis is units of cockatiel antigen in dust samples. One unit is equal to $1 \mu\text{g}$ of extracted standard cockatiel droppings. Significant antigen levels were detected from carpets in homes 1-5 after removal of the birds. Sample 6 was collected from a house in which the bird was kept in a room with wooden floors and only trace amounts of antigen were detected.

DISCUSSION

As evident from our data, bird antigen persists in the home environment for a prolonged period of time after the bird has been removed. Environmental cleanup, including rug cleaning, failed to eradicate the antigen. Unlike *Fel d 1* antigen which is light and permeates the home,⁵ reduced amounts of bird antigen were detected away from the bird's room, suggesting that the antigen may be less buoyant. A second explanation is that, unlike cats, the bird is usually restricted to a cage. The one house with a free flying bird had large amounts of antigen detected in both rooms sampled. It also appears that vigorous cleaning of the bird cage, along with housing the bird in an isolated room that can be easily cleaned (wooden instead of carpeted floors), may decrease the amount of residual bird antigen. Last, it appears that significant amounts of bird antigen can be found in homes without birds if wild bird excrement is heavily deposited outside the house. It is pre-

sumed that the antigen is tracked in on shoes.

Treatment of HP requires antigen avoidance and sometimes corticosteroids. If the persistence of serology and symptoms is secondary to persistence of antigen, more aggressive therapy than just removing the bird from the home would be essential. Our data suggest that refractory cases may be due to persistence of the bird antigen in the home, and in severe cases, the patient may need temporary living quarters until vigorous environmental cleanup is done. Even after cleanup, avoidance of the room in which the bird was kept may be of benefit. In patients with bird-induced HP who fail to respond adequately to therapy, a home visit with assessment of dust for bird antigen may be warranted.

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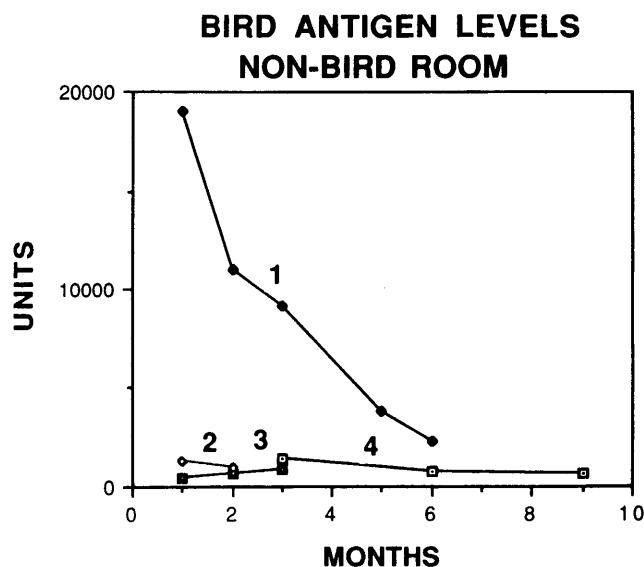


Figure 4. Time (in months) since bird was removed, plotted against amount of cockatiel antigen (in units) in rooms other than that in which bird was kept. One unit is equal to $1 \mu\text{g}$ of extracted standard cockatiel droppings. Generally, less antigen is found in rooms that did not house the bird (compare with figure #3). The exception is sample #1, where the bird had free access to all rooms in the house.

Request for reprints should be addressed to: Timothy Craig, LCDR, USN
Department of Allergy-Immunology
Walter Reed Army Medical Center
Washington, DC 20307-5000