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EVALUATING ALLERGIC RESPONSES TO BIRD ALLERGENS

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Inhalation of large amounts of organic dust for long and intense periods can cause an inflammation of the lung parenchyma in the terminal bronchioles and alveoli previously referred to as extrinsic allergic alveolitis, and now termed allergic alveolitis (AA).[1,2] The condition is often known by the more general name of 'Bird fancier's (breeder's) lung', with symptom prevalence of up to 16% among bird owners.[3] This condition may occur in pigeon breeders after close contact with pigeons (Fig. 1), but other birds such as budgerigars, parakeets, cockatiels and parrots can also cause it.

Fig. 1. Homing pigeon or rock dove (Columba *spp.*).

PATHOLOGY AND CLINICAL MANIFESTATIONS OF PIGEON BREEDER'S DISEASE

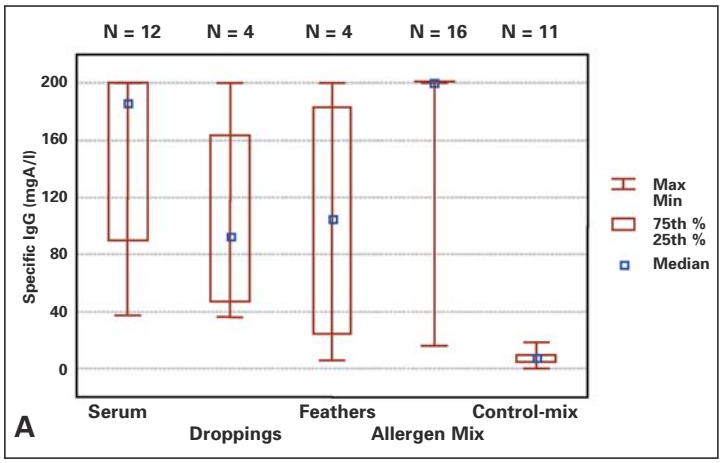
The presence of IgG antibodies in symptomatic people and a delay between exposure and onset of symptoms suggest complex pathogenic immune mechanisms. However, the involvement of cellular immune responses was also demonstrated by the presence of sensitised lymphocytes in both circulation and bronchoalveolar lavage (BAL) fluids,[4] but their role in the disease development remains unclear. B-cell involvement is also suggested by the characteristically strong IgG antibody response in blood, BAL fluid, sputum and saliva among exposed individuals.[5,6] Nevertheless, one has to bear in mind that during exposure to offending antigens, antibodies of different types of immunoglobulins can be generated in addition to IgG antibodies, such as IgE,[7] IgM[8] and IgA,[9-11] which are usually not quantified.

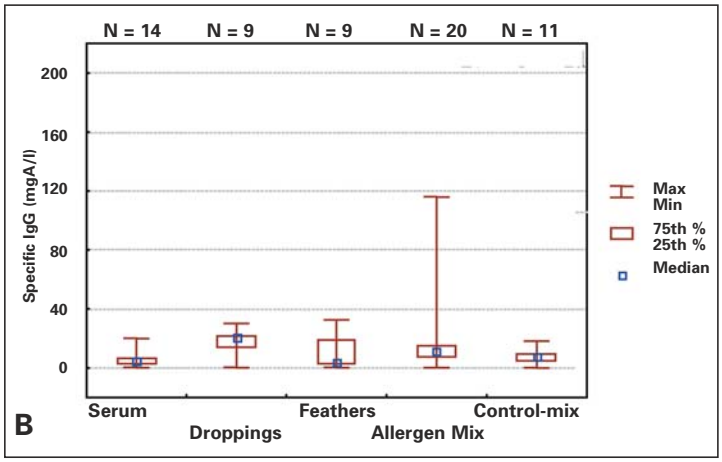
Symptoms may start soon after allergen exposure or even after many years and include breathlessness, cough, and occasionally chills and fever; they can even lead to death.[12] Symptoms typically commence a few hours after close contact with the birds (such as dipping, cleaning the loft, etc.) even if people take the precaution of wearing a facemask. Keeping pigeons is an occupation with an ancient and respected history, but there is a lack of awareness of associated infections and hypersensitivity disorders. Exposure can cause a heterogeneity of diseases such as asthma, ornithosis (microbial infections of birds which can be transferred to humans), lung inflammation from inhaling irritant dusts and AA. The latter is defined as an interstitial inflammatory disease associated with restricted ventilatory defect, reduced lung compliance and diffusion abnormality. AA is a pulmonary hypersensitivity caused by a variety of sources, e.g fungal, bacterial and animal proteins.

EXPOSURE TO BIRD ANTIGENS

Exposure to dust in the pigeon loft can be considerable, as pigeon fanciers spend on average 20 hours per week in their lofts.[13] Seasonal differences in dust levels and the airborne antigen concentrations have been measured. Dust levels are high during activity in the loft, with medians for respirable and non-respirable dusts of 12.11 mg/m^3 and 1.58 mg/m^3 respectively.[14] The airborne particles are generally 1-3 microns in diameter which is of aerodynamic size likely to settle in the alveoli. The antigen content in the dust ranges from 0 to 40 µg/ml (median 0.55 µg/ml), as measured by enzyme-linked immunoassay (ELISA), and makes up on average 0.8% of the total dust. This information confirms that individuals are exposed to considerable amounts of dust and antigens in pigeon-breeding areas, particularly during the autumn moult of the birds, which in turn is correlated with periodicity in the serological responses seen in pigeon fanciers.

Only recently has it been recognised that a low degree of environmental exposure to bird antigens can stimulate the immune response and result in hypersensitivity reactions. One of the very few studies evaluating environmental exposure demonstrated bird antigens in the indoor environment.[15] The highest levels of antigens of pigeon droppings, analysed in 115 dust samples, were found in pigeon coops, and surprisingly in pigeon-infested schools and to a lesser extent in homes and hospitals. However, over 50% of the samples from homes and hospitals demonstrated increased levels above the detection limit. A family in Holland was exposed to wild pigeons on the outdoor ledge of their house and developed shortness of breath, alveolitis and high antibody titres to pigeon allergens.[16] Subsequently the mother died. Two case reports from Japan demonstrated that patients, who had previously kept birds (pigeons, budgerigar), subsequently developed AA after using feather duvets or pillows,[17] and this after long periods of not keeping birds. Cross-reactivity among bird antigens has been previously demonstrated and could account for this incident. Furthermore, exposure to bird antigens is also seen in the occupational setting of chicken breeders. A previous cross-sectional study among South African poultry workers by Rees *et al.*[18] showed a high prevalence of exposure-related symptoms. However, the immunological assays employed at this time could not demonstrate association between disease and specific IgG antibody titres.





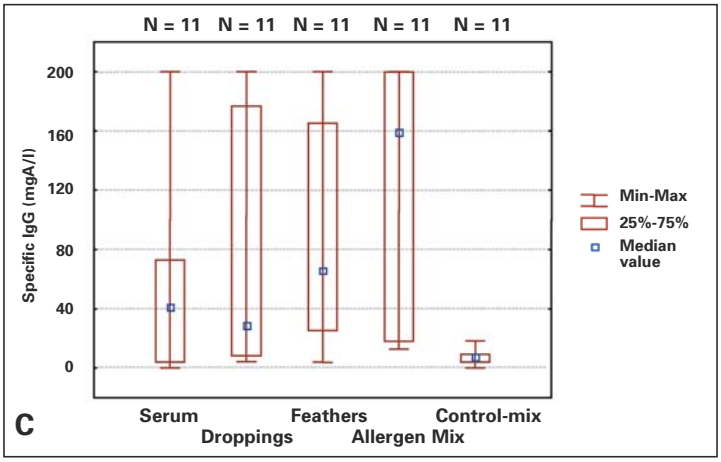


Fig. 2. Evaluation of specific IgG antibodies to different allergen sources of pigeons. Group A (PP group; Fig. 2A), symptomatic subjects with positive Ouchterlony; Group B (non-PP group; Fig. 2B), symptomatic subjects with negative Ouchterlony; Group C (Fig. 2C), exposed pigeon breeders. Specific IgG levels were quantified using the UniCAP system and specific immunoCAPs for pigeon serum, droppings and feathers, and compared with the pigeon-mix (RGe91), which contains antigens from serum, droppings and feathers. Specific IgG antibody values are expressed in mg/l and the median values calculated for each antigen and group. N = number of subjects.

ALLERGEN SOURCES

Allergenic proteins are found in bird droppings, skin scales and feather bloom.[19] The feather bloom of birds contains large quantities of keratin granules (1 micrometer diameter), which can act not only as a vehicle to take the soluble antigens to the lower airways, but might also act as an adjuvant. Birds such as pigeons, parakeets, budgies or cockatiels produce particularly large amounts of bloom, whereas poultry such as chicken and ducks produce very little bloom.

Pigeon droppings are another important source of antigens, particularly in environmental exposure. Secreted IgA antibodies are a major protein antigen and intestinal mucin is a major carbohydrate antigen. However, bird droppings contain many other substances with non-specific biological activity (e.g. endotoxins) as well as microbes (e.g. *Chlamydia, Mycoplasma*), fungi (e.g. *Aspergillus, C. albicans*) and viruses (e.g. adenovirus, influenza). The main source of clean pigeon-derived soluble antigens for serological and skin tests is the serum of the birds, which is also used for the qualitative Ouchterlony assay. The major antigens are immunoglobulins (IgA); albumin and other proteins, however, are also known to cross-react with albumin from other avian species.

ALLERGEN SOURCES IN PIGEON EXPOSURE

The level of serum IgG antibodies against bird antigens reflects the level of antigen exposure; higher levels are usually associated with more severe disease. To demonstrate the presence of antigens in different bird sources, we analysed serum samples from four different groups of individuals. All sera were tested for precipitating antibodies by Ouchterlony (see below) and specific IgG antibodies quantified in the following groups: the first group (Group 0) consisted of asymptomatic non-exposed blood donors (BD group; n=11).[20] The next two groups (Groups A and B) consisted of symptomatic patients with precipitating antibodies (PP group; n=16) or without precipitating antibodies (non-PP group; n=20). In addition a group of exposed pigeon breeders (Group C) (n=11) was analysed.

IgG antibodies were quantified by ImmunoCAP (specific IgG) using the UniCAP® system (Pharmacia Diagnostics AB, Uppsala, Sweden).[21] All serum samples were analysed for the presence of IgG antibodies against serum, droppings and feather antigens from pigeons (research ImmunoCAPs). In addition all sera were tested for three bird-antigen mixes (serum, droppings and feather antigens combined) from pigeons (RGe91), budgerigar (RGe90) and parrot (RGe92) respectively.

The mean specific IgG values to pigeon serum, droppings and feathers are generally lower than the IgG response to the pigeon mix in all three groups analysed. In the PP group (Fig. 2A) only the IgG levels to pigeon serum demonstrated similar high values compared with the mix-antigens, with 92% above 50 mg/l. In the non-PP group 6/9 (67%) demonstrated levels above 20 mg/l to pigeon droppings (Fig. 2B), considerably more than for pigeon antigen-mix (15%). In the group of pigeon breeders (Fig. 2C) 2/11 sera were negative for pigeon serum and only 55% of sera were above 20 mg/l compared with the mix-antigens (64%). However, 82% (9/11) of the serum samples had IgG values above 20 mg/l to feathers.

All sera were analysed for IgG to the three different bird-antigen mixes. The blood donors had, as expected, very low concentrations of IgG antibodies against all mixes: 6.6, 3.0 and 2.6 mg/l for pigeons, budgerigar and parrots respectively (data not shown). Similar low levels were seen in the non-PP group with mean values below 9 mg/l. As expected the IgG antibody concentrations in the PP group demonstrated a strong increase with titres of up to 7 280 mg/l. The mean values for pigeons, budgerigar and parrots were 897 mg/l, 448 mg/l and 319 mg/l respectively. Interestingly the pigeon breeders, even though not symptomatic for AA, demonstrated highly elevated levels to pigeon antigens. The distribution of concentrations varied over a wide range; 13-4 680, 5-1 150 and 5-1 230 mg/l for pigeon, budgerigar and parrot, respectively. Nevertheless, the mean values were considerably lower than those of the PP group: 168, 70 and 56 mg/l respectively.

There seems to be considerable antigenic cross-reactivity, confirming previous reports for pigeon, hen and duck.[22]

COMPARISON OF PRECIPITATION ASSAY (OUCHTERLONY) WITH SPECIFIC IgG TITRES

Currently the most widely used method to detect AA in routine clinical laboratories is immunoprecipitation of IgG antibodies with the antigens (Ouchterlony assay). The precipitation assay is performed by standard Ouchterlony double-diffusion technique, based on the immunodiffusion and precipitation of antigen-antibody complexes in agarose gels, and is currently used in most laboratories to detect precipitating IgG antibodies in sera.[23] All serum samples were tested for precipitating antibodies by Ouchterlony and regarded as having precipitation (PP group) or non-precipitating (non-PP) antibodies.

Control sera and sera with known concentrations of pigeon-specific IgG antibodies were compared on the Ouchterlony assay (Fig. 3). The negative control sera did not demonstrate any precipitation lines. However, sera with low concentrations of IgG antibodies (5.4 and 35.9 mg/l) show similar precipitation lines.

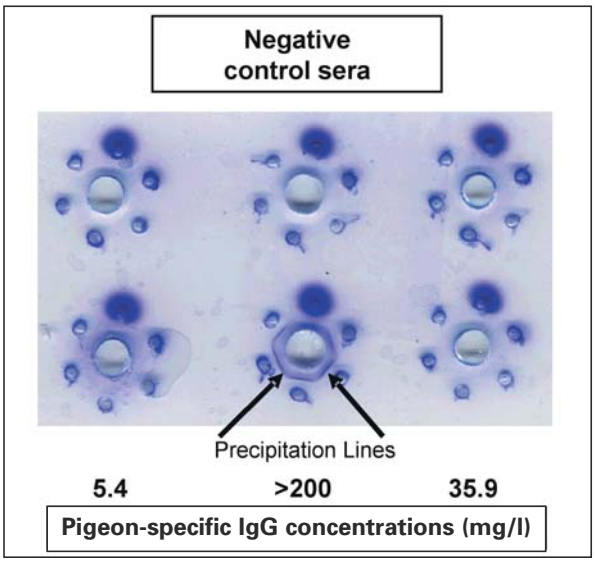


Fig. 3. Ouchterlony assay to demonstrate the precipitation of patients' pigeon-specific IgG antibodies (in large centre well) with pigeon antigens (serum) in doubling dilutions (in small wells). The precipitation lines are visualised by staining with the protein dye Coomassie Blue. IgG antibody concentrations of positive sera are expressed in mg/l (mg antigen-specific IgG antibody); precipitation lines are indicated with arrows.

Owing to the difficulties in obtaining antigenic extracts, studies aimed at quantifying antibodies to different antigenic sources of birds are very limited. The results of different assays in many laboratories are often not conclusive in reporting of antibody levels in quantitative terms;[24] therefore the Ouchterlony assay uses pigeon serum as antigen source. However, previous comparative studies of ELISA and Ouchterlony in serum and BAL of patients with AA demonstrated a much higher sensitivity (up to 1 000 times more) than the precipitation assay.[25] This was supported by a comparative study conducted in our laboratories[26] demonstrating the high level of agreement (94.1%) between the Ouchterlonly results and the ImmunoCAP.

CONCLUSIONS

In conclusion, it has been demonstrated that bird antigens from different sources such as serum, droppings and feathers can stimulate immune responses. Occupational and environmental exposure to pigeon is best quantified using the UniCAP system for mixed-bird antigens and can assist in the diagnosis of AA. In addition it is valuable in monitoring circulating antibody levels as a measure of exposure and as a useful guide to the effectiveness of avoidance measures. However, the diagnosis of AA must be based on a detailed clinical history supported by the quantification of IgG antibodies to the suspected antigen.

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