

REVIEW

A review of antigen diversity causing lung disease among pigeon breeders

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Introduction

Keeping pigeons is an occupation with an ancient and venerable history [1]. There is an equally long-standing awareness of associated infections and hypersensitivity disorders. In this review we consider the different antigens likely to be encountered and how these might help to explain the range of disorders and the diversity of symptoms associated with pigeon exposure.

Antigen exposure in a pigeon loft

The inside of a pigeon loft is a dusty environment. This dust is derived mainly from pigeons and consists of desiccated pigeon droppings (guano), feather dust (bloom), and dust from grain feed. Exposure to these dusts can be considerable; in surveys we have found that pigeon fanciers will spend on average 20 h per week in their lofts looking after approximately 60 birds. There are two loft management systems which have a bearing on dust levels. The 'deep-litter system' allows droppings to accumulate, dry out and become a fine chalk-like powder which then absorbs fresh droppings. This system can generate substantial dust [2,3] and is the minority practice. Normally droppings are cleaned out daily as part of the general husbandry and therefore tend not to constitute a great source of dust. In these situations the dust produced copiously from pigeon feathers, known as bloom, is predominant. This has the appearance of talcum powder coating surfaces of pigeon lofts, and can be transported on hands, hair and clothing after working with the birds.

There is a seasonal periodicity of antigen exposure related to the increased time spent training the birds during the summer racing season, together with the increasing amount of bloom produced by each bird as it matures into peak

racing condition. This is followed by increased dust exposure in autumn when pigeons moult and shed feathers. Corresponding seasonal differences in respirable and non-respirable dust levels and the airborne antigen concentration in pigeon lofts have been measured [4]. In this study of 21 pigeon lofts, the number of pigeons correlated significantly with the respirable dust levels when sampling was done in undisturbed lofts. However, when there was activity in lofts the dust levels were significantly higher (medians for respirable and nonrespirable dusts were 12.11 mg/m³ and 1.58 mg/m³, respectively), but these did not correlate with the number of pigeons in the loft. The antigen content in the dust was measured by enzyme immunoassay (median 0.55 µg/mL, [range 0–40]), and this made up, on average, 0.8% of the total dust. The antigen content correlated with the respirable dust level, and this was significantly higher during the autumn moult. It is also known that there is an annual periodicity in the serological response around the time of this maximum antigen exposure [5]. This information confirms that individuals are exposed to considerable and variable amounts of dust and antigens in pigeon lofts, with changing levels at different times of the year. The clinical consequence of these different antigen exposure patterns is still to be resolved, but expression of symptoms among pigeon fanciers is most frequent in late Autumn.

Disease spectrum associated with pigeons

The list of diseases associated with pigeon exposure includes extrinsic allergic alveolitis/hypersensitivity pneumonitis (EAA/HP), asthma, ornithosis (microbial infections of birds which can be transferred to man), and lung inflammation from inhaling irritant dusts. The disease most closely associated with avian exposure is EAA/HP, however, surveys of pigeon breeders usually reveal a rich heterogeneity of symptoms. One interpretation is that there

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are many sources of antigenic sensitization, infection or inflammation associated with pigeons giving rise to symptoms, some of which are similar to EAA/HP. A proper epidemiological study may help unravel some of the symptoms and causes.

Alveolitis is by definition an interstitial inflammatory disease most evident at the alveolar level, associated with a restrictive ventilatory defect, reduced lung compliance and a diffusion abnormality. However the spectrum of disease reported among pigeon fanciers also includes bronchiolitis [6,7], bronchitis with obstruction [8,9], asthma [10,11] and rhinitis [12]. There is therefore evidence of inflammation throughout the respiratory tract in pigeon breeders.

Extrinsic allergic alveolitis/hypersensitivity pneumonitis (EAA/HP)

EAA/HP is a pulmonary hypersensitivity disease caused by repeated inhalation of immunogenic dusts from a wide variety of sources mainly fungal, bacterial and animal (glyco-) proteins. The most commonly studied syndromes are Pigeon Breeders Disease (bird fancier's lung) and Farmer's Lung. The symptoms in the acute phase include shortness of breath without wheeze, general malaise and aching discomfort in the joints and muscles. There is associated pyrexia with rigors and sweating and an irritating unproductive cough. These symptoms are most noticeable shortly (generally 4–8 h) following exposure to the offending antigenic material. The symptoms are often described as 'flu-like' by affected subjects, however, unlike an acute viral illness, these symptoms usually resolve within 24 h. The more insidious forms of the disease may follow prolonged exposure to smaller amounts of antigen or may be a consequence of prolonged subclinical (subacute) disease [13]. This chronic form of disease is characterized by a slow progressive increase in shortness of breath and reduction in exercise tolerance, often with chronic cough and fibrotic changes.

EAA/HP caused by avian exposure

The first description of EAA/HP caused by exposure to pigeons was documented in 1965 [14]. This clinical report however, was not the first recognition of the problem because the pigeon fanciers themselves had long known about the intolerance of their birds which was different from ornithosis [15,16] and the typical disease pattern had already been described in subjects exposed to avian antigens [17–21]. Long before these reports however, there was a typical description of the disease in 1897 among the 'gaveurs de pigeons'. These were agricultural workers who daily force-fed thousands of pigeons for market in Paris [22]. Nevertheless, Charles Reed and colleagues [14]

gave the first full clinical and serological report and proved the association of the disease with pigeons by provoking the symptoms in one subject after contact with his pigeon in a controlled environment. The disease was subsequently reported worldwide [23,24].

The nature of the antigens in EAA/HP

The range of antigens which can cause alveolitis forms an ever increasing and diverse list [25]. Each of these different sources of antigen is associated with similar clinical problems, and these are grouped loosely into a disease syndrome of EAA/HP. Despite the range of antigens the clinical presentation of affected subjects is similar. This suggests that although the initial antigen recognition is specific for each situation the subsequent hypersensitivity reactions are common to all, irrespective of the antigen. For this simple reason it could be argued that there can be no 'disease-specific' antigen as such, although there are some immunologically specific and nonspecific factors common to all these antigens (a summary of these is listed on Table 1).

(a) Non-specific factors The airborne particles which can cause alveolitis are generally of the order of one to three microns in diameter [26] which is the correct aerodynamic size to reach and settle in the alveoli [27], and it is at the alveolar level that the disease process is most evident [28,29]. Pigeon bloom is of considerable importance in this regard. It is the fine powder down which is a waterproofing agent on the flight feathers and is produced in copious quantities by healthy flying birds. It consists of particles of approximately this size demonstrated by scanning electron microscopy (Fig. 1), and this is in accordance with the estimates of Stettenheim [30]; an ornithologist who described bloom as being composed of granules of keratin approximately 1 micron in diameter. Keratin is a highly hydrophobic protein very resistant to enzyme degradation and therefore may act as an inflammatory foreign body causing granuloma in the lung. Therefore in addition to bloom acting as a vehicle to get the soluble antigens to the lower airways, it may also act as an irritant or adjuvant.

Among the range of avian species associated with the development of EAA, the group which comprise 'fancy' birds (parakeet [21], pigeon *Columba livia* [14], budgerigar *Melopsittacus undulatus* [31], lovebirds [32], dove [33], cockateil *Nymphicus hollandicus* [34]) all produce copious amounts of bloom, whereas poultry; (duck [18], chicken [35] and turkey [36]) produce very little bloom. The disproportionately high prevalence of EAA in breeders of fancy birds probably reflects the relatively greater amount of bloom produced by birds that fly and are in peak condition. This can be compared with the minimal amounts of bloom

Table 1. Summary of pigeon-associated antigens

Pigeon-derived antigens	
Serum	This is the main source of clean soluble antigens for serological, skin test and inhalational challenge studies. The major antigens are gamma-globulin proteins (pigeon immunoglobulin, which occurs also as pigeon IgA and IgA fragments in droppings and bloom). Albumin is also a major antigen, which is cross-reactive with albumin from other avian species. The other serum proteins seem to be immunogenic in accordance with their relative abundance.
Bloom	Inert keratin particles, produced copiously from feathers, which are the correct aerodynamic size to transport serum proteins to peripheral airways. May act as a local irritant or adjuvant to promote an immune response to these antigens.
Droppings	A source of excreted serum protein antigens which are difficult to categorize because of enzyme degradation. IgA is a major protein antigen and intestinal mucin is a major carbohydrate antigen. Droppings are a source of bacterial endotoxin and teichoic acid, and fungal β 1 $\rightarrow$ 4 glucan, both of which are pro-inflammatory. Extract from droppings are likely to contain many other substances with nonspecific biological activity as well as microbes, which limit their use in serological, skin and inhalation challenge testing.
Pigeon-associated organisms	
Bacteria	<i>Chlamydia</i> sp., <i>Mycoplasma</i> , <i>M. avium</i> , <i>E. coli</i> , <i>Pasteurella</i> , <i>Strep bovis</i>
Fungi	<i>C. albicans</i> , <i>Aspergillus</i> , <i>Mucor</i> , <i>Crypt neoformans</i>
Viruses	<i>Adenovirus</i> , <i>Herpesvirus</i> , <i>Influenza</i>
Parasites	<i>Trichomonads</i> , <i>Hexamita</i>
Mites	<i>Diplaegidia columbae</i>

found in those birds that do not fly and the fact that EAA/HP is uncommon among poultry workers despite close contact with large numbers of birds often in confined spaces.

Animal models of EAA/HP have demonstrated that a simple immunological stimulus is not sufficient to establish experimental disease [37], and that a vigorous adjuvant [38], or inflammatory [39] stimulus is also required. For EAA/HP in man, a similar need for intrinsic immunological adjuvant activity was postulated by Pepys [40]. In farmer's lung, mitogenic and adjuvant activity of spores of *M. faeni* have been described [41,42], and the disease process of EAA in maltworkers may involve the nonspecific alveolitis-inducing activity of glycoprotein antigens from *A. clavatus* [43].

The antigens associated with EAA/HP have been reported to directly activate complement [44,45]. Activation via the alternative pathway has been described, although there is some evidence to suggest that the antigens can act directly at the level of C4 of the classical pathway [46]. The proteolytic enzymes from bacteria and fungi and the hydrolytic enzymes from avian droppings were implicated in the activation sequence [47]. Bacterial teichoic acid as well as 'C-substance like' carbohydrate material have been identified in various dust extracts and these can cause classical pathway activation by binding C-reactive protein, and also cause calcium ion-dependent false-positive precipitin reactions with sera of patients under investigation for EAA [48].

(b) *Specific factors* Alveolitis-inducing dusts have soluble components which are highly immunogenic, and the specific nature of these antigens relates with disease, although the nonspecific activities may have some importance in initiating and determining the extent of this specific response.

Many sources of antigen have been described. Antigens have been extracted from pigeon droppings, feathers, serum, egg yolk and white, crop fluid and gut wall [49–51], and these are a rich heterogeneous mixture of soluble proteins, glycoproteins, polysaccharides and protein fragments. These various antigens were characterized by cross absorption and found to occur primarily in pigeon droppings, and this has been described as the 'complete' source of antigens concerned with the disease [50].

In pigeon droppings extract (PDE), the major antigen had gamma mobility on immuno-electrophoresis and was detected by precipitin formation using sera from pigeon breeders. This acidic glycoprotein of molecular weight 200 kDa was identified as pigeon IgA and was quantitatively the major antigen of PDE, comprising about 24% of the dry weight. The pigeon IgA antigen in pigeon droppings cross-reacted with the main serum gamma-globulin antigen [52–54]. Pigeon IgA and IgG were also shown to be the major antigenic components of the soluble antigens in pigeon bloom [55,56]. Recently, a disease-specific 21-kd protein from PDE was described which caused lymphocyte transformation only of subjects with EAA/HP [57]. In

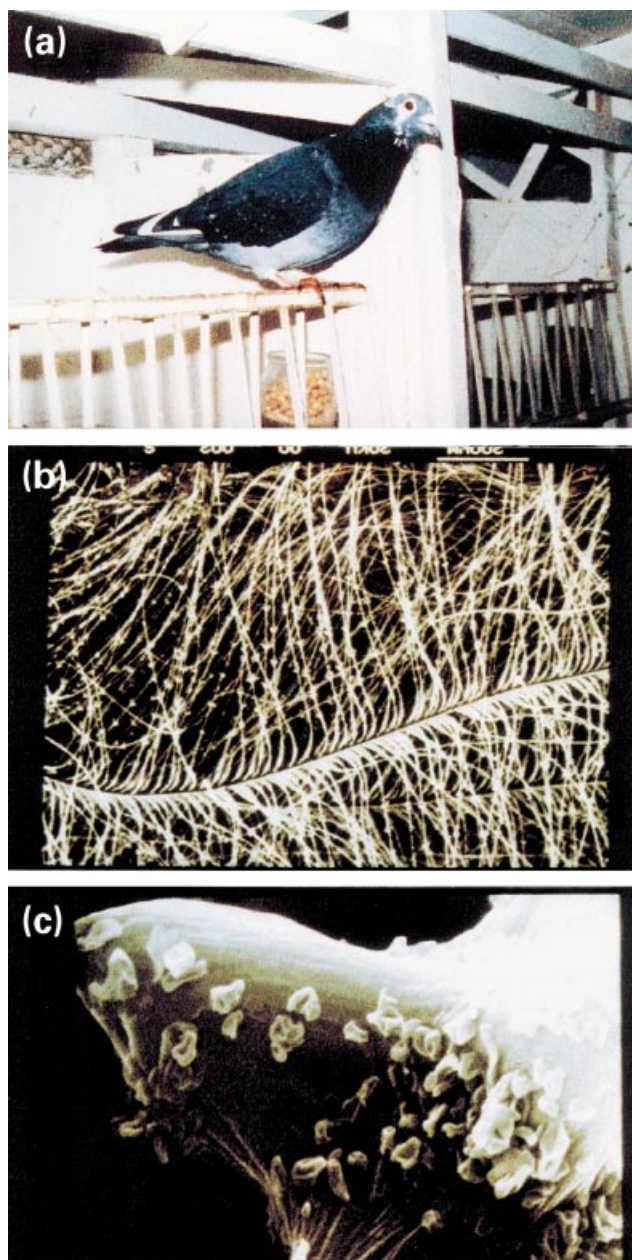


Fig. 1 (a) A racing pigeon (*Columba livia*) in its cot. These birds are larger than their more common feral counterparts, with better muscle definition and a luxuriant bloom coating when in peak flying condition. (b) A scanning electron micrograph of a small breast feather showing the barbs for feather ruffling to trap air for warming, together with the bloom particles. (c) A micrograph of bloom particles showing good uniformity of structure and size of around 1 micron.

addition to these antigens, PDE contains many other less well defined antigenic substances. We find that using PDE extracts for testing pathological sera from different diseases can show false-positive precipitin reactions. We would

recommend that the use of PDE for the routine serological investigation of EAA/HP be discontinued.

PDE contains two well-defined glycolipid (the P1 blood group substance) and glycoprotein (pigeon intestinal mucin) antigens which may provide clues to the immunopathogenesis of the disease. These will allow structural analysis of the immunological interactions at the molecular level. There is a glycolipid found in PDE which occurs on pigeon erythrocytes and which shows identity with the human P1 blood group substance [58,59]. The antigenic structure of P1 is α galactose (1–4) β galactose (1–4) β N-acetylglucosamine (1–3) β galactose (1–4) glucose ceramide. Sensitization to this antigen among pigeon breeders has been shown to decrease the survival of donated P1 red blood cells post transfusion. Recently in PDE an important new glycoprotein antigen, pigeon intestinal mucin, was described which stimulates high antibody titres [60]. Carbohydrate epitopes on this molecule were identified as 'disease-associated' for pigeon fanciers lung [61]. Antigenic fragments of these are relatively resistant to biochemical breakdown, as with IgA, and both are likely to be stable in dust in the pigeon loft environment.

Working with PDE presents difficulties with standardization and with the stability of the preparations, along with toxic and nonspecific biological activity of some of the constituent components. As an alternative, pigeon serum has been recognized as a major source of clinically important antigens by serological and by inhalation provocation tests [14,62]. It has become the most readily available antigen source for investigating this condition because it contains the major antigens and avoids the problems of contamination of droppings or bloom extracts by microorganisms or other agents.

Avian serum contained many antigenic proteins detected by the sera of sensitized pigeon fanciers, and chromatography of pigeon serum generated many protein fractions of different molecular sizes. Most of these had complement-depleting activity when incubated with the serum of pigeon fanciers [63]. Similarly, most of these antigen fractions also caused proliferation of lymphocytes from subjects with pigeon breeders disease [64].

The sera of many avian species have proteins with antigenic identity [65,66] which could explain the positive precipitin reactions to pigeon serum in subjects with exposure to other avian species, or in patients with inflammatory bowel disease e.g. coeliac disease in which serum antibody against avian antigens is caused by immune responses to ingested hens' egg proteins [66]. There were species-specific serum antigens in the gamma-globulin fraction [66,67] and therefore pigeon serum gamma-globulin was considered to be the best specific antigen associated with pigeon breeders' disease, and this antigen was further demonstrated to be highly immunogenic in man, more so

than serum albumin [68]. A summary of these various antigens is listed on Table 1.

Is there a disease-specific antigen for EAA/HP?

There is a range of diverse antigens which can cause EAA/HP [25], and it is unlikely that between them there is a significant common antigen. It is also unlikely that of the many antigenic components associated with any one disease syndrome, there is only one responsible for disease. Some antigens are however, immuno-dominant. With pigeons, soluble components from serum, droppings and bloom can all cause acute symptoms after inhalation challenge [69], and the major common antigenic component in these is the gamma-globulin proteins, and the common component is the immunoglobulin light chain [69].

Pigeon-derived antigens will induce an antibody response as a consequence of their abundance and immunogenicity. Moreover, the antibody titre to the major and minor antigens is generally more abundant among individuals with EAA, but antibody can also be readily detected in relatively lower titres among those without symptoms.

There are claims that certain antigens in EAA are 'disease-specific' [61,64,70–73], based on the observation that they form precipitin reactions only with sera from symptomatic subjects. It is noteworthy that these are generally minor antigens which are likely to produce only a relatively modest or low antibody response. Antibody against these minor antigens can be detected in those with EAA by virtue of the fact that these subjects tend to have higher antibody titres to all antigens, whereas the antibody titres against these minor antigens would fall below the precipitin detection threshold in sera from most other subjects who would have relatively lower titres to all antigens.

We have had the opportunity to demonstrate this. We found that antibody of low titre in the serum of asymptomatic pigeon breeders could be detected by using a sensitive enzyme-immunoassay using a supposedly disease-specific antigen from droppings extract (a generous donation from Dr Nick Calvanico [73]). The fact that precipitating antibody against this antigen could only be detected in the serum of EAA/HP patients was simply a reflection of the relative insensitivity of precipitin tests compared with enzyme-immunoassay. Furthermore, the relative titres between symptomatic and asymptomatic pigeon breeders was the same when using this or any of the other avian antigens (our unpublished observations).

Asthma and rhinitis

Asthma [9–11] and rhinitis [12] are commonly reported in surveys and case reports of symptoms amongst pigeon breeders. Despite this, in our experience, IgE antibody to

avian antigens as measured by positive skin prick tests or by serological tests is uncommon [69–74]. Other antigens may be involved. Pigeon feather mites, *Diplaegidia columbae*, are abundant in pigeons and can constitute up to 10% of the weight of the feather. These are antigenically distinct from other mites and may be clinically important. In one clinical study, IgE antibody to these allergens was associated with significant symptoms [75]. A similar investigation of sensitivity to storage dust mites, which could infest grain feed or litter, may be indicated [76].

Inflammatory (irritant) lung disease

Various bacterial and fungal products have been identified in the dust of pigeon lofts, including bacterial lipopolysaccharide [77], teichoic acid and C-substance-like carbohydrate material [48], and fungal beta-glucan [77]. Like endotoxin-rich dusts and aerosols in other environments [78,79] these materials can cause a symptom profile which is similar to EAA, but which is unrelated to specific sensitization to pigeons. This may be the cause of symptoms in poultry farms where endotoxin exposure is high but allergic sensitization is minimal [76].

The husbandry of pigeons requires storage and handling of substantial amounts of grain feed. Inflammatory reactions have been described following inhalation of crude organic grain dust [80,81] which was due to the irritant nature of the dusts rather than a specific hypersensitivity reaction. We have found an unusual presentation of chronic bronchitis in nonsmoking pigeon breeders [9]. This was associated with sero-positivity to avian antigens [8], but the toxic effects of grain dust may also contribute to this bronchitis, and this requires investigation.

There is further potential for confusion when investigating the immuno-pathogenesis of these reactions because soluble extracts from grain dust can form nonspecific in-vitro precipitin reactions with serum. Grain may also harbour *Aspergillus* and *Thermoactinomyces* spores and these are known pathogens for pigeons and humans, and sensitivity to these organisms among pigeon breeders requires investigation.

Cross-infections from pigeons

Human ornithosis following contact with pigeons has been reported [82], and despite the fact that pigeon breeders maintain healthy birds, widespread serological sensitization to *Chlamydia psittaci* among pigeon breeders has been demonstrated [83]. It is important not to over-interpret this sero-positivity to *Chlamydia* and recognize that symptoms of acute EAA/HP can resolve within 24 h, whereas *Chlamydial* infection may be more prolonged. This sero-positivity does suggest that cross infection can occur, but

what is unclear is whether symptoms result from this. Similarly, avian species harbour many viruses which are potentially pathogenic for humans [84] and serological sensitization to many respiratory virus has been described [23] but explained as an inflammation-associated anamnestic response [23]. Examples of pigeon pathogenic and commensal organisms are listed on Table 1. Evidence for other sources of infection or sensitization should be sought and any association with symptoms investigated.

Controlling antigen exposure

The skill and experience of the pigeon fancier is a result of extensive investment in time spent in contact with the birds, breeding and training them in order to develop their remarkable homing instinct which is central to the sport. This is part of a considerable social, financial and emotional commitment which exists among pigeon fanciers for their birds. This is more a way-of-life rather than a hobby, and pigeon racing is a highly organized and competitive sport, with considerable financial rewards.

It is not surprising then that many pigeon fanciers with symptoms are reluctant to seek medical advice because they do not want to be told to give up their birds. We are trying to encourage a culture among the pigeon fanciers to come for advice at an earlier stage of symptoms when a process of reduction in antigen exposure may be of benefit. This can be done by several means. Firstly by reducing the time spent with the birds, secondly by wearing a suitable mask, thirdly by wearing a dust hat and coat, and lastly by promoting hygiene and ventilation measures. We have found that this strategy can work, for example, using a positive pressure respirator caused a fall in the serum antibody titres reflecting reduced avian antigen exposure [85]. In a 10-year follow-up study of 24 patients with EAA, there was a benign course of disease with clinical improvement in most subjects who used masks and reduced exposure time [86]. By contrast, in a study of 15 subjects who did not use this protection or exposure modification there was an accelerated decline in pulmonary function monitored over 18 years at 3–4 years intervals [87].

Although controlling the antigen exposure in lofts is crucial, there are other sources of avian antigens which should be considered. As well as in the pigeon loft, antigenic dusts from pigeons have been measured within the home environment. These may be carried into the home from the loft on overalls and hair of the pigeon breeders which may sensitize other family members [88], and there may be additional exposure to avian antigens from bird feathers in duvets, pillows and soft furnishings [89,90]. This may explain disease which can affect other family members, especially children, who are not directly involved with handling pigeons [91–94]. These antigenic dusts can persist for up to 18 months in the home environment after removal

of birds [95], and this may explain situations where there is no resolution of avian-specific serology or if clinical progress is slow.

Conclusions and future studies

There is considerable diversity of symptoms reported by pigeon breeders, and the symptom profiles often do not fit easily into recognizable disease categories. This is likely to reflect the wide range of dusts and antigens in the pigeon loft. Many of the dusts, e.g. endotoxin and grain dust can cause nonspecific acute inhalational fever which may be indistinguishable from antigen-specific acute EAA. Obstructive airways disease and chronic bronchitis has been described as part of the disease spectrum of EAA/HP specifically related to avian antigen exposure, but these could also be caused by other allergic materials associated with pigeons; mainly mites, or may be a nonspecific consequence of long-term exposure to high levels of respirable irritant dusts.

Several areas for further work can be identified. Air sampling studies are surprisingly uncommon in this area despite the clear need to understand the amounts and dynamics of various dust and antigen exposure. Studies should include seasonal levels and levels associated with different loft management strategies. These could be altered if necessary, and better loft ventilation could be designed as part of an overall improvement in environmental hygiene. Epidemiological studies on defined cohorts (selected and unselected) of pigeon breeders, along with air sampling, associated serology, lung function and clinical follow-up would help in the interpretation of some of the clinical profiles described.

The definition of EAA/HP perhaps should perhaps be reviewed. This disease syndrome has been defined in early epidemiological studies [96,97], and again more recently by committee [98]. However, additional descriptions of asthma and bronchitis, along with the recognition of the extent of nonspecific dust exposure has meant that symptoms and pulmonary function among pigeon fanciers require to be revisited. Better categorization of disease is required if progress is to be made in the study of the immuno-pathogenic mechanisms of pigeon breeders disease. Anomalies exist in the measurement of various immunological mediators and this may well be because the disease categories in study groups are more heterogeneous than we currently appreciate.

Studies of symptom prevalence may be confounded by symptoms from co-existing infections and therefore the possibility of ornithosis should be considered. Such infections or other viral influences may also be of importance in facilitating or potentiating immunological sensitization, analogous to that which seems to be necessary for initiating experimental alveolitis, and the adjuvant activity from avian mycobacteria should be considered in this respect.

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References

- 1 The Magna Illustrated Guide to Pigeons of the World. McNeillie A, Magna Books, Leicester, 1993.
- 2 Leung D. Pigeon racers will not want to reduce time spent in lofts. *BMJ* 1997; 315:1311.
- 3 Edwards JH, Trotman DM, Mason OF, Davies BH, Jones KP, Alzubaidy TS. Pigeon breeders lung – the effects of loft litter materials on airborne particles and antigens. *Clin Exp Allergy* 1991; 21:49–54.
- 4 Morris G, Anderson K, McSharry C, Boyd G. Gravimetric determination of dust and antigen exposure in pigeon breeders. *Ann Occup Hygiene* 1994; 48 (Suppl. 1):919–22.
- 5 McSharry C, Banham SW, Lynch PP, Boyd G. Seasonal variation of antibody levels among pigeon fanciers. *Clin Allergy* 1983; 13:293–9.
- 6 Perez-Padilla R, Gaxiola M, Salas J, Mejia M, Ramos C, Selman M. Bronchiolitis in chronic pigeon breeder's disease: Morphologic evidence of a spectrum of small airway lesions in hypersensitivity pneumonitis induced by avian antigens. *Chest* 1996; 110:371–7.
- 7 Scadding JG. Lung biopsy in the diagnosis of diffuse lung disease. *BMJ* 1970; 2:557–64.
- 8 Bourke SJ, Anderson K, Lynch P, Boyd J, King S, Banham S, Boyd G. Chronic simple bronchitis in pigeon fanciers. Relationship of cough with expectoration to avian exposure and pigeon breeders' disease. *Chest* 1989; 95:598–601.
- 9 Bourke SJ, Carter R, Anderson K, Boyd J, King S, Douglas B, Boyd G. Obstructive airways disease in non-smoking subjects with pigeon fanciers' lung. *Clin Exp Allergy* 1989; 19:629–32.
- 10 Tauer-Reich I, Fruhmann G, Czuppon AB, Baur X. Allergens causing bird fancier's asthma. *Allergy* 1994; 49:448–53.
- 11 Pelikan Z, Schot JDL, Koedijk FHJ. The late bronchus-obstructive response to bronchial challenge with pigeon faeces and its correlation with precipitating antibodies (IgG) in the serum of patients having long-term contact with pigeons. *Clin Allergy* 1983; 13:203–11.
- 12 Pelikan Z, Pelikan-Filipek M. Nasal form of pigeon breeder's disease, a new disease picture *Nederlands Tijdschrift Voor Geneeskunde* 1988; 132:657–9.
- 13 Fabbri LM, Caramori G, Beghe B, Papi A, Ciaccia A. Physiologic consequences of long-term inflammation. *Am J Resp Crit Care Med* 1998; 157:S194–8.
- 14 Reed CE, Sosman AJ, Barbee RA. Pigeon breeders lung – a newly observed interstitial pulmonary disease. *JAMA* 1965; 193:261–516.
- 15 Lamers JJH, Maesen FPV. Pigeon breeders lung. *Radiologia Clinica* 1976; 45:183–6.
- 16 Lynch PP. Health Centre, Stenhousmuir. Personal communication, 1998.
- 17 Fawcitt R. Fungoid conditions of the lung. *B J Radiol* 1936; 9:172–95 and 354–78.
- 18 Feldman HA, Sabin AB. Pneumonitis of unknown aetiology in a group of men exposed to pigeon excreta. *J Clin Invest* 1948; 27:533.
- 19 Emmons CW. Saprophytic sources of *Cryptococcus neoformans* associated with the pigeon (*Columba livia*). *Am J Hygiene* 1955; 62:227–32.
- 20 Plessner MM. Une maladie des tireurs de plumes; la fièvre de canard. *Societe Do Med Du Travail Bordeaux de Sa Region Mai* 1959. *Archives des Maladies Professionnelles* 1960; 21:67–9.
- 21 Pearsall HR, Morgan EH, Tesluk H, Beggs D. Parakeet dander pneumonitis: acute psittaco-kerato-pneumoconiosis: report of a case. *Bulletin Mason Clinic* 1960; 14:127–37.
- 22 Renon L. Etude sur l'Aspergillose chez l'animal et chez l'homme. *Masson et Cie, Paris*, 1897: pp 166–287.
- 23 Boyd G, Dick HW, Lorimer AR, Moran F. Bird breeders lung. *Scot Med J* 1967; 12:69–71.
- 24 Molina C, Cheminant JC, Brun J, Aiache JM, Jeanneret A. Bird-fanciers disease. New clinical, diagnostic and medico-legal aspects. *Nouv Presse Med* 1976; 5:784–6.
- 25 Calvert JE, Baldwin CI, Allen A, Todd A, Bourke SJ. Pigeon fancier's lung: a complex disease? *Clin Exp Allergy* 1999; 29:166–75.
- 26 Austwick PKC. The role of spores in the allergies and mycoses of man and animals. *Colston Papers, Proceedings 18th Symp Colston Res Soc Bristol* 1966; 18:321.
- 27 Brain J, Valberg PA. Deposition of aerosol in the respiratory tract. *Am Rev Respir Dis* 1979; 120:1325–73.
- 28 Hensley GT, Garancis JC, Cherayil GD, Fink JN. Lung biopsies of pigeon breeders disease. *Arch Pathol* 1969; 87:572–9.
- 29 Seal RME. Pathology of extrinsic allergy bronchiolo-alveolitis. In: *Alveolar Interstitium of the Lung Progress Respir Research* 1975; 8:66–73.
- 30 Stettenhein P. The integument of birds. In: *Avian Biology Volume II* Farmer, King, eds. Academic Press, 1972: p 14.
- 31 Hargreave FE, Pepys J, Longbottom JL, Wraith DG. Bird breeders (fanciers) lung. *Lancet* 1966; 1:445–9.
- 32 Fink JN. Hypersensitivity pneumonitis: A case of mistaken identity. *Hospital Practice* 1974; 9:119–24.
- 33 Cunningham AS, Fink JN, Schleuter DP. Childhood sensitivity pneumonitis due to dove antigens. *Pediatrics* 1976; 58:436–42.
- 34 Jongerius CM. Allergische alveolitis bij duivenmelkers. *Nederlands Tijdschrift Voor Geneeskunde (Amsterdam)* 1969; 113:2130–1.
- 35 Korn DS, Florman AK, Gribetz J. Recurrent pneumonitis with hypersensitivity to hen litter. *JAMA* 1968; 205:44–5.
- 36 Boyer RS, Klock LE, Schmidt CD, Hyland L, Maxwell K, Gardner RM, Renzetti AD. Hypersensitivity lung disease in the turkey raising industry. *Am Rev Respir Dis* 1974; 109:630–5.
- 37 Salvaggio JE. Recent advances in pathogenesis of allergic alveolitis. *Clin Exp Allergy* 1990; 20:137–44.

- 38 Richerson HB, Seidenfeld JJ, Ratajczak HV, Richards DW. Chronic experimental interstitial pneumonitis in the rabbit. *Am Rev Respir Dis* 1978; 117:5–13.
- 39 Peterson LB, Thrall RS, Moore VL, Stevens JO, Abramoff P. An animal model of hypersensitivity pneumonitis in the rabbit. Induction of cellular hypersensitivity to inhaled antigens using carrageenan and BCG. *Am Rev Respir Dis* 1977; 116:1007–12.
- 40 Pepys J. Hypersensitivity disease of the lungs due to fungi and other organic dusts. *Monogr Allergy* 1969; 4:44–51.
- 41 Bice DE, McCarron K, Hoffman ED, Salvaggio JE. Adjuvant properties of *Micropolyspora faeni*. *Int Arch Allergy Appl Immunol* 1977; 55:267–74.
- 42 Smith SM, Snyder IS, Burrell R. Mitogenic response to *M. faeni* cell walls. *J Allergy Clin Immunol* 1980; 65:298–304.
- 43 Blyth W. The occurrence and nature of alveolitis-inducing substances in *Aspergillus clavatus*. *Clin Exp Immunol* 1978; 32:272–82.
- 44 Marx JJ, Boren MN, Moore VL. Antibody independent activation of the complement cascade by a purified component of pigeon dropping extract (PDE). *Clin Immunol Immunopathol* 1977; 8:487–93.
- 45 Berrens L, Guikers CLH, Van Dijk A. The antigens of pigeon breeders disease and their interaction with human complement. *An N Y Acad Sc* 1974; 221:153–62.
- 46 Marx J. Involvement of the complement system in hypersensitivity pneumonitis. *J Allergy Clin Immunol* 1978; 61:208–10.
- 47 Berrens L, Guikers CLH. An immunological study of pigeon breeders disease. III. The interaction of pigeon intestinal enzyme with human serum and a study of serum inhibitors. *Int Arch Allergy Clin Immunol* 1972; 43:337–46.
- 48 Faux JA, Holford-Strevens V, Wells ID, Pepys J. False-positive precipitation reactions of organic dust due to a teichoic acid from *S. Aureus* *Clin Exp Immunol* 1970; 7:897–902.
- 49 Barboriak JJ, Sosman AJ, Reed CE. Serological studies in pigeon breeders disease. *J Lab Clin Med* 1965; 65:600–4.
- 50 Edwards JH, Barboriak JJ, Fink JN. Antigens in pigeon breeders disease. *Immunol* 1970; 19:729–34.
- 51 Sennekamp J, Vogel F, Steins R. Detection of IgG antibodies against pigeon intestinal mucosa antigens in pigeon breeders sera using the immunofluorescent technique. *Int Arch Allergy Appl Immunol* 1976; 50:674–83.
- 52 Edwards JH, Fink JN, Barboriak JJ. Excretion of pigeon serum proteins in pigeon droppings. *Proc Soc Exp Biol Med* 1969; 132:907–10.
- 53 Goudswaard J, Noordzij A, Stam JWE. Pigeon IgA. A major antigen in pigeon breeders disease. *Immunol Comm* 1978; 7:661–8.
- 54 De Ridde G, Goudswaard J, Berrens L. Antibodies against purified IgA in pigeon breeders disease. *Z Immunoforsch Immunobiology* 1979; 155:223–31.
- 55 Banham SW, McKenzie H, McSharry C, Lynch PP, Boyd G. Antibodies against a pigeon bloom-extract: a further antigen in pigeon fancier's lung. *Clin Allergy* 1982; 12:173–8.
- 56 Longbottom JL. Pigeon breeders' disease: quantitative immunoelectrophoretic studies of pigeon bloom extract. *Clin Exp Allergy* 1989; 19:619–24.
- 57 Hisauchi-Kojima K, Sumi Y, Miyashita Y, Miyaki S, Toyoda H, Kurup VP, Yoshizawa Y. Purification of the antigenic components of pigeon dropping extract, the responsible agent for cellular immunity in pigeon breeders disease. *J Allergy Clin Immunol* 1999; 103:1158–65.
- 58 Radermecker M, Bruwier M, Francois-Gerard C, Brocteur J, Salmon J, Andre A, Van Caudwenberge H. Anti-P1 activity in pigeon breeders' serum. *Clin Exp Immunol* 1975; 22:546–9.
- 59 Munro AC, Inglis G, Lynch PP, Boyd G. A survey of P1-antibodies in Scottish pigeon fanciers. *Clin Allergy* 1980; 10:643–50.
- 60 Todd A, Coan RM, Allen A. Pigeon breeders' lung: pigeon intestinal mucin, an antigen distinct from pigeon IgA. *Clin Exp Immunol* 1991; 85:453–8.
- 61 Baldwin CI, Todd A, Bourke SJ, Allen A, Calvert JE. Pigeon fanciers lung: identification of disease-associated carbohydrate epitopes on pigeon intestinal mucin. *Clin Exp Immunol* 1999; 117:230–6.
- 62 Banham SW. Reaginic antibody in Pigeon Breeders Disease. A clinical and serological study concerning the humoral immune response to avian antigens in pigeon fanciers. MD Thesis, Dept Medicine, University of Edinburgh, 1982.
- 63 Berrens L, Guikers CLH. An immunological study of pigeon breeders disease. iv. A highly selective in vitro test of pigeon fanciers lung based on complement (C3) inactivation. *Int Arch Allergy Clin Immunol* 1972; 43:347–59.
- 64 Mendoza F, Melendro EI, Baltazares M, Banales JL, Ximenez C, Chapela R, Selman M. Cellular immune response to fractionated avian antigens by peripheral blood mononuclear cells from patients with pigeon breeder's disease. *J Lab Clin Med* 1996; 127:23–8.
- 65 Sennekamp J, Lange G, Nerger K, Berdel D, Meier-Sydon J. Human antibodies against antigens of the sparrow, blackbird, weaver, finch, canary, budgerigar, pigeon and hen using the indirect immunofluorescence technique. *Clin Allergy* 1981; 11:375–84.
- 66 Faux JA, Hendrick DJ, Anand BS. Precipitins to different avian serum antigens in bird fanciers lung and coeliac disease. *Clin Allergy* 1978; 8:101–8.
- 67 Fink JN, Tebo T, Barboriak JJ. Characterisation of human precipitating antibody to inhaled antigens. *J Immunol* 1969; 103:244–51.
- 68 Fink JN, Tebo T, Barboriak JJ. Differences in the immune responses of pigeon breeders to pigeon serum proteins. *J Lab Clin Med* 1969; 74:325–30.
- 69 McSharry C. Immunological studies in extrinsic allergic alveolitis. PhD Thesis, Glasgow University, 1984.
- 70 Barboriak JJ, Fink JN, Scribner GH. Antigenic specificity in a hypersensitivity pneumonitis. *Int Arch Allergy Appl Immunol* 1968; 33:473–82.
- 71 Peeters PA, Brombacher PJ, Maesen FP. Specific and non-specific precipitins in the serum of patients suffering from 'pigeon breeder's disease'. *Clin Chim Acta* 1971; 34:467–73.

- 72 de Beer PM, Bouic PJ, Joubert JR. Identification of a 'disease-associated' antigen in pigeon breeder's disease by western blotting. *Int Arch Allergy Appl Immunol* 1990; 91:343–7.
- 73 Calvanico NJ. A component of pigeon dropping extract that reacts specifically with sera of individuals with pigeon breeders disease. *J Allergy Clin Immunol* 1986; 77:80–6.
- 74 McSharry C, Banham SW, Lynch PP, Boyd G. Skin testing and extrinsic allergic alveolitis. *Clin Exp Immunol* 1983; 54:282–8.
- 75 Colloff MJ, Merrett TG, Merrett J, McSharry C, Boyd G. Feather mites are potentially an important source of allergens for pigeon and budgerigar keepers. *Clin Exp Allergy* 1997; 27:60–7.
- 76 Thelin A, Tegler O, Rylander R. Lung reactions during poultry handling and bacterial endotoxin levels. *Eur J Respir Dis* 1984; 65:266–71.
- 77 Cuthbert OD, Jeffrey IG. Storage mites responsible for occupational asthma among farmers. *Clin Exp Allergy* 1995; 25:382–3.
- 78 Rylander R, Goto H, Yuasa K, Fogelmark B, Polla B. Bird droppings contain endotoxin and (1 (r) 3) - β -D-glucan. *Int Arch Allergy Immunol* 1994; 103:102–4.
- 79 Mattsby I, Rylander R. Clinical and immunological findings in workers exposed to sewage dust. *J Occup Med* 1978; 20:690–2.
- 80 Anderson K, McSharry C, Clark C, Clark CJ, Barclay GR, Morris GP. Sump bay fever: inhalational fever associated with a biologically contaminated water aerosol. *Occup Environ Med* 1996; 53:106–11.
- 81 Broder I, McAvoy D. Characterisation of precipitation reaction between grain dust and normal human serum and comparison of reactive and non-reactive grain handlers. *Clin Immunol Immunopathol* 1981; 21:141–53.
- 82 May JJ, Stallones L, Darrow D, Pratt DS. Organic dust toxicity (pulmonary mycotoxicosis) associated with silo unloading. *Thorax* 1986; 41:919–23.
- 83 Bourke SJ, Carrington D, Frew CE, Stevenson RD, Banham SW. Serological cross-reactivity among chlamidial strains in a family outbreak of psittacosis. *J Infect* 1989; 19:41–5.
- 84 Bourke SJ, Carrington D, Frew CE, McSharry C, Boyd G. A comparison of the sero-epidemiology of chlamidial infection in pigeon fanciers and farmers in the UK. *J Infect* 1992; 25:91–8.
- 85 Oxford JS, Daniels RS. The holy grail of influenza virologists. *Nature Med* 1999; 5:484–5.
- 86 Anderson K, Walker A, Boyd G. Specific antibody response in pigeon breeders. *Clin Exp Allergy* 1989; 19:45–9.
- 87 Bourke SJ, Banham SW, Carter R et al. Longitudinal course of extrinsic allergic alveolitis in pigeon breeders. *Thorax* 1989; 44:415–8.
- 88 Schmidt CD, Jensen RL, Christensen LT et al. Longitudinal pulmonary function changes in pigeon breeders. *Chest* 1988; 93:359–63.
- 89 Riley DJ, Saldana M. Pigeon breeders lung. Subacute course and the importance of indirect exposure. *Am Rev Respir Dis* 1973; 107:456–60.
- 90 Haitjema T, Van VelzenBlad H, Van den Bosch JMM. Extrinsic allergic alveolitis caused by goose feathers in a duvet. *Thorax* 1992; 47:990–1.
- 91 Seaton A. Hypersensitivity Pneumonitis. In *Occupational Lung Disease* 3rd edn. A. Seaton, K. Morgan, eds. Saunders, Philadelphia, London. Chapter 20, 1995, p 556.
- 92 Grammer LC, Roberts M, Lerner C et al. Clinical and serologic follow-up of four children and five adults with bird-fanciers lung. *J Allergy Clin Immunol* 1990; 85:655–60.
- 93 Wolf SJ, Stillerman A, Weinberger M, Smith W. Chronic interstitial pneumonitis in a 3 year old child with hypersensitivity to dove antigens. *Pediatrics* 1987; 79:1027–9.
- 94 Kruger M, Henschen M, Forster J, Brandis M. Problems of diagnosis and therapy in extrinsic allergic alveolitis (EAA). *Monatsschrift Fur Kinderheilkunde* 1995; 143:245–9.
- 95 Krasnick J, Patterson R, Stillwell PC, Basaran MG, Walker LH, Kishore R. Potentially fatal hypersensitivity pneumonitis in a child. *Clin Pediatrics* 1995; 34:388–91.
- 96 Craig TJ, Hershey J, Engler RJM, Davis W, Carpenter GB, Salata K. Bird antigen persistence in the home environment after removal of the bird. *Ann Allergy* 1992; 69:510–2.
- 97 Christensen LT, Schmidt CD, Robbins L. Pigeon breeders disease – a prevalence study and review. *Clin Allergy* 1975; 5:417–30.
- 98 Banham SW, McSharry C, Lynch PP, Boyd G. The relationship between avian exposure, humoral immune response and pigeon breeders disease amongst Scottish pigeon fanciers. *Thorax* 1986; 41:274–8.
- 99 Richerson HB, Bernstein IL, Fink JN et al. Guidelines for the clinical evaluation of hypersensitivity pneumonitis. Report of the Subcommittee on Hypersensitivity Pneumonitis. *J Allergy Clin Immunol* 1989; 84:839–42.
- 100 Pennycott T. Veterinary Investigation Centre, Auchincruive, Ayrshire. Personal communication, 1998.
- 101 Miyagawa T, Ochi T, Takahashi H. Hypersensitivity pneumonitis with antibodies to *Cryptococcus neoformans*. *Clin Allergy* 1978; 8:501–9102.