

Allergy to laboratory animals among animal handlers

David H Bryant, Lynette M Boscato, Paul N Mboloi and Margaret C Stuart

Because of the need for animal models in medical research, large numbers of small mammals (such as mice, rats, rabbits and guinea pigs) are often held in research facilities. Their handlers may become sensitised to the allergens of these animals¹ and often report allergic symptoms, most commonly nasal symptoms (reported by more than 90% of those with allergies).² In some instances, symptoms become so severe that workers are forced to change jobs, sometimes after many years of specialised training. It would be desirable to identify those at most risk of developing sensitivity to laboratory animals and to advise them on ways to minimise the risk. With the exception of a 1981 survey of individuals working in a mouse animal house,³ there has been no published report of the problem in Australia.

We conducted a cross-sectional survey of laboratory animal handlers to determine the prevalence of allergy to laboratory animals and asthma and to assess the characteristics associated with the development of laboratory animal sensitivity.

Subjects and methods

Two hundred and twenty-eight subjects were surveyed between January 1989 and December 1992. They comprised teaching and research staff, animal house workers and animal husbandry students and teachers from Macquarie University, the universities of Sydney and New South Wales, the Garvan Institute, St Vincent's Hospital, the Westmead Centre, the Children's Medical Research Foundation and Department of Technical and Further Education (TAFE) institutions in Sydney.

Permission for the study was obtained

Abstract

Objective: To determine the prevalence among laboratory animal handlers of allergy to laboratory animals and of asthma and the factors associated with their development.

Design: A cross-sectional survey.

Setting: Teaching and research institutions in Sydney, between January 1989 and December 1992.

Participants: Laboratory animal handlers (teaching and research staff, animal house workers and animal husbandry students and teachers).

Main outcome measures: Duration of exposure to laboratory animals, allergic symptoms on contact, skin reactivity to laboratory and domestic animal allergens and evidence of current asthma.

Results: 228 subjects were surveyed. Allergy symptoms occurred in 73 (56%) of the subjects exposed to laboratory animals for three months or more. This group also had significantly higher prevalences of skin reactivity to laboratory animal allergens (62%) and bronchial hyperresponsiveness (21%) than those with shorter exposure (14% and 8%, respectively). Atopic subjects exposed to laboratory animals (particularly those sensitised to domestic animals) and animal attendants (with a high intensity of exposure to laboratory animals) had significantly higher frequencies of skin reactivity to laboratory animals and asthma than other subjects (77% and 30%, respectively, among exposed atopic subjects and 84% and 33%, respectively, among animal attendants).

Conclusions: Allergy to laboratory animals is an occupational hazard among laboratory animal handlers, especially for those who are atopic and sensitised to domestic animals, and may lead to the development of asthma. Screening for atopy and skin reactivity to laboratory animals before and during employment would enable those at risk to take precautions.

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from the Department of TAFE and the institutions. The study was approved by the human ethics and research committees of St Vincent's Hospital, the University of New South Wales, Macquarie University and the Westmead Centre. All subjects gave informed consent and were visited at their place of work or study. Personnel were informed of the study content and asked to participate. Participation rates varied between 100% (in most centres) and 60%.

Classification of animal exposure

Subjects were classed as exposed to laboratory animals if they had worked with them for three months or longer, and as not exposed if they had had less than three months' contact.

Questionnaire

All participants completed a questionnaire, based on that of Dales et al.,⁴ which asked for data on demography, smoking habits, duration of contact with laboratory animals, duties performed and hours spent per week in the animal house, previous medical history, and symptoms on contact with laboratory animals. Subjects were specifically asked, both directly and indirectly, about nasal symptoms (sneezing, itch-

St Vincent's Hospital, Sydney, NSW.

David H Bryant, MD, FRACP, Associate Professor of Medicine.

Lynette M Boscato, MSc, Senior Scientific Officer, Division of Chemical Pathology, Institute of Laboratory Medicine.

Paul N Mboloi, MMed, Visiting Fellow, Respiratory Medicine.

School of Biological Sciences, Macquarie University, Sydney, NSW.

Margaret C Stuart, PhD, Senior Lecturer in Biotechnology.

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ing, nasal obstruction or rhinorrhoea), chest symptoms (cough, breathlessness, chest tightness or wheezing), eye symptoms (redness, irritation, itching or lachrymation), and skin symptoms (rash or itching).

Skin reactivity tests

Skin prick testing to determine skin reactivity to allergens was done by the standard technique, with buffered normal saline and 5% histamine as negative and positive controls, respectively.^{5,6} Wheal diameter was measured after 15 minutes: diameters more than 2.5 mm were considered positive reactions.

Skin reactivity to laboratory animals was tested with extracts of laboratory animal hair (Hollister-Stier Laboratories, Division of Cutter Laboratories, Spokane, WA, USA) and urine. Urine extracts were prepared from urine collected from male and female adult Wistar rats, mice (Fullensdorf and BALB/c) and New Zealand white rabbits. Pooled urine from each species was concentrated in an Amicon ultrafiltration unit with a membrane with a 10 000 Da cut-off. The concentrates were dialysed against normal saline at 4°C, heated at 56°C for 30 minutes, filtered through 0.2 µm filters into sterile containers and stored frozen. When thawed for use, concentrates were diluted 1:2 with glycerol, and phenol was added to a concentration of 0.4%.

Preliminary tests with these animal extracts showed that they produced positive results in subjects known to be sensitised, and negative results in control subjects. Subjects with positive reactions to any laboratory animal extract were classed as reactive to laboratory animals.

Atopy was tested by skin prick testing with common environmental allergens (house dust, house dust mite [*Dermatophagoides pteromyssinus*], pollens [from Timothy grass, ryegrass and plantain], fungi [*Alternaria tenuis*, *Aspergillus fumigatus*, and *Hormodendrum clavatus*] and cows' milk) and domestic animal allergens (from cat, dog and horse hair; Hollister-Stier Laboratories, Division of Cutter Laboratories, Spokane, WA, USA). Subjects with positive reactions to at least one extract were classed as atopic.

Spirometry

Airflow limitation was assessed by spirometry with standard techniques and a Vitalograph dry bellows spirometer (Vitalograph Ltd, Buckingham);⁷ forced vital capacity (FVC) and forced expiratory volume in one second (FEV₁) were measured at body temperature and saturated pressure and compared with values predicted for sex, age and height.⁸ Airflow limitation was defined as FEV₁ less than 80% of predicted value and was classed as reversible if it increased by 12% or more after inhalation of 200 µg salbutamol.

Bronchial responsiveness to histamine was determined by the Yan method.⁹ Hyperresponsiveness was defined as a 20% decline in FEV₁ at a cumulative dose of histamine less than 4.0 µmol.

Subjects were classed as having asthma if they gave a history of episodic wheezing within the last two years or had evidence of reversible airways obstruction.

Statistical methods

Student's *t* test was used to assess observed differences, the χ^2 test to detect differences of association and the *z* test to compare proportions.

Results

The 228 subjects comprised 140 women and 88 men, aged 15–58 years. Most animal attendants were women (70%), while most teaching and technical staff were men (71%). Gloves were worn by 51% of subjects, face masks by 44% and helmet and filter systems by 16% of the subjects while working with laboratory animals.

One hundred and thirty subjects were classed as exposed and 98 as not exposed to laboratory animals. Characteristics of the two groups are shown in Table 1. They were similar in age and prevalence of cigarette smoking. Exposed subjects had significantly higher frequencies of allergic symptoms on contact with laboratory animals, skin reactivity to laboratory animals, atopy and asthma than those not exposed ($P < 0.005$). They also had a significantly higher frequency of bronchial hyperresponsiveness and significantly lower FEV₁ measurements ($P < 0.05$). Among

Table 1: Comparison of subjects exposed and not exposed to laboratory animal allergens

Characteristic	Not exposed (n = 98)	Exposed (n = 130)
Mean age (years) (SD)	26 (8)	29 (8)
Cigarette smoking	44 (45%)	48 (37%)
Allergic symptoms on laboratory animal exposure	0	73 (56%)*
Skin reactivity to laboratory animals	14 (14%)	80 (62%)*
Atopy	43 (44%)	93 (72%)*
Asthma	5 (5%)	28 (22%)*
Mean FEV ₁ (L) (SD)	3.71 (0.56)†	3.50 (0.71)‡§
Mean FVC (L) (SD)	4.27 (0.67)†	4.33 (0.77)†
Bronchial hyperresponsiveness	8 (8%)	27 (21%)§

* Significantly different from the not exposed group ($P < 0.005$). † $n = 80$. ‡ $n = 112$. § Significantly different from the not exposed group ($P < 0.05$).

the 33 who reported symptoms of asthma within the last two years, all but one (in the exposed group) had bronchial hyperresponsiveness to histamine. In the not exposed group, three with hyperresponsiveness had no symptoms of asthma but reported hay fever.

Among exposed subjects, 73 (56%) had allergic symptoms on contact with laboratory animals. All of these had nasal symptoms, with many reporting at least one other symptom: chest symptoms in 34 (46%); eye in 46 (63%); and skin symptoms in 30 (41%). None of those exposed for less than three months had symptoms on animal contact ($P < 0.005$).

The associations between symptoms

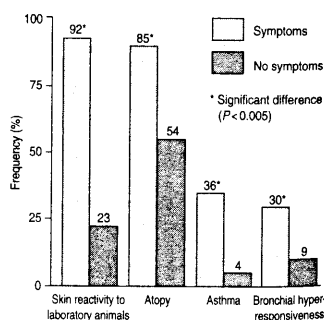


Figure 1: Frequency of allergy and asthma in symptomatic and asymptomatic subjects exposed to laboratory animals for three months or more.

Table 2: Percentage of subjects with skin reactivity to laboratory animal allergens in each group (number with skin reactivity/total in group)

Atopy	Exposed			Not exposed No symptoms
	Symptoms	No symptoms	Total	
Atopic	100% (62/62)	32% (10/31)	77% (72/93)	33% (14/43)
Reactive to domestic animal allergens	100% (59/59)	42% (5/12)	90% (64/71)	65% (11/17)
Reactive to environmental allergens alone	100% (3/3)	26% (5/19)	36% (8/22)	12% (3/26)
Non-atopic	45% (5/11)	12% (3/26)	22% (8/37)	0 (0/55)

and aspects of allergy and asthma in the exposed group are shown in Figure 1. Most of those with symptoms were sensitive to laboratory animal allergens (92%) and atopic (85%). Those with symptoms had significantly higher frequencies of skin reactivity to laboratory animals, atopy, asthma and bronchial hyperresponsiveness than those exposed but without symptoms. The frequencies of all these variables, except bronchial hyperresponsiveness, did not differ significantly between the latter and those not exposed.

The association between skin reactivity to laboratory animals and atopy is shown in Table 2. The prevalence of skin reactivity to laboratory animals was significantly higher among atopic subjects (exposed and not exposed) than non-atopic subjects ($P < 0.005$). It was also more common among atopic subjects who reacted to domestic animal allergens than among those who reacted to moulds, pollens or dust alone. This difference was more pronounced among those not exposed to laboratory animals than among those exposed.

All subjects with asthma were atopic and asthma was more common in the exposed group (30% of exposed atopics versus 12% of not exposed atopics; $P < 0.05$). All 28 exposed subjects with asthma had skin reactivity to laboratory animal allergens (86% also reacted to domestic animal allergens and the remaining 14% to common environmental allergens alone) and most reported symptoms on contact with laboratory animals. The skin reactivities of the five unexposed subjects with asthma were to: laboratory animal allergens in two, domestic animal allergens in four and common environmental allergens alone in one.

The frequencies of allergy in different types of laboratory personnel are shown in Figure 2. Animal attendants had the highest intensity of contact with laboratory animals and also had significantly higher ($P < 0.05$) frequencies of allergic symptoms on contact, skin reactivity to laboratory animals and asthma than other types of animal handlers.

Discussion

This study clearly shows a high prevalence of allergy to laboratory animals among exposed laboratory personnel in the Sydney region between 1989 and 1992. Over half those exposed for three months or more reported symptoms, ranging from rhinitis to skin irritation, on contact with laboratory animals, and 22% had evidence of asthma.

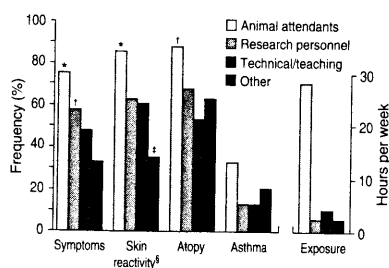
The prevalence of allergy was higher than that found in other studies. In the only other published Australian study,³ 32% of 121 subjects had allergic symp-

toms on contact with mice. In other multisite studies 7%–44% of people exposed to several species of small mammals reported allergic symptoms.^{10–15} However, cross-sectional studies are likely to underestimate the extent of the problem, as more severely affected workers may seek alternative employment. We suspect that the true prevalence of allergy to laboratory animals and of associated asthma is even higher than detected.

Immediate hypersensitivity seemed to be the major cause of symptoms, as 92% of those with symptoms showed skin reactivity to laboratory animal allergens on skin prick testing. In the remaining six subjects who had no evidence of sensitisation, symptoms may have been caused by physical irritation to airborne dust, detergents or disinfectants. However, it is possible that in-vitro testing for specific IgE antibody could have detected lower levels of reactivity than those detectable by skin testing.

Sensitisation to laboratory animals was more common among those exposed for three months or more than among those exposed for less than three months, among both atopic and non-atopic subjects. Animal attendants were more at risk than other laboratory animal handlers, which is consistent with the increased prevalence of sensitisation found with increasing intensity of exposure in other studies.^{12,15,16} Laboratory animal allergens are usually found in the dust in animal houses and can remain airborne for long periods after disturbance.^{17,18} The main route of immunisation appears to be via the lungs. Animal attendants are exposed for long periods to potentially high levels of antigens by activities such as cage cleaning. Research personnel generally spend less time in the facilities, handle fewer animals and are involved in activities such as injections, surgery and killing, which are less likely to produce aerosols. Nevertheless, the high level of sensitisation among these personnel suggests that these allergens are highly antigenic and remain airborne at high levels.

We also found that, although many subjects wore gowns and gloves, few protected themselves against aerosols. Devices to reduce dust inhalation and procedures to



* Significantly different from frequencies in other groups ($P < 0.005$) and from total frequency ($P < 0.05$).

† Significantly different from frequencies in other groups ($P < 0.05$).

‡ Significantly different from total frequency ($P < 0.05$).

§ To laboratory animal allergens.

Figure 2: Allergy, asthma and weekly exposure to animals in different groups of animal handlers exposed to laboratory animals for three months or more.

minimise dust in the work environment have been used in the United Kingdom, with an accompanying reduction in the frequency of allergic symptoms.¹⁹ Reductions in stock density and use of filter-top cages and non-contact absorbent pads as cage bedding (instead of sawdust or woodchips) have also been recommended to reduce levels of airborne allergens.²⁰

The strong association between atopy and sensitisation to laboratory animal allergens found in this study is consistent with the results of many other studies,^{1,3,10,13,14,16} but conflicts with others.^{11,12,19} It is thus uncertain if testing people for atopy before they start a career working with laboratory animals would identify all those at risk of developing allergy, as some may develop atopy only after exposure to the animals. Longitudinal studies are needed to resolve this.

Our data are also consistent with the suggestion of Lutsky et al.¹⁰ that atopic subjects exposed to laboratory animals may develop asthma, particularly if they become sensitive to laboratory animals. We may have underestimated the problem, as self-reporting of asthma and single measurements of spirometry and bronchial histamine responsiveness may not reflect the true frequency of asthma.

Most subjects who were atopic, sensitive to laboratory animal allergens and asthmatic were also sensitive to domestic animal allergens on skin prick testing. This extends previous findings of associations between skin reactivity to domestic animal antigens and asthma²¹ and between allergy to domestic and to laboratory animals.^{2,22,23} The high correlation between sensitivities to the two groups of animals may result from: cross-reactive antigenic determinants on their allergens,^{23,24} or concurrent exposure and sensitisation to both types of animal.

The greater prevalence of atopy among exposed subjects is a novel finding. Possible reasons include:

- Allergic individuals have preferentially volunteered for the study. This is unlikely as a high proportion of those working in the centres (both exposed and not exposed) were tested.
- Environmental levels of mould and dust allergens in animal houses are higher than in the general environment, leading to increased sensitisation and hence development of atopy.

Recommendations for reducing allergy to laboratory animals

- Pre-employment screening for atopy and allergy to laboratory animals (to enable appropriate risk management advice);
 - Use of protective clothing when handling laboratory animals; and
 - Introduction of measures to reduce dust in laboratory animal facilities (e.g., filter-top cages, non-contact absorbent pads as cage bedding, decreased stock density).
- Laboratory animal allergens presented to the immune system on particles via the lung act as adjuvants, stimulating an immune response to antigens that are not normally allergenic.
 - Those working with laboratory animals have a higher level of atopy than the general population and exposure to laboratory animals has no effect on the degree of atopy.
- Longitudinal studies would help to clarify this.
- We conclude that exposure to laboratory animals is a significant risk factor for the development of allergy to laboratory animals among atopic subjects, and that subjects who become sensitised to laboratory animals have a significantly increased risk of developing asthma. Doctors should question patients presenting with asthma or allergic rhinitis about contact with laboratory animals.
- Atopic subjects reactive to domestic animal allergens may be at higher risk of developing allergy to laboratory animals. Possible measures to reduce the frequency of allergy to laboratory animals are shown in the Box.

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