

## **Evidence of allergic hypersensitivity to chironomid midges in an English village community**

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*(Received in original form 12 June 1987 and in revised form 8 December 1987;  
accepted for publication 15 December 1987)*

### **Summary**

Some chironomid (non-biting) midges contain potent human allergens, principally in the form of insect haemoglobins. In view of previously demonstrated immunological cross-reactivity between some different midge species, the possible role of British midges in allergic disease was studied. The chosen site, Farmoor near Oxford, is a village that has been subject to several annual nuisance midge swarms since the construction of an adjacent water-supply reservoir in 1964, which was extended in 1975. The incidence and severity of hypersensitivity in the community was assessed by questionnaire survey, skin-prick testing, radio-allergosorbent tests (RAST) and RAST inhibition, and enzyme-linked immunosorbent assays (ELISA) and ELISA inhibition, using extracts of locally caught adult midges (*Tanytarsus sylvaticus*, *T. bathophilus* and *Chironomus anthracinus*) and laboratory cultured larvae (*C. riparius*). These tests revealed a low but significant level of midge-related hypersensitivity with skin-prick results showing seasonal fluctuations, but this seasonality was not confirmed with an IgE RAST. A correlation between IgE levels and relevant symptoms was found ( $P < 0.01$ ) using RAST, but not between IgE and skin-test results. Enzyme-linked immunosorbent assay results did not correlate with any other parameter measured. The incidence and severity of allergic reactions to the midges tested in this community was low.

### **Introduction**

Adult non-biting midges (Insecta: Chironomidae) have been shown to cause immediate type IgE-mediated hypersensitivity in man in Sudan [1,2], U.S.A. [3], Sweden [4] and Japan [5,6]. In Britain, 14% of patients attending a dermatology clinic

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were found to have a positive skin reaction when tested with larval midge allergens (E. Young, personal communication). In Germany, larvae used commercially as fish food cause occupational allergic complaints in handlers [7]. Thus, both larval and adult midges have been implicated in allergic disease, and the major allergens appear to be larval haemoglobins, which can be retained into the adult midge [8,9]. Furthermore, immunological cross-reactivity has been shown between *Cladotanytarsus lewisi* Freeman, the major environmental allergen in Sudan, and the distantly related *Chironomus* species responsible for the occupational allergy in Germany [8,10].

In many countries, including Britain, rivers, lakes and reservoirs are artificially enriched with nutrients ('eutrophicated') in the form of washed-out fertilizers and sewage effluents. This process leads to the increased production of the aquatic biota, including chironomid midges, which have aquatic larval stages. Consequently, swarms of midges are increasingly the source of complaints by people who reside close to such water bodies. Exposure to midge allergens is thought to be either by direct contact with midges, causing immediate hypersensitivity reactions, or by inhalation of allergenic fragments in dust, resulting in immediate and delayed respiratory tract and mucosal reactions. Since midges have been predicted to be environmental health hazards elsewhere in the world, we selected a site with documented midge nuisance, Farmoor near Oxford, to assess the possible significance of these midges as environmental allergens in the U.K.

Farmoor has a historical record of seasonal emergences of midge swarms from a local water-storage reservoir. Major emergences occur in most years in spring, in early April, when large swarms of several different species have previously caused considerable nuisance to residents close to the reservoir. Midge emergence continues throughout the summer, although in smaller numbers, until a final emergence occurs, often at nuisance levels, in autumn [11].

## Subjects and methods

### *Questionnaire/symptomology*

A questionnaire survey of the whole village population was carried out in December 1984 to obtain records of allergy-related symptoms, and to assess the community awareness of the possible link between midges and hypersensitivity reactions. The frequency, periodicity and seasonality of common symptoms associated with allergic conditions, such as streaming or itchy eyes or nose, sneezing, wheezing and skin rashes, were determined in a carefully constructed questionnaire. Questions were asked to establish the frequency of symptoms, in the previous 2 years, associated with hay fever-type allergic reactions, asthma and, more generally, the likely proportion of atopics in the population. Midge nuisance was judged seasonally on a subjective 1-5 scale with 1 being no problem and 5 a very serious problem. This was done on a household basis, although individuals could be identified from the answers given. Residents were invited to attend a preliminary trial where blood (serum) samples, for IgE determination, were taken and skin-prick tests to cultured midge larvae (haemoglobin-rich) were conducted. On the basis of the results obtained, a second similar study was carried out using extracts of adult midges caught near Farmoor during the springtime midge emergence. The symptoms of those who participated were determined by direct questioning. Symptoms were assigned an arbitrary value ranging from 0 to 3, covering

the range from no symptoms to very serious, for each respondent. These values were used in correlations with radio-allergosorbent tests (RAST), enzyme-linked immunosorbent assay (ELISA) and skin-test weal area.

#### *Preparation of the antigens*

Midges were collected by netting, dried in air, and sorted into species. *Tanytarsus sylvaticus* (Wulp), *T. bathophilus* Kieffer and *Chironomus anthracinus* Zetterstedt numerically dominated the catches in the sequence given, although the appreciably larger *C. anthracinus* occurred in the greatest biomass. The midges were defatted with ether and extracted in Coca's solution [12,13], filtered (0.45  $\mu$ m Millipore), dialysed (Visking tubing 24/32), and lyophilized. Laboratory cultured *Chironomus riparius* Meigen (synonymous with some earlier European workers *C. thummi thummi* Kieffer) larvae were extracted in the same manner. The protein concentration of the extracts varied between 68% and 74% dry weight, as determined by the Folin-phenol reagent test [14].

#### *Skin-prick tests*

Skin-prick tests were performed on Farmoor resident volunteers on two occasions; November 1985 and July 1986. Logistics and screening of test solutions prevented testing closer to the peak emergence period of midges in 1985. Therefore, a second testing session was organized in July of the following year, in which many people from the first session were retested, along with some additional volunteers. In both sessions, patients were tested with extracts of grass pollens (GRP. B.2. (Bencard, Brentford, Middx)), cat fur (Bencard), *Dermatophagoides pteronyssinus* (Bencard) and *Chironomus riparius* larvae. Patients who developed positive skin reactions (as defined below) to pollen, cat fur and house dust mite were considered as 'atopic' for the purpose of this study. In November, patients were additionally tested with extracts of mixed adult midges collected in Farmoor. In the second session in July, individual extracts of *T. bathophilus*, *T. sylvaticus* and *C. anthracinus* replaced the mixed midge extract. The method of preparation of midge extracts was identical to that previously described [8]. All skin-test solutions were made up in 50% Coca's solution and 50% glycerol to a concentration of 1 mg/ml extract. Histamine (dihydrochloride) at 1 mg/ml was used as a positive control, and the diluent used as a negative control. Any skin reaction measuring >2 mm diameter was considered a positive result. This small size was chosen due to the low level of reactivity initially detected against midge allergens. Weals were ringed in ballpoint pen and recorded on clear adhesive tape for subsequent measurement.

#### *Human sera*

During both testing sessions, 20-ml blood samples were taken from every willing volunteer who had been previously skin tested. The serum was aliquoted and stored at -20°C until required. There were ninety-six serum samples from the first session (November) and seventy from the second (July), of which forty were from the same patients. In practice this was just over 90% of those who volunteered for skin testing.

#### *Radio-allergosorbent tests and RAST inhibition*

Circulating midge-specific IgE levels were measured using the RAST (Pharmacia, Uppsala, Sweden), as described [8] and are expressed in Phadebas RAST Units (PRU).

The binding concentration of unfractionated extract was 10 mg/g unexpanded CNBr-activated Sepharose 4B (Pharmacia). The RAST assay was carried out with two overnight incubations using 50  $\mu$ l serum with 100  $\mu$ l of allergen polymer complex (APC) (6.25% concentration) and 50  $\mu$ l  $^{125}$ I-anti-IgE (Pharmacia), respectively. Human cord blood serum was used in all assays as a negative control. Radio-allergosorbent test inhibition studies were conducted on pools of positive sera by overnight pre-incubation with serial concentrations of the extracts. Preliminary screening to *C. riparius* was carried out using a serum pool derived from European patients (Department of Allergy and Clinical Immunology, Brompton Hospital, London, U.K.) with positive skin tests and RASTs to *Cladotanytarsus lewisi*, most of whom were unlikely to have been naturally exposed to this midge. Subsequent RASTs were conducted using a mixed antigen of equal parts of *T. bathophilus*, *T. sylvaticus* and *C. anthracinus* on serum collected from patients from Farmoor.

#### *Enzyme-linked immunosorbent assay*

In order to establish if the ELISA technique could be used as a more rapid and cheaper alternative to RAST, an indirect double antibody ELISA was used, similar to that described by Sepulveda *et al.* [15]. Briefly, plastic microtitre plates (Immulon M129A, Dynatec, Billingshurst, Sussex) were coated with 100  $\mu$ g/ml antigen in carbonate buffer (pH 9.6), overnight at 4°C, using a working volume of 150  $\mu$ l/well. The volumes and dilutions used were those that gave optimum results from a chequer-board dilution assay. The wells were aspirated and washed three times with phosphate buffered saline (PBS) + 0.05% v/v Tween 20, then blocked for 1 hr by incubation with 1% w/v bovine serum albumin (BSA) in PBS-Tween 20, followed by three washes with PBS-Tween 20. Seventy-five microlitres of the patient's serum plus 75  $\mu$ l PBS-BSA were added to each well and incubated at room temperature for 3 hr. After aspiration and three washes in PBS-Tween 20, 150  $\mu$ l peroxidase-conjugated goat-anti-human IgE serum (Fc specific) (Nordic, Maidenhead, Berks.) were added to each well and incubated for a further 2 hr at room temperature. The plates were again washed three times and 150  $\mu$ l peroxidase-conjugated rabbit-anti-goat IgG (H + L) serum (Nordic) were added to each well and incubated for 1 hr. Both antisera were peroxidase conjugated in order to achieve maximum amplification. After a final series of three washes, 150  $\mu$ l of the enzyme substrate ortho-phenylene diamine (OPD) were added to each well and incubated for 30 min in the dark. The reaction was stopped with 25  $\mu$ l 8N H<sub>2</sub>SO<sub>4</sub> and the absorbance at 492 nm was recorded for each sample. For comparative purposes the same tests were performed with ELISA as with RAST above. Blanks (no serum), negative controls (human cord serum) and known positive sera were run in each assay to allow direct comparison between the plates.

#### *Total IgE*

Total IgE was not measured in this study because previous results have shown no correlation between total and midge allergen-specific IgE [8].

## **Results**

#### *Questionnaire*

Responses were elicited from approximately 75% of the village population (total population c. 550 individuals). The results of the questionnaire are shown in Table 1.

Approximately 14% of the respondents reported allergy-related symptoms, including 3–4% who reported having asthma.

There was a clear association between perceived nuisance and season, with most complaints concerning spring emergence (Table 2).

#### *Skin-prick tests*

Skin-prick test responses to midge extracts, even in atopic individuals, were often less than those recorded for histamine. This was particularly noticeable in November 1985, some months removed from peak midge emergences (Fig. 1). Repeat skin tests in July 1986 showed increased responses in some individuals such that the weal area provoked was greater than that for histamine. However, these results should be treated with caution since three extracts of different midge species were used in the second testing session (Fig. 2). The number of patients tested in the first session was 126; in the second session it was eighty-one, of which forty-six were repeats, giving a total of 161 separate skin-test responses. Additional data were also available for a few patients tested during a preliminary trial in June 1985.

#### *Radio-allergosorbent tests and RAST inhibition*

Radio-allergosorbent test dose-response and inhibition assays for a serum pool from donors (see above) who were skin reactive to *C. lewisi* were performed using *C. raparius* larval antigen. Similar assays were also conducted on a serum pool from patients from Farmoor who were skin-test positive to one or more of the *T. bathophilus*, *T. sylvaticus* or *C. anthracinus* adult midge extracts. In each case inhibition was carried out using the homologous allergens to validate the assay (Table 3). No cross-reactivity studies are reported here. The RAST inhibition studies indicate that the homologous antigen is an efficient inhibitor, with approximately 68% inhibition at 2 mg/ml in both the *C.*

**Table 1.** Questionnaire survey of Farmoor residents, December 1984

| Symptom                            | % of population |      |
|------------------------------------|-----------------|------|
| Itchy or runny eyes                | (*)             | 12.7 |
| Blocked or itchy nose              | (*)             | 12.4 |
| Sneezing, or runny nose            | (*)             | 14.4 |
| Nausea or vomiting                 |                 | 1.7  |
| Chest tightness                    | (**)            | 4.4  |
| Pain in chest                      |                 | 2.9  |
| Difficulty in breathing            | (**)            | 2.9  |
| Wheezing in chest                  | (**)            | 4.4  |
| Headache or dizziness              |                 | 10.5 |
| Itchy bumps on skin                | (***)           | 11.4 |
| Poor concentration                 |                 | 3.2  |
| Hay fever, asthma or other allergy |                 | 13.6 |
| No symptoms                        |                 | 64.8 |

Positive questionnaire responses showing frequency of rhinitis (\*), asthma (\*\*) and skin hypersensitivity (\*\*\*) in a discrete village population at Farmoor near Oxford, U.K. Total number of responses = 410.

**Table 2.** Seasonally perceived midge nuisance

| Perceived nuisance | Spring                   | Summer                   | Autumn                   | Winter                   |
|--------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| None (1)           | 138·00<br>13·06<br>33·66 | 259·00<br>24·50<br>63·17 | 266·00<br>25·17<br>64·88 | 394·00<br>37·28<br>96·10 |
| Slight (2)         | 61·00<br>42·66<br>14·88  | 44·00<br>30·77<br>10·73  | 26·00<br>18·18<br>6·34   | 12·00<br>8·39<br>2·93    |
| Moderate (3)       | 105·00<br>44·30<br>25·61 | 62·00<br>26·16<br>15·12  | 66·00<br>27·85<br>16·10  | 4·00<br>1·69<br>0·98     |
| Serious (4)        | 75·00<br>51·37<br>18·29  | 33·00<br>22·60<br>8·05   | 38·00<br>26·03<br>9·27   | 0·00<br>0·00<br>0·00     |
| Very serious (5)   | 31·00<br>54·39<br>7·56   | 12·00<br>21·05<br>2·93   | 14·00<br>24·56<br>3·41   | 0·00<br>0·00<br>0·00     |

The levels of perceived nuisance, on an arbitrary scale, in each season of the year. Contingency Chi-square analysis reveals a significant ( $P < 0·001$ ) trend, indicating that over 50% (summation of column %) of the population regarded midges as a moderate to very serious nuisance problem in spring, and 25–30% regarded them as a problem during winter. Total sample size = 410.

*raparius* and mixed adult assay (Table 3). The specificity of the RAST response, therefore, would appear to be adequate, although not as good as reported for *C. lewisi* [8].

Individual serum samples demonstrated over 10% binding (or up to 2·80 PRU/ml), using 6·25% APC with a combined defined species extract consisting of equal parts of the three midges above (Table 3). Only 1–2% of those tested gave moderate to high RAST results, whereas approximately 8% (14/167; Table 4) did have detectable amounts. There was no evidence of seasonal fluctuation in the IgE levels detected in this small proportion. However, higher scores were recorded (up to approximately 4 PRU/ml) in some *C. lewisi*-sensitive patients in *C. raparius* RAST (data not shown).

#### *Enzyme-linked immunosorbent assays and ELISA inhibition*

Similar dose–response and inhibition experiments to the RAST above are illustrated in Tables 3 and 4. The ELISA did appear to give an allergen-specific response, as indicated by the inhibition, although the technique suffered from a lack of sensitivity, as can be seen in the positive pool titration (Table 3). In a non-parametric Wilcoxon's ranked pair test, RAST and ELISA were significantly different ( $P < 0·01$ ), thus lacking any correlation. In the two separate trials ( $n = 96$ ;  $n = 70$ , respectively) the RAST data exhibited a characteristically overdispersed distribution, whereas the ELISA data was normally distributed (histograms not shown).

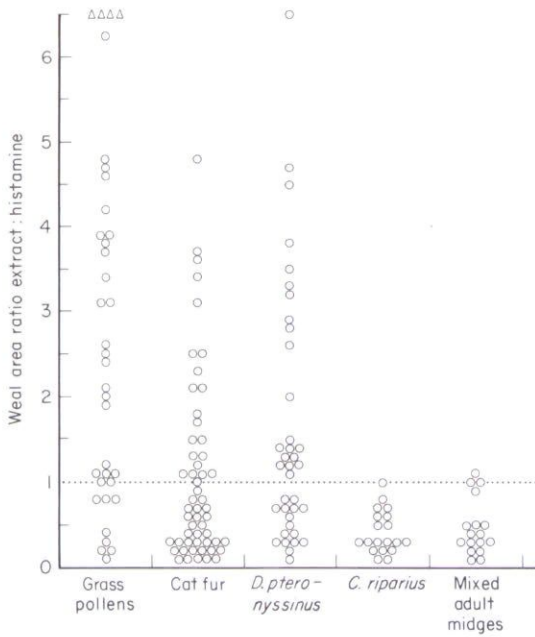


**Fig. 1.** Skin-test results from Farmoor residents 1/11/85. Each symbol (O) represents the result from one individual. Total number of people tested = 126. (....) Standardized histamine control response. ( $\Delta$ ) = Response greater than 7. Only positive results are shown.

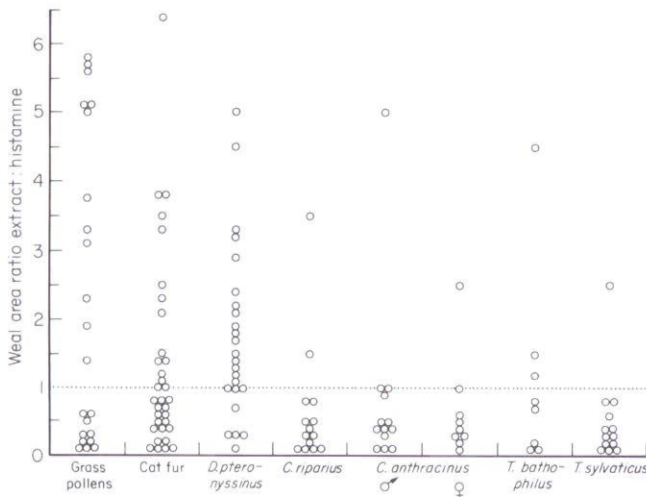


**Fig. 2.** Skin-test results from Farmoor residents 18/6/86. Each symbol (O) represents the result from one individual. Total number of people tested = 81. Note increased response to midge extracts in a few cases. All midge weals > standardized histamine response (....), except for one in *T. bathophilus* column, were from people also tested in the first session (Fig. 1). Only positive results are shown.

**Table 3.** RAST and ELISA inhibition and titration

| Inhibitor<br>conc. mg/ml | Inhibition           |       | Serum<br>dilution | Titration         |                    |
|--------------------------|----------------------|-------|-------------------|-------------------|--------------------|
|                          | RAST<br>% inhibition | ELISA |                   | RAST<br>% binding | ELISA<br>OD 492 nm |
| 2                        | 67.2                 | 81.3  | Neat              | 4.5               | 0.54               |
| 1                        | 60.4                 | 70.5  | 1/2               | 2.9               | 0.45               |
| 0.5                      | 58.6                 | 68.7  | 1/4               | 2.2               | 0.36               |
| 0.25                     | 49.1                 | 62.4  | 1/8               | 1.6               | 0.26               |
| 0.125                    | 47.1                 | 57.0  | 1/16              | 1.4               | 0.20               |
| 0.0625                   | 40.9                 | 47.5  | 1/32              | 0.9               | 0.16               |
| 0.03125                  | 31.0                 | 45.5  | 1/64              | 1.1               | 0.08               |
| 0.015625                 | 27.6                 | 39.0  | 1/128             | 1.1               | n.d.               |
| 0.0078125                | 26.5                 | n.d.  |                   |                   |                    |
| 0.00395625               | 12.7                 | n.d.  | Cord              | 0.7               | 0.03               |

RAST and ELISA inhibition and titration of positive serum pools using a mixed adult midge antigen of *T. bathophilus*, *T. sylvaticus* and *C. anthracinus*.

### Symptomology

All the patients who responded to midge extracts were atopic; however, no patients ascribed their allergic symptoms to midges. There was a strong positive association between reported relevant symptoms with midge extract RAST (Kruskal-Wallis,  $H=20.03$ ,  $k=4$ ,  $n=96$ ,  $P<0.01$ ; and  $H=7.80$ ,  $k=2$ ,  $n=71$ ,  $P<0.01$  for the two sessions, respectively) but not between symptoms and skin-test results, or between symptoms and ELISA scores.

### Discussion

Haemoglobins have been identified as the major allergenic component in proteinaeous midge extracts [16,17]. In our survey three dominant species were identified in the spring emergence of midges at Farmoor, all of which contain haemoglobins in their larval stages. Many of the sub-dominant species identified from Farmoor reservoir may also contain haemoglobin, at least in their larval forms, and it seems likely that haemoglobin antigenic determinants might well persist in adult midges [9,18].

Although the questionnaire revealed that the residents of Farmoor were concerned with the nuisance value of the midges when they occurred in large numbers, they did not ascribe or associate allergy-related symptoms with their seasonal emergence. The first survey of the community provided a basis for further investigation since symptoms often associated with exposure to environmental allergens were reported by a significant proportion (12–15%; Table 1) of the population.

Preliminary RAST tests indicated that a low proportion of the population had circulating midge-specific IgE, albeit in low titres. The first session of skin tests seemed to confirm this, although the responses to midge allergens were considerably lower than those against more common environmental allergens in most cases. Retesting a section of the community during the summer, having allowed sufficient time to ensure a fully developed immunological response to spring and summer emerging midges,

**Table 4.** RAST, ELISA, skin test and symptoms

| RAST      |        | ELISA     | Skin                  | Symptom |
|-----------|--------|-----------|-----------------------|---------|
| % binding | PRU/ml | OD 492 nm | reactivity            | score   |
| 10.04     | 2.15   | 0.810     | —*                    | 1       |
| 9.92      | 2.80   | 0.809     | ++++                  | 2       |
| 6.83      | 1.20   | 0.550     | +                     | 3       |
| 5.91      | 0.86   | 0.380     | —*                    | 0       |
| 4.68      | 0.58   | 0.721     | +                     | 1       |
| 4.57      | 0.55   | 0.675     | +                     | 0       |
| 3.94      | 0.42   | 0.661     | +                     | 3       |
| 3.65      | 0.43   | 0.458     | ++--                  | 0       |
| 3.38      | 0.32   | 1.020     | —*                    | 3       |
| 3.34      | 0.38   | 1.121     | +- - -                | 2       |
| 3.20      | 0.30   | 0.395     | —*                    | 2       |
| 3.15      | 0.35   | 0.948     | - - - -               | 1       |
| 2.87      | <0.30  | 0.529     | +                     | 1       |
| 2.83      | 0.30   | 0.733     | ++++                  | 0       |
| 2.64      | 0.30   | 0.593     | —*                    | 1       |
| 2.57      | <0.30  | 0.808     | ++-+                  | 1       |
| 2.54      | <0.30  | 0.594     | ++++                  | 0       |
| 2.54      | <0.30  | 0.861     | —*                    | 0       |
| 2.38      | <0.30  | 1.009     | - - - -               | 0       |
| 2.18      | <0.30  | 0.338     | —*                    | 0       |
| 2.13      | <0.30  | 0.394     | —*                    | 0       |
| 0.73      | —      | 0.530     | (Cord serum; $n=24$ ) |         |

A RAST value of 0.3 PRU was chosen to represent a positive score. Wilcoxon's non-parametric ranked pair correlation test indicated no correlation between RAST and ELISA scores. Total sample size = 167. Skin reactivity was designated as + = positive and — = negative. Skin reactivity to four midge extracts (*C. riparius*, *T. bathophilus*, *T. sylvaticus* and *C. anthracinus*) were recorded for some patients, whilst others were only tested with *C. riparius* (\*). Symptoms were scored 0–3 (see text).

revealed increased levels of skin reactivity to midge allergens, both in terms of the proportion of the people tested and the magnitude of the reactions (Figs 1 and 2).

It is interesting that only moderate levels of circulating IgE against midge antigens were detected by the RAST, and then only in a few cases (Table 4). Unlike skin reactivity, the IgE levels in the same individual did not fluctuate greatly between the two test dates. The RAST results, however, correlated better with reported symptoms than did the skin-test results. Since the skin-prick results to other more common environmental allergens were considerably greater than the responses to midges, the correlation between midge RAST and symptomology must be interpreted with caution. It seems probable that many of the symptoms reported may be associated with other allergens.

The specificity of the ELISA for the detection of allergen-specific IgE has been demonstrated to be very high by some workers [15, 19, 20], whereas in common with others we have found no correlation between ELISA and the more conventional

RAST [21, 22]. Although the inhibition data suggests specificity, the normal distribution of the ELISA data suggests the opposite. These equivocal findings might be resolved by the use of more defined allergen extracts [19] or by selecting or developing different reagents. The reported ELISA is not a viable alternative to the conventional RAST.

In conclusion we have found that a proportion (approximately 8%) of the English community studied, which has been seasonally exposed to nuisance midges, developed low to moderate levels of specific IgE directed against midge allergens. Skin reactions showed seasonal fluctuations in intensity, but no evidence was obtained for a similar fluctuation in circulating IgE levels. However, chironomid allergens in this community should still be considered as potential environmental allergens, but their clinical importance should not be overestimated. The lower allergenicity of the three British midge species studied, as compared with *C. lewisi* in Sudan [23], and *Chironomus* species in Germany [7], may be related to any or all of the following conditions; less intensive exposure to midges *per se*, reduced exposure to allergenic determinants derived from midges (such as dust in the environment), a diminished amount of larval allergenic haemoglobin retained into the adult midge species studied [18], or a reduced allergenicity of the haemoglobin in the species studied.

### Acknowledgments

This work was funded by the Wellcome Trust and approved by the Brompton Hospital Ethics Committee. We gratefully acknowledge the co-operation and help given by Dr A. J. Newman Taylor (Consultant Physician and Head of the Department of Occupational Medicine, Brompton Hospital) and the practical assistance of Dr B. Graneek (Brompton Hospital) and Dr N. Slater (Oxford). Thanks are also extended to Dr K. Venables, Miss R. Hawkins and Mr T. Betts of the Brompton Hospital, and to the residents and Residents Association of Farmoor.

### References

- 1 Lewis DJ. Chironomidae as a pest in Northern Sudan. *Acta Trop* 1956; 13:142–58.
- 2 Cranston PS, Gad El Rab MO, Kay AB. Chironomid midges as a cause of allergy in the Sudan. *Trans R Soc Med Hyg* 1981; 75:1–4.
- 3 Kagen SL, Yunginger JW, Johnson R. Lake fly allergy: incidence of chironomid sensitivity in an atopic population. *J Allergy Clin Immunol* 1984; 73:187 (abstr).
- 4 Eriksson NE, Petersson I, Vedal S, Hogstedt B, Belin L, Johansson SGO. Allergy among farmers 9 Stockholm: *Trans EAACI*, 1985:119.
- 5 Sasa M. Chironomid midges as the cause of bronchial asthma. *Kanko Eisei* 1985; 32:8–14.
- 6 Yamashita N, Ito K, Nakagawa T *et al.* IgG and IgE antibodies to Chironomidae in asthmatic patients. *Clin Exp Immunol* 1987; 68:93–9.
- 7 Baur X. Chironomid hemoglobin—a major allergen for humans. *Chironomus* 1982; 2:24–5.
- 8 Tee RD, Cranston PS, Dewair M, Prelicz H, Baur X, Kay AB. Evidence for haemoglobins as common allergenic determinants in IgE-mediated hypersensitivity to chironomids (non-biting midges). *Clin Allergy* 1985; 15:335–43.
- 9 Prelicz H, Baur X, Dewair M *et al.* Persistence of hemoglobin allergenicity and antigenicity during metamorphosis of Chironomidae (Insecta, Diptera). *Int Arch Allergy Appl Immunol* 1986; 79:72–6.
- 10 Baur X, Dewair M, Haegerle K, Prelicz H, Scholl A, Tichy H. Common antigenic determinants of haemoglobin as basis of immunological cross-reactivity between chironomid species (Diptera, Chironomidae): studies with human and animal sera. *Clin Exp Immunol* 1983; 54:599–607.
- 11 Bawden AN. The ecology of some reservoir Chironomidae. D. Phil thesis, University of Oxford, 1976.
- 12 Phillips GL Preparation of allergenic extracts for testing and treatment. In: Sheldon JM, Lovell RG, Mathews KP, eds. *A Manual of Clinical Allergy*. 2nd ed. Philadelphia: Saunders Company, 1967:507.

- 13 Tee RD, Cromwell O, Longbottom JL, Cranston PS, Kay AB. Partial characterization of allergens associated with hypersensitivity to the 'green nimitti' midge (*Cladotanytarsus lewisi*, Diptera: Chironomidae). Clin Allergy 1984; 14:117-27.
- 14 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ Protein measurement with the Folin-phenol reagent. J Biol Chem 1951; 193:265-75.
- 15 Sepulveda R, Longbottom JL, Pepys J. Enzyme linked immunosorbent assay (ELISA) for IgG and IgE antibodies to protein and polysaccharide antigens of *Aspergillus fumigatus*. Clin Allergy 1979; 9:359-71.
- 16 Baur X, Dewair M, Fruhmman G, Aschauer H, Pfletschinger J, Braunitzer G. Hypersensitivity to chironomids (non-biting midges). Localization of the antigenic determinants within certain polypeptide sequences of hemoglobins (erythrocruorins) of *Chironomus thummi thummi* (Diptera). J Allergy Clin Immunol 1982; 69:66-76.
- 17 Cranston PS, Tee RD, Credland PF, Kay AB. Chironomid haemoglobins: their detection and role in allergy to midges in the Sudan and elsewhere. Mem Am Ent Soc 1983; 34:71-84.
- 18 Cranston PS. Allergens of non-biting midges (Diptera, Chironomidae)—a systemic survey of chironomid haemoglobins. Med Vet Entomol 1988, in press.
- 19 Hoffman DR. Estimation of serum IgE by an enzyme-linked immunosorbent assay (ELISA). J Allergy Clin Immunol 1973; 51:303-7.
- 20 Negrini AC, Troise C, Voltolini S. ELISA in diagnosis of respiratory allergy. A comparison with RAST and skin tests. Allergy 1985; 40:238-41.
- 21 Eriksson NE, Ahlstedt S. Diagnosis of reaginic allergy with house dust, animal dander and pollen allergens in adult patients. V. A comparison between ELISA, provocation tests, skin tests and RAST. Int Arch Allergy Appl Immunol 1977; 54:88.
- 22 Bamdad S, Goodwin BFJ, Hill JE. IgE antibodies to food allergens detected by ELISA, RAST and monkey PCA. Clin Allergy 1983; 13:89-97.
- 23 Cranston PS, Gad El Rab MO, Tee RD, Kay AB. Immediate-type skin reactivity to extracts of the 'green nimitti' midge, (*Cladotanytarsus lewisi*), and other chironomids in asthmatic subjects in the Sudan and Egypt. An Trop Med Parasitol 1983; 77:527-33.

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