Immediate-type skin reactivity to extracts of the 'green nimitti' midge, \( \text{Cladotanytarsus lewisi} \), and other chironomids in asthmatic subjects in the Sudan and Egypt

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Received 4 May 1982

Skin 'prick' tests with an extract of the 'green nimitti' midge, \( \text{Cladotanytarsus lewisi} \) (Freeman), a potent airborne allergen associated with asthma and rhinitis in the Khartoum area, were undertaken on asthmatic subjects in several localities in the Sudan and Egypt in order to provide further information on the extent of this allergic problem. In the Sudan skin-test-positive individuals were identified in the Khartoum area, Kosti, Sennar, Wad Medani, Shendi and Atbara, and in Egypt at Aswan, Luxor and Qena.

A number of asthmatic individuals giving a positive reaction to \( C. \text{lewisi} \) were also tested with extracts of seven sub-dominant species of Nilotic Chironomidae. There appeared to be a limited degree of cross-reactivity with these species, particularly \( \text{Dicrotendipes fusconotatus} \) (Kieffer), \( \text{Procladius noctivagus} \) (Kieffer) and \( \text{Conchapelopia cygnus} \) (Kieffer). The numbers of \( C. \text{lewisi} \) sensitive subjects giving positive skin-tests to these other species were ten out of 20, 12 out of 28 and 12 out of 29 respectively. In contrast, only five out of 31 reacted to \( \text{Paracladopelma graminicolor} \) (Kieffer), five out of 24 to \( \text{Cladotanytarsus pseudornancus} \) (Goetghebuer), three out of 32 to \( \text{Nanocladius vitellinus} \) (Kieffer) and two out of 28 to \( \text{Cryptochironomus neonilicola} \) (Freeman).

These results indicate that \( \text{Cladotanytarsus lewisi} \) is a major source of chironomid allergen(s) in these asthmatic subjects and that hypersensitivity to \( C. \text{lewisi} \) is probably far more widespread than originally envisaged.

It has been known for many years that Chironomidae, non-biting midges, are abundant in various parts of northern Sudan (Lewis, 1956; Rzozka, 1961; Satti and Abdel Nur, 1974; Cranston et al., 1981). The problem is thought to be associated with the working of dams which produce lake-like conditions when the Nile discharge is falling, so providing optimal breeding conditions. In some areas close to the River Nile the numbers of midges, principally \( \text{Cladotanytarsus lewisi} \) (Freeman), may be so high that physical distress is caused and outdoor activity prevented. The widespread belief that these midges are responsible for...
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Allergic symptoms is supported by observations of Kirk (reported in Lewis, 1956) who showed that a crude extract of the midges produced positive immediate-type skin-tests in Sudanese asthmatics. Kay et al., (1978) demonstrated that human lung fragments could be passively sensitized by anti-C. lewisi IgE for the antigen-induced release of mediators of hypersensitivity. Gad El Rab and Kay (1980) developed a serological radioallergosorbent diagnostic (RAST) test and partially chemically characterized a major allergen (Gad El Rab et al., 1980).

Although skin-test-positive asthmatics have been identified in the Khartoum area (Jebel Auliya, Kalakla and Khartoum) and Wadi Halfa (Gad El Rab and Kay, 1980; Lewis, 1956) the true extent of the problem along the length of the Nile is unknown. In an attempt to get some appreciation of the numbers of affected individuals, skin tests have been performed on asthmatics living in close proximity to the Nile in both Sudan and Egypt. The results of these preliminary investigations are reported herein.

Analysis of the species composition of nuisance swarms of Chironomidae in the Sudan showed that Cladotanytarsus lewisi, termed 'green nimitti', was dominant (Freeman, 1950; Kay et al., 1978). Walker (1963) suggested that further species might be involved in the problem, and continuous monitoring by light-trapping showed that other species of Chironomidae do occur in varying proportions. Antigen was extracted from each of the seven most abundant species of Chironomidae and used to test for cross-reactivity by skin 'prick' testing asthmatics previously shown to be hypersensitive to the dominant species, C. lewisi.

MATERIALS AND METHODS

Species used in Investigation

Chironomidae were collected in a trapping programme using modified Monks Wood light-traps at Kalakla (30 km) and Jebel Auliya (60 km) south of Khartoum on the White Nile (Cranston et al., 1981). The following dominant species of Chironomidae were separated from collections preserved in 70% ethanol:

- Procladius noctivagus (Kieffer) (Tanypodinae)
- Conchapelopia cygnus (Kieffer) (Tanypodinae)
- Nanocladius vitellinus (Kieffer) (Orthocladiinae)
- Dicrotendipes fusconotatus (Kieffer) (Chironominae)
- Paracladopelma graminicolor (Kieffer) (Chironominae)
- Cryptochironomus neonilicola (Freeman) (Chironominae)
- Cladotanytarsus lewisi (Freeman) (Chironominae)
- Cladotanytarsus pseudomancus (Goetghebuer) was found to be the dominant species in light-trap catches made by Dr. P. Mellor (Animal Virus Research Institute, Pirbright) at Wad Medani on the Blue Nile. Specimens of C. pseudomancus were separated from these collections, preserved in 70% ethanol, and used, with the above species, in cross-reactivity studies. Extracts were made from specimens of each species and from both dried and alcohol-stored C. lewisi for studies on the distribution of hypersensitivity.

Preparation of C. lewisi Antigen Extract from Dried Material

Adult midges were collected by light-trap in the Sudan and identified (Cranston) as comprising over 99% C. lewisi. These were dried at 26°C for 48 hours and stored in sealed plastic bags at 4°C until use.

The midges were defatted with three changes of ether over 24 hours and air dried. An extraction was made in ten volumes of Coca’s solution (5 g sodium chloride, 2.75 g sodium bicarbonate and 4 g of phenol made up to 1 litre with distilled water) for 48 hours. The
extract was then centrifuged and filtered through a 0·45 μm Millipore membrane (Millipore Ltd, Bedford, USA), dialysed against six changes of 0·05 M ammonium bicarbonate over 48 hours and freeze dried.

**Preparation of Antigenic Extracts from C. lewisi and Related Species Stored in Ethanol**
The ethanol was decanted off and the specimens defatted in ether for five hours and air dried. Extraction was made directly into ten volumes of 0·05 M ammonium bicarbonate for 72 hours because of the small numbers of insects available. The supernatants were passed through a 0·45 μm Millipore filter and freeze dried.

**Preparation of Skin Test Solutions**
The material used for skin testing was prepared at 1 mg ml⁻¹ in 50% Coca's solution: 50% glycerol and passed through a 0·45 μm Millipore filter into a glass bottle with plastic cap and applicator (Bencard Ltd, Worthing, England). Histamine at 1 mg ml⁻¹ was used as a positive control solution and 50% Coca's solution: 50% glycerol as a negative control.

**Studies on Distribution of Hypersensitivity**
Four hospitals and clinics were visited in the Khartoum area and five elsewhere in the Sudan. These sites were selected for the size of population, proximity to potential nuisance numbers of midges and/or previous unconfirmed reports of midge hypersensitivity. The numbers of centres visited had to be restricted because of logistical problems.

Two clinics were visited in Upper Egypt, and C. lewisi antigen was left with Professor Soliman Diaa El Din (Ain Shams University Hospital, Cairo) for use in skin testing in other parts of Egypt.

At each locality medical staff provided a number of patients with a clinical history of bronchial asthma. These patients were skin-prick tested with extracts of dried C. lewisi antigen, histamine and the negative control. A positive response was taken as a wheal size, at 15 minutes, of at least 2 × 2 mm greater than the control solution.

**Cross-Reactivity Studies**
At several localities, but principally at Kalakla and Shaggara (south of Khartoum on the White Nile), patients who gave a positive reaction to extracts of dried C. lewisi, and a zero response to the negative control, were selected for cross-reactivity tests using the seven sub-dominant species. Since the related species of Chironomidae had been stored initially in alcohol, an extract was also made of C. lewisi similarly stored in alcohol, to allow direct comparison of patient's skin test reactivity.

**RESULTS**

**Distribution of Hypersensitivity to C. lewisi**
The results of the skin-prick tests undertaken at nine clinics in the Sudan, two in Upper Egypt and at Ain Shams University Hospital Allergy Clinic, Cairo, are shown in Table 1. The distributional information is summarized in Fig. 1.

Hypersensitive individuals were identified as far south as Kosti on the White Nile, as far east as Sennar on the Blue Nile and as far north as Qena in Upper Egypt. None of the 25 asthmatics tested living in middle and Lower Egypt showed sensitivity to C. lewisi.

The additional localities shown in Fig. 1 related to the domicile of skin test positive asthmatics tested at clinics some distance from their homes. The open circles on the map
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refer to localities where there is strong circumstantial evidence of hypersensitivity, but where logistical problems prevented confirmation by skin testing.

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number and proportion of asthmatics giving positive skin-prick tests (wheal size—2 × 2 mm) with C. lewisi antigen in Sudanese and Egyptian clinics</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. tested</th>
<th>No. positive</th>
<th>% positive</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SUDAN</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Khartoum area</td>
<td></td>
<td></td>
</tr>
<tr>
<td>University Clinic</td>
<td>30</td>
<td>28</td>
</tr>
<tr>
<td>Kalakla</td>
<td>81</td>
<td>74</td>
</tr>
<tr>
<td>Jebel Auliya</td>
<td>41</td>
<td>38</td>
</tr>
<tr>
<td>Shaggara</td>
<td>40</td>
<td>37</td>
</tr>
<tr>
<td>Kosti (White Nile)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Sennar (Blue Nile)</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Wad Medani (Blue Nile)</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Shendi (Nile)</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>Atbara (Nile)</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td><strong>EGYPT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aswan (Nile)</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>Luxor (Nile)</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>*Upper Egypt (between Qena and Aswan)</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>*Middle &amp; Lower Egypt</td>
<td>25</td>
<td>0</td>
</tr>
</tbody>
</table>

* Tested at Ain Shams University Hospital asthma clinic by Professor Soliman Diaa El Din.

Cross-Reactivity

The results of the skin tests using extracts from seven sub-dominant species of Chironomidae are shown in Fig. 2 and Table 2. At least some individuals gave a positive result with each extract, but in no case was a wheal size greater than 4 × 3 mm observed.

The possibility of antigen(s) of C. lewisi contaminating the sub-dominant species collected and stored together in alcohol is considered unlikely because: the alcohol supernatant gave a negative skin test on a known C. lewisi hypersensitive patient and there was no residue in the freeze-dried supernatant. Additional evidence comes from the degree of variability in individual responses to extracts of sub-dominant species with several instances of negative skin tests for each extract in patients highly sensitive to C. lewisi antigen.

These results show that there is a limited cross-reactivity with the sub-dominant species, greatest for the more abundant species D. fusconotatus, P. noctivagus and C. cygnus, though the wheal was never as large as that produced by C. lewisi.

DISCUSSION

Previous reports have suggested that hypersensitivity to Chironomidae may be found in a number of Nilotic districts in the Sudan (Gad El Rab and Kay, 1980). By skin-prick tests with allergen extracted from Cladotanytarsus lewisi (Freeman) (the dominant species in nuisance swarms), we were able to confirm that there are hypersensitive individuals in communities on the Blue Nile north from Sennar, on the White Nile north from Kosti and on the main Nile as far north as Qena (Fig. 1, Table 1). Although the sensitivity of some
individuals may not have arisen in the area in which they were tested, we believe that these results indicate that the problem of hypersensitivity exists in a much larger area than was previously suspected.

![Map showing areas of the Nile basin where chironomid hypersensitivity is confirmed or suspected.](image)

**Fig. 1.** A map showing areas of the Nile basin where chironomid hypersensitivity is confirmed or suspected.

**TABLE 2**

Cross-reactivity. Number and percentage of Cladotanytarsus lewisi-hypersensitive asthmatics giving positive skin-prick tests with sub-dominant species of Nilotic Chironomidae

<table>
<thead>
<tr>
<th>Species of Chironomidae tested</th>
<th>No. of patients tested</th>
<th>No. skin test positive</th>
<th>% skin test positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dicrotendipes fusconotatus</td>
<td>20</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>Procladius noctivagus</td>
<td>28</td>
<td>12</td>
<td>42</td>
</tr>
<tr>
<td>Conchapelopia cygnus</td>
<td>29</td>
<td>12</td>
<td>41</td>
</tr>
<tr>
<td>Cladotanytarsus pseudomancus</td>
<td>24</td>
<td>5</td>
<td>21</td>
</tr>
<tr>
<td>Paracladopelma grammicoscola</td>
<td>31</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>Narwcladius vitellinus</td>
<td>32</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>Cryptochironomus neonicola</td>
<td>28</td>
<td>2</td>
<td>7</td>
</tr>
</tbody>
</table>
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For reasons of time, and local organizational difficulties, we limited the present study to asthmatics rather than extending the tests to subjects with rhinitis or to normal, symptomless individuals who might have become sensitized to chironomid allergens. It was clear that the medical attendants at the various clinics could more readily recruit known asthmatics than rhinitis sufferers, and that a study of normal individuals would have resulted in too few numbers for meaningful conclusions to be drawn. It should be emphasized that the diagnosis of bronchial asthma was made solely on the clinical history and therefore, in the patients studied we cannot completely exclude (a) other causes of ‘wheeze’, (b) so-called ‘intrinsic’ (or non-allergic) asthmatics and (c) the effect of medication on skin test reactivity. The latter variable was controlled, to a certain extent, by the exclusion of individuals who failed to give at least a 2 x 2 mm wheal when skin-prick tested with histamine at 1 mg ml⁻¹.

Massive emergences of midges have been observed in the Khartoum area each winter and are reported from Wadi Halfa (Lewis, 1956), Lake Nasser (Satti and Abdel Nur, 1974) and Sennar (Gad El Rab, personal communication). Only in Khartoum and the surrounding area has the dominance of *C. lewisi* been confirmed, and the evidence for the scale and species composition of nuisance midge swarms in other localities visited is confused and sometimes contradictory. Some doctors and patients in every town visited believed that midges (known as ‘nimitti’ or ‘hamoosh’) caused problems, but in common parlance, however, it is not possible to separate biting blackflies (Simuliidae) from the non-biting Chironomidae.

Massive emergence of nematocerous flies (referred to as ‘lake-flies’) occur on the Ugandan shores of Lake Victoria, and show lunar periodicity (Corbet, 1958; Macdonald, 1953, 1956). These flies may be implicated in allergic reactions (Gillett, 1978 and personal communication) but since these swarms contain several species of Chironomidae as well as Chaoboridae, the cause of any allergy is uncertain. The finding of a single individual hypersensitive to *C. lewisi* extract in Kisumu, on the Kenyan shore of Lake Victoria, together with a report of *C. lewisi* from Ugandan Lake Victoria (Mclachlan in litt. to Hutson), indicates that further investigation is warranted.
In Egypt Chironomid midges are not reported as causing nuisance swarms in areas close to the Nile, but may cause problems in irrigated fields and ditches. Observations on the distribution of adult Chironomidae in the Nile Valley (Cranston, in preparation) show that C. lewisi is present in Kosti, Wad Medani, Shendi, Atbara and Umbenane (near Singa, Blue Nile) in the Sudan, and is recorded for the first time in Egypt, from Luxor. The results of the cross-reactivity studies show clearly that C. lewisi antigen extract provokes the largest response, but that three further species elicit a weaker, but positive response (Table 2, Fig. 2). These species, Dicrotendipes fusconotatus, Procladius noctivagus and Conchapelopia cygnus, are amongst the most frequent of the sub-dominant species in many parts of the Nile. Other, scarcer, sub-dominant species provoke a weak positive response in a few patients. However, none of the extracts of sub-dominant species provoked a wheal size greater than 4 × 3 mm, while over 60% of patients tested with alcohol-stored C. lewisi extracts produced a wheal of at least this size. Whether the cross-reactivity is associated with the major allergen(s) described by Gad El Rab et al. (1980), or other, as yet unidentified components, requires further investigation.

ACKNOWLEDGEMENTS. We are grateful to Professor Hashim H. Erwa, University of Khartoum, for encouraging this work and allowing access to facilities. The skilled technical help of Mr. Abdel Moneim is acknowledged as are the numerous medical personnel who assisted P.S.C. with travel and work in the Sudan and Egypt. We also wish to thank Dr. P. Mellor (Animal Virus Research Institute, Pirbright, UK) for some light-trap catches; Dr. M. T. Gillies (University of Sussex, UK) for collections made on Lake Nasser and Dr. A. G. Hildrew for hospitality in Kenya and organization of the visit to Lake Victoria.

The authors are grateful to Professor Soliman Diaa El Din, Ain Shams University, Cairo, Egypt, for permitting the inclusion of his skin test results in this paper. The details of this preliminary clinical study in Egypt will be published elsewhere.

This work was supported by the Wellcome Trust.

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