

# Occupational asthma caused by champignon flies

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**Key words:** Diptera; fly hypersensitivity; IgE immunoblotting; mushroom worker; occupational asthma; Phoridae.

**Background:** Occupational bronchial asthma in mushroom (champignon) workers is unusual, although reports on it appeared in 1938 and 1951; we have not found any others since those dates. Here we report the case of a 52-year-old man who works as a champignon cultivator. He suffered rhinoconjunctivitis and asthma attacks whenever he entered the champignon culture caves. We studied flies as a possible antigen source. We collected these insects from the growing sites in order to identify them, and then prepare an extract; the samples turned out to be of two families of insects of the order Diptera, 98% from the Phoridae family (Brachycera suborder) and 2% from the Sciaridae (Nematocera suborder).

**Methods:** Skin prick tests, conjunctival provocation tests, serum specific IgE, specific IgE-binding fractions in immunoblotting, and monitoring of PEFr (at work and off work) were performed.

**Results:** IgE-mediated hypersensitivity to these flies was demonstrated by skin prick test, conjunctival provocation test, serum specific IgE, and IgE-binding fractions in immunoblotting. Monitoring of PEFr both at work and off work showed a clear relationship between symptoms, or fall in PEFr, and the workplace.

**Conclusions:** We report the case of a patient suffering from asthma and rhinoconjunctivitis caused by hypersensitivity to fly proteins.

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The cultivation of mushrooms in Spain is normally a family business; usually both champignon (*Agaricus bisporus*) and other mushrooms (*Pleurotus ostreatus*) are cultivated by the same worker. Publications on occupational asthma due to *Pleurotus* spores appeared in 1991 (1, 2).

Hypersensitivity pneumonitis in champignon growers has been described since 1959. Occupational bronchial asthma in these workers is unusual; although reports from 1938 and 1951 have been found, no cases have been identified subsequently (3, 4). We knew that small flies often invade champignons, and so we examined them as a possible antigen source after eliminating other causes. Insects have been previously referred to as causative agents in occupational asthma (5, 6).

Here we report the case of a patient suffering from asthma and rhinoconjunctivitis caused by hypersensitivity to champignon fly proteins.

## Case report

For 2 years, a 52-year-old man who had been a mushroom cultivator for 20 years had begun to present with rhinoconjunctivitis and asthma attacks whenever he entered the champignon culture caves. No other systemic or organic symptoms were present. His condition would worsen in the spring and autumn when champignon production was at its height, and would improve during holidays when he was away from his hometown.

Auscultation revealed bilateral inspiratory and expiratory wheezes. Total IgE was 32 IU/ml. Serial forced basal spirometry showed moderate to severe bronchial obstruction ( $FEV_1$  50%), which improved after inhalation of a  $\beta_2$ -agonist. Chest radiography was normal.

## Material and methods

### Extract preparation

We collected the insects mentioned from the culture sites in order to identify them and prepare an extract for tests. Entomologists from the biology department at the Complutense University of Madrid identified the specimens. The sample revealed two families of insects of the Diptera order; 98% were from the Phoridae family (Brachycera suborder) and 2% from the Sciaridae (Nematocera suborder). Samples of these insects were obtained throughout different seasons, but the ratio between these families always remained the same. Since separating the Sciaridae flies from the others was technically impossible, and considering that they represented only 2% of the total, only one extract was prepared.

The collected flies were extracted with PBS (phosphate-buffered saline) at a 10% (w/v) ratio for 24 h at room temperature. After centrifugation, the extract was dialyzed against pure water and stored in aliquots at  $-20^{\circ}\text{C}$  until used. The protein content of the extract as determined by the Lowry method was 0.92 mg/ml.

### *In vivo* tests

#### Skin tests

Skin prick and intradermal tests were performed according to the Subcommittee on Skin Tests of the European Academy of Allergology and Clinical Immunology. Histamine hydrochloride at 10 mg/ml and 0.9% saline solution were used as positive and negative controls, respectively. Tenfold dilutions (from  $10^{-6}$  to  $10^{-1}$ ) of fly extract were prepared for skin testing. A battery of commercially available inhalants, as well as *Pleurotus* spores and *Culex* and red-midge larvae extracts, were also used. *Actinomyces* extract was also used to perform prick and intradermal tests. (Extracts were provided by Abelló ALK and IFIDES A Aristegui.)

#### Conjunctival provocation test

For the conjunctival provocation tests, 10-fold dilutions (from  $10^{-6}$  to  $10^{-1}$ ) of the fly extract were prepared. End-point criteria were the evaluation of two main symptoms, ocular itching and conjunctival redness ( $>50\%$  of the area).

One drop of diluent was placed in the lower conjunctival sac of one eye. After 10 min, the eye was examined. If there was no reaction, one drop of the lowest allergen concentration was placed in the other eye. If there was no reaction within 10 min, the next allergen concentrations were used and the results entered onto a record sheet.

Controls were performed with the fly extract in 10 atopic and 10 nonatopic patients.

#### Monitoring of peak expiratory flow rate

Serial determinations of the patient's peak expiratory flow rate (PEFR) were performed with a Mini-Wright peak flow meter (Clement Clarke International, London, UK). PEFR was measured every 2 h from waking until bedtime, during both a working and a holiday period of the patient. The use of an inhaled  $\beta_2$ -agonist (terbutaline) was recorded daily.

### In vitro tests

We used RAST to determine specific IgE against insect extracts. Paper disks were activated with BrCN and coupled with fly extract, as described by Ceska & Lundqvist (7). Fly disks were incubated overnight with the patient's serum and then, after washing, were incubated with 100 000 cpm of  $^{125}$ I-labeled mouse antihuman IgE mAb (8). *Lolium* disks and four dilutions of a grass-specific serum pool which had been calibrated against the Pharmacia Phadebas RAST system were used as reference.

Specific IgE to mites, pollen, fungus spores, and cat and dog dander was determined by the CAP system (Pharmacia, Uppsala, Sweden) according to the manufacturer's instructions.

### SDS-PAGE and IgE immunoblotting

SDS-PAGE was carried out according to Laemmli (9), using nonreducing conditions and a 15 % polyacrylamide running gel. The fly-extract proteins separated by SDS-PAGE were transferred onto PVDF membranes (0.4 × 5 cm), as described by Towbin et al. (10). After blocking with 5 % BSA in PBS, immunodetection of IgE-binding proteins was achieved by incubating with a 1/5 dilution in BSA/PBS, of patient serum and then with 1/3000 dilution of mouse anti-human IgE mAb HE-2 ascitic fluid. Immunodetection of IgE-binding proteins was achieved by enhanced chemiluminescence according to the manufacturer's instructions (ECL, Amersham, UK). As controls, two blots with extract proteins of red-midge larvae and *Calliphora* fly larvae were also incubated with patient serum, and one blot was incubated with dilution buffer instead of patient serum (negative control).

## Results

### Skin tests

The only positive prick test was the one performed with fly extract at  $10^{-6}$  dilution. The battery of common inhalants, *Pleurotus* spores, *Culex*, red-midge larvae, and *Actinomyces* were negative in both prick and intradermal tests.

### Conjunctival provocation tests

The conjunctival provocation test performed with fly extract was also positive at the  $10^{-6}$  dilution.

The results of the same tests performed in the control group were negative with the fly extract.

### In vitro test

The RAST to fly extract was class 3 (given in Phadebas RAST units). No specific IgE against red-midge larvae, fly larvae (*Calliphora*), or common inhalants was present in the patient's serum.

Two IgE-binding fractions of about 33 and 29 kDa were detected specifically in the champignon fly extract (Fig. 1); no IgE-binding fractions were found in the other extracts (*Calliphora*, red midge).

### Symptoms and monitoring of peak expiratory flow rate

The patient showed rhinoconjunctivitis and asthma symptoms a few minutes after entering the culture caves. The longer he was in the caves, the worse his symptoms became, to the point of being unable to remain inside. Symptomatic treatment was unsatisfactory; indeed, hospitalization was required. He improved only during holidays when he has away from his hometown, but only then to the point of not requiring terbutaline.

Monitoring of PEFR and the patient's terbutaline score when at work and off work are shown in Fig. 2. During the working period, the drop in PEFR reached 56% of baseline values, terbutaline did not afford sufficient restoration of

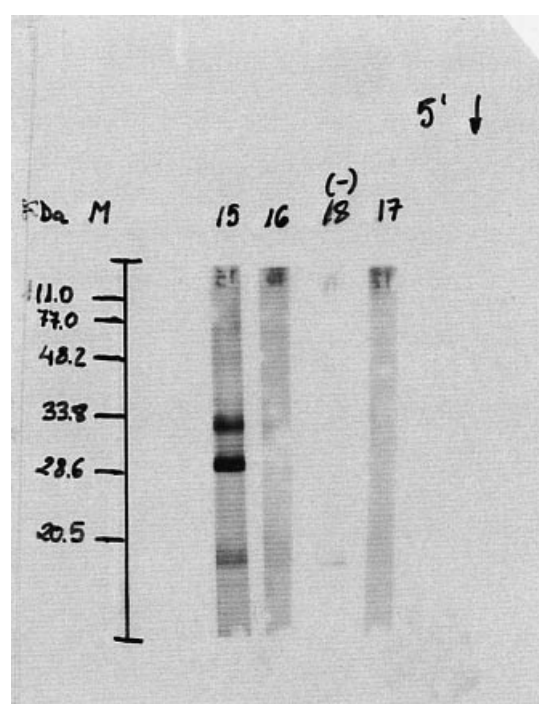


Figure 1. IgE immunoblotting. Lane 15) fly extract (Phoridae); 16) red-midge larvae; 17) fly larvae (*Calliphora*); 18) negative control.

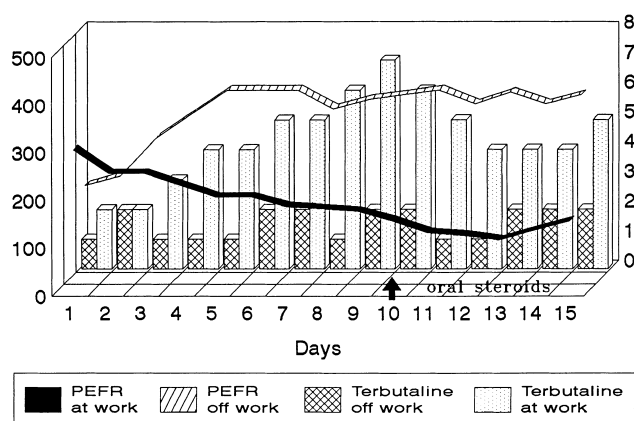


Figure 2. Daily monitoring of peak expiratory flow rate (mean daily values) and terbutaline (number of inhalations) score when off and at work.

PEFR, and on day 10 of work he began to take oral steroids. During the period off work, there was a significant improvement, but he still had to take terbutaline once or twice a day.

## Discussion

Hypersensitivity pneumonitis has been almost the only occupational disease described in champignon cultivators (11, 12). The only two references to occupational asthma in these workers are dated some 50 years ago (3, 4), and no further studies have been published since then. In these reports, flies are considered to be the antigen source (they are termed “champignon flies”), but there was no further biologic identification of them and no *in vitro* specific IgE was performed.

Identification of the causative agent was difficult. First of all, we tested *Thermoactinomyces*, champignon spores (although in Spain champignons are usually picked while still closed), and also *Pleurotus* spores, since the patient sometimes worked with these mushrooms, and we had already described occupational asthma due to *Pleurotus* spores (2). It was our own visit to the cultivation regions, and seeing the vast swarms that invade the champignon growing

areas, that made us think of champignon flies as the possible antigen source.

IgE-mediated hypersensitivity to these flies was proved by skin prick test, conjunctival provocation test, serum specific IgE, and specific IgE-binding fractions in immunoblotting. The monitoring of PEFR at work and off work showed a clear relationship between symptoms, or drop in PEFR, and the workplace.

The numerous culture caves are situated near the villages, and flies have been observed in homes from spring to autumn. This may well explain why our patient did not improve as markedly as was expected when away from his work. Of course, this could have been due to the natural course of such an illness, but it is striking that he did not need terbutaline when absent from the village. The bronchial provocation challenge could not be performed because the FEV<sub>1</sub> never exceeded 70%. The conjunctival test was markedly positive at very low concentrations, and given the results of other tests such as the specific IgE tests and peak flow monitoring, it was decided not to run the risk of a bronchial provocation test for this patient.

The Phoridae family had been described as a cause of only urogenital, pulmonary, and intestinal myiasis (13–16). Adult midges and flies causing environmental allergies have been reported in swampy areas of Japan and India (17–19). Larvae of various insect species are causative agents of type I respiratory allergic disorders in people with contact (20–22).

Occupational asthma and rhinitis due to exposure to various flies has previously been described, and IgE-mediated hypersensitivity has also been proved with cross-reactivity between asthma-inducing flies and other flies or insects (5, 6, 23). In our particular patient's serum, no IgE-binding fractions were detected in the red-midge larvae or *Calliphora* fly extracts. Further study of possible cross-reactivity is clearly necessary.

Flies, midges, and Diptera species in general, are frequently involved in allergic disease. They produce strong allergens, which should always be tested whenever present in the workplace or any other environment.

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