

## Case report

# Specific sensitization to the common housefly (*Musca domestica*) not related to insect panallergy

**Background:** Allergy to houseflies is rare. We report a case of respiratory allergy from occupational exposure to houseflies in a farmer.

**Case report:** A 30-year-old female farmer with a long-standing history of grass pollen allergy observed for 2 years rhino-conjunctivitis and mild asthma when entering livestock stables and barns. Allergy retesting revealed sensitization to various pollens but not to animal danders. Houseflies (*Musca domestica*) occurring on the farm in great quantity were suspected by the farmer herself as the causative agent.

**Results:** Skin prick testing with housefly was positive in the patient and negative in four controls. Experimental radioallergosorbent test was class 3 positive. Sensitization to house dust mite, storage mites and cockroach was not detectable. Western blots with housefly extracts revealed immunoglobulin E (IgE)-binding to bands of 70, 50, and approximately 16 kDa. Tropomyosin in the housefly extract (35 kDa) was recognized by a tropomyosin reference serum but not by the patient. In enzyme-linked immunosorbent assay (ELISA) inhibition assays using housefly as the solid phase, IgE-binding of the patient was inhibited by 75% by *M. domestica* and by 44% by the closely related lesser housefly (*Fannia canicularis*), but not by extracts from blowfly (*Lucilia* spp.), fruit fly (*Drosophila* spp.), horsefly (*Haematopota pluvialis*) and mosquito (*Culex pipiens*). The IgE-binding of the tropomyosin control serum was inhibited by 60–80% by all species.

**Conclusions:** In accordance with previous reports, this case demonstrates that respiratory sensitization to insects may be highly specific. According to ELISA inhibition, cross-sensitization in the present case was restricted to species of the family of true flies (*Muscidae*).

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Respiratory sensitization to insect allergens has been described from many insect orders, including moths and butterflies, caddies flies, beetles, cockroaches, locusts, flies, and many others. Although the clinical relevance of sensitization to these insects often remains uncertain, it may be concluded from these data that all insects are allergenic provided there is adequate contact. This is supported by the fact that occupational exposure to insects in breeding and research establishments is associated with a substantial risk of sensitization in atopics as well as nonatopics (1–8). In beekeepers and their families, respiratory sensitization to honeybee body proteins is not uncommon (9). Also insect larvae used as fishing bait (10) or pet food (5, 11) may cause sensitization.

The Common housefly (*Musca domestica*) has very rarely been described as a cause of inhalant allergy. We report a clinically relevant sensitization to housefly in a female farmer heavily exposed to these insects in livestock stables and her living rooms.

## Case report

A 30-year-old female patient presented with a long-lasting history of hay fever with rhino-conjunctivitis and sporadic cough from May to September. The patient was an occupational farmer and had close contact with cattle kept for dairy farming. For 2 years she noticed spontaneous onset or worsening of ocular itch, rhinitis and breathing difficulties when entering cowsheds, barns and living rooms infested by the Common housefly (*M. domestica*). The patient herself suspected the flies as the cause of her complaints. Foods were well tolerated apart from raw carrots, which led to mild oral symptoms. Serological allergy screening performed before presentation revealed sensitization to grasses and *Compositae* pollens.

## Material and methods

Skin prick testing was performed with common inhalant allergens including pollens, molds, animal danders and house dust mites (all

ALK, Copenhagen, Denmark). Fruits, vegetables and spices were tested by the prick-to-prick method. Radioallergosorbent test (RAST) was performed with selected allergens (UniCAP, Pharmacia-Upjohn, Uppsala, Sweden). Sensitizations detected by skin testing or RAST were confirmed by nasal provocation test. Houseflies were collected in the patient's farm and a crude alcoholic extract (35% ethanol) was prepared for prick testing.

#### Allergen extracts

An aqueous extract from adult *M. domestica* specimens was prepared by homogenizing the animals under liquid nitrogen and extracting proteins at 4°C overnight in phosphate-buffered saline (PBS). After centrifugation, the supernatants were dialyzed and lyophilized. Extracts were prepared after the same protocol also from adult specimens of the closely related Lesser housefly (*Fannia canicularis*), a blowfly (*Lucilia spp.*), a fruitfly (*Drosophila spp.*), a horsefly (*Haematopota pluvialis*), and a mosquito (*Culex pipiens*). Honeybee and *Vespa* venom was purchased from Vespa (Vespa Laboratories, Spring Mills, PA).

#### SDS-PAGE

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was carried out with 12% homogenous gels with 5% stacking gels at 25 mA and constant voltage. Lyophilized proteins were resuspended in distilled water to give a final protein concentration of 15 µg per lane, and separated without further denaturation and reduction.

#### Western blotting

Proteins were blotted onto nitrocellulose in a buffer system following Towbin et al. (12). After transfer, sheets were cut into strips of 3 mm and blocked with bovine serum albumin (BSA) in buffer (50 mmol/l sodium phosphate, 0.5% (v/v) Tween 20, 0.5% (w/v) BSA and 0.05% (w/v) sodium azide, giving a final pH of 7.5) for 30 min. Each strip was incubated overnight with 1 ml of serum (diluted 1 : 10) at 4°C under continuous shaking, washed twice in buffer for 30 min and incubated overnight at room temperature with <sup>125</sup>I-labeled rabbit antihuman IgE (dilution 1 : 10, Kallestad, Germany).

Serum from a nonatopic healthy subject and a patient with grass pollen allergy served as negative controls. Additionally, the serum from a patient with house dust mite allergy and a history of food allergy to shrimp (RAST class 3) was tested. This patient was assumed to be sensitive to the panallergen tropomyosin.

#### ELISA inhibition

Enzyme-linked immunosorbent assay plates were coated with 10 µg per well of *M. domestica* extract, and blocked with BSA for 1 h. Patients' sera (final dilution 1 : 5) were preincubated with the inhibitor solutions overnight at 4°C at an inhibitor concentration of 100 µg/ml. Bound IgE was detected by alkaline phosphatase-labeled antihuman IgE (1 : 1000; PharMingen, San Diego, CA) at 405 nm using a Dynatech MR7000 (Dynatech Laboratories, Alexandria, VA) plate reader.

## Results

Skin prick testing with standard allergens was positive to grass pollens and negative to all other allergens. Of the

tested foods only carrot was positive. Radioallergosorbent test was positive to grass pollens (class 2), weakly positive to mugwort pollen (class 1), and negative to animal danders, *Blattella germanica*, *Dermatophagoides pteronyssinus*, and the storage mites *Acarus siro* and *Lepidoglyphus destructor*. The total IgE was 358 kU/l. Upon rhinomanometry, the patient reacted with a 51% fall in nasal flow after provocation with grass pollen, and with a 23% fall after mugwort pollen.

Skin prick testing with the crude *M. domestica* extract in the patient gave a 3-mm weal with surrounding erythema of 15 mm diameter, and was negative in four controls. Experimental CAP to housefly (Pharmacia-Upjohn) was class 3 positive.

Western blots with the patient's serum revealed bands of 70, 50, and approximately 16 kDa (Fig. 1, lane 4). A 35-kDa band (presumably tropomyosin) was recognized by the tropomyosin reference serum but not by the patient's serum. A band around 75 kDa detected by all sera as well as the buffer control was considered nonspecific. Western blots with honeybee and *Vespa* venom in the patient were negative.

Enzyme-linked immunosorbent assay IgE-binding to *M. domestica* was inhibited in the patient by 75% by *M. domestica*, and by 44% by *Fannia canicularis*. No inhibition was obtained with the other insects. In contrast, binding of the tropomyosin control serum was inhibited by 60–80% by all insects (Fig. 2).

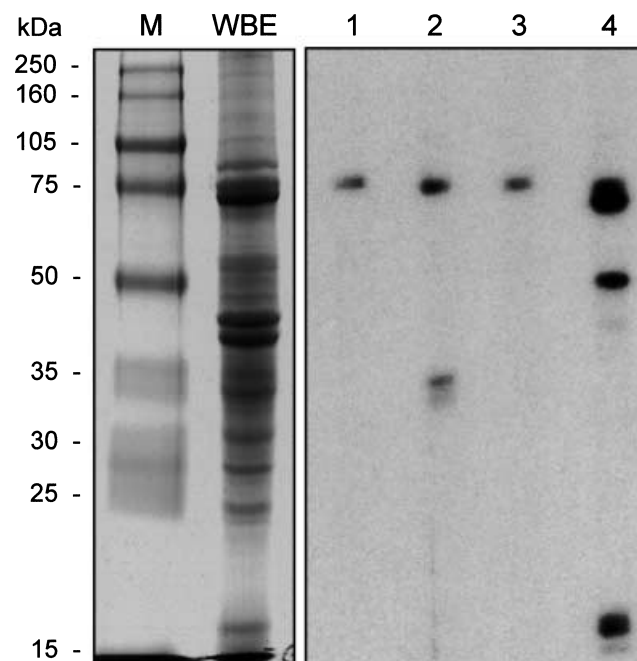


Figure 1. Commassie blue staining of Common house fly (*Musca domestica*) whole body extracts (WBE) and IgE-binding to blotted allergens. Lane 1: nonatopic control, lane 2: tropomyosin reference serum, lane 3: atopic control with grass pollen allergy, lane 4: patient, M: marker proteins.

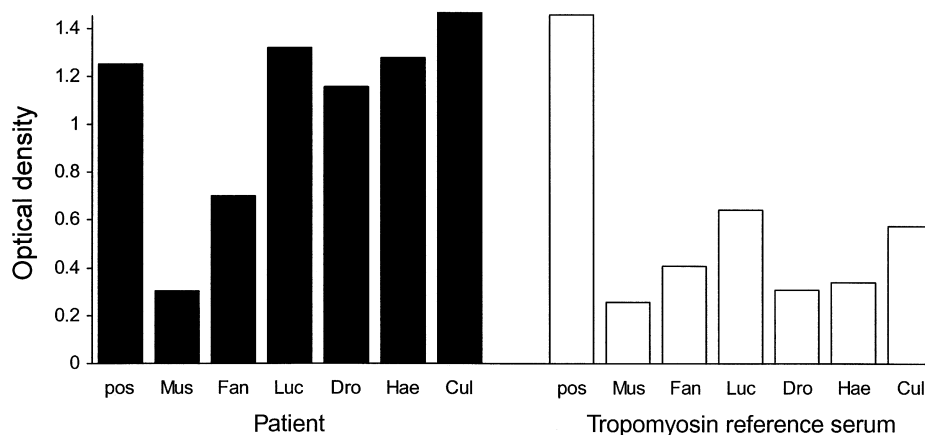


Figure 2. Enzyme-linked immunosorbent assay inhibition of IgE-binding to *Musca domestica* allergens in the patient (left side) and a tropomyosin reference serum (right side). Sera were preincubated with 100 µg/ml of extracts from six different Diptera species. pos: noninhibited positive control, *Mus*: *Musca domestica* (Common house fly), *Fan*: *Fannia canicularis* (Lesser house fly), *Luc*: *Lucilia* spp. (blowfly), *Dro*: *Drosophila* spp. (fruit fly), *Hae*: *Haematopota pluvialis* (horsefly), *Cul*: *Culex pipiens* (mosquito).

## Discussion

Despite the worldwide distribution of houseflies, reports about clinical allergy to these insects are exceptionally rare. Sensitization to the closely related blowflies (*Calliphoridae*) has been described more frequently, mostly from occupational exposure (1, 8) or from contact with fly larvae in hobby fishermen (10). The first case of housefly allergy was described by Jamieson 60 years ago (13). More recently, another two reports about a worker occupationally exposed to houseflies in a fly-rearing unit and a farmer developing symptoms when entering barns and stables, were published (14, 15). As in our patient, nasal discharge and ocular itch were the main symptoms in these patients.

There is little data on the prevalence of sensitization to housefly. Studies having considered fly extracts in screening programs found prevalences up to 30% (16, 17). The clinical significance of these findings remains uncertain because many of the identified subjects reacted with more than one insect species and also with dust mites, crustaceans and spiders, implying extensive cross-sensitization (18–23). There is good evidence now that panallergens like tropomyosin are responsible for this multiple reactivity (22, 24), arguing for a concept of ‘insect panallergy’ (25).

However, insect panallergy is opposed by a number of reports similar to the present one suggesting that, in patients with manifest insect allergy, sensitization may be more specific. Sensitization to house dust mite was absent in our patient and in the previously described cases of housefly allergy (14, 15). Such lack of cross-sensitization is also evident from several other studies concerning locusts (2, 7), mealworms (5), crickets (3), fruit flies (6), coffin flies (*Phoridae*) (26), lady beetles (27), and others.

Galindo et al. (28) observed that, among subjects sensitized to *Chironomus thummi*, those actually exposed to the midges had IgE against the unique *Chironomus* allergen Chi t 1, whereas those not exposed had IgE against tropomyosin or other arthropod panallergens. To date, little is known about the diversity and the nature of the insect allergens bound by the IgE from specifically exposed patients and about the taxonomic level where cross-reactivity will be found. In the present study, sensitization was confined to the family level of ‘true flies’ (*Muscidae*) without cross-sensitization to the closely related blowflies (*Calliphoridae*) or other more distant species.

The nature of the housefly allergens identified by our patient’s IgE in the Western blots is unknown. Some *M. domestica* skin test-positive patients investigated by Martinez et al. (22) reacted with similar proteins of 17, 20, 49, and 70 kDa, in addition to tropomyosin. Baldo et al. (8) observed IgE-binding to comparable proteins in *M. vestutissima* extracts in patients sensitized to the blowfly *Lucilia cuprina*. However, at the time being there is no other data except protein size to speculate on the homology between these allergens and those detected by us. We wondered if the 16 kDa allergen bound by our patient’s IgE corresponds to allergens of similar size, possibly erythrocyruorin-like proteins related to *Chironomus* hemoglobins, that have been described as cross-reactive components in oyster, shrimp, caddis fly, and hymenoptera venoms (29). Our patient, however, did not react with blotted hymenoptera venoms.

In summary, we describe a rare case of respiratory allergy from housefly not related to tropomyosin sensitization and with limited cross-reactivity with other Diptera species.

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