

Short Report

The occurrence of mite-containing wheat flour

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Anaphylaxis after eating mite-infested wheat flour has been recently reported. This paper is to describe two cases and examine the occurrence of mite contamination in wheat flour in Japan. Packages of wheat flour from retail outlets and homes were examined microscopically for the presence of mites. Three of 176 packages from retail outlets and seven of 127 from homes were infested with mites, and it seems likely that the mite contamination takes place in most cases at homes after the packages have been opened. No mites were found in packages stored in a refrigerator, therefore, mite-sensitive patients must be advised to store wheat flour products in a refrigerator.

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Since 1993, a systemic allergic reaction because of the ingestion of mite-contaminated wheat flour foods has been reported, including four cases who visited our clinic (1–5). We recently observed two more cases, suggesting that we are perhaps dealing with a medical problem that may occur more frequently than initially supposed. The occurrence and magnitude of mite contamination of processed flour foods have not been extensively studied, therefore, this study was undertaken to examine a wide range of wheat flour foods from factories, food retail outlets and homes for the presence of mites.

Case report

A 13-yr-old boy experienced a sore throat and chest tightness almost 1 min after eating homemade hotcakes, followed by a stuffy throat and paleness of the face. Physical examination in the ambulance 15 min later revealed wheezing in all lung fields, skin flushing and streaming eyes. He had not suffered from any allergic disorder before this episode.

A 7-yr-old girl experienced coughing, wheezing, streaming eyes and skin flushing 1 h after

eating homemade 'tako-yaki'. Fifteen months later, she again noticed sneezing, coughing, wheezing, an itchy body and streaming eyes immediately after eating homemade buns. She has allergic rhinitis with dust mite sensitivity.

Both patients have eaten stored but already opened flour-containing foods several times, but no adverse reaction has been observed. The offending flour products had been purchased previously, opened once, and then had remained uncooked in a cupboard until the day of the reaction.

Skin prick tests (SPT) with a usual panel of food extracts (Torii, Tokyo, Japan) were performed. Dilutions (1:20, w/v) of the remaining mixes and unopened commercial mixes from both patients were also examined. Positive SPT reactions were observed in the both patients with the remaining mixes. The SPT reactions of both patients to individual ingredients of the hotcakes or 'tako-yaki', and the unopened commercial mixes were negative, and no adverse reaction was observed in oral provocation tests with the unopened mixes, using exactly the same amounts and methods as in the case of the anaphylactic episodes, respectively. Oral provocation tests

with the remaining mix were not conducted, since the anaphylactic reaction observed in the boy patient was life-threatening. Approximately 320 mites were found in 100 mg of the boy patient's hotcake. The mites were identified as *Dermatophagoides farinae*. Unfortunately, the remaining 'tako-yaki' mix from the girl patient was all used for the skin test. The result with the CAP FEIA system (Pharmacia Diagnostics, Uppsala, Sweden) in the sera from both patients was class 6 for *D. farinae*.

Materials and methods

Sample preparations

The offending flour packages had been manufactured in factory A in Tokyo for the boy patient and the factory B in Kumamoto for the girl patient. The wheat flour in the mills is submitted to impact treatment in order to destroy any live insects or arthropods and their eggs, and to aspiration for the exclusion of mature insects or arthropods from the line. Dust samples were collected around the warehouse, flour combination room, sifter, aspirator, tanks for drying and refrigeration, blizzard room, lights, air-conditioner, dusting equipment, mops, employee's resting room, office, and dressing room at the factory A and the factory B. Flour samples were also obtained from the factories, including wheat flour, dried bonito, granulated sugar, glucose, shortening, salt, expanders, skim milk, and coloring agents. These samples were obtained in May 2001 from factory A and in October 2001 from factory B.

A total of 176 wheat flour packages (97 hotcake, 55 okonomi-yaki, 13 steamed bread, and 11 others) were obtained in September 2001 from food retail outlets. All the flour foods were packed by factory B during the period September 2000 through August 2001. In addition, mite contamination of randomly selected flour packages was studied in 127 unrelated urban families in January 2001. Each home donated one flour package, which had been already opened there. All samples (47 wheat flour, 41 tempura-powder, 19 okonomi-yaki, 11 kara-age powder, and nine others) had been packed by factory A during the period February 1995 through December 2000, opened once, and then had remained uncooked for periods ranging from 2 to 13 wk in the homes. About 114 packages had been kept at room temperature (74 in a cupboard, 32 under a sink, two in a basket, one above a sink, one in a cardboard, one on a kitchen shelf, one in a container under the floor, one in a bow window, one beside a cupboard), and

13 had been stored in a refrigerator. One hundred packages had been kept sealed and/or folded with or without rubber bands or a clip. Twenty-six packages had been kept in a firmly sealed container or a plastic bag. One package had been stored opened in a cupboard.

Mite examinations

The mites were analyzed by flotation technique. In brief, the dust and flour samples were mixed with a saturated salt solution, followed by separation by centrifugation. After aspiration onto filter paper, the mites were examined morphologically and counted under a light microscope. Some samples were cultured at 25°C in a 75% humidified chamber for 4 wk. Mite identification and counting were confirmed by Kokusai Eisei, Tokyo.

Statistical analysis

For statistical analysis, the Fisher direct test was used, and differences with a p-value of 0.05 or less were considered statistically significant.

Results

Mature mites were not found in any of the dust and flour samples from the factories, therefore, the samples were cultured for 4 wk and then screened again for mites. Microscopic examination again revealed no live or dead mites in any of the samples. Mature mites were found in three unopened hotcake packages from food retail outlets. Approximately 63 and three *Aleuroglyphus ovatus* were found in 10 g from two packages, respectively, and three *Tyrophagus putrescentiae* in 10 g of the other package. All the flour foods infested by mites were packed in June 2001 at factory B. Mature mites were found in seven packages (four wheat flour, two tempura-powder and one hotcake) obtained from the homes. The mites in the infested flour foods were counted the numbers, ranging from 1 to 6 per 10 g, respectively. The mites were all identified as *D. farinae*. All the infested packages had been stored in a cupboard at the homes, the occurrence being significantly higher than in any other places at room temperature (7/74 vs. 0/40, $p = 0.044$). No mites were found in the packages stored in a refrigerator, although the occurrence was not statistically different compared with those stored at room temperature. Two of 26 packages kept in a firmly sealed container or a plastic bag were contaminated by mites, therefore, the mite contamination was almost the same as in the case

of no such storage. One package that had been stored opened was contaminated by mites, this occurrence being statistically significant compared to packages stored with the top sealed and/or folded (1/1 vs. 4/100, $p = 0.050$).

Discussion

Four species of mites have been determined so far to cause immunoglobulin E (IgE)-mediated anaphylaxis when ingested, i.e., *D. farinae*, *T. putrescentiae*, *Suidasia pontifica*, and *Thyreophagus*, *Entomophagus* (1–5). In this study, *D. farinae* was found in seven of 127 (5.5%) flour packages that had been previously opened in urban homes in central Japan. *T. putrescentiae* was also detected in one of 176 (0.6%) wheat flour packages obtained from food retail outlets. Although there are no statutory limits as to the number of mites that may be present in food in Japan, the present evidence supports that *D. farinae* and *T. putrescentiae* are a potential food safety issue in our country.

This study clearly demonstrates that mite contamination could have occurred before the flour products were packed. The mite-contaminated flour packages from food retail outlets had been manufactured during June by factory B in Kumamoto. Kumamoto is located in the southern part of Japan. The weather in June is warm and highly humid, and is therefore ideal for mite development.

Since *D. farinae* is known as a species naturally occurring in Japanese homes (6), it seems likely that the initial mite contamination takes place in most cases at home after the

packages have been opened. Two risk factors for mite contamination at homes were confirmed by this study, i.e., (i) storing flour packages in a cupboard and (ii) storing flour packages opened. No mites were found in packages stored in a refrigerator, therefore, we advise mite-sensitive patients to store wheat flour products in a refrigerator.

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