

Detection of snow-crab antigens by air sampling of a snow-crab production plant

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Summary

Background and objective We previously assessed the prevalence of occupational asthma (OA) to snow-crab in production plant workers. We also showed that this type of OA is related to immediate immunological reactivity as demonstrated by skin reactivity and increased specific IgE antibodies. However, we did not show that snow-crab antigens causing immunological reactivity and OA could be found in the air sampling in the plants atmosphere. This was the purpose of the current work.

Material and methods Area air samples worn by workers at four different worksites of a snow-crab producing plant were obtained on PVC filters with an SKC pump run at 1.5 L/min for 2 h. Snow-crab was being boiled and processed during the air sampling periods. Filters were analysed by RAST inhibition in a blind manner (i.e. without knowledge of the worksite where the filter originated).

Results Eluate from one of the four sites (#2) had the highest protein concentration and yielded the highest per cent inhibition of RAST — 13% inhibition with the snow-crab meat, 23% and 28% inhibition with the snow crab water RAST in two separate assays. An eluate taken from a filter at another site (#1) showed borderline reactivity (1% and 10% inhibition in two assays) whereas the two other ones and a control filter were negative. The two filters that contained snow-crab proteins were the ones nearest the boiling process, site #2 being the nearest followed by site #1. It was estimated that a 28% inhibition corresponded to ~8.6 µg of proteins and to ~1.5 µg of allergens on the filter.

Conclusion This study suggests that airborne snow-crab-derived proteins, released during the boiling process, are the cause of immunological reactivity and of OA to snow-crab.

Keywords: occupational asthma, environmental monitoring, aeroallergens, bronchial diseases, occupational exposure

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Introduction

Occupational asthma (OA) with a latency period can be caused by high- and low-molecular weight agents [1]. Seafoods are common causes of OA [2]. We previously demonstrated that snow-crab caused OA in 15% of food processors and that atopy is a predisposing factor [3]. The disease is IgE-mediated and is due to crab proteins present

in the boiling water [4]. Extracts of snow-crab meat and boiling water cause immediate skin reactivity in sensitized workers; moreover, elevated specific IgE levels have been demonstrated in subjects with OA due to snow-crab [4]. However, snow-crab antigens have not been detected in the working environment atmosphere. Wet aerosolization of snow-crab antigens can be suspected in the boiling process.

The purpose of this work is to look for the presence of snow-crab antigens in environmental sampling of a snow-crab processing plant.

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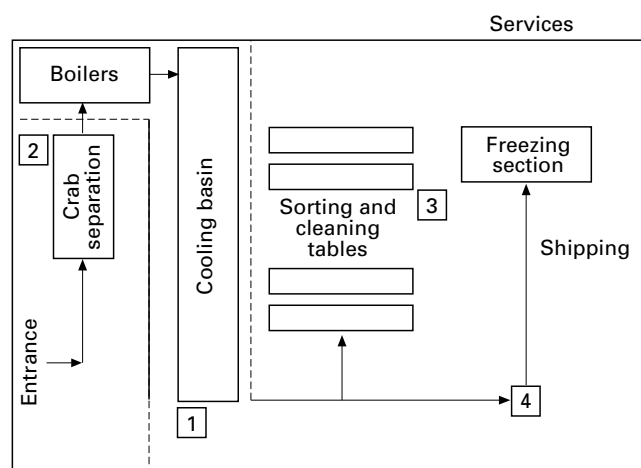


Fig. 1. General scheme of the processing plant. The arrows indicate the direction of snow-crab processing, from first the entrance to crab separation, then boiling followed by cooling in the basin and finally to either the sorting and cleaning tables or directly to the freezing section, before shipping. The area samplers worn by workers are illustrated by boxes and numbered according to the presentation of the results in the text. The dotted lines represent plastic curtains, whereas continuous lines represent walls.

Material and methods

Snow-crab producing plant

The scheme of the snow-crab producing plant that was investigated is shown in Figure 1. After being separated into sections (legs, claws, bodies), the crab was boiled, after which it was sent to a cooling basin. After being sorted and cleaned, snow-crab was frozen and shipped. Air sampling was carried out at four different stations (Fig. 1). Station 2 was the closest to the boilers.

Area sampling

For the area air sampling, an SKC pump run at a constant flow rate of 1.5 L/min for 2 h was used. Filters were type A/E glass fiber filters (SKC Inc., Eighty Four, PA). Samples were obtained from four places in the processing plant (Fig. 1). Filters were coded and analysed blind (without prior knowledge of the sampling site where filters were placed).

Snow crab allergen extracts and immunological testing

Two extracts were prepared of snow-crab. One extract (meat) was obtained from snow crab meat removed from boiled claws and body, homogenized in phosphate-buffered saline, centrifuged, and the supernatant fluid

concentrated by ultrafiltration on an Amicon YM1 filter (molecular weight exclusion 1000) (Amicon Corp., Lexington, Massachusetts). The second extract (water) was prepared from snow crab cooking water collected in the cooking pots in the plant, centrifuged, and the supernatant fluid concentrated (Amicon YM1). Extracts used for skin tests were sterile filtered, dry weight was determined, and concentrations adjusted to 10 mg/ml concentration. These extracts were stabilized with 50% sterile glycerol.

Following sampling, environmental sampling filters were cut into approximately 2 × 3 mm pieces and sonicated (Braunsonic 1510) in 1.5 ml, phosphate buffered saline (0.01 M, pH 7.2) on an ice pack for 30 s/100 watts. The sonicate was centrifuged (20 min, 100 000 × g) and the supernatant stored at −4°C.

For RAST inhibition 50 µl of pooled sera of workers with occupational asthma and high serum specific IgE levels to snow-crab extracts [4], and 50 µL containing sample buffer (negative control), increasing concentrations of snow-crab meat or water extract, or filter eluates, were simultaneously added to RAST discs coated with snow-crab extract in duplicate. The samples were incubated at room temperature overnight; the next day the discs were washed with 0.9% saline, radiolabelled anti-IgE was added (15 000 counts/min), the discs were washed again and the amount of label bound to the discs determined. The reduction in the amount of label binding to discs by snow-crab or sample extracts was expressed as a per cent inhibition. The RAST was performed essentially as described by Ceska and co-workers [5].

We estimated the amount of allergen/protein by per cent inhibition of the RAST in the following fashion. A pool of sera containing IgE antibodies to snow-crab allergens (from sensitized workers) was used in a RAST inhibition using increasing concentrations (0.001, 0.01, 0.1, 1, 10, 100 µg) of snow crab extract to inhibit the binding of IgE antibodies in the sera of sensitized snow-crab workers. At the same time, 50 µL of the filter eluate was also tested for inhibition. With the results, an inhibition curve for snow-crab allergen was plotted; from the inhibition by the filter eluates, we were able to extrapolate the amount of allergen from the linear portion of the curve. The total amount of allergen eluted was then calculated.

Results

The eluates of sampling discs were concentrated and analysed for snow-crab allergen by RAST inhibition, first using discs coated with snow-crab meat or snow-crab boiling water and second using snow crab water only (Table 1). We found a significant inhibition activity with an eluate of filter from site # 2 but not with the other filter eluates from other sites. The test was repeated using greater amounts of

Table 1. Analysis of snow-crab allergens on discs

Inhibitor	First analysis			Second analysis	
	Protein ($\mu\text{g}/50\ \mu\text{l}$)	Snow-crab meat (% inhibition)	Snow-crab water (% inhibition)	Protein ($\mu\text{g}/50\ \mu\text{l}$)	Snow-crab water (% inhibition)
Filter no. 1	0.105	0	1	0.189	10
Filter no. 2	0.49	13	23	0.882	28
Filter no. 3	<0.5	0	0	<0.09	0
Filter no. 4	0.07	0	0	0.126	0
Control filter	0.05	0	3	<0.09	0
Peanut	1.0	0	0	0.9	0
PBS	—	20.5%*	19.3%*	—	8%*

*% of total I^{125} added. Control filter was obtained from sampling in the offices of the production plant.

filter eluates and the results, shown in Table 1, demonstrate that filter from site #2 again gave the greatest inhibition; there was borderline inhibition by filter from site #1. As shown in Figure 1, filter from site # 2 was taken from a working place which was the closest to the boilers, just separated by plastic curtains, and where vapour originated.

It was estimated that the snow-crab allergen on filter no.2 was approximately $1.657\ \mu\text{g}$ allergen ($\sim 8.6\ \mu\text{g}$ protein).

Discussion

This study suggests that the mechanism of sensitization to snow-crab in processing workers occurs through an airborne dispersion of snow-crab antigen during the process of boiling. We indeed showed snow-crab antigens in filters obtained from area samplers worn by workers. We previously showed that sensitization to snow-crab occurs through an IgE-dependent mechanism. The route of sensitization was, however, unknown. We now demonstrate that the antigen is aerosolized. Although it can be suspected that this principally occurs through inhalation — subjects indeed experience nasal and respiratory symptoms — introduction of the antigen through the skin or the gastrointestinal gut cannot be excluded. In a previous study, we showed that one-third of affected workers also reported having digestive symptoms on eating crab [4].

This study also shows that the part of the working place where generation takes place seems to be the boiling section. We did not find detectable levels of antigens in other parts of the building where crab is handled and separated into sections after being cooled. It is not excluded, however, that some crab antigens are also present in this section, albeit not detectable due to lack of sensitivity of our sampling process and/or immunological assay. Our observations therefore warrant being confirmed by repeating the

sampling in the same or another plant. Finally, we did not have a sufficient amount of proteins to allow for identification of the responsible proteins by electrophoresis and immunoblotting. Other sampling would therefore also need to be carried out for this purpose.

This study has practical implications. It has been estimated that there are 21 plants processing snow-crab in which 2000 workers are exposed seasonally (1–2 months per year) on the North shore of the St Lawrence river, Gaspésie and Iles-de-la-Madeleine. The prevalence of occupational asthma was shown to reach as much as 16% in the workforce in a similar snow-crab processing plant at Iles-de-la-Madeleine in the South part of the gulf of St Lawrence [3]. Reduction of airborne antigen should be obtained by enclosing the boiling process and removing any generated aerosol through an extractor system, therefore eliminating exposure of the workforce. Immunological assays carried out on area sampling filters can help in monitoring the efficacy of attempts of reduction in wet aerosol generation which, in their own right, should lead to less likelihood of immunological sensitization and of developing asthma. This has indeed been shown in the case of other protein-derived occupational antigens [6].

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