

ORIGINAL ARTICLE

Work-related allergic respiratory disease and asthma in spice mill workers is associated with inhalant chili pepper and garlic exposures

Anita van der Walt,¹ Tanusha Singh,^{2,3} Roslynn Baatjies,^{1,4}
Andreas Ludwig Lopata,^{5,6} Mohamed Fareed Jeebhay¹

¹Faculty of Health Sciences, Centre for Occupational and Environmental Health Research, School of Public Health and Family Medicine, University of Cape Town, Cape Town, South Africa

²National Health Laboratory Services, National Institute for Occupational Health (NIOH), Johannesburg, South Africa

³Department of Immunology, School of Pathology, University of the Witwatersrand, Johannesburg, South Africa

⁴Department of Environmental and Occupational Studies, Faculty of Applied Sciences, Cape Peninsula University of Technology, Cape Town, South Africa

⁵Faculty of Medicine, Health and Molecular Sciences, School of Pharmacy and Molecular Science, James Cook University, Townsville, Australia

⁶Faculty of Health Sciences, Institute of Infectious Diseases and Molecular Medicine (IIDMM), University of Cape Town, Cape Town, South Africa

Correspondence to

Professor Mohamed F Jeebhay, Faculty of Health Sciences, Centre for Occupational and Environmental Health Research, School of Public Health and Family Medicine, University of Cape Town, Room 4.47, Fourth Level, Falmouth Building, Anzio Road, Observatory, Cape Town 7925, South Africa; Mohamed.Jeebhay@uct.ac.za

Received 6 September 2012

Revised 24 January 2013

Accepted 20 February 2013

ABSTRACT

Objective The aim of the study was to determine the prevalence and risk factors for allergic respiratory disease in spice mill workers.

Methods A cross-sectional study of 150 workers used European Community Respiratory Health Survey questionnaires, Phadiatop, serum specific IgE (garlic, chili pepper), spirometry and fractional exhaled nitric oxide (FeNO). Personal air samples (n=62) collected from eight-hour shifts were analysed for inhalable particulate mass. Novel immunological assays quantified airborne garlic and chili pepper allergen concentrations.

Results Mean dust particulate mass (geometric mean (GM)=2.06 mg/m³), chili pepper (GM=0.44 µg/m³) and garlic allergen (GM=0.24 µg/m³) were highest in blending and were highly correlated. Workers' mean age was 33 years, 71% were men, 46% current smokers and 45% atopic. Spice-dust-related asthma-like symptoms (17%) were common, as was garlic sensitisation (19%), with 13% being monosensitised and 6% cosensitised to chili pepper. Airflow reversibility and FeNO>50 ppb was present in 4% and 8% of workers respectively. Spice-dust-related ocular-nasal (OR 2.40, CI 1.09 to 5.27) and asthma-like (OR 4.15, CI 1.09 to 15.72) symptoms were strongly associated with airborne garlic in the highly exposed (>0.235 µg/m³) workers. Workers monosensitised to garlic were more likely to be exposed to higher airborne chili pepper (>0.92 µg/m³) (OR 11.52, CI 1.17 to 113.11) than garlic allergens (OR 5.08, CI 1.17 to 22.08) in this mill. Probable asthma was also more strongly associated with chili pepper than with garlic sensitisation.

Conclusions Exposure to inhalable spice dust (GM >2.06 mg/m³) containing garlic (GM>0.24 µg/m³) and chili pepper (GM >0.44 µg/m³) allergens increase the risk of allergic respiratory disease and asthma.

BACKGROUND

Spices are derived from taxonomically different species of plants that harbour allergenic potency and can induce mild local to severe systemic reactions.¹ Inhalation is the main route of exposure among workers in the spice trade (farms, factories, groceries).² Workers in the spice-related industry are exposed to a variety of different respiratory sensitisers, and aerosolisation of spice dust has been implicated in inhalant-related allergic manifestations of rhinoconjunctivitis and asthma. Occupational asthma has been reported linked to

What this paper adds

- ▶ Inhalable particulate dust levels as low as 2 mg/m³ are not protective, since airborne concentrations of garlic (>0.24 µg/m³) and chili pepper (>0.44 µg/m³) allergens are associated with an increased risk of probable asthma and obstructive lung disease.
- ▶ Inhalant chili pepper exposure appears to amplify the allergic respiratory response.
- ▶ This paper identifies the need for specific occupational airborne exposure limits (OEL) for spice allergens to protect workers in high-risk exposure settings in the food processing industry.

a range of spices, including cinnamon, paprika, mace, coriander, aniseed, garlic and onion.^{3–6} Irritant reactions in addition to these predominantly allergic responses in workers handling spices have also been reported.⁷ Spices cross-reacting with other spices, certain pollens and/or foods can also result in allergic respiratory disease.² In a previous case series, we identified a 50 kDa cross-reactive allergen from garlic and onion, as well as other allergens of approximately 40 kDa and 52 kDa from chili pepper causing work-related allergy and asthma in spice mill workers.⁸ Furthermore, IgE reactivity to multiple spice allergens, birch and mugwort pollens, as well as a variety of plant profilins, was also demonstrated in the presence of sensitisation to chili pepper in the affected workers who were investigated. Garlic and chili pepper allergens were identified as the main sensitisers in the case series of work-related asthma reported. Epidemiological studies exploring the correlation between workplace exposure inhalant factors (particulates, allergens) and respiratory disease among spice dust-exposed workers are few, suggesting the need for more detailed investigation of this group of workers.⁹

The aim of this study was to determine the prevalence and the risk factors (host and environmental) associated with occupational allergic respiratory disease in spice mill workers using novel exposure assessment approaches for airborne spice allergens (garlic, chili pepper) responsible for the sentinel cases of occupational asthma observed in this mill.

To cite: van der Walt A, Singh T, Baatjies R, et al. *Occup Environ Med* Published Online First: [please include Day Month Year] doi:10.1136/oemed-2012-101163

MATERIALS AND METHODS

Study design and population

A cross-sectional epidemiological study of 150 currently employed spice mill workers was conducted. Ethical approval for the study was obtained from the University of Cape Town (Cape Town, South Africa).

Environmental assessment

Work processes in this mill predominantly involve weighing, blending and packing of various spices. Dry raw materials, such as paprika and chili peppers (*Capsicum annuum*) are separately ground by a milling process. Dried garlic flakes, powder or granules, which are independently manufactured, are essential ingredients of most spice recipe products.

Environmental sampling

Environmental exposure assessment entailed the collection of 62 full-shift (8 h) airborne personal samples on randomly selected individuals employed in various departments of the spice mill using the National Institute for Occupational Safety and Health (NIOSH) occupational exposure sampling strategy manual (top 10% of highly exposed workers in each department with a confidence level of 0.90). A PAS6 sampling head, connected to a Gillian GilAir pump with constant-flow calibrated at 2 L/min, was used to collect samples as an inhalable dust sampling method. Glass fibre filters (Whatman GF/A; pore size 1.0 µm, 25 mm diameter) were used as the filter medium. Field blanks were used for each sampling day. Filters were weighed before and after sampling using a microbalance and acclimatised prior to weighing for 24 h with controlled humidity and temperature to ensure standard weighing conditions. The samples were analysed for inhalable particulate mass and occupational airborne allergen exposure (garlic, chili pepper) using novel immunological ELISA inhibition techniques (using-antigen specific IgE antibodies from sensitised subjects) as described below. None of the samples collected had values below the limit of detection for total inhalable dust particulate.

Immunological analysis

Extracts were prepared from raw garlic and chili pepper obtained from the spice mill. Homogenised garlic and chili pepper were extracted in phosphate buffered saline overnight at 4°C. The spice extracts were then centrifuged to remove large particulate matter. Concentrations of proteins in the spice extracts (garlic and chili pepper) were quantified using the Bio-Rad DC protein assay kit (Bio-Rad, Hercules, USA), and used for the ImmunoCAPs and the standard curve (100; 10; 1; 0.1; 0.01; 0.001) (1 µl/ml). The garlic and chili pepper extracts (1 mg/ml) were individually labelled with Biotin-7-National Health Service (NHS) and coupled to Streptavidin ImmunoCAPs as described by Perborn (2004).¹⁰ One hundred (100) µl of the spice (garlic or chili pepper) standard dilutions and filter extracts (unknown concentrations) were added in microtubes. Buffer was used as a negative control and a concentrated spice extract for the positive control. Pooled allergen-specific positive sera from index cases (100 µl) were added to all tubes and incubated using a shaker for 1 h at room temperature. The criteria for pooled sera included individuals who were sensitised to the respective airborne spice allergen tested (either garlic or chili pepper) which was determined by a positive ImmunoCAP (Phadia, Uppsala, Sweden). The inhibition tests on the pooled serum were conducted using the spice extracts (garlic and chili pepper) obtained from raw material from the spice mill, to

ensure that cross-reactivity between the allergens quantified and the one potentially present could not influence the results. Samples were centrifuged and the supernatant was used. The specific IgE was measured using the biotin-labelled allergen extract coupled to the streptavidin CAP using the ImmunoCAP 100 System (ImmunoCAP 100 System; Phadia, Uppsala, Sweden) (Pharmacia Diagnostics document 300512/1.0). The concentration of airborne allergen was calculated from all samples:

$$\% \text{Inhibition at conc } x = \frac{(\text{Response}_{(\text{blank})} - \text{Response}_{(X)})}{(\text{Response}_{(\text{blank})} - \text{Response}_{(\text{concentrated})})} \times 100$$

The % inhibitions of known allergen concentrations were plotted against the concentration of the diluted standards. A log transformation (logconc) was performed, and these were plotted against the % inhibition. A linear equation was derived (a log transformation (logconc); logconc = $\beta_0 + (\beta_1 \times \% \text{ inhibited})$) and the concentrations of the unknown filter samples extrapolated from the model by substituting the value of the % inhibition in the model.

Health assessment

Questionnaire

Each worker completed an interviewer-administered European Community Respiratory Health Survey questionnaire designed for the investigation of asthma,¹¹ and adapted for the spice work environment. Additional questions relating to current and previous employment and degree of exposure to spice dust were included. Smoking status was classified into the following three categories: never-smoker (lifelong abstinence), ex-smoker (defined as having quit completely >1 month prior to survey) and current smoker. Work-related upper respiratory symptoms due to spice inhalation were defined as work-related ocular-nasal symptoms (sneezy/itchy/runny nose or red/itchy/watery eyes) specifically associated with spice dust exposure in the workplace. Work-related lower respiratory symptoms due to spice inhalation were defined as symptoms of work-related chest tightness or wheeze specifically associated with spice dust inhalation during work.

Immunological tests

Serum analyses for common inhalants (Phadiatop test) as well as specific IgE for garlic (f47) (*Allium sativum*) and chili pepper (f279) (*Capsicum frutescens*) were carried out using the UniCAP assay procedure (ImmunoCAP 100 System; Phadia, Uppsala, Sweden) according to the manufacturer's instructions. An ImmunoCAP value of ≥ 0.35 kU_A per litre was considered positive.

Spirometry

Lung function was measured by spirometry according to ATS/ERS guidelines.¹² A flow-volume Koko Spirometer was used. Reference values of the European Community for Coal and Steel were used for spirometry interpretation, with lower limits corresponding to the 95th percentile.¹³ The spirometry was done before and after inhalation of a short-acting β_2 -agonist salbutamol (400 µg) using a metered dose inhaler. Significant airway reversibility suggestive of asthma was defined as an absolute increase in FEV₁ $\geq 12\%$ and ≥ 200 ml postbronchodilator. Probable obstructive lung disease was defined as FEV₁/Forced vital capacity (FVC) ratio postbronchodilator <5th percentile.¹⁴

Fractional exhaled nitric oxide

Fractional exhaled nitric oxide (FeNO) was determined prior to spirometry using ATS/ERS criteria.¹⁵ A hand-held portable nitric oxide sampling device (NIOX MINO Airway Inflammation Monitor, Aerocrine AB, Solna, Sweden) was used. FeNO measurements were taken for each participant (mean of three efforts) on a Monday during the eight-hour work shift after having confirmed that there was no prior spice dust exposure 48 h before testing. Information on recent chest infections, smoking, alcohol consumption, green vegetable consumption, medication usage, recent exercise and previous spirometry was collected. Tested individuals were required not to smoke tobacco, eat or drink at least 1 h, nor have an upper airway infection within 4 weeks, prior to the test.¹⁶ FeNO > 50 ppb was considered to indicate eosinophilic airway inflammation suggestive of probable underlying asthma.¹⁷

Statistical analysis

Statistical analyses were performed using STATA V.8 computer software (StataCorp, College Station, Texas, USA). Key associations of interest involved investigating relationships between risk factor variables (exposure status, host factor attributes) and health outcomes (respiratory symptoms, immunological status, pulmonary function, FeNO). These associations were explored using bivariate analysis initially, and only those associations commensurable with a priori hypothesis were pursued further with multivariate analyses. Skewed variables, such as exposure metrics and IgE levels, were log transformed (natural log, ln) before model development. Saturated multivariate logistic regression models were developed to examine the relationship between the exposure metrics and clinical endpoints of interest. Exposure metrics used in the models were based on the current exposure categories divided into tertiles based on the exposure assessment measurements for inhalable particulate mass (mg/m³), garlic and chili pepper allergen concentrations (µg/m³). Models were adjusted for age, atopy, smoking status and a history of allergic reactions to spices/spicy foods upon ingestion. Construction of other more refined multivariate models that used composite outcome variables, which included symptoms in addition to pulmonary function and FeNO indices, were not possible due to lack of power issues.

RESULTS

All current permanent (n=139) and casual (n=11) workers in the spice mill were investigated having a mean duration of employment of 6.9 years in the factory and 3.2 years in the current job.

Exposure assessment

The mean dust particulate levels were 2.06 mg/m³, with peak levels 48 mg/m³ (table 1). Mean airborne chili pepper allergen (geometric mean (GM) 0.44 µg/m³, 0.01–73.99) were twice as high as the garlic allergen (GM 0.24 µg/m³, 0.02–43.29). Airborne garlic and chili pepper allergen were up to 10 times higher in the blending/sifting area than other areas. Dust particulate correlated strongly with airborne garlic and chili pepper allergens (r=0.70). Airborne chili pepper and garlic allergen levels were also strongly correlated (r=0.74).

Health outcome assessment

The mean age of this predominantly male (71%) workforce was 33 years, 46% were current smokers, 45% were atopic as determined by Phadiatop, and 35% reported allergic symptoms due to common inhalants (table 2). Work-related ocular-nasal symptoms due to spice dust were more common (43%) than asthma-like (17%) symptoms. Five per cent of workers with asthma symptoms reported chest symptoms related to ingestion of spicy foods.

Sensitisation patterns revealed that among the 150 workers, 19% were sensitised to garlic (G++C+), among whom 13% were monosensitised to garlic (G++), the other 6% were cosensitised to chili pepper (C++G+). All the individuals in the latter group were atopic. Sensitisation patterns also showed that garlic and chili pepper were highly correlated (r=0.91) (figure 1). Between 4% and 8% had evidence supportive of probable asthma based on significant airflow reversibility, or the presence of high FeNO (>50 ppb), respectively (table 2). There were 6% of workers who had evidence of probable obstructive lung disease (FEV₁/FVC ratio postbronchodilator <5th percentile). Host factors were strongly associated with respiratory outcomes (table 3). Atopy was positively correlated with high FeNO. As expected, smoking was negatively associated with high FeNO. Ingestion-related spice allergy was positively associated with work-related asthma-like symptoms (OR 15.37, CI 2.79 to 84.71) due to spice dust and airway obstruction (OR 10.92, CI 1.66 to 71.85).

Table 1 Personal inhalable dust particulate, garlic and chili pepper allergen exposure levels in spice mill workers

Department	n	Particulate mass concentration (mg/m ³)				Garlic allergen concentration (µg/m ³)				Chili pepper allergen concentration (µg/m ³)			
		AM	GM	GSD	Range	AM	GM	GSD	Range	AM	GM	GSD	Range
Blending/sifting	10	11.81	5.78	4.47	0.23–47.64	10.68	3.71	7.91	0.05–43.29	21.33	6.53	10.77	0.13–43.81
Weighing	4	4.87	4.32	1.72	2.49–9.19	2.13	0.76	6.47	0.13–6.01	9.89	7.18	2.80	1.85–18.53
Packing	17	4.53	3.48	2.05	1.37–15.24	3.79	0.32	7.99	0.04–40.02	6.15	0.65	8.79	0.01–73.99
Milling	5	3.91	3.87	1.18	3.22–4.59	0.28	0.19	2.86	0.04–0.65	2.12	0.88	5.06	0.14–6.39
Maintenance (production)	4	1.33	1.03	2.49	0.31–2.36	0.63	0.18	6.41	0.03–2.20	0.09	0.08	2.15	0.03–0.17
Stores	6	1.20	1.06	1.75	0.49–2.22	0.05	0.04	1.50	0.03–0.07	0.19	0.11	3.09	0.02–0.65
Receiving/despatch	7	0.84	0.74	1.70	0.33–1.57	0.05	0.05	1.60	0.03–0.09	0.04	0.03	2.09	0.02–0.13
Food laboratory	6	0.57	0.60	1.91	LOD–1.16	0.09	0.07	2.10	0.03–0.22	0.22	0.11	3.26	0.04–0.84
Administration	3	0.54	0.37	2.82	0.17–1.19	0.03	0.03	1.48	0.02–0.05	0.05	0.04	2.40	0.02–0.10
Overall	62	4.15	2.06	3.33	LOD–47.64	2.97	0.24	8.42	0.02–43.29	5.99	0.44	11.63	0.01–73.99

AM, arithmetic mean; GM, geometric mean; GSD, geometric SD; LOD, limit of detection; n, number of workers.

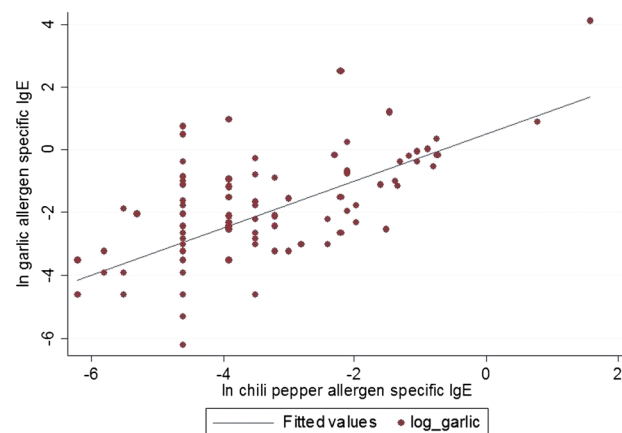
Workplace

Table 2 Demographic and employment characteristics, respiratory symptom experience and pulmonary function indices in spice mill workers

Characteristics	(%)
Demographic and employment characteristics (n=150)	
Age (years)	33.4±7.7
Sex (% male:female)	71 : 29
Smoking status	
Current smokers	69 (46)
Ex-smokers	15 (10)
Non-smokers	66 (44)
Employment status	
Employment duration in current spice mill (years)	6.93±5.6
Employment duration in current job (years)	3.23±3.4
Permanent (vs casual job status)	139 (93)
Past history of allergy (self-reported)	
Hay fever	36 (24)
Asthma	14 (9)
Allergic symptoms related to common inhalants	52 (35)
Respiratory symptom experience (n=150)	
Upper respiratory symptoms	
Work-related ocular-nasal symptoms caused by inhaling spice dust	64 (43)
Lower respiratory symptoms	
Asthma history	
Doctor diagnosed asthma	13 (9)
<17 years	8 (5)
>17 years	6 (4)
Wheezing or whistling in chest in the past year	17 (11)
Woken up with a tight chest in the past year	15 (10)
Work-related asthma-like symptom experience	
Work-related asthma symptoms (tight chest or wheeze)	26 (17)
Work-related asthma symptoms caused by inhaling spice dust	25 (17)
Ever inhaled an excessive amount of dust/vapours/mist	25 (17)
Job change due to work-related chest symptoms	10 (7)
Pulmonary function indices (n=143)	
FEV ₁ (litres)	3.5±0.73
FVC (litres)	4.32±0.89
PEFR (l/s)	8.06±1.70
FEV ₁ % predicted	95.31±13.73
FVC % predicted	100.55±13.78
PEFR % predicted	94.06±14.80
FEV ₁ /FVC	0.80±0.06
FEV ₁ <80% predicted	19 (13)
FEV ₁ reversibility (≥12% and ≥200 ml postbronchodilator)	6 (4)
FeNO >50 ppb	12 (8)
FEV ₁ /FVC ratio postbronchodilator (<5th percentile)	8 (6)

Continuous variables, mean±SD; categorical variables, number (%).
Reference values used European Community for Coal and Steel, 1993.
FeNO, fractional exhaled nitric oxide; PEFR, Peak expiratory flow rate.

In multivariate models, work-related ocular-nasal (OR 2.40, CI 1.09 to 5.27) and asthma-like (OR 4.15, CI 1.09 to 15.72) symptoms due to spice dust were strongly associated with airborne garlic allergen levels when comparing upper versus lower tertiles exposure groups (>0.235 vs <0.066 µg/m³) (table 4). Garlic monosensitisation (G++) was more strongly associated with airborne chili pepper when comparing upper versus lower tertiles (>0.92 vs <0.09 µg/m³) (OR 11.52, CI 1.17 to 113.11) than garlic (OR 5.08, CI 1.17 to 22.08) allergen levels. Garlic monosensitisation (G++) was also strongly associated with

**Figure 1** Correlation between chili pepper IgE (ln chili allergen kU_A per litre) and garlic IgE (ln garlic allergen kU_A per litre) in spice mill workers (n=150). (ln, natural log).

airborne particulate mass when comparing upper versus lower tertiles (>3.78 vs <1.18 mg/m³) (OR 11.89, CI 1.25 to 113.19). Due to the small numbers of subjects in the medium categories, no significant associations were demonstrable when comparing medium versus lower tertiles exposure groups, although the ORs were in the similar direction of effect.

The prevalence of work-related ocular-nasal symptoms was higher among workers sensitised to garlic (G++C+) (9%) than those cosensitised to chili pepper (C++G+) (2%). Garlic monosensitisation (G++) demonstrated a strong association with spice dust-related asthma-like symptoms, even after adjusting for host factors (age, atopy, smoking status and spices/spicy foods reaction) (OR 3.16, CI 0.99 to 10.06). A similar trend was noted among workers sensitised to garlic and chili pepper (G++C+) after adjusting for confounders (OR 3.86, CI 1.29 to 11.47) (data not shown). Individuals sensitised to garlic and chili pepper (G++C+), showed a stronger association with elevated FeNO >50 ppb (OR 5, CI 1.48 to 16.88) than those individuals with garlic monosensitisation (G++). This strong association was further observed for either elevated FeNO >50 ppb or airway reversibility indices in relation to chili pepper sensitisation. A similar pattern was observed for probable obstructive lung disease (FEV₁/FVC ratio postbronchodilator <5th percentile) (OR 4.87, CI 1.13 to 20.90) in individuals sensitised to garlic and chili pepper (G++C+), which was independent of smoking. These associations were even more pronounced in atopic workers. After adjusting for host factors, the strong association observed with elevated FeNO >50 ppb, or probable obstructive lung disease, remained in those cosensitised to chili pepper (C++G+).

DISCUSSION

This is the first detailed epidemiological study of spice mill workers documenting specific exposure to garlic and chili pepper allergens which demonstrates strong dose-response associations with allergic respiratory disease and probable obstructive lung disease.

Sensitisation patterns in our study revealed that a larger proportion of workers were monosensitised to garlic (13%) than those having cosensitisation to chili pepper (6%), since garlic was an essential ingredient in most spice recipes. IgE reactivity to multiple spice allergens associated with acute respiratory symptoms and lung function changes have been previously demonstrated in a group of Croatian spice mill workers.⁷ The

Table 3 Host factors associated with work-related symptoms, lung function and FeNO levels among spice mill workers

Outcome	Age OR (95% CI)	Gender male	Atopy	Smoking	Spices/spicy foods reactions
WRURS reported due to spice	0.98 (0.94 to 1.02)	0.66 (0.32 to 1.33)	0.94 (0.49 to 1.80)	2.31 (1.19 to 4.48)	1.8 (0.40 to 8.5)
WRLRS reported due to spice	1.00 (0.94 to 1.05)	0.56 (0.23 to 1.37)	1.73 (0.73 to 4.11)	2.41 (0.99 to 5.88)	15.37 (2.79 to 84.71)
FeNO >50 ppb	1.02 (0.94 to 1.09)	1.27 (0.33 to 4.92)	16.11 (2.02 to 128.30)	0.09 (0.01 to 0.74)	5.32 (0.91 to 30.96)
FEV ₁ increase ≥12% and ≥200 ml postbronchodilator	0.96 (0.85 to 1.08)	*	0.57 (0.10 to 3.22)	1.11 (0.22 to 5.68)	*
FEV ₁ /FVC ratio postbronchodilator (<5th percentile)	1.09 (1.00 to 1.18)	1.09 (0.21 to 5.65)	2.02 (0.46 to 8.80)	0.65 (0.15 to 2.81)	10.92 (1.66 to 71.85)

*OR not calculable; OR: each OR is a separate unadjusted regression model.

FeNO, fractional exhaled nitric oxide; WRLRS, work-related lower respiratory symptoms; Spice/spicy foods reactions: asthma symptoms associated with ingestion of spices/spicy foods; WRURS, work-related upper respiratory symptoms.

prevalence of sensitisation to chili pepper in Croatian workplaces (13.3%) was higher than that found in the current study (6%). However, while the current study demonstrated the presence of sensitisation using chili pepper-specific IgE, generally regarded as a more specific test, the Croatian study used skin-prick tests that are more sensitive, but less specific for detecting sensitisation to food allergens. In this current study, workers with cosensitisation to chili pepper were more likely to have elevated FeNO >50 ppb suggestive of probable asthma compared with the garlic sensitised group, despite the higher prevalence of sensitisation in the latter

group. This may be related to the relatively higher chili pepper exposures (GM=0.44 µg/m³) compared with garlic (GM=0.24 µg/m³), which may have contributed to the additional non-allergic inflammatory responses.¹⁸

In this study, a sensitive novel assay was developed for quantifying garlic and chili pepper allergens using antibodies of sensitised workers from this mill which enabled a more detailed characterisation of exposures beyond dust particulate. Airborne garlic and chili pepper allergen levels were particularly high in the blending area where bulk spices were manually added into

Table 4 Multivariate logistic regression model of environmental factor associated with respiratory and sensitisation outcome measures

Outcome	Particulate mass concentration (mg/m ³) OR (95% CI)	Chili pepper allergen concentration (µg/m ³) OR (95% CI)	Garlic allergen concentration (µg/m ³) OR (95% CI)
WRURS due to spice			
Med vs low	0.27 (0.07 to 0.99)	0.82 (0.25 to 2.65)	2.28 (0.56 to 9.27)
High vs low	1.29 (0.56 to 2.95)	1.85 (0.73 to 4.67)	2.40 (1.09 to 5.27)
WRLRS due to spice			
Med vs low	0.44 (0.03 to 5.54)	0.31 (0.02 to 3.87)	*
High vs low	3.83 (0.85 to 17.07)	3.18 (0.69 to 14.64)	4.15 (1.09 to 15.72)
Garlic sensitised (G++C+)			
Med vs low	1.24 (0.25 to 6.2)	0.86 (0.17 to 4.31)	*
High vs low	2.21 (0.64 to 7.55)	1.88 (0.53 to 6.61)	1.64 (0.59 to 4.59)
Garlic monosensitisation (G++)			
Med vs low	4.03 (0.28 to 56.74)	3.53 (0.24 to 50.90)	*
High vs low	11.89 (1.25 to 113.19)	11.52 (1.17 to 113.11)	5.08 (1.17 to 22.08)
Chili pepper cosensitisation (C++G+)			
Med vs low	0.62 (0.09 to 4.18)	0.29 (0.04 to 2.11)	*
High vs low	0.34 (0.06 to 2.0)	0.23 (0.04 to 1.38)	0.31 (0.06 to 1.54)
FeNO >50 ppb			
Med vs low	0.19 (0.01 to 2.28)	0.24 (0.03 to 1.91)	0.90 (0.07 to 11.36)
High vs low	0.91 (0.20 to 4.09)	0.72 (0.14 to 3.78)	1.42 (0.32 to 6.32)
FEV ₁ increase ≥12% and ≥200 ml postbronchodilator			
Med vs low	*	*	*
High vs low	*	*	2.66 (0.28 to 24.83)
FEV ₁ /FVC ratio postbronchodilator (<5th percentile)			
Med vs low	6.98 (0.80 to 60.83)	5.09 (0.55 to 46.37)	*
High vs low	0.50 (0.06 to 4.25)	0.47 (0.05 to 4.17)	0.19 (0.03 to 1.08)
WRLRS+garlic Sensitisation			
Med vs low	0.39 (0.03 to 5.34)	0.29 (0.02 to 4.03)	*
High vs low	0.94 (0.18 to 4.74)	0.82 (0.15 to 4.23)	1.08 (0.24 to 4.77)

*OR not calculable; OR: OR derived using low exposure category as reference and was adjusted for age, atopy, smoking status and spices/spicy foods reaction.

Particulate mass concentration (mg/m³) tertiles: high >3.78; medium >1.18 <3.78; low <1.18.

Chili pepper allergen concentration (µg/m³) tertiles: high >0.92; medium >0.09<0.92; low <0.09.

Garlic allergen concentration (µg/m³) tertiles: high >0.235; medium >0.066<0.235; low <0.066.

FeNO, fractional exhaled nitric oxide; WRLRS, work-related lower respiratory symptoms; WRURS, work-related upper respiratory symptoms.

Workplace

mechanical blenders and correlated strongly with particulate dust ($r=0.70$). The highly correlated airborne allergen ($r=0.74$) and allergic sensitisation ($r=0.91$) patterns in workers suggest that most workers were either simultaneously exposed to both allergens during their work and that there exists potential cross-reactivity between these allergens.

This study has demonstrated that spice mill workers are at an increased risk of becoming sensitised to airborne allergens (garlic, chili pepper). While no specific international occupational exposure limits exist for these allergens, the 3 mg/m^3 (total inhalable particulates) proposed by the Control of Substances Hazardous to Health regulations of the Health and Safety Executive in the UK for irritant spice dusts, such as garlic, ground chillies and mustard, is unlikely to protect against IgE sensitisation in spice mill workers.^{19 20} The current study clearly demonstrated that inhalable particulate dust levels of even 2 mg/m^3 (the mean overall exposures observed) are not protective, since adverse respiratory health outcomes were observed even at such low levels.

The findings of this study suggest that both probable asthma and obstructive lung disease were present among this group of spice mill workers. Host-related factors, such as atopy, smoking and possible ingestion-related spice food allergy were strongly associated with the respiratory outcome measures in this study. Atopy was positively associated with sensitisation to occupational allergens (garlic, chili pepper) and with elevated FeNO ($>50 \text{ ppb}$) levels. This is consistent with other studies reporting the risk for high molecular weight (protein) allergen-specific sensitisation and asthma is increased in atopics.²¹ In our study, smokers were also more likely to present with spice dust-related upper (OR 2.31, CI 1.19 to 4.48) and lower (OR 2.41, CI 0.99 to 5.88) respiratory symptoms which were independent of sensitisation. This association between smoking and respiratory symptoms has been observed in other spice workers.²² Interestingly, cumulative smoking exposures have also been associated with a higher cough threshold to capsaicin among workers chronically exposed to hot chili peppers.²³

Evidence of a positive exposure-response relationship was observed for particulate mass and allergen exposures in this group. Workers exposed to relatively higher airborne garlic allergen levels ($>0.235 \text{ } \mu\text{g/m}^3$) were also more likely to have work-related ocular-nasal as well as asthma-like symptoms due to spice dust inhalation. Interestingly, workers were more likely to be monosensitised to garlic in the group exposed to relatively higher levels of airborne chili pepper (>0.92 vs $<0.09 \text{ } \mu\text{g/m}^3$) than garlic (>0.235 vs $<0.066 \text{ } \mu\text{g/m}^3$). Furthermore, even though a higher prevalence of allergic respiratory disease was observed in the garlic (G++C+) sensitised group, chili pepper sensitisation appears to modify the IgE response to garlic in a synergistic manner causing a stronger association between elevated FeNO $>50 \text{ ppb}$ and chili pepper sensitisation which persisted even after adjusting for atopy and other host factors. A similar pattern was observed for probable obstructive lung disease in relation to chili pepper sensitisation which was independent of smoking status.

Studies of lower respiratory symptoms and lung function associated with organic dust exposures in the food processing industry, including spice handling, have consistently demonstrated a higher prevalence of both acute and chronic respiratory symptoms.²⁴ In this current study, 17% of workers reported asthma-like symptoms associated with the inhalation of an excessive amount of spice dust in the past year. However, only up to 8% were found to have elevated FeNO $>50 \text{ ppb}$ suggestive of probable occupational allergic asthma in the presence of

allergic sensitisation. It is likely that the remaining proportion had asthma-like symptoms due to irritant exposures in the mill resulting in increased bronchial hyper-responsiveness. Work-related respiratory symptoms of an irritant nature have been previously reported in epidemiological studies of chili grinders.^{25 26} Lundberg *et al*²⁷ found that neuropeptide substance P (SP) and capsaicin (an active ingredient in chili and paprika) induced a dose-dependent contraction of human segmental bronchi in vitro. In animal studies, different spices (chili pepper, paprika, caraway, coriander leaves, coriander seeds, cinnamon, ginger, onion, curry and parsley) have demonstrated a dose-related contractile response of isolated guinea pig tracheal smooth muscle.⁷ This finding suggests that exposure to spice dust in these factories can result in a highly efficient direct bronchoconstrictor challenge of the respiratory tract, aside from other allergic inflammatory mechanisms.

Aside from chili pepper sensitisation being a major determinant of probable obstructive lung disease and chronic airway inflammation, involvement of non-IgE-mediated mechanisms, such as receptor-mediated activation of nerves and cells in the airways should also be considered. Chili pepper contains several potential allergens, such as profilin,²⁸ osmotin and high concentration of capsaicin.²⁹ The latter protein is a potent and well-characterised stimulator of a calcium activating receptor, Transient Receptor Potential Vanilloid-1 (TRPV1).³⁰ TRPV1 is specifically located in sensory nerve cells, responsible for the typical cough response to inhaled chili dust, as well as bronchoconstriction and mucus hypersecretion.³¹ In addition, TRPV1 has been increasingly implicated in 'neurogenic inflammation' through indirect activation of mast cells, eosinophils and lymphocytes, and is markedly elevated in patients with asthma or airway inflammation.¹⁸ In this current group of workers, activation of these receptors via inhalant exposure to capsaicin in chili pepper, in the presence of the potent allergens from garlic, could enhance the airway hyper-reactivity, and also induce the production of allergen-specific IgE. Since inflammatory mediators are elevated in atopic individuals, this could occur at even lower concentrations of capsaicin for TRPV1 activation.³²

Other immunological and biochemical characteristics of spices could also be involved in promoting allergic sensitisation in spice mill workers. This includes the increased cellular permeability documented for 'hotter' spices, such as chili pepper, which facilitates the transport of molecules less than 70 kDa through the epithelial barrier.^{1 33} This may result in a higher dose of garlic allergen being presented to the immune system increasing the risk of subsequent allergic sensitisation which merits further detailed investigation. Data from other studies also suggest that sensitisation to spices is more complex due to polysensitisation to various plant profilins, as has been observed in the pollen-food syndrome.^{34 35} Furthermore, the highly correlated sensitisation patterns to garlic and chili pepper may also be due to cross-reactive allergens present in chili pepper and garlic ($50\text{--}52 \text{ kDa}$).⁸

In conclusion, this study has demonstrated, using a sensitive novel allergen detection assay for garlic and chili pepper, that spice mill workers exposed to inhalable spice dust particulate (mean $>2 \text{ mg/m}^3$) containing garlic and chili pepper allergen levels of $0.24 \text{ } \mu\text{g/m}^3$ and $0.44 \text{ } \mu\text{g/m}^3$, respectively, have an increased risk of work-related lower respiratory symptoms, allergic sensitisation, probable asthma and obstructive lung disease. It is likely that both immunological (IgE) responses to chili pepper in combination with non-IgE-mediated respiratory mechanisms are responsible for the airway reversibility and enhanced airway inflammatory response.

Acknowledgements We would like to thank Alta Olckers from the Lung Institute (UCT) for conducting the spirometry testing; Natalie Nieuwenhuizen from the IIDMM (UCT) for preparing the spice extracts; Dawn Venter (UCT) for assisting with the questionnaires and fractional exhaled nitric oxide testing; Edith Ratshikhopha (NIOH) for assisting with the immunological analysis. Our appreciation to all the workers, without whose willingness to participate, this study would not have been possible. The Allergy Society of South Africa-Glaxo Smith Kline Research Award, the Medical Research Council of South Africa and the employer who provided financial support for the project.

Contributors AvdW developed the protocol, study instruments and oversaw the fieldwork, and took the lead in writing the article. TS supervised the laboratory analysis of the environmental samples for particulate mass, allergen as well as endotoxin concentrations. TS was also responsible for the analysis of the serum samples for determination of allergen-specific IgE and contributed towards writing the article. ALL is an ARC Future-Fellow. ALL developed the methodological approaches of the immunoassays used for the detection of the allergens in environmental samples together with TS. ALL provided specific input into the interpretation of the immunological results and writing the article. RB was primarily involved in the statistical analysis of the data including the model development and writing of the article. MFJ was the principle investigator and project leader responsible for conceptualising the study design, interpretation of the statistical models and the drafting of this article. He also supervised AvdW's master's dissertation—MPhil Occupational Health.

Competing interests None.

Patient consent Obtained.

Ethics approval Ethics approval was obtained from the Research Ethics Committee, University of Cape Town prior to the study being conducted.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement The remaining data is currently being written up for another article, therefore additional data sharing is not applicable.

REFERENCES

- Schöll I, Jensen-Jarolim E. Allergenic potency of spices. Hot, medium hot, or very hot. *Int Arch Allergy Immunol* 2004;135:247–61.
- Chen JL, Bahna SL. Spice allergy. *Ann Allergy Asthma Immunol* 2011;107:191–9.
- Uragoda CG. Asthma and other symptoms in cinnamon workers. *Brit J Ind Med* 1984;41:224.
- Sastre J, Olmo M, Novalvos A, et al. Occupational asthma due to different spices. *Allergy* 1996;51:117–20.
- Fraj J, Lezaun A, Colás C, et al. Occupational asthma induced by aniseed. *Allergy* 1996;51:337–9.
- Mansoor N, Ramafi G. Onion Allergy—a case report. *Curr Allergy Clin Immunol* 2000;13:14–15.
- Zuskin E, Kanceljak B, Skuric Z, et al. Immunological and respiratory findings in spice-factory workers. *Environ Res* 1988;47:95–108.
- Van der Walt A, Lopata AL, Nieuwenhuizen NE, et al. Work-related allergy and asthma in spice mill workers—the impact of processing dried spices on IgE reactivity patterns. *Int Arch Allergy Immunol* 2010;152:271–8.
- Van der Walt A, Jeebhay MF. Work-related allergy and asthma to inhaled spices—a review. *Curr Allergy Clin Immunol* 2010;23:186–92.
- Perborn H. ImmunoCap Rast-Inhibition protocol. *Pharmacia Diagnostics AB*; 2004-06-03.
- Burney PGJ, Luczynska C, Chinn S, et al. The European Community Respiratory Health Survey. *Eur Respir J* 1994;7:954–60.
- Pellegrino R, Viegi G, Brusasco V, et al. Interpretative strategies for lung function tests. Series "ATS/ERS Task Force: standardisation of lung function testing." Edited by V Brusasco, R Crapo, G Viegi. Number 5 in this Series. *Eur Respir J* 2005;26:948–68.
- Quanjer PH, Tammeling JE, Cotes OF, et al. Lung volumes and forced ventilatory flows. *Eur Respir J* 1993;6(S16):5–40.
- Hansen JE, Sun XG, Wasserman K. Spirometric criteria for airway obstruction: use percentage of FEV₁/FVC ratio below the fifth percentile, not <70%. *Chest* 2007;131:349–55.
- American Thoracic Society/European Respiratory Society. ATS/ERS Recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med* 2005;171:912–30.
- Kharitonov S, Alving K, Barnes PJ. ERS Task Force Report: exhaled and nasal oxide measurements: recommendations. *Eur Respir J* 1997;10:1683–93.
- Dweik RA, Boggs PB, Erzurum SC, et al. American Thoracic Society documents: an official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FeNO) for clinical applications, 2011. *Am J Respir Crit Care Med* 2011;184:602–15.
- Doherty MJ, Mister R, Pearson MG, et al. Capsaicin responsiveness and cough in asthma and chronic obstructive pulmonary disease. *Thorax* 2000;55:643–9.
- ACGIH 2011 TLVs and BEIs. *Threshold limit values for chemical substances and physical agents*. Cincinnati, OH: American Conference of Governmental Industrial Hygienists 2011.
- Health and Safety Executive, HSE 2001. Agriculture and Food Sector - Sector Information Minute 01/2001/51 Version 1: Managing Health Risks Arising from the Use and Handling of Spices and Seasonings - Formerly SIM 05/2001/14. <http://products.ihh.com/ohsis-seo/1015818.html> (accessed 7 Mar 2013).
- Jeebhay MF, Quirce S. Occupational asthma in the developing and industrialised world: a review. *Int J Tuberc Lung Dis* 2007;11:122–33.
- Zuskin E, Schachter EN, Kanceljak B, et al. Organic dust disease of airways. *Int Arch Occup Environ Health* 1993;65:135–40.
- Blanc P, Liu D, Juarez C, et al. Cough in hot pepper workers. *Chest* 1991;99:27–32.
- Zuskin E, Mustajbegović J, Schachter EN, et al. Respiratory function in female workers occupationally exposed to organic dusts in food processing industries. *Acta Med Croatica* 2000;54:183–91.
- Uragoda CG. Symptoms among chilli grinders. *Brit J Indus Med* 1967;24:162.
- Uragoda CG. A comparative study of chilli grinders with paprika splitters. *J Soc Occup Med* 1983;33:145–7.
- Lundberg JM, Martling CR, Saria A. Substance P and capsaicin-induced contraction of human bronchi. *Acta Physiol Scand* 1983;119:49–53.
- Santos A, Van Ree R. Profilins: mimickers of allergy or relevant allergens? *Int Arch Allergy Immunol* 2011;155:191–204.
- Mueller-Seitz E, Hiepler C, Petz M. Chili pepper fruits: content and pattern of capsaicinoids in single fruits of different ages. *J Agric Food Chem* 2008;56:12114–21.
- Lee L-Y, Gu Q. Role of TRPV1 in inflammation-induced airway hypersensitivity. *Curr Opin Pharmacol* 2009;9:243–9.
- Banner KH, Igney F, Poll C. TRP channels: Emerging targets for respiratory disease. *Pharmacol Ther* 2011;130:371–84.
- Chuang HH, Prescott ED, Kong H, et al. Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P₂-mediated inhibition. *Nature* 2001;411:957–62.
- Jensen-Jarolim E, Gajdzik L, Haberl I, et al. Hot spices influence permeability of human intestinal epithelial monolayers. *J Nutr* 1998;128:577–81.
- Jensen-Jarolim E, Leitner A, Hirschwehr R, et al. Characterization of allergens in Apiaceae spices: anise, fennel, coriander and cumin. *Clin Exp Allergy* 1997;27:1299–306.
- Egger M, Mutschlechner S, Wopfner N, et al. Pollen-food syndromes associated with weed pollinosis: an update from the molecular point of view. *Allergy* 2006;61:461–76.



Work-related allergic respiratory disease and asthma in spice mill workers is associated with inhalant chili pepper and garlic exposures

Anita van der Walt, Tanusha Singh, Roslynn Baatjies, et al.

Occup Environ Med published online March 14, 2013

doi: 10.1136/oemed-2012-101163

Updated information and services can be found at:

<http://oem.bmj.com/content/early/2013/03/13/oemed-2012-101163.full.html>

These include:

References

This article cites 32 articles, 6 of which can be accessed free at:

<http://oem.bmj.com/content/early/2013/03/13/oemed-2012-101163.full.html#ref-list-1>

P<P

Published online March 14, 2013 in advance of the print journal.

Email alerting service

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

Advance online articles have been peer reviewed, accepted for publication, edited and typeset, but have not yet appeared in the paper journal. Advance online articles are citable and establish publication priority; they are indexed by PubMed from initial publication. Citations to Advance online articles must include the digital object identifier (DOIs) and date of initial publication.

To request permissions go to:

<http://group.bmj.com/group/rights-licensing/permissions>

To order reprints go to:

<http://journals.bmj.com/cgi/reprintform>

To subscribe to BMJ go to:

<http://group.bmj.com/subscribe/>