Original articles

Clinical and immunologic studies among egg-processing workers with occupational asthma

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Twenty-five workers in an egg-processing factory were evaluated for respiratory sensitization to inhaled egg proteins by a physician evaluation, serial peak expiratory flow rate (PEFR) measurements for a 1-week period, and immunologic tests. Immunologic studies included skin prick tests, serum-specific IgE (RAST), and specific IgG (ELISA) to solutions prepared from commercial food allergens: factory-powdered egg white and yolk products and purified egg white fractions, including ovalbumin, ovomucoid, lysozyme, and conalbumin. Six workers had significant daily PEFR lability (>20%) of whom five had associated cutaneous reactivity to at least one egg allergen. A diagnosis of "definite asthma" was established in five workers suspected by the physician of having asthma. These five workers exhibited significant decrements in daily PEFR that were accompanied by bronchial symptoms. Occupational asthma was diagnosed by the physician in four of the five latter workers. Definite asthma was significantly associated with both cutaneous reactivity to egg allergens ($p < 0.01$) and RAST binding ($p < 0.01$). Of eight workers with cutaneous reactivity to at least one egg reagent, four workers (50%) were positive to only purified egg white fractions. The highest levels of RAST binding were detected in four workers, and the best binding activity was to ovomucoid and ovalbumin fractions. Elevated specific IgG responses were significantly higher in egg-factory workers to whole egg ($p < 0.005$), lysozyme ($p < 0.002$), and conalbumin ($p < 0.002$) allergens compared to responses of nonexposed control subjects. However, no differences in specific IgG were detected between symptomatic and asymptomatic workers. Thus, in four workers, the association of cutaneous reactivity with pulmonary function changes observed at work confirmed the physician-derived diagnosis of occupational asthma caused by inhalation of egg protein.
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Human hypersensitivity to egg proteins is commonly encountered in individuals with food-induced allergic reactions. This group is comprised largely of atopic individuals who develop food sensitivity early in life.[1] Problems associated with ingestion of eggs may include urticaria, atopic dermatitis, gastrointestinal complaints, bronchoconstriction, or generalized anaphylaxis. Immunologic characterization of egg white proteins in food-sensitive subjects have identified ovalbumin and ovomucoid as major allergens.[2-5] Previously, OA has been associated with inhalation of

Abbreviations used
PEFR: Peak expiratory flow rate
PBS: Phosphate-buffered saline
OA: Occupational asthma

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TABLE I. Ranges of daily percent variability in PEFR performed every 3 hours during 1 work week and physician diagnoses in 25 egg workers

Worker No.	Range of* % variability	Physician diagnosis
4	2.1-9.6	OA vs A
7	2-6.1	Irr
8	4.9-12.1	A vs Br
9†	5.6-49.0	OA
10†	10.5-30.9	A vs Irr
12†	18-51.7	OA
13†	11.3-25.4	OA
25†	2-21.2	OA
28	1.7-3.7	A vs Br
30	0-6.4	OA vs Irr
14	6.2-54.5	—
1	5.7-7.4	—
5	1.8-11.1	—
6	2.8-12.8	—
11	2.2-4.4	—
15	0-6.3	—
16	5.7-16.4	—
17	0-6.3	—
18	0-4.8	—
20	0-5.2	—
21	1.6-7.9	—
26	3.2-8	—
27	4.9-9.3	—
29	3.3-9.8	—
31	3.2-8.3	—

A = nonoccupational asthma; Irr = irritant respiratory symptoms; Br = chronic bronchitis; — = no significant respiratory symptoms.

*Ranges of percent variability between daily maximum and minimum PEFRs performed every 3 hours during 1 week.

†"Definite asthma" established by criteria of physician diagnosis of asthma and >20% PEFR variability associated with bronchial symptoms.

egg products in bakers.[6] However, IgE-mediated respiratory sensitization to egg proteins could not be confirmed in the latter group of symptomatic workers. Immunologic studies are presented in this article from a cross-sectional evaluation of egg-processing workers. Inhalation of egg proteins resulted in allergic respiratory sensitization and work-related changes in pulmonary function.

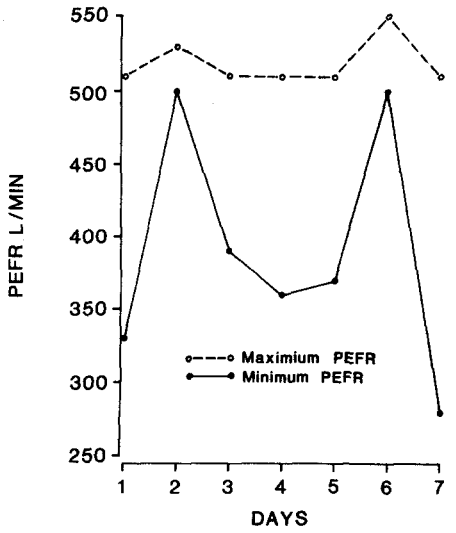
METHODS

Thirty-one employees from a factory that processed raw eggs into powdered egg yolk, powdered whole egg, and liquid egg white were invited to participate in a set of examinations.[7] All participants signed an informed consent statement previously approved by an institutional review board. Not every invitee participated in every procedure, which included a physician's interview and examination (31 participants), skin testing (31 participants), serial determination of PEFR (25 participants), and serologic testing (25 participants, 19 of whom also had PEFR determinations). It was not possible to perform bronchoprovocation testing to egg allergens in this group of workers. The physician examiner who obtained the occupational histories was blinded to the results of all other examinations. OA was diagnosed by the physician if a subject reported increased wheezing, dyspnea, or chest tightness while the subject was at work and a decrease in symptoms away from work, at night, on the weekend, or on vacation. Non-OA was diagnosed if the latter symptoms were reported by workers but without a clear history of exacerbation of symptoms at work or improvement away from the plant. Serial PEFRs were obtained with portable mini-Wright peak flow meters (Airmed, London, England) every 3 hours while the subjects were awake for 7 days. The best result of three maneuvers at each determination was recorded. Participants were requested to keep the peak flow meters at their bedsides and to perform a determination if they were awakened from sleep for any reason. The presence or absence of wheezing, shortness of breath, chest tightness, and cough were recorded on diary cards at the time of each determination. Workers also documented days when they were exposed and days when there was no exposure to egg processing. The difference between the daily maximum and minimum of PEFRs was calculated with a difference of ≥20% during the day consistent with significant nonspecific bronchial hyperresponsiveness.[8, 9]

A worker was classified with "definite asthma" if he or she satisfied the following three criteria: (1) the physician suspected OA or non-OA based on clinical interview and examination, (2) the participant demonstrated >20% variability in PEFR on at least 1 day, and (3) the participant recorded symptoms of wheezing, shortness of breath, or chest tightness at the same time that a significant fall in PEFR was recorded. The classification of "definite asthma" did not establish an occupational cause but simply identified those workers with significant bronchospasm associated with asthmatic symptoms during 1 work week.

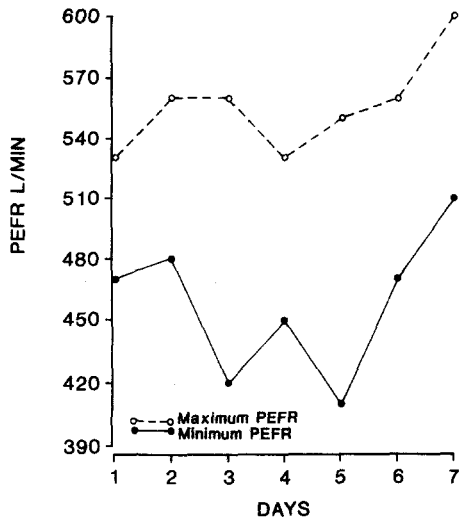
Immunologic studies

To determine epicutaneous sensitization to egg proteins, a variety of skin test reagents were prepared. The egg-allergen skin test panel comprised nine reagents from several sources that included commercial-food skin test reagents, egg white fractions, and extracts of powdered egg products obtained from the factory. The three commercial-food reagents (1:10 wt/vol) consisted of whole egg, egg yolk, and egg white purchased from Hollister-Stier (Spokane, Wash.). Purified lyophilized egg fractions (Sigma Chemical Co., St. Louis, Mo.) consisted of ovalbumin, conalbumin, lysozyme, and ovomucoid. Before testing, these fractions were diluted in PBS to a final concentration of 10 mg/ml of protein and sterilized through a Millipore filter (Millipore Corp., Bedford, Mass.).



Egg Exposure	+	–	+	+	+	–	+
Medication	–	–	+	+	–	–	+
% PEFR Variability	35.2	5.7	23.5	29.4	27.4	12.0	49

FIG. 1. Daily maximum and minimum PEFR plotted in worker No. 9. Percent variability in PEFR, concommitant asthma medications, and daily record of egg-processing exposure are illustrated.



Egg Exposure	+	+	+	+	+	+	+
Medication	+	+	+	–	–	–	–
% PEFR Variability	11.3	14.3	25	18	25.4	16.1	10

FIG. 2. Daily maximum and minimum PEFR plotted in worker No. 13. Percent variability in PEFR, concommitant asthma medications, and daily record of work exposures are presented.

Whole egg and egg yolk products obtained from the egg factory were dissolved in PBS at a concentration of 100 mg/ml (wt/vol). Undissolved material was removed by centrifugation. The supernatants were then filtered through a 0.45 μm Millipore filter followed by filtration through a 0.22 μm Millipore filter into sterile vials. The resultant supernatants were quantitated for protein content by the Lowry method. The factory egg yolk and whole egg reagents contained 8.5 and 9.0 mg/ml of protein, respectively.

Skin prick testing was performed with a panel of commercial aeroallergens consisting of short ragweed, mixed trees, mixed grasses, cat, and house dust mites (Hollister-Stier). Control skin tests included PBS, histamine (1 mg/ml), and human serum albumin. A positive skin test was defined as a wheal measuring ≥3 mm than the saline control wheal.

Specific IgE antibody to the various egg allergens was determined with a modification of the RAST.[10] The RAST disks were prepared with factory-powdered whole egg reagent (9 mg/ml) and egg white fractions of ovalbumin, lysozyme, conalbumin, and ovomucoid (10 mg/ml). A commercial-food egg white allergen disk (Pharmacia, Piscataway, N. J.) was also included in the RAST panel. Briefly, approximately 10 mg/ml of protein antigen was coupled to methylcellulose disks activated with cyanogen bromide dissolved in acetonitrile. After washing with PBS, the disks were incubated with worker's serum for 3 hours at room temperature. This was followed by incubation with 50,000 cpm of ^{125}I-labeled horse antihuman IgE for 16 hours. After a final wash with PBS, the counts per minute of ^{125}I–anti-IgE bound was measured. Specific IgE was also expressed as percent binding or counts per minute of ^{125}I–anti-IgE bound divided by total counts per minute added and multiplied by 100. A positive test was defined as counts per minute bound that was ≥4 SD than the mean binding of 16 laboratory control subjects who were never exposed to egg powder by inhalation.

Specific IgG to egg allergens

The ELISA method of Voller et al.[11] was used. Aliquots of 0.15 ml of allergen (200 μg/ml) diluted in 0.1 mol/L of $NaHCO_3$ (pH 8.6) were incubated in micro-ELISA plates (Dynatech, Alexandria, Va.) at 4° C for 18 hours. After washing with PBS and Tween, a 1:100 dilution of test serum diluted in 5% bovine serum albumin was incubated for 1 hour at room temperature. Goat antihuman IgG alkaline phosphatase conjugates (Sigma Chemical Co.) diluted 1:100 in PBS were then added for 1 hour at room temperature. Finally, 0.15 ml of 0.006 mol/L of p-nitrophenyl phosphate disodium diluted in glycine buffer (pH 10.4) was added. Enzymatic activity was terminated at 10 minutes with 2 N NaOH. Optical density, read on a micro-ELISA MR 592 spectrophotometer was considered significant if readings were more than three times the mean of eight nonegg-exposed laboratory control subjects.

RESULTS

The data presented here are limited to the 25 egg-processing factory workers in whom serial PEFR mea-

TABLE II. Skin prick test responses, PEFR variability status (+ equals ≥20% daily variability), and physician-derived diagnoses in 25 egg-processing workers

Worker	Egg skin tests*	PEFR variability†	Physician diagnosis‡
4	—	−	OA vs A
7	FWE	−	Irr
8	—	−	A vs Br
9†	CON, L	+	OA
10†	FWE, OVA, L, OVO	+	A vs Irr
12†	L, OVO	+	OA
13†	WE, EY, Con, L, OVO	+	OA
25†	CON, OVA	+	OA
28	—	−	A vs Br
30	—	−	AO vs Irr
14	—	+	NS
1	—	−	—
5	FWE, CON	−	—
6	—	−	—
11	—	−	—
15	—	−	—
16	—	−	—
17	—	−	—
18	—	−	—
20	OVO	−	—
21	—	−	—
26	+	−	—
27	—	−	—
29	—	−	—
31	—	−	—

*Skin test antigens that resulted in positive prick tests: FWE, factory powdered whole egg; WE, whole egg (Hollister-Stier); EY, egg yolk (Hollister-Stier); CON, conalbumin; OVA, ovalbumin; L, lysozyme; OVO, ovomucoid.
†Pressure (+) or absence (−) of ≥20% variability between daily minimum and maximum PEFR.
‡A nonoccupational asthma; Irr, irritant respiratory symptoms; Br, chronic bronchitis; —, no significant respiratory symptoms.

surements could be corroborated with the physical history and skin testing. Nineteen of these 25 workers also provided blood for serologic assays.

Clinical evaluation

As presented in Table I, the physician diagnosed unequivocal OA in four of 25 completed workers. There were five other workers in whom OA or non-OA was suspected. The first worker could not be differentiated from non-OA (No. 4), and one other worker (No. 30) could not be distinguished from irritant respiratory symptoms. Based on history, three other workers were suspected of having non-OA but could not be distinguished from either bronchitis (Nos. 8 and 28) or irritant respiratory symptoms (No. 10). In the remaining 16 patients, no significant respiratory symptoms were elicited by the physician.

In all four cases in which OA was the only diagnosis assigned by the physician, significant PEFR variability (>20%) was demonstrated on at least 1 of 7 test days. However, significant PEFR variability was also detected in one (No. 10) of five workers in whom OA or non-OA was suspected but in whom the physician could not make a diagnosis with certainty. There was only one worker (No. 14) of 16 who reported no symptoms but who had significant variability in PEFR. "Definite asthma" was established in five cases based on the aforementioned criteria of a suspected diagnosis of asthma and PEFR variability associated with asthmatic symptoms recorded on diary cards. It was noteworthy that four of five "definite asthma" cases had been diagnosed as having OA by the physician.

PEFR studies

Daily PEFRs were analyzed by determining the percent difference between the maximum and minimum measurements on each test day.

TABLE III. Summary of RAST binding expressed as counts per minute in four workers with elevated serum-specific IgE to egg allergens

Worker	FWE	Egg white	Allergen disks			
			L	OVO	CON	OVA
9	1150	629	1080*	1093	966*	1437
10	1463	815	895*	3605	1255	1450
12	3007	1904	1315*	3448	3742	2401
13	5068	2031	4512	5305	2062	1258
Control subjects (N = 16)	$\bar{X}$ = 636 (± 82)	$\bar{X}$ = 297 (± 62)	$\bar{X}$ = 756 (± 140)	$\bar{X}$ = 644 (± 66)	$\bar{X}$ = 522 (± 143)	$\bar{X}$ = 522 (± 143)

FWE = factory powdered whole egg; L = lysozyme; OVO = ovomucoid; CON = conalbumin; OVA = ovalbumin.
*NS = not significant or <4 SD above the mean.

The five "definite asthma" cases had a mean age of 31.8 ± 15.9 years, compared to 30.7 ± 11 years for the 20 nonasthma cases. The duration of employment at the plant for the five cases was 25.4 ± 11.4 months, compared to 20.2 ± 14 months for the 20 nonasthma cases. The five "definite asthma" cases had maximum PEFR variabilities that ranged from 21.5% to 51.7% with a mean of 35.6 ± 13.9%. The maximal percent variability in the remaining 20 participants ranged from 3.7% to 54.5%. The mean maximal PEFR of all 20 participants who were not "definite asthma" cases was 10.5 ± 10.8%. Less than 16.4% PEFR variability was obtained in all workers except the one individual who was diagnosed as normal by the physician but nevertheless had asymptomatic bronchial lability.

Two different patterns of positive responses observed in two workers who demonstrated significant variability during 1 work week are illustrated in Figs. 1 and 2. The first pattern of response was demonstrated in worker No. 9 (Fig. 2). As illustrated, the variability between daily maximum and minimum PEFR decreased <10% on days when there was no exposure to egg processing but increased dramatically from 23.5% to 45.1% when that worker was working in the egg factory. Another pattern of PEFR response observed in worker No. 13 is presented in Fig. 2; there was significant variability in PEFR on 2 of 7 days. This occurred despite the fact that this worker was self-medicated with bronchodilators. Apparent failure to demonstrate sustained improvement in PEFR away from egg exposure in this individual reflected our inability to obtain measurements during more prolonged periods when worker No. 13 was not exposed to egg processing.

Immunologic studies

Epicutaneous reactivity to one or more of the egg allergens was detected in eight of 25 workers. This group included all five "definite" asthma workers, of whom four had been diagnosed as unequivocal OA by the physician. In contrast, three of 20 patients without asthma had positive skin tests to egg allergens. A significant association between "definite asthma" and cutaneous reactivity to egg allergens was demonstrated by Fisher's exact test ($p < 0.01$). Thus, skin testing to relevant egg allergens exhibited a sensitivity of 100% for identifying workers with "definite asthma." It was also noteworthy that five of six workers with 20% PEFR variability had cutaneous reactivity to the egg allergens. The skin test negative worker (No. 14) reported no symptoms to the physician nor did he report symptoms associated with decrements in PEFR. Table II also illustrates that four of eight or 50% of workers who had cutaneous reactivity to egg allergen reacted only to the egg fractions: ovalbumin, ovomucoid, conalbumin, or lysozyme but not to the commercial food or factory-derived reagents. Thus, it was essential to use egg white fractions to identify correctly all workers with cutaneous egg sensitization. There were only two of 25 workers (Nos. 10 and 26) with at least one positive skin test to the panel of common inhalant aeroallergens. One of these workers (No. 10) also exhibited cutaneous sensitivity to egg allergens.

The RAST was positive to one or more of the egg allergens in four workers but negative in 21 other egg workers tested (Table III). All RAST positive workers also exhibited good binding to factory whole egg, egg white (Pharmacia), ovomucoid, and ovalbumin. In this group, the best overall direct binding activity was to ovomucoid. Significant but lesser RAST activity was detected to lysozyme in one worker and conalbumin allergens in three workers. All four workers with elevated RAST binding to egg allergens also had "definite asthma." The association between "definite asthma" and RAST reactivity was significant (χ^2; $p < 0.01$).

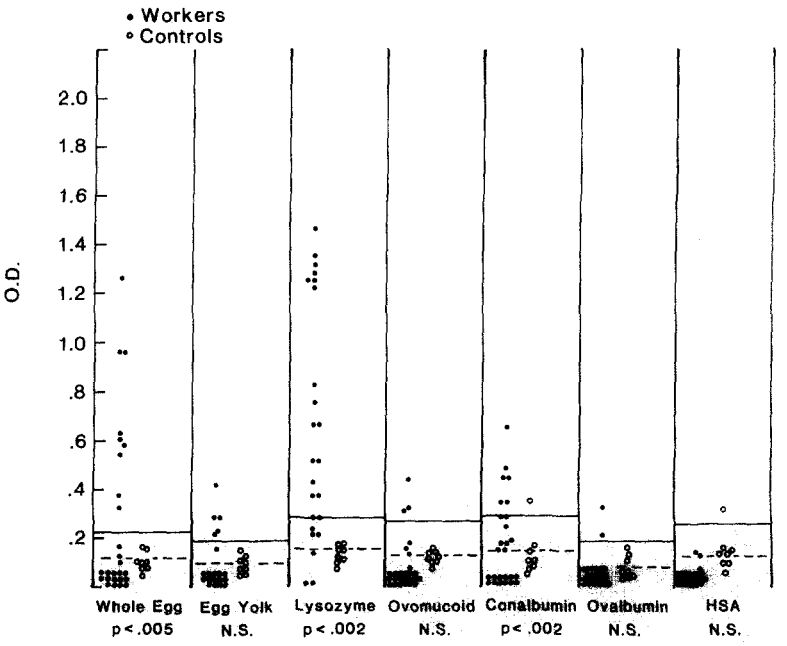


FIG. 3. ELISA-specific IgG to crude and egg white protein allergens performed at a serum dilution of 1:100.

Results of serum-specific IgG determinations are presented in Fig. 3. ELISA results are expressed as the optical density at a serum dilution of 1:100. In egg-exposed workers, the IgG responses to lysozyme ($p < 0.002$), whole egg ($p < 0.005$), and conalbumin ($p < 0.002$) were elevated compared to responses of eight nonexposed laboratory control subjects. There were no significant differences in IgG levels between egg-exposed symptomatic subjects with asthma and nonsymptomatic workers.

DISCUSSION

This study is the first to document IgE-mediated sensitization to egg proteins in egg-processing workers. Allergic sensitization was confirmed by the presence of cutaneous reactivity and serum-specific IgE to factory egg products, as well as egg white protein fractions. Specific IgE binding to a commercial egg white food allergen was detected in all subjects with RAST reactivity to the factory-powdered egg. Thus, these findings suggested that the processed powdered eggs and the standard food egg white possessed common allergens.

The presence of cutaneous sensitivity to egg antigens was not significantly associated with reactivity to common aeroallergens. Thus, atopic status did not appear to be a significant risk factor for the development of egg-induced OA. It also indicated that environmental aeroallergens could not have caused changes in PEFR, observed in this group of symptomatic workers.

In this study, diurnal variability in PEFRs was used to document significant bronchial obstruction. Differences in daily PEFR of >20% have also been demonstrated to correlate highly with increased bronchial hyperresponsiveness to histamine.[9] In five egg-processing workers, the clinical suspicion of asthma combined with the presence of variability in PEFRs >20% concurrent with reported bronchial symptoms during the work week was sufficient to establish a diagnosis of definite asthma. In four of five of these workers, OA was diagnosed with certainty by the physician. Furthermore, cutaneous reactivity to egg allergens was detected in all five of these workers. Thus, the egg skin tests were highly sensitive for identifying both workers with definite asthma and workers with unequivocal OA diagnosed by the physician. The failure to elicit a positive history and PEFR changes in three other workers with skin reactivity to eggs indicated that cutaneous sensitization at work may actually precede the onset of clinical sensitivity.

Worker No. 9 clearly demonstrated decrements in PEFR only on days when there was exposure but not on days when there was no exposure to egg processing. This observation combined with significant variability in PEFR and cutaneous sensitization to egg proteins in three other workers who were diagnosed by the physician with unequivocal OA confirmed the

diagnosis of work-related asthma in four workers induced by sensitization to powdered egg proteins.

As previously mentioned, only 50% of workers reacting to one or more egg fractions exhibited positive skin tests to factory or commercial whole egg and egg yolk extracts. Failure to use individual egg fractions would have decreased the overall sensitivity of skin testing in this study. Previous unsuccessful attempts to demonstrate cutaneous sensitization to eggs in bakers reporting asthmatic symptoms associated with inhalational exposure to eggs could possibly be explained by a failure to use purified egg allergens for skin testing.[6] Moreover, prior heating of egg protein reagents in this study could have denatured important allergenic determinants and decreased the prevalence of positive skin tests.

RAST binding activity to egg allergen was demonstrated in four workers. In comparison to RAST, skin testing exhibited greater sensitivity (100% versus 80%) for identifying those workers with "definite asthma." The highest degree of RAST binding among all four workers was detected to the heat-stabile fraction, ovomucoid. This could be attributed to possible increased resistance of ovomucoid to the factory processes of heating and drying that could have resulted in denaturation of heat-labile fractions, such as conalbumin or lysozyme. This could increase the workers' potential exposure to ovomucoid and thereby provide more opportunity for subsequent sensitization to this protein.

Specific IgG responses were not predictive of clinical sensitization. The significance of an unusually high IgG response to lysozyme compared to the other three fractions in this group of workers is uncertain.

In conclusion, inhalational exposure to egg proteins was demonstrated to cause IgE-mediated OA. Skin prick testing was the best predictor of clinical sensitivity. Specific IgE and IgG responses to multiple egg allergens were observed. Serial PEFR measurements, skin testing, and RAST were useful diagnostic methods for performing this cross-sectional study and identifying symptomatic workers with allergic sensitization to egg proteins. Based on this investigation, it is evident that prospective surveillance of egg-processing plants should determine the prevalence and scope of this problem.

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