Workplace-Related Chronic Cough on a Mushroom Farm*

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Background: Mushroom spores have frequently been associated with respiratory allergy. The aims of this study were to elucidate the incidence and causes of chronic cough in a mushroom farm.

Methods: Participants were 69 mushroom workers who produce Hypsizigus marmoreus (Bunashimeji) and 35 control subjects. We excluded six workers because they had had asthma or allergic rhinitis before working. Participants completed a cross-sectional health survey 2 years after starting work at the mushroom farm.

Results: The mean airborne endotoxin levels in the harvesting and packing rooms were approximately 60-fold higher than those in the offices. Of 63 workers, 42 workers (67%) reported chronic cough after working on this farm, 19 workers had no cough, while 2 workers had hypersensitivity pneumonitis develop to the spore, which has been previously reported by us. Of the 42 workers with cough, 6 workers had organic dust toxic syndrome (ODTS), 18 workers had postnasal drip syndrome, 15 workers had cough variant asthma, and 3 workers had eosinophilic bronchitis. Seventy-one percent of the workers noticed the cough in the first 3 months, and the mean latent period in ODTS workers was the shortest. The cough had a trend to improve or disappear after weekend holidays. Bronchial hyperresponsiveness but not FEV1/FVC% in the 42 workers with cough was significantly (p < 0.001) increased as compared with the control subjects.

Conclusions: Working on a mushroom farm carries a significant risk for chronic cough from inhalation of mushroom spores, and we suggest that elevated airborne endotoxin on this farm is the cause.

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Key words: chronic cough; endotoxin; mushroom spore; respiratory allergy

Abbreviations: BHR = bronchial hyperresponsiveness; CVA = cough variant asthma; Grs = respiratory conductance; HP = hypersensitivity pneumonitis; ODTS = organic dust toxic syndrome; PD_{35Grs} = cumulative dose of methacholine that decreased respiratory conductance to 65% of its baseline level; Rrs = respiratory resistance

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ccupational respiratory diseases associated with exposure to mushroom spores have been reported in recent years.1–6 An edible mushroom, Hypsizigus marmoreus (Bunashimeji), is cultivated in windowless rooms during all seasons in Japan. When mature, the cultivating, harvesting, and packing rooms are filled with the spores. As the size of the spore is 4 to 6 μm, it can reach the alveolus of the lung. We previously reported two cases of hypersensitivity pneumonitis (HP) caused by the spore in a Bunashimeji mushroom farm.7 We also observed that chronic cough and other respiratory allergic symptoms occurred frequently, and that some mushroom workers left because of intolerable work-related respiratory symptoms.8 However, there has been only one previous report1 on the incidence of cough in mushroom farms.

Chronic cough is defined as a cough persisting for
at least 3 weeks\textsuperscript{10} or 8 weeks,\textsuperscript{11} with no abnormal shadow evident on chest radiography. Organic dust toxic syndrome (ODTS) is a noninfectious, febrile illness associated with chronic cough, malaise, and headache that occurs after heavy organic dust exposure.\textsuperscript{12} ODTS shares many clinical features with HP, but ODTS differs from it in several respects: negative chest radiographic findings, absence of severe hypoxemia, and no need for prior sensitization to antigens in the organic dust. Airborne endotoxin and mycotoxins have been proposed as causes of ODTS.\textsuperscript{13} Dose-response relationships were observed between dust (including endotoxin) and respiratory symptoms in the indoor environment of a swine confinement building.\textsuperscript{14} In the paper industry, a relationship was found between exposure to airborne endotoxin and bronchial hyperresponsiveness (BHR).\textsuperscript{15} There has been only one previous report\textsuperscript{16} measuring airborne endotoxin in a mushroom farm, but respiratory symptoms were not evaluated.

We speculated that mushroom farms are workplaces where various allergies might be common, although little is documented about the incidence and cause of chronic cough in such environments. To clarify this problem, we conducted a cross-sectional health survey and measured the airborne endotoxin level on a Bunashimeji mushroom farm. This is the first prospective study of the frequency of causes of chronic cough in such an environment.

### Materials and Methods

#### Study Population

We examined one mushroom farm producing a single variety of mushroom (Bunashimeji). On this farm, there were 4 office workers (1 woman and 3 men) and 69 mushroom workers (all women). The 69 mushroom workers (mean ± SD age, 48 ± 12 years; range, 34 to 66 years) and 35 age-matched healthy female control subjects (mean age, 48 ± 11 years; range, 31 to 66 years) participated in the study after giving informed consent. The control subjects were recruited from Sapporo Medical University Hospital and had never cultivated Bunashimeji mushrooms. There were no subjects with heartburn or hypertension receiving angiotensin-converting enzyme inhibitors in either group. We excluded 6 subjects from the 69 workers, because they had been treated for bronchial asthma or allergic rhinitis before entering the mushroom farm.

#### Exposure

Measurements of airborne endotoxin levels were made by drawing air through a 110-mm glass fiber of 0.6 \( \mu \)m porosity (GB-100R; Toyo Roshi; Tokyo, Japan) at a flow rate of 500 L/min for 30 min using a high-volume air sampler (HVC-500; Shihata Kagaku; Tokyo, Japan). The air inlet of the sampler was attached to the subject's nose level. Sampling involved three filters in cultivating rooms, three filters in the harvesting and packing rooms, and three filters in an office room (control). The filters were shaken for 10 min in 10 mL of distilled water. The solution from the filters was analyzed for endotoxin using specific limulus lysates. Fifty microliters of filter extract and 50 \( \mu \)L of endotoxin-specific lyase (Endospec SP test; Seikagaku Kogyo; Tokyo, Japan) were placed in a microwell plate. The plate was incubated in a spectrometer, and the kinetics of the ensuing color reaction was read photometrically (Wellreader, SK-601; Seikagaku Kogyo). The values were expressed as nanograms per milliliter of liquid, and this level was transformed to nanograms per cubic meter using the flow rate of the air sampler. The limit of detection for endotoxin is 10 pg/mL.

#### Study Design

A cross-sectional examination was performed in June 1997, 24 months after mushroom production began. We applied a questionnaire and gathered information about symptoms, atopic status, chest radiographs, pulmonary function tests, and serum precipitins to mushroom antigens in the 63 workers and 35 control subjects. Each subject was interviewed by trained personnel to ascertain name, age, gender, smoking status, symptoms that newly appeared after working in the factory (cough, phlegm, shortness of breath, fever and runny nose), and whether the subjects noticed or disappeared after weekend holidays. If they had any symptoms, we asked them to state when they first noticed them. Subjects were defined as nonatopic asthmatics when they had no positive response to antigen-specific serum IgE, and were defined as atopic when they had at least one or more positive antigen response to 12 common antigens tested with a commercial kit (CAP Phadiatop FEIA; Pharmacia and Upjohn; Uppsala, Sweden). Chest radiographs were reviewed by two chest radiologists. Pulmonary function tests were performed using a desktop pneumotachospirometer (Chest Graph HI-701; Chest; Tokyo, Japan). The subjects performed at least three trials, and the highest value of FEV\textsubscript{1} was recorded. Preparation of Bunashimeji antigens and the method for detecting serum precipitins to the Bunashimeji spore were reported previously.\textsuperscript{7} Briefly, the Bunashimeji spores were cultured in Sabouraud glucose broth, and the proteins were extracted with 50% ammonium sulfate. The sample was centrifuged at 3,000 revolutions per minute for 15 min, and the pellet was dialyzed with distilled water. Fifty microliters of antigen and/or serum were added to opposite wells of 1% agarose plates and monitored for 72 h at room temperature.

First, we excluded noncoughers and patients with HP (Fig 1). If reticulonodular shadows on chest radiographs were found, we

![Figure 1](http://publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21982/ on 06/17/2017)
determined whether the subjects had HP, using the following criteria: (1) cough, dyspnea, and fever some hours after work; (2) reticulonodular opacities on chest radiography and CT scans; (3) reduced vital capacity and diffusing capacity of the lung for carbon monoxide; (4) positive serum precipitin findings and in vitro lymphocyte blastogenesis for Bunashimeji antigen; (5) increase in the number of lymphocytes in BAL fluid; (6) interstitial pneumonitis, frequently with granuloma formation in a pathological specimen; and (7) resolution of episodic respiratory symptoms after ceasing exposure to mushroom spores.

Second, BHR was examined in all the remaining cough workers by the continuous methacholine inhalation method with simultaneous measurement of respiratory resistance (Rrs) developed by Takishima et al.17 (Astograph Jupiter 21; Chest). Briefly, we prepared 10 twofold incremental concentrations of methacholine chloride diluted in physiologic saline solution from 49 to 25,000 µg/mL. Each methacholine aerosol was inhaled for 1 min using Bird micronebulizers (Bird Corporation; Palm Springs, CA) with an output of 0.25 L/s. Rrs was measured by the 3-Hz forced oscillation method. After recording baseline Rrs (physiologic saline solution inhalation), methacholine aerosols were inhaled during tidal breathing without a pause while Rrs was continuously measured. Inhalation continued until the measured Rrs exceeded by twofold the baseline Rrs level. Respiratory conductance (Grs [the reciprocal of Rrs]) was plotted, and the cumulative dose of methacholine that decreased Grs to 65% of its baseline level (PD35Grs) was obtained from each dose-response curve. This parameter was measured in terms of a unit defined as 1-min inhalation of 1 mg/mL of methacholine. The values were expressed as logPD35Grs. Ishii et al.18 reported that the logPD35Grs values in this simple continuous inhalation provocation test were significantly correlated (r = 0.80) with those in a more complex standard intermittent method, in which methacholine aerosols of stepwise incremental concentrations are inhaled during tidal breathing for 2 min, and each increment is followed by 5 min of rest after the end of each inhalation period by means of the forced oscillation method.

Third, we defined ODTS according to clinical criteria as a noninfectious, febrile illness associated with chronic cough, with or without BHR, dyspnea, headache, and malaise, which occurs after heavy organic dust (endotoxin) exposure (Fig 1).12 ODTS shares many clinical features with HP, but ODTS differs from HP in having negative chest radiographic findings, absence of severe hypoxemia, and no need for prior sensitization to antigens in the organic dust.

Next, we diagnosed postnasal drip syndrome (PND) from the questionnaire responses (Fig 1). Spontaneous sputum samples were obtained in the morning during the working period in the remaining workers without PND. Patients were asked to rinse their mouths, swallow the water, and blow their nose to minimize contamination with saliva and postnasal drip. The method of assessing sputum eosinophils was previously reported by Pin et al.29 The smear samples were reviewed by one clinical pathologist.

Finally, we attempted to distinguish bronchitis from asthma at the workplace; workers with BHR are presented as asthma, and employees without BHR are presented as bronchitis (Fig 1). In workers with bronchitis, when sputum eosinophils (>3% nonsquamous cells) were present, the workers received a diagnosis of eosinophilic bronchitis. Eosinophilic bronchitis presents with chronic cough and is characterized by sputum eosinophilia without evidence of airflow obstruction or BHR.30 We examined a shift change at the workplace on Monday using a desktop pneumotachospirometer in asthma workers. We assessed cough and peak expiratory flow rate and FEV1 before, and 1 h, 3 h, and 8 h after working. When a >15% decrease in FEV1 from baseline was obtained, the workers were defined as having classical asthma. If there was no such decrease in FEV1 but a cough developed, the workers were defined as having cough variant asthma (CVA).

### Statistical Analysis

All data are presented as mean ± SD. Comparisons between two groups were made using the Mann-Whitney U test. Statistical significance was assumed at p < 0.05.

### Results

Airborne endotoxin levels in each room are shown in Table 1. The endotoxin concentration was greater in the harvesting and packing rooms, being approximately 60-fold higher than that in the office.

Of 63 workers, 2 workers were patients with HP caused by the mushroom spore, who have already been reported.7,42 workers complained of chronic cough at the workplace, and 19 workers had no cough (Fig 1). As shown in Table 2, 71.4% of the 42 subjects had a cough develop within the first 3 months of work. None of the 42 workers had reticulonodular shadows on chest radiography or abnormal respiratory sounds on chest examination.

A schematic drawing of the differential diagnosis of the 42 workplace-related chronic coughs is shown in Figure 1. BHR (logPD35Grs) was significantly (p < 0.001) increased in the 42 workers as compared with the control subjects, but FEV1/FVC% was not (Table 3). Six people had symptoms similar to HP having a noninfectious febrile illness associated with chronic cough without phlegm and general malaise. The mean latent period of the cough in these six subjects was the shortest (Table 3). As described above, airborne endotoxin levels in this farm were as high as those found in the paper industry, in which ODTS has been reported. Therefore, we diagnosed ODTS in these six workers. Of the remaining 36 workers, 18 workers had a runny nose with phlegm, in whom we diagnosed PND. Sputum eosinophilia (>3% nonsquamous cell) was present in the remain-

| Table 1—Concentrations of Airborne Endotoxin in the Cultivating Room, the Harvesting and Packing Rooms, and the Office Room |
|---|---|---|
| Workplace | Filters, No. | Mean Endotoxin Level, ng/m³ (Range) |
| Cultivating room | 3 | 3.2 (1.5–5.3) |
| Harvesting and packing rooms | 3 | 12.1 (3.8–20.1) |
| Office room | 3 | 0.2 (0.1–0.31) |

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ing 18 subjects, of whom 3 subjects had a normal BHR (logPD$_{35}$Grs is within mean ± 2 SD of control subjects), and 15 workers had an increased BHR (logPD$_{35}$Grs less than mean ± 2 SD of control subjects; Table 3). We diagnosed eosinophilic bronchitis in these three workers. In the remaining 15 workers, no one had a > 15% of decrease in FEV$_1$ from baseline in the provocation test, and all workers complained of cough after work. Based on these results, we diagnosed CVA in these 15 workers. The severity of cough in the 42 workers with chronic cough tended to improve or disappear after weekend holidays. Therefore, a temporary absence from the workplace improves the respiratory symptoms.

Thirty-one of 42 cough workers had positive serum precipitin findings to the mushroom spores, whereas none of control subjects did (Table 3). There were no significant differences in percentage of vital capacity, percentage of FEV$_1$, and logPD$_{35}$Grs values between the 31 precipitin-positive and the 11 precipitin-negative workers, and between the 17 smokers and the 25 nonsmokers.

**DISCUSSION**

Our results indicate that mushroom workers have a significant risk for allergic diseases, and 67% of the employees had a chronic cough after working for 2 years. Mushroom antigens are known to produce allergic reactions of all the Gell and Coombs types I to IV, and allergic symptoms appear 5 to 10 h after antigen provocation. There has been only one cross-sectional health study on occupational allergy in mushroom farms; but, to the best of our knowledge, no data exist regarding the causes of chronic cough. We were surprised that as many as 71.4% of the cough workers experienced the cough within 3 months, and some workers noticed it within 1 week or 2 weeks after starting work at this Bunashimeji farm. This short latent time makes a sensitization-induced allergic reaction by inhaled mushroom antigens unlikely. However, it is known that workers with ODTS had chronic dry cough, fever, dyspnea, and chest tightness after exposure to organic dust on dairy farms. Because workers with ODTS generally have no precipitins to antigens of mold, endotoxins and fungal toxins of moldy hay were proposed as causes of ODTS. In swine and dairy farms, where airborne endotoxin levels were high, farmers complained of more respiratory allergic symptoms and had greater BHR than workers in greenhouses, where the endotoxin levels were low. Exposure to airborne endotoxin and BHR appear to be related in the paper industry. There has been only one report about airborne endotoxin measurements at a mushroom farm; and at the preflush and chicken manure area, the levels were as high as those in silo or grain-handling workplaces, wherein exposed workers had ODTS develop. In our measurements, the airborne endotoxin levels in the picking and harvesting rooms were similar to levels in workplaces where many workers complained of symptoms of ODTS. Therefore, the environmental conditions on this mushroom farm may induce ODTS in workers. From these results, chronic cough and increased BHR in mushroom workers might partly result from inhalation of irritant endotoxin in the workplace.

**Table 2—Months After Employment When Workers First Noticed Chronic Cough, Obtained by Questionnaire (n = 42)**

<table>
<thead>
<tr>
<th>Months</th>
<th>Cumulative</th>
<th>Employees, No.</th>
<th>Cumulative, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤1</td>
<td></td>
<td>13</td>
<td>30.9</td>
</tr>
<tr>
<td>≤3</td>
<td></td>
<td>30</td>
<td>71.4</td>
</tr>
<tr>
<td>≤6</td>
<td></td>
<td>37</td>
<td>88.0</td>
</tr>
<tr>
<td>≤12</td>
<td></td>
<td>41</td>
<td>97.6</td>
</tr>
<tr>
<td>≤24</td>
<td></td>
<td>42</td>
<td>100</td>
</tr>
</tbody>
</table>

**Table 3—Characteristics of Workers With Chronic Cough at the Workplace (n = 42) and Control Subjects (n = 35)†**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Age, yr</th>
<th>Smokers, No.</th>
<th>Atopy, No.</th>
<th>Onset of Cough, mo</th>
<th>FEV$_1$/FVC%,</th>
<th>Log(PD$_{35}$Grs), U</th>
<th>Precipitins Positive, No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control subjects</td>
<td>48 ± 11</td>
<td>12</td>
<td>17</td>
<td>None</td>
<td>95.5 ± 10.5</td>
<td>2.0 ± 0.21</td>
<td>0</td>
</tr>
<tr>
<td>Cough workers</td>
<td>48 ± 12</td>
<td>17</td>
<td>20</td>
<td>4.2 ± 6.0</td>
<td>83.2 ± 7.8</td>
<td>1.27 ± 0.43</td>
<td>31</td>
</tr>
<tr>
<td>ODTS (n = 6)</td>
<td>51 ± 10</td>
<td>3</td>
<td>5</td>
<td>1.5 ± 1.3</td>
<td>90.9 ± 11.6</td>
<td>1.56 ± 0.30</td>
<td>5</td>
</tr>
<tr>
<td>PND (n = 18)</td>
<td>48 ± 12</td>
<td>7</td>
<td>8</td>
<td>7.8 ± 7.4</td>
<td>81.0 ± 7.9</td>
<td>1.24 ± 0.33</td>
<td>14</td>
</tr>
<tr>
<td>EB (n = 3)</td>
<td>48 ± 8</td>
<td>1</td>
<td>1</td>
<td>3.5 ± 3.2</td>
<td>82.4 ± 12.1</td>
<td>1.80 ± 0.17</td>
<td>2</td>
</tr>
<tr>
<td>CVA (n = 15)</td>
<td>47 ± 12</td>
<td>6</td>
<td>6</td>
<td>4.1 ± 3.2</td>
<td>80.1 ± 7.1</td>
<td>1.04 ± 0.30</td>
<td>10</td>
</tr>
</tbody>
</table>

*EB = eosinophilic bronchitis.
†p < 0.05.
‡p < 0.001 vs control subjects.
However, we were unable to evaluate dose-response relationships between airborne endotoxin levels, and a direct causation was difficult to prove in this study.

In the present study, 15 workers had CVA and 18 workers had PND. CVA exists when cough is the principal symptom with normal respiratory sounds and spirogram accompanied by increased BHR and principal symptom with normal respiratory sounds. Workers had PND. CVA exists when cough is the first reported six patients with CVA, in whom coughing disappeared after starting treatment with a bronchodilator or inhaled corticosteroids. Beneficial effects of specific therapy strengthen the diagnosis of CVA and PND. However, in this study, only eight workers wished to receive medication. This point is a limitation of this study. Bronchodilator or inhaled corticosteroids were administered to three CVA workers, and antihistamines were administered to five workers with PND. After these treatments, the cough gradually decreased.

Studies suggest that occupational anaphylactic reaction, asthma, and extrinsic allergic alveolitis due to the inhalation of mushroom spores are frequent among atopic asthmatics. The BHR observed among mushroom workers might be related to the atopic state. For this reason, we measured serum-specific IgE antibodies to 12 common allergens, but there was no significant difference in PD25Grs and FEV1/FVC% between atopic workers and nonatopic subjects. These results agree with those in a previous study in dairy farmers. We suggest that airway hyperresponsiveness in mushroom workers might not relate to IgE-dependent responses.

In conclusion, of 63 Bunashimeji mushroom workers, 42 workers (67%) had a work-related chronic cough, 19 workers had no cough, and 2 workers had HP caused by mushroom spores. In the 42 workers with chronic cough, 18 workers had PND, 15 workers had CVA, 6 workers had ODTS, and 3 workers had eosinophilic bronchitis. We suggest that mushroom farms carry a significant risk for chronic cough, and inhalation of mushroom spores might be important in its development. Elevated environmental endotoxin is also suggested as a candidate etiology of chronic cough.

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