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Investigation of an Outbreak of "Humidifier Fever" in a Print Shop

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An outbreak of "humidifier fever" affected 16 (57%) of 28 workers in a print shop. The most common symptoms were myalgia, chills or subjective fever, and cough. Illness began 5–13 hours after entering the workplace, and lasted 2–24 hours. A humidifier in use the day of the outbreak was found to be contaminated with fungi, amebae, and Gram-negative bacteria. The risk of illness was highest for those who had been on the job 3 months before the outbreak, a time when the humidifier was in constant use. Serologic studies of print shop workers showed positive reactions to extracts of organisms isolated from the humidifier, but could neither distinguish ill from well workers, nor identify causative organisms. The presence of endotoxin-producing bacteria and the clinical syndrome are consistent with an organic dust toxic syndrome. Previous exposure appeared to be the major risk factor for illness.

Key words: organic dust toxic syndrome, hypersensitivity pneumonitis, bacterial endotoxin, humidifier fever, aerosol contamination

INTRODUCTION

Acute febrile illnesses encountered in the workplace can be caused by a variety of occupational exposures. Syndromes such as "metal fume fevers" are caused by fumes from zinc and other metals and polymers. Similar acute and recurrent illnesses result from reactions to airborne microorganisms and microbial particles. Occupational diseases such as farmer's lung have been characterized as a hypersensitivity pneumonitis, which may progress to chronic fibrotic lung disease. Similar syndromes have been given other descriptive names: mushroom worker's lung, bird fancier's lung, and maple bark stripper's disease [Fink, 1986].

Humidifier fever has also been considered in some studies to be a type of hypersensitivity pneumonitis caused by various aerosolized microorganisms growing in the humidifier water [Ganier et al., 1980; Banaszak et al., 1970; Woodard et al.,

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1988]. An alternative explanation has been that humidifier fever and similar acute illnesses are caused by an inflammatory reaction to airborne microbial endotoxin produced by Gram-negative bacteria [Rylander et al., 1978]. Bacterial endotoxin has been isolated from a water spray humidifier [Flaherty et al., 1984], and pathogenic levels of airborne endotoxin from a contaminated humidifier have been quantified [Rylander and Hagland, 1984].

Previous reports on the epidemiology of humidifier fever have tended to focus on chronically exposed populations. Serologic studies have demonstrated antibodies in exposed individuals to a variety of microbial organisms and to endotoxin [Banaszak et al., 1970; Finnegan et al., 1987; Ganier et al., 1980; Flaherty et al., 1984], although there has been inconsistent correlation of the results with symptomatic disease. While acute humidifier fever is usually self-limited, the epidemiology and pathophysiology of the illness are still under investigation.

We report a study of an outbreak of acute illness in a print shop in Vermont. The outbreak affected a majority of the workers in a shop that had no previous reports of occupational disease. The worker population included recent, as well as long-term, employees. We attempted to characterize the epidemiology of the illness and to identify the causative microbial agents.

BACKGROUND

On June 9, 1988, Vermont Occupational Safety and Health Administration (VOSHA) industrial hygiene consultants were called by the owner of a small print shop, who reported that he and many of his employees had become ill the previous day. Common symptoms were myalgia, subjective fever or chills, cough, and shortness of breath. Most workers returned to work the day after the outbreak.

Since the printing process requires a constant level of humidity in the air, a humidifier was located in the press room. The spring rains had recently stopped and a period of dry weather began. The humidifier, which had not been in use in over two months, was activated on June 8 by the low ambient humidity.

MATERIALS AND METHODS

Epidemiologic

We interviewed 28 (90%) of the 31 employees who had worked on the day of the outbreak. Workers were asked about the time of onset and duration of any symptoms experienced on the day of the outbreak and in the previous month. The length of employment at the print shop and in their current job position were also ascertained. Since most of the jobs required going to various locations in the facility, we asked how much of the workday each worker spent in the press room where the humidifier was located. Further information elicited from each worker included a history of smoking, asthma, and/or allergies; exposure to farm work where organic dusts might have been encountered; and the extent of any symptoms experienced away from work.

A case was defined as a worker who reported 2 of the following 3 symptom groups on the day of the outbreak: (1) myalgia, (2) subjective fever or chills, or (3) respiratory symptoms of cough, chest tightness, or shortness of breath.

Blood specimens were collected from 26 workers for serologic analysis 5 days after the onset of the illness, and convalescent-phase sera were collected 4 weeks later. Sera were also collected from a convenience sample of seven health department employees to serve as a comparison group.

The EpiInfo program was used to calculate relative risks, confidence limits, and p values for univariate and stratified analyses of risk factors [Dean et al., 1990].

Environmental

Environmental inspection of the facility by the industrial hygienists focused on a search for any chemical or physical agents that could have caused an acute febrile illness. The ventilation system, which was designed to maintain negative pressure in the printing press room, was evaluated. The humidifier was dismantled and inspected, and water and sludge samples were taken for culture.

Laboratory

Water and sludge samples collected from the humidifier were cultured for bacteria, thermophilic actinomycetes, and fungi on suitable media at 25, 35, and 55°C. The bacterial isolates were maintained on trypticase soy agar (TSA) slants. Bacterial antigens for serologic studies were prepared by culturing the isolates for 24–48 hours in TSA broth, transferring 1.0 ml of the suspension to TSA plates, and 24–48 hours later washing the bacteria from the plate with carbonate buffer (pH 9.5). The bacterial suspensions were diluted in the same buffer to a turbidity of 0.5 at 450 nm for use in serologic analyses.

Fungal isolates from the humidifier sludge were subcultured on malt agar, transferred to malt broth, and cultured at 37°C for 4–6 weeks until confluent, mature cultures were obtained. The mycelial mats and spent media were homogenized, and antigen extracts were prepared as previously described [Olenchock et al., 1989].

An ameba isolated from the sludge was cultured in a cell-free medium for 8 weeks at 30°C. The ameba culture was disrupted by sonication, clarified by centrifugation, and used as an antigen extract for serologic analyses.

The serum samples were analyzed for antibodies to the bacterial isolates by enzyme-linked immunosorbant assay (ELISA) [Voller and Bidwell, 1986], modified for bacteria. Briefly, the bacteria were allowed to adhere to ELISA plates, the plates were washed extensively, and serum samples diluted 1:100 were added to each well. After a 1 hour incubation, the plates were washed, and polyvalent antisera to human immunoglobulins conjugated with horseradish peroxidase (Sigma Chemical Co., St. Louis, MO) were added to each well. The plates were developed using ABTS substrate, and the absorbance at 410 nm was measured. All samples were tested in duplicate. The mean absorbance for the seven unexposed comparison subjects was determined, and serum samples from workers were considered positive, if the absorbance value of the sample was more than 2 standard deviations above the mean of the unexposed comparison subjects.

The sera were tested for precipitating antibodies to the fungal and ameba isolates by counterimmunoelectrophoresis [Gordon et al., 1971]. One or more lines of precipitation was considered positive. An immunofluorescent antibody test (IFA) was used to test for serologic reactions to *Legionella*.

TABLE I. Symptoms Reported by 16 Case-Patients With Humidifier Fever in Print Shop, Vermont, 1988

Symptom	No. (%) reporting
Myalgia	15 (94)
Fever/chills	15 (94)
Cough	8 (50)
Headache	7 (44)
Chest tightness/shortness of breath	6 (38)
Nausea	5 (31)
Coryza	3 (19)
Wheezing	2 (13)
Sore throat	1 (6)
Diarrhea	1 (6)
Skin rash	0 (0)

RESULTS

Epidemiologic

Sixteen (57%) of 28 workers met the case definition, and their symptoms are shown in Table I. Generalized muscle aches (myalgia) and fever and/or chills were the most common symptoms reported by ill workers. Three workers measured their own temperatures at 100°–101°F. Half of the ill workers reported cough, which was characterized as nonproductive. Other respiratory symptoms were reported by fewer than 20% of ill workers, and none reported skin rashes.

The onset of symptoms ranged from 5 to 13 hours after entering the workplace, with the median onset of illness being 7 hours after the start of the day shift on June 8. The illness lasted from 2 to 24 hours; 75% of the ill workers recovered fully overnight. None of the workers consulted a physician, and all but 2 workers returned to work the following day. Symptoms did not recur on return to work, and none of the workers reported having had a similar illness in the previous month.

Table II shows results of the analysis of potential risk factors for developing humidifier fever. All workers spent some time each workday in the press room where the humidifier was located. Workers who reported spending >50% of their workday in the press room did not have a greater likelihood of experiencing illness than workers who reported spending less time in the press room. Ten (63%) of the ill workers reported spending <25% of their workday in the press room. However, workers who had been employed at the print shop for ≥3 months were more likely to become ill (relative risk [RR] = 4.1; 95% confidence limits [CL] 0.9, 148.6; p = 0.06).

Workers' ages ranged from 20 to 54 years, with a median age of 26.5 years. There were equal numbers of men and women. Workers over the median age and women showed a tendency toward an increased risk of illness (RR for each factor = 1.7, 95% CL 0.8, 3.3; p = 0.13). A history of smoking, asthma, or reported allergies did not appreciably alter the risk of illness. Only 2 of the workers reported any farm work in the previous 5 years.

To evaluate the potentially confounding factors of age and gender on the effect of having been employed in the print shop for ≥3 months, stratified analyses were done. Adjusting for age or gender did not substantially change the relative risk (crude 4.1, age adjusted 4.0, 95% CL 0.6, 29.1; gender adjusted 4.8, 95% CL 0.8, 29.9).

TABLE II. Risk Factors for Developing Humidifier Fever in Print Shop, Vermont, 1988

	Workers w/factor		Workers w/o factor				
Factor	Cases/total	AR[a]	Cases/total	AR	RR[b]	95% CI	p value
>50% work day in press room	5/8	63	11/20	55	1.1	(0.4,2.0)	1.00
≥3 mo. on job	15/22	68	1/6	17	4.1	(0.9,149)	0.06
Female gender	10/14	71	6/14	43	1.7	(0.8,3.3)	0.13
Age ≥27	10/14	71	6/14	43	1.7	(0.8,3.3)	0.13
Allergies	6/10	60	10/18	56	1.1	(0.5,2.0)	1.00
Smoker	5/11	46	11/17	65	0.7	(0.3,1.5)	0.44

[a]AR, attack rate (%).
[b]Crude relative risk.

Environmental

Environmental investigation of the work site did not reveal any possible source of metal or polymer fumes that might have caused an acute febrile illness. There were visible quantities of settled paper dust in the press room. On examination of the humidifier in the press room, the water reservoir, and the baffles through which the atomized water passed were covered with brownish-grey sludge. There was no record of the humidifier having been cleaned in the 19 months since it was purchased. The humidifier was set to operate automatically when the ambient humidity was too low for optimal printing conditions. Press room workers first noticed the humidifier to be running on the morning of June 8, the day of the outbreak. They had not noted it to be in use for the previous 2 months.

The morning of June 9, the owner discovered that the ventilation system had not been functioning. The intake filter was plugged, causing the ventilation system to shut down. This was quickly remedied, and the negative air pressure in the room with the presses was restored before the arrival of VOSHA consultants.

Laboratory

Organisms isolated from the humidifier sludge included 3 species of the fungus *Fusarium,* ameba of the genus *Acanthoemaeba,* and Gram-negative bacteria. The bacteria isolated were *Acinetobacter calcoaceticus, Pseudomonas acidovorans* (2 strains), *Enterobacter cloacae, Pseudomonas luteola,* species of *Flavobacteria* and *Alcaligenes,* and a bacterium identified as CDC group IV C-2.

The results obtained by the ELISA assay for serologic reaction to bacterial extracts are shown in Table III. The prevalence of antibodies in acute-phase sera from workers as a group reflected their exposure to humidifier organisms, when compared with sera from the unexposed comparison group. However, there were no significant differences seen at the $p < 0.05$ level between the prevalence of antibacterial antibodies in sera from workers who became ill, and in sera of workers who did not, nor when comparing acute-phase sera with convalescent-phase sera from ill workers.

In regard to nonbacterial organisms, serologic results did not reliably reflect exposure or illness status. The sera of more ill print shop workers showed at least one positive precipitin reaction to the fungal extracts (6/15, 40%) than did the sera from non-ill workers (3/11, 27%). However, this difference was not significant at the $p < 0.05$ level, and unexposed comparison group sera also showed precipitin reactions

TABLE III. Prevalence of Antibodies to Extracts of Humidifier Organisms in Sera From Workers and From Unexposed Comparison Group as Determined by ELISA; Print Shop, Vermont, 1988

	Ill workers N = 15	Non-ill workers N = 11	Comparison group N = 7
Bacterium	No. (%)	No. (%)	No. (%)
Acinetobacter calcoaceticus	10 (67)	6 (55)	0
Alcaligenes sp.	7 (47)	6 (55)	0
CDC group IV C-2	1 (7)	2 (18)	0
Enterobacter cloacae	6 (40)	3 (27)	0
Flavobacterium sp.	5 (33)	3 (27)	0
Pseudomonas acidovorans 1	0	0	1 (14)
Pseudomonas acidovorans 2	3 (20)	3 (27)	0
Pseudomonas luteola	3 (20)	3 (27)	0

(3/7, 43%). The sera from only four workers showed any reaction to the ameba extract, three of whom met the case definition. None of the serum pairs showed evidence of exposure to *Legionella* by IFA.

DISCUSSION

The illness experienced by the majority of print shop employees working on June 8 appears to have been associated with the resumption of operation of the humidifier in the press room on that day. There was no other environmental agent found that would have been likely to cause the typical short-lived respiratory and systemic reaction seen. There have been no further reports of illness in the print shop, since the humidifier has been cleaned and disinfected on a regular basis.

As is common in outbreaks of illness in a workplace, a set of unusual circumstances contributed to the problem. The unusually dry weather following spring rains caused the poorly maintained humidifier to dispel an aerosol of biologically contaminated water after at least two months of inactivity. The same day, the ventilation system, which usually maintained negative pressure in the press room, was not functioning. Industrial processes involving paper products which produce a dust, have been reported previously as sources of organic material [Pickering et al., 1976; Rylander and Haglind, 1984]. The standing water mixed with cellulose in the reservoir of the humidifier provided a favorable medium for the growth of numerous microorganisms.

While humidifier fever attributed to a hypersensitivity pneumonitis has been reported in association with amebae [Finnegan et al., 1987] as well as with airborne fungi [Banaszak et al., 1970], the humidifier sludge in this outbreak grew numerous species of endotoxin-producing Gram-negative bacteria. It has been shown that the quantity of endotoxin in humidifier reservoir water increases with periods of disuse [Rylander and Haglind, 1984]. Unfortunately, we did not have the opportunity to measure airborne levels of bacterial endotoxin or other airborne microbial agents at the time of the outbreak.

Reports of the clinical effects of endotoxin inhalation on naive subjects in both experimental [Rylander et al., 1989] and outbreak settings [Brinton et al., 1987] have

shown that illness resulted without the subjects having had previous exposure. A dose-response relationship was observed in terms of endotoxin concentration or amount of exposure time in these settings.

The clinical symptoms and the high attack rate for workers in the Vermont print shop were consistent with an organic dust toxic syndrome [doPico, 1986]. An inflammatory reaction to airborne endotoxin from the humidifier was the most likely cause of the illness. However, the results of our epidemiologic investigation suggest the importance of previous exposure to the humidifier contaminants as a risk factor for acute illness. Those who had worked in the print shop for at least 3 months showed a 4-fold increase in relative risk. Since the humidifier was in constant use during the dry winter months and not during the humid spring weather prior to the time of the outbreak, it is possible that the 3-month threshold represents a marker for prior exposure and sensitization to endotoxin or other microbial agents. Further, we were not able to establish a toxic dose-response relationship by comparing primary work station proximity to the humidifier, or in terms of time spent in the press room where the humidifier was located.

The results of our serologic studies reflect previous worker exposure to several bacterial isolates, when compared to unexposed comparison group sera. In reported studies of hypersensitivity pneumonitis, up to 50% of those in exposed populations may show serologic reactions to certain microbial agents, while, generally, $<15\%$ show radiologic or other clinical evidence of pneumonitis [Fink, 1986]. Antibody reactions to environmental microbial antigens may often be seen in completely asymptomatic persons [Burrell and Rylander, 1981; Pickering et al., 1976]. In this investigation, we could not use the serologic data to identify a specific causative agent for the illness, nor to distinguish symptomatic from asymptomatic workers.

The acute illness caused by the inhalation of endotoxin and other microbial agents has been suggested to result from a combination of acute inflammatory and immune-mediated reactions [Holt, 1990]. Threshold values of airborne endotoxin, which cause the specific symptom of chest tightness, have been suggested to be 4–10 times higher for naive subjects than for chronically exposed cotton workers [Rylander, 1990].

The epidemiology of the acute illness that resulted from a single peak exposure to humidifier contaminants in the present worker population supports the relative importance of prior sensitization, while a dose-response effect was not observed. In this print shop setting, further exposure can be minimized by using preventive industrial hygiene measures.

VOSHA consultants recommended that the humidifier be disassembled and thoroughly cleaned with a dilute 1:10 chlorine solution on a weekly basis. The ventilation system would be checked daily to assure proper functioning, and any worker who had a recurrence of symptoms would be advised to see a physician.

Future studies of workers exposed to organic dusts and airborne endotoxin may help to further elucidate the pathophysiologic mechanisms involved, and the role of sensitization in the development of acute pulmonary and systemic reactions.

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