An investigation of operating theatre staff exposed to humidifier fever antigens

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ABSTRACT Sixty staff working in a hospital operating theatre, where a case of humidifier fever had been identified, were studied together with 49 subjects working in other parts of the hospital. They each had a blood test for serology, a skin test, and a chest radiograph and completed a questionnaire. The theatre staff also had pulmonary function tests. The theatre humidifier was found to contain several organisms including amoebae and antigens cross-reacting highly with those implicated in previous outbreaks of humidifier fever. Of the 60 exposed subjects, 25 had developed antibodies, nine had probable symptoms of humidifier fever, and six possible symptoms. There was a strong association between symptoms and antibodies ($p = 6 \times 10^{-5}$ by Fisher's exact test). The development of antibodies was also related to duration of exposure in the theatre ($p < 0.01$ by χ^2 test for trend) and inversely to smoking ($p = 0.0073$ by Fisher's exact test) but not to history of atopy. Because of the presence of antigens and because certain biocides added were ineffective in controlling antigenic build-up the humidifier was switched off. Eight months later specific IgG levels in the theatre staff sera, estimated by an enzyme-linked immunosorbent assay technique, had fallen on average by 25%. Inhalation challenge with humidifier water was performed in eight subjects. Four subjects reacted to the challenge, including both those with antibodies and previous symptoms.

Humidifier fever presents as an influenza-like illness in sensitised individuals some hours after exposure to antigenic material from contaminated humidifiers. It has been considered an example of extrinsic allergic alveolitis¹ in that the time course and certain features of an acute attack are similar and precipitins are present against offending antigens in the humidifier water. Acute attacks of humidifier fever described in published reports, however, have not been accompanied by radiological change,^{1,2} and there is no evidence, as yet, of any chronic changes following the acute disease in Britain. Humidifier fever has also been compared with byssinosis since in both illnesses symptoms tend to occur particularly on a Monday,¹⁻⁴ or on the first day back at work after a break. Byssinosis lacks the characteristic pyrexial episode and serological findings of humidifier fever, however.

Several reports of illness due to exposure to organisms in air-conditioning systems have come

from the United States.⁵⁻⁸ These may well be describing a different disease, since acute, insidious, and chronic forms were found with accompanying radiological changes.^{9,10} Thermophilic organisms were incriminated in these outbreaks,^{5,11,12} but the serological and other responses obtained could be accounted for by false-positive reactions leading to erroneous conclusions.¹³

A nursing sister in our hospital operating theatre was diagnosed as having humidifier fever on the basis of symptoms, lung function changes after working in the theatre, and the presence in her serum of antibodies to antigens from the contaminated water from the humidifier serving the theatre ventilation system.¹⁴ This case led us to investigate the working environment of the operating theatre and the clinical and immunological features of the other staff working there. The aims in the investigation presented here were to determine the proportion of the exposed population that had developed antibodies or symptoms, or both, the clinical and radiological features in those affected, and the long-term effects on serology when antigenic exposure

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Fig 1 Diagram of theatre humidifier.

ceased. We also attempted to study the acute illness in more detail by performing inhalation challenge tests under controlled conditions.

Materials, subjects, and methods

HUMIDIFIER

A single humidifier supplied air at 21°C to four operating theatres and their associated rooms. It was of the static spray type where water taken from a sump reservoir (capacity 600 l) was forced through small orifices forming sprays (fig 1). Filtered air passed through the spray system and water not evaporated fell back into the sump and was recycled. Larger droplets were removed by baffle plates. The system was routinely cleaned every three months but no biocides were added to the water; the intake air filters were changed once a year. The usual temperature of the water in the reservoir tank was about 14°C. Air was taken in from outside and not recycled. The apparatus, installed in 1959, was due for replacement with a steam humidification system.

When the humidifier was initially inspected the tank water appeared reasonably clear, although a carpet of light brown, fluffy material that was slimy to the touch was present on the bottom of the tank. This material and 20 l of the humidifier water were removed and kept at 4°C.

Antigen preparation—Water taken when the humidifier was first inspected was concentrated $\times 1000$ by air dialysis. The concentrate was used per se for gel diffusion studies, diluted $\times 10$ in saline for inhalation challenge, $\times 100$ in carbonate/bicarbonate buffer pH 9.5 for enzyme-linked immunosorbent assay (ELISA) and $\times 330$ in saline for skin testing. Sterilisation was achieved by filtration through a

Millipore (0.22 μ m) filter.

Microbial analysis—Humidifier water was analysed for fungi on Sabouraud's agar and corn meal agar at room temperature; for bacteria on nutrient agar at room temperature, 37°C, and 50°C; and for amoebae by placing a drop of the water on a lawn of *Klebsiella aerogenes* and incubating at room temperature. The water was also observed directly at $\times 100$ magnification for the presence of ciliates, nematodes, and mites, since these have been observed in humidifier sludge from other outbreaks.

Remedial action—Attempts were made to prevent the build-up of antigenic material by mechanical and chemical means. Firstly, simple cleaning was used. This was followed by running water continually out of the sump as quickly as it ran in from the mains supply. It was not possible to run the water to waste since the pressuring pump would have run dry and burnt out. Another method tried was to drain the sump every day and replace with fresh water. These actions were all taken while the humidifier sprays were operational. Biocides were added to the sump when the sprays were not operating thus preventing biocide contamination of the operating theatre atmosphere. Each biocide was left in the water for 12 hours, then the sump was drained and filled with fresh water and the humidifier sprays switched on. Samples were taken three and seven days afterwards for testing. The build-up of antigens was determined as previously described by double gel diffusion. Only partial elimination of antigens was achieved, and the spray system had to be switched off one month after the study began.

STUDY POPULATION

All 60 nursing and technical staff, excluding the index case, were studied. Many of them worked

only part time but some worked 40 hours a week or more in the theatre.

Questionnaires—One physician (AC) interviewed all the staff, using the MRC questionnaire on respiratory symptoms and including an occupational history. In particular the staff were asked about episodes of fever, chills, malaise, arthralgia, or breathlessness after work, and on the basis of these interviews the physician, blind of other results, divided the staff into those with no symptoms, those with possible symptoms, and those with probable symptoms of humidifier fever. Probable symptoms were fever, chills, arthralgia, and general malaise occurring in the evenings after work. Possible symptoms were less specific, such as excessive tiredness or aching legs after work. The staff were interviewed again after the humidifier had been switched off and asked whether their symptoms, if any, had changed.

Radiology—All the theatre staff had a chest radiograph taken. The films were mixed randomly with those from the control population and read, blind of group, by a panel of three experienced readers.

Blood samples—Ten millilitres of venous blood was taken from each subject at the beginning of the study, and the serum was separated and tested as described. The major portion of the serum was stored at -70°C and then compared with a second sample taken nine months later.

Skin tests—Intradermal skin tests were performed on 56 of the staff using 0.05 ml of three times concentrated humidifier water (in practice $\times 1000$ water diluted $\times 330$ in saline). The results were read at 15 minutes, two hours, six hours, and 24 hours.

Pulmonary function tests—The theatre staff undertook pulmonary function tests at the start of the study and three months after the humidifier had been switched off. The tests included measurement of forced expiratory volume in one second and forced vital capacity using a McDermott bellows spirometer, lung volumes by helium dilution, and single breath gas transfer factor.

CONTROL POPULATION

The same doctor interviewed a control group of 49 non-exposed hospital staff. Blood samples and chest radiographs were taken and skin tests performed as for the theatre staff. Pulmonary function tests were not undertaken.

SEROLOGICAL TESTS

Gel diffusion—The sera of both theatre staff and controls were tested by double gel diffusion in agar against a battery of antigens, including antigenic material from previous outbreaks of humidifier

fever, antigenic material from the operating theatre humidifier, and amoebal extracts. The reactions were read as a whole and graded negative, weakly positive, positive, moderately positive, or strongly positive. Weakly positive reactions were barely visible and are of doubtful significance. In addition to the sera from the hospital control group 400 consecutive sera from the area blood bank were tested against the same antigens.

IgG measurement—The first serum samples from the theatre staff were stored at -70°C and then re-tested alongside the samples taken after nine months. On this occasion a single antigenic preparation, that produced multiple precipitin lines on gel diffusion against positive sera, was used in both gel diffusion and ELISA tests.

ELISA technique¹⁵—Micro ELISA trays (Dynatech) were coated with $\times 10$ humidifier water in 0.1M carbonate/bicarbonate buffer pH 9.5 (0.2 ml per well overnight at 4°C) and washed thrice in phosphate buffered saline pH 7.2 with Tween 20 at 0.05% (PBS-Tween). Sera were diluted 1/1000 in PBS and incubated (0.2 ml per well) for 30 minutes at room temperature, then washed thrice with PBS Tween. Alkaline phosphatase-linked rabbit anti-human IgG (Miles-Yeda Ltd), diluted 1/100 in PBS was added for 30 minutes at room temperature and then washed thrice with PBS Tween. Substrate for the enzyme, p-nitrophenyl-phosphate, was added (0.2 ml at 1 mg/ml in pH 9.8 diethanolamine) and incubated for 45 minutes at room temperature. The reaction was stopped by 0.05 ml of 3M sodium hydroxide and the colour produced read by diluting one in five in water and determining absorbance at 400 nm in 2 cm pathlength 1.0 ml volume quartz cells using an SP 800 twin beam spectrophotometer.

INHALATION CHALLENGE

Eight subjects undertook inhalation challenge with humidifier water. There were two non-exposed control subjects and six exposed subjects: two without antibodies or symptoms, two with antibodies but without symptoms, and two with antibodies and with a history of symptoms of humidifier fever. They were each challenged on a double-blind basis with 2 ml of $\times 100$ humidifier water and 2 ml of sterile water on consecutive days in random order. Lung function, body temperature, and white cell count were measured and chest radiographs were taken before challenge and at intervals afterwards.

Results

HUMIDIFIER

Antigens and microbial analysis—Antigenic material in the humidifier water concentrate reacted with

positive sera from previous outbreaks of humidifier fever, and cross-reacted highly with antigens from these outbreaks. Organisms were identified in the tank water, but the plethora of species found in previous outbreaks was not present. In general *Cephalosporium* was the main fungal element. A few Gram-negative organisms were detected but no thermophiles. Amoebae of several varieties were detected, differing in cyst size and in their spreading ability on the *K aerogenes* lawn. Mites, nematodes, and ciliate protozoa, seen in previous outbreaks, were not present.

Remedial action

Mechanical—Draining the humidifier twice a week and cleaning it once a fortnight was not effective in preventing the build-up of antigenic material. The process of running out as much water as was run in was effective but not practicable because of loss of cooling effect when the ambient temperature reached about 21°C. Draining the tank daily and replenishing with fresh water was also effective but labour intensive, therefore impracticable.

Biocides—A biocide with a wide spectrum of activity, Halophan (a bismethylene chlorophenol compound), was used at a concentration greater than that effective against *Pseudomonas aeruginosa*—that is, 250 ppm for 12 hours. This was doubled in a second test but still only partially prevented antigen build-up. Resiguard (a mixture of picloxydine, octyl phenoxy polyethoxyethanol, and benzalkonium chloride) at 250 ppm for 12 hours was also ineffective under the same conditions. Metronidazole, used clinically as an antiamoebic drug, was ineffective at 30 ppm under the same conditions.

STUDY AND CONTROL POPULATIONS

Clinical and immunological findings

Ten of the theatre staff described symptoms considered to be probable attacks of humidifier fever and a further 12 had less specific "possible" symptoms. Some people thought their symptoms were worse at the end of their first day at work after a break but many had not noticed this. Nine of the ten people with probable symptoms and six of the 12 with possible symptoms experienced definite improvement after the humidifier was switched off. Only these 15 cases who improved were counted as probable or possible cases when analysing the results.

Of the 60 theatre staff 25 had definite antibodies to the battery of humidifier fever antigens, 13 had weakly positive antibodies, and no antibodies were detected in the remaining 22 people. There was a strong association between the presence of antibodies

Table 1 Presence of antibodies against humidifier fever antigens and history of symptoms of humidifier fever in 60 subjects

	Antibodies against humidifier fever antigens		
	Negative/weak positive	Positive	Moderate/strong positive
Symptoms			
Negative	33	9	3
Possible	2	3	1
Probable	0	0	9

and a history of symptoms of humidifier fever (table 1) ($p = 6 \times 10^{-5}$ by Fisher's exact test).

Ten of the 60 theatre staff had a history of asthma or hayfever, but there was no association between such a history and the presence of antibodies. People with a history of asthma or hayfever seemed slightly more likely than those without to have symptoms of humidifier fever; five out of 10 compared with 10 out of 50, but this difference was not significant at the 5% level. The staff comprised 27 non-smokers, 17 ex-smokers, and 16 current smokers. Smoking history did not appear to be associated with a history of symptoms of humidifier fever, but fewer current smokers had antibodies than ex- or non-smokers ($p = 0.0073$ by Fisher's exact test) (table 2).

Duration of exposure in the operating theatre, estimated from the number of years worked in the theatre with a correction for working part-time, was associated with the development of antibodies ($p < 0.01$ by χ^2 test of trend) (table 3). The trend was not statistically significant if exposures of less than one year were excluded. Age was associated both with duration of exposure and with development of antibodies. Further analysis showed that even when age was taken into account duration of exposure was still significantly associated with the development of antibodies ($p < 0.05$ by extended Mantel-Haenszel procedure).¹⁶

Concentrated humidifier antigens given intradermally produced reactions in several volunteer non-exposed subjects; when the material was diluted $\times 330$ with sterile saline these subjects

Table 2 Smoking history related to antibodies against humidifier fever antigens in 60 subjects

	Smoking history		
	Non-smokers	Ex-smokers	Current smokers
Antibodies			
Negative/weak positive	14	7	14
Positive	6	4	2
Moderate/strong positive	7	6	0

Table 3 Duration of exposure in operating theatre and antibodies against humidifier fever antigens in 60 subjects

Duration of exposure (yr)	Antibodies against humidifier fever antigens		
	Negative/ weak positive	Positive	Moderate/ strong positive
< 1	17	2	0
1-4	10	6	5
5-9	7	3	5
≥ 10	1	1	3

did not react. This antigenic preparation, equivalent to $\times 3$ humidifier water, was then used to skin test the theatre staff and hospital control group. Of the 60 theatre staff, 56 had a skin test with the $\times 3$ humidifier water preparation. The reaction was positive at 15 minutes, with swelling and erythema in 14 people; doubtful in 13; and negative in the remaining 29. No delayed or dual reactions occurred. All but two of the positive skin reactions were in people with strongly positive antibodies and the association between skin reactivity and serology was statistically significant ($p < 0.01$ by χ^2). Skin tests and serology combined correlated no better with symptoms than did serology alone.

The control group of 49 non-exposed hospital staff were similar to the theatre staff in age, sex, and smoking habits. Seven of them described symptoms such as vague tiredness or aching legs after work, but none described probable humidifier fever symptoms. The sera of five of them gave weakly positive reactions against humidifier fever antigens, and the remaining 44 did not react. None of the control subjects gave a positive reaction to intradermal $\times 3$ humidifier water. Of the 400 blood bank sera tested, less than 4% gave weakly positive reactions against humidifier fever antigens, the remainder giving no reaction.

Pulmonary function and radiology

The results of the pulmonary function tests in the theatre staff were within normal limits initially and were unchanged when repeated three months after the humidifier had been switched off. Two cases, both known to have chronic bronchitis and neither with symptoms or antibodies of humidifier fever, had a mild obstructive ventilatory defect. One man had abnormal results related to previous thoracic surgery.

The panel of three experienced readers reported the chest radiographs as normal in all subjects except in six cases (three theatre staff and three controls) with previously known lung disease, such as old tuberculosis. No chest radiograph in either

group was considered to show features of interstitial disease.

CHANGES IN ANTIBODY LEVELS

Fifty-four of the theatre staff had a repeat blood sample taken after nine months, six having left the hospital. By this time there had been no antigenic exposure in the theatre for eight months. In 50 of these cases most of the initial serum sample had been stored at -70°C and was available to test alongside the second sample.

The gel diffusion studies performed with a single antigenic preparation gave results similar to those performed using a battery of antigens, although the results for individuals tended to be lower as one would expect.

There was a good correlation between the results of gel diffusion tests and the "levels" of specific IgG antibody estimated by the ELISA technique ($p < 0.01$ by a median test) (fig 2).¹⁷ The tests were performed concurrently, using the same antigenic preparation. Comparison of parts (a) and (b) of fig 2 shows that the levels of antibody as estimated

Fig 2 Antibodies estimated by gel diffusion and specific IgG antibody levels estimated by ELISA technique in 50 cases with paired sera. (a) Initial sera, (b) sera at nine months. White blocks represent negative and weak positive precipitins on gel diffusion, hatched blocks positive precipitins, and black blocks moderate and strong positive precipitins.

Fig 3 Specific IgG antibody levels, estimated by ELISA technique, in 50 theatre staff with paired sera. Initial values v values at nine months. Solid line represents initial mean level v nine-month mean level. Dashed line is line of equality.

by the ELISA method fell noticeably over the nine-month period between the serum samples, whereas the numbers of sera in each precipitin category as estimated by gel diffusion were little changed. Thus the ELISA method seems the more sensitive index of changes in antibody levels, as might be expected.

Figure 3 illustrates the fall in antibody levels as estimated by the ELISA technique over the nine-month period in the 50 subjects from whom paired sera were available. With the exception of 11 subjects with low levels, all the subjects had a fall in antibody levels. The average level fell from 0.8 to 0.61 absorbance units at 400 nm, a mean fall of almost 25% in eight months without antigenic exposure.

INHALATION CHALLENGE TESTS

The inhalation of humidifier water produced reactions in four of the eight subjects. Neither of the non-exposed controls reacted, one of the exposed subjects without antibodies or symptoms reacted, one of the exposed subjects with antibodies but without previous symptoms reacted, and both of the exposed subjects with antibodies and with previous symptoms reacted. The reactions were mainly systemic with fever, chills, arthralgia, headache, and general malaise, maximal between 10 and 12 hours after challenge. Accompanying changes in lung function were relatively mild and consisted

mainly of a fall in vital capacity without evidence of large airways obstruction and with no fall in gas transfer factor. These results are reported in more detail elsewhere.¹⁸ Chest radiographs taken at the height of the reaction showed no abnormalities.

Discussion

In this investigation 25 people in a population of 60 exposed to humidifier fever antigens developed precipitins; 15 had probable or possible symptoms of the illness; and all but two of these had antibodies (see table 1). Of the exposed people with antibodies, 12 had no symptoms of the illness. Thus 42% of the exposed population developed antibodies, and about half of these had symptoms. The development of antibodies was unaffected by atopic status, as judged by questionnaire, but precipitins were more often present in non- or ex-smokers than in current smokers, as has been found by Belin.¹⁹ This may reflect some reduction in the capacity of smokers to respond to antigenic stimuli^{20 21} and is in agreement with findings in farmer's lung.²² Increasing duration of exposure also increased the likelihood of developing antibodies independent of the effect of age. This has been shown in pigeon breeders²³ but has not previously been shown with regard to humidifier fever.

Studies of previous outbreaks of humidifier fever in Britain have been reported. In a printing works in Nottingham, Pickering *et al*² found nine cases of illness associated with a contaminated air conditioning system. All nine cases had antibodies to humidifier material as did 75% of similarly exposed workers who were asymptomatic. Friend *et al*¹ reported on 24 cases of humidifier fever in a stationery factory in Aberdeen: 23 had antibodies to freeze-dried extracts of contaminated water from vacuum pumps used in the factory. Five out of 30 exposed asymptomatic workers also had antibodies. In an outbreak of humidifier fever in a factory using rayon,³ 20 of 50 office workers had symptoms but there were no symptoms among the factory-floor workers. Sixteen out of 18 of the symptomatic individuals had antibodies to material from the contaminated humidifier, and 44% of the exposed, asymptomatic factory-floor workers also had antibodies.

All these reports have indicated an association between symptoms and antibodies, almost all symptomatic cases having antibodies and a varying proportion of exposed, asymptomatic individuals having antibodies. Our results are in general agreement with the previous reports but, in addition, have shown a negative association between smoking

and the development of antibodies. The varying proportions of exposed individuals developing antibodies and symptoms may be related to the form and intensity of antigen exposure.

It seems that the antigens implicated in the various outbreaks are similar, as materials from different outbreaks show a high degree of cross-reactivity.²⁴ Although the antigens are not identical they are related and possibly come from a common source. The most likely organisms seem to be water-borne, cyst-forming amoebae.²⁵ Previous outbreaks have implicated organic materials, such as paper or rayon, on which the colonising organisms can become established. In the present case no such organic material was present, which may explain the relative paucity of contaminating organisms compared with other outbreaks.

The previously reported outbreaks of humidifier fever in Britain have noted the periodicity of symptoms in affected individuals.¹⁻³ The symptoms tended to be worse on a Monday evening or on the evening of the first day back to work after a break. In the theatre staff with symptoms there was usually no clear periodicity. This is probably related to the part-time nature of the work for many of them, who might, for example, work Monday, Thursday, and Friday each week. The index case, who worked Monday to Friday, had symptoms mainly on Monday evenings.

The condition that has been described in association with contaminated air conditioning systems in the United States seems to be different from the disease "humidifier fever" described in Britain. In American reports thermophilic organisms have been isolated from the air conditioning systems and implicated as allergens in the disease.^{11,12} It is possible that erroneous conclusions with respect to thermophilic organisms causing humidifier fever have been drawn, as reported elsewhere.¹³ The water in the humidifier in the present outbreak was cold (<20°C), and thermophilic organisms were, not surprisingly, absent. The water temperature in at least one of the other described British outbreaks was also low.¹ The illness described in the United States resembles an extrinsic allergic alveolitis with x-ray changes during acute attacks^{5,6} and progression to chronic disease with x-ray and lung function abnormalities after prolonged exposure.¹⁰ No x-ray changes have been noted during natural or induced attacks of humidifier fever, and there is no evidence so far of any persisting lung function or radiological abnormalities even after exposures of some years.^{1,2} In the present outbreak very few people with exposures of less than one year (at 40 hours a week) developed antibodies (table 3). It is not possible to estimate accurately

when the humidifier became contaminated, but antigenic exposure has probably been occurring for several years.

The occurrence of humidifier fever in a further type of work place, previously unsuspected, raises the question of how common the condition may be. We found no definite antibodies in 400 blood bank sera, so exposure to antigens in the general population is not very common. The condition, however, could be responsible for ill health in populations working in environments served by humidifiers. The symptoms may be mild and sufficiently non-specific to escape much mention. When the condition is suspected the humidifier should first be examined and tested for the presence of humidifier fever antigens, and if these are present the exposed population should then be examined clinically and immunologically. Preventing further antigenic exposure can be achieved, but ultimately it may be necessary to install a different type of humidifier.

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