NAVIGATING THE COMPLEXITIES OF MOULD EXPOSURE IN DAMP BUILDINGS: A CASE REPORT ON CHALLENGES AND POTENTIAL SOLUTIONS

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ABSTRACT

The increasing presence of moulds in workplaces poses significant occupational health risks, particularly in poorly maintained structures. Insufficient attention is given to dealing with this emerging issue; therefore, it is imperative to understand mould-related health effects and remediation strategies to ensure a safe and healthy work environment. This case investigation aimed to establish an association between employee symptoms and moulds in a damp building. An environmental assessment was undertaken to identify visible signs of water damage and identify mould species in air and surface samples. Information on mould exposure, building-related symptoms and predisposing factors was gathered through an online self-administered questionnaire. Serum samples were collected from the index cases and controls to determine possible atopy and hypersensitivity reactions to moulds. The walkthrough revealed water-damaged walls, visible mould growth and suboptimal maintenance of the plumbing system. Environmental mould species, including *Cladosporium*, *Aspergillus* and *Penicillium*, were identified. The most common symptom reported was headache, followed by a pressing sensation on the scalp, a lack of concentration and fatigue. Most of the workers were atopic, and specific IgE tests yielded negative results for all workers except one positive for *Alternaria alternata*. Elevated sIgG antibody levels were detected for *Cladosporium* and *A alternata* species, linking exposure to at least one mould species identified in the work environment. This case highlights the importance of employing appropriate serological tools to investigate mould exposure. Furthermore, it underscores the challenge of interpreting laboratory results without standardised reference values, which may have an impact on accurate diagnosis and case management, in turn emphasising the need to establish local IgG reference ranges. The investigation also raises awareness of effective case management to prevent adverse health effects related to mould sensitisation in occupational settings.

Keywords: indoor air quality, bio-aerosols, mould sensitisation, water-damaged building, atopy, exposure assessment

INTRODUCTION

Occupational and environmental health professionals often encounter building-related complaints with symptoms arising from biological exposures in indoor environments. Moulds proliferate in building structures with high humidity or dampness, poor ventilation, structural deficiencies, water damage and leaks.[1] Among these exposures, indoor moulds have gained prominence due to their multifarious and ubiquitous nature, the four genera that are most commonly associated with health effects are *Alternaria, Cladosporium, Penicillium* and *Aspergillus*.[2] Mould exposure in the workplace is considered to be prolonged and extensive, raising concerns about occupational risks and health effects.[3] Whereas some conditions such as allergies, infections, sick building syndrome (SBS) and building-related illness (BRI) are well understood, others such as neurological effects, mixed mould toxicosis (MMT), and dampness and mould hypersensitivity syndrome (DMHS) remain the subject of ongoing research.[4]

Health complaints often encompass symptoms such as mucous membrane discomfort, headaches and fatigue. These symptoms are collectively recognised as manifestations of SBS, particularly when no specific diagnoses such as asthma or rhinitis can be established. Conversely, BRI occurs when one or more building occupants experience health problems and the clinical causality is linked to indoor environment factors. These

conditions often improve with allergen avoidance and appropriate medical treatment.[4] Mould spores and/or hyphae may cause immunoglobulin E (IgE)-mediated reactions, leading to conditions such as allergic rhinitis (AR), rhinosinusitis, conjunctivitis or allergic asthma.[5,6] Previous studies have estimated a population prevalence of 10% IgE antibodies to inhalant moulds, with 5% likely to develop clinical symptoms.[7] Allergic occupational rhinitis (AOR) is a public health concern due to its prevalence, costs and the negative impact it has on quality of life (QoL) and work abilities.[8,9] AR is a symptomatic IgE-mediated inflammatory disease of the nose that is consistently associated with dampness and indoor mould exposure in epidemiological studies.[1] Allergic reactions to moulds can range in severity and may be aggravated by the irritant effects of exposure. Asthma triggered by mould allergens generally manifests an hour after exposure, featuring symptoms such as chest tightness, wheezing, coughing and dyspnoea that worsen with allergen exposure.[2] Allergic bronchopulmonary aspergillosis (ABPA) represents a rare immunologic and inflammatory lung disease associated with sensitisation to *Aspergillus* species, typically *Aspergillus fumigatus*, which proliferates in the bronchi of individuals with asthma.[1,2] ABPA has been reported in cases of BRI, affecting individuals with conditions such as immunosuppression and chronic obstructive pulmonary disease (COPD).[2]

Beyond these, other immunological responses include non-IgE reactions such as hypersensitivity pneumonitis (HP), infectious diseases such as aspergillosis, and irritant and toxic effects stemming from mycotoxins, microbial volatile organic compounds (MVOCs) and fungal glucans.[3,6,8,10,11] HP, a complex lung condition primarily associated with mould exposure in occupational settings, involves an exaggerated immune reaction upon inhaling mould spores or their constituents. The ensuing lung-tissue inflammation is characterised by a combination of type-III and type-IV hypersensitivity reactions that result in lung parenchymal inflammation, granuloma formation and, in some cases, perpetual damage and fibrosis in response to the repetitive inhalation of a sensitised allergen.[12–16] Moulds such as *A alternata, Aspergillus, Cladosporium* and *Mucor* are common causative agents of HP, of which *A alternata* is mostly involved in occupational HP.[14]

In certain instances, some moulds can produce various metabolites, including MVOCs, which may lead to central nervous system (CNS) symptoms such as headaches, dizziness or impaired concentration.[2] Exposure to toxin-producing moulds indoors has been associated with neurotoxic effects; however, there is no consistent evidence, and cognitive problems have been causally linked to mycotoxins.[1,17] Although in vivo studies have demonstrated that mycotoxins have immunomodulatory and concentration-dependent cytotoxic effects, the exact mechanisms linking mould exposure to these symptoms are not well established; therefore, further research is needed to confirm these associations.[1] Notably, conditions such as MMT and DMHS are not widely accepted in the scientific community, which sparks ongoing debate about their validity. The term 'MMT' is used in Functional Medicine to describe a collection of symptoms attributed to exposure to various moulds and mycotoxins. In some cases, symptoms begin initially with mild mucosal irritation; however, the disease may become chronic over time, presenting challenges in diagnosing DMHS.[18] Some individuals claim to experience a combination of symptoms, including those related to respiratory, allergic and neurological issues in damp or mouldy environments. Therefore, the specific clinical definitions and mechanisms underlying these conditions are still subject to investigation.[19] Owing to a lack of evidence of the causal relationship and accepted diagnostic criteria, mould toxicosis and DMHS remain contentious debates.[1,2,17]

The susceptibility of individuals to fungal spore exposure varies greatly and atopic patients are generally more sensitive to moulds than non-atopic individuals.[1,4] While a linear causal relationship between mould species and health complaints is rare, indoor mould growth must be considered a potential health risk and the immediate remediation of water moisture or water damage is crucial to effective prevention and health promotion.[1,18] The immunological reaction can involve an allergic hypersensitivity, usually IgE-mediated, but may also involve immunoglobulin G (IgG) antibodies.[20] Therefore, quantifying serum precipitin (IgG antibodies) levels against potential mould via technologies such as ImmunoCAP may provide useful information on exposure in patients with hypersensitivity symptoms and evidence of mould growth in the working or living environment.[20]

Understanding mould exposure scenarios, typical symptoms and the association pattern between exposure and symptoms enables patients to be managed clinically in a timely manner. Environmental assessment and immunological tests can be useful tools with which to measure the potential risk of exposure, especially for sensitised and vulnerable workers.

The following case report highlights the complexity of linking identified moulds in a water-damaged workplace to reported symptoms and serum antibody levels among office-bound workers. It also emphasises the importance of appropriate laboratory test selection in identifying the source of exposure, which can often be challenging. The clinical diagnosis of disease related to the occupational exposure of affected workers is not covered by this case report, but the possible effects are mentioned where it appeared reasonable.

CASE REPORT

The Occupational Allergy Clinic of the National Institute for Occupational Health (NIOH) investigated a case involving two medical practitioners presenting with respiratory symptoms, headache, fatigue and intracranial congestion. They provided medical consultation services in an office-bound setting, conducting weekly clinic consultations. The employees spent a significant portion of their workday in the office, which was located in close proximity to the clinic and was suspected to be the cause of their health problems. The following sections detail the occupational and environmental health investigation conducted from April to July 2022, including the workplace characteristics, reported health complaints, environmental assessments and immunological tests conducted to explore the potential impact of mould exposure on their health.

ENVIRONMENTAL ASSESSMENT

The investigation was conducted in two buildings, categorised as the 'index building' (moisture damaged) and the 'reference

TABLE I: CHARACTERISTICS OF THE PARTICIPANTS INVESTIGATED AS REPORTED IN THE QUESTIONNAIRE

VARIABLE DESCRIPTION	STATISTIC
Gender (*n*, %)	
Male	3 (37.5)
Female	5 (62.5)
Age in years (m, SD)	46 (11.4)
Smoker (*n*, %)	
Yes	0 (0.0)
No	8 (100.0)
Type of profession (*n*, %)	
Medical practitioner	4 (50.0)
Nurse	2 (25.0)
Administrator	1 (12.5)
Researcher	1 (12.5)
Duration in current position (m, SD)	7 (5.08)
Complaint (*n*, %)	
Intermittent odour	3 (37.5)
Volatile organic compounds	1 (12.5)
Chemical vapour	1 (12.5)
Ventilation (*n*, %)	
Windows	7 (87.5)
Doors	1 (12.5)

Key: m – mean, SD – standard deviation

building' (no water damage), based on visible observations of moisture and mould growth. A site inspection was conducted by a multidisciplinary team comprising microbiologists, a health and safety officer and an occupational medicine specialist. A walkthrough assessment was done in the basement of the four-floor index building and on the second floor of the reference building. In the index building, five areas, including three offices, one X-ray data-filing room, and two clinics, exhibited water damage on the walls, whereas the reference building – an office that showed no signs of water damage – was selected for sampling. The identified areas in both buildings depend on air conditioning and natural ventilation.

MICROBIAL CHARACTERISATION

Surface and air samples were collected to confirm the presence and source of viable mould. The MAS 100 air sampler (Merck Pty, Germany) was used for the collection of moulds by an impaction method on Sabouraud dextrose agar and chloramphenicol agar (SDA+ chlor) (Diagnostic Media Products, South Africa). The total sampling flow rate was 100 L/min. A total of 14 duplicate samples were collected, including the two reference samples (outdoor and office considered low/no risk in the reference building). Wall scrapings from the water-damaged areas with visible mould were collected in sterile specimen bottles (Lasec, South Africa). A swab sample was also taken from a wall in the reference building.

HEALTH ASSESSMENT

The health assessment included the two index cases and six controls, that is, workers in the same work vicinity. Individuals were classified as controls if they were employed in the same department as the cases in the index building and did not report any work-related symptoms through the company's self-reporting occupational health and safety information system.

QUESTIONNAIRE

An online self-administered questionnaire using Google Forms was issued to cases and controls.[21] The tool included questions on medical, smoking and occupational history. The questionnaire was administered in English. In accordance with a previous study, symptoms were considered 'while at work' if they occurred once a week or more at work for the past four weeks. Symptoms that occurred once a week or more for the past four weeks but improved when away from the building were considered 'building-related'.[22] The following four symptom categories were used for symptoms reported 'while at work' or being 'building-related':

1. 'Multiple atopic symptoms' (all three of the following: sneezing, itchy eyes, runny nose).
2. 'Multiple sick-building syndrome symptoms' (at least three of the following: headache, sore or dry throat, nasal congestion, unusual fatigue, irritated eyes).
3. 'Multiple respiratory symptoms' (at least three of the following: shortness of breath, cough, chest tightness, wheezing).
4. 'Multiple neurologic symptoms' (at least two of the following: headache, concentration problems, dizziness).

IMMUNOLOGICAL TESTS

The immunological assessment was performed on five symptomatic individuals identified through the questionnaire. Blood samples (serum-separating tube (SST) gel tubes, Lasec, South Africa) from five cases and controls were sent to the NIOH Occupational Allergy laboratory for specific (sIgE) and specific IgG (sIgG) testing. Sera were separated from the blood samples by centrifuging at 1 200 g for 10 minutes and stored at –20 ºC until analysis was undertaken for sIgE and sIgG antibodies against commercial and workplace-specific moulds.

LABORATORY ANALYSIS

MOULD CULTURE IDENTIFICATION

After air sampling, the agar plates were incubated at 25 ºC ± 2 ºC for seven days to allow mould growth. Wall scrapings containing 2 mL sterile water were mixed for 1.5 minutes before culturing onto Sabouraud Dextrose Agar and chloramphenicol (SDA + Chlor) (Diagnostic Media Products, South Africa). The surface sample plates were also incubated at 25 ºC ± 2 ºC for seven days until growth was observed, and moulds were identified at genus and species level using lactophenol cotton blue stain, macroscopic (visual morphological characteristics) and phenotypical characteristics using an Olympus microscope (Olympus BX43F, Japan) at 100× magnification. The presumptive identification of mould species was made using reference books.[23] Negative control (sterile agar media) and positive control (*A niger*) for moulds were included in the analysis for quality control. The outdoor sample was used as a reference sample as there is no occupational exposure limit (OEL) for biological agents.

SERUM ANALYSIS FOR SPECIFIC IgE AND IgG DETECTION

The employees' sera were tested for sIgE and sIgG to mould allergens (*Penicillium chrysogenum* (m1), *Cladosporium*

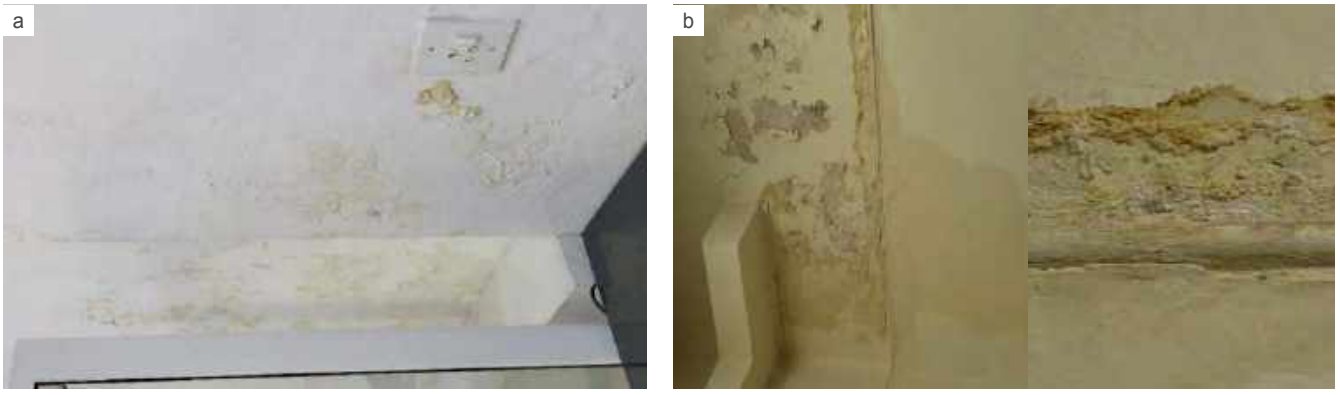

Figure 1: Photographs of visible mould on wall surfaces of areas investigated: (a) clinic room wall with mould growth and (b) X-ray file data storage room.

herbarum (m2), *A fumigatus* (m3), *A alternata* (m6), *Aspergillus flavus* (m228)) following the manufacturer's instructions using the Phadia 250 system, which employs an automated fluorescent enzyme immunoassay (FEIA) (Thermo Scientific®, Uppsala, Sweden).[24] The atopic profile was assessed with the Phadiatop mix following the manufacturer's instructions. Individual sIgE allergen results are expressed in kilounits of allergen (kUA) and those of the Phadiatop mix in Phadia Arbitrary Units per litre (PAU/L). Antibody concentrations ≥0.35 are considered to be positive results. The IgG concentrations are expressed in milligrams of antigen-specific IgG per litre (mgA/l). The manufacturer does not have a dogmatic interpretation of sIgG antibody test results and recommends that laboratories establish their reference ranges. In this case, a report published on expected sIgG reference ranges for m1, m2, m3 and m6 used was consulted to compare our results.[13] The specific IgG cut-off value for *A flavus* (m228) reported by Huang et al was used in this study.[25]

INHIBITION ANALYSIS

In order to determine cross-reactivity between identified mould species, antibody inhibition of *A alternata* positive serum was performed against the other mould species (*Cladosporium species*, *A fumigatus*, *A flavus*, *Penicillium* spp) isolated from the workplace, according to the method described by Phadia.[26] Protein from the mould samples was extracted in PBS pH 7.4 using the standard laboratory method. Briefly, 500 µl of PBS was added to 500 µg of lyophilised samples of each mould. These were extracted by shaking overnight at 500 rpm at 2 to 8 °C. The extracts were centrifuged at 1 200 g for 15 minutes. Protein concentrations of the supernatant were estimated using the Bio-Rad method.[26] Equal amounts of 100 µg/mL extracts of the four moulds and buffer samples were incubated with the *A alternata* positive serum for 60 minutes. The combined sera and extracts were tested for sIgE to *A alternata* (m6) using the abovementioned method.

The percentage inhibition was calculated as follows:

$$\text{Inhibition, \% at conc } \chi = \frac{(\text{Response}_{(0\%)} - \text{Response}_{(\chi)})}{(\text{Response}_{(0\%)} - \text{Response}_{(100\%)})} \times 100 \text{ (Phadia, 2004)}$$

RESULTS

DEMOGRAPHIC CHARACTERISTICS OF CASES AND CONTROLS

Of the eight individuals, 37.5% were male and 62.5% female. All the respondents (100%) reported that they were non-smokers. The job types were variable and included: medical practitioners (50%), nurses (25%), administrators (12.5%) and researchers (12.5%) (see Table I). The cases spent most of their time performing administrative work, including computer work, phone calls and consulting patients on clinic days. The environmental complaints included intermittent smell or unidentifiable odour and volatile organic compounds or chemical vapour. The symptomatic individuals indicated that their symptoms improved when away from and soon after leaving the building, at home or over the weekend (37.5%). Ventilation was reportedly mainly through doors (75%) and windows (25%).

None of the cases nor any of the controls had multiple atopic symptoms (sneezing, itchy eyes, runny nose) or multiple respiratory symptoms (shortness of breath, cough, chest tightness, wheezing). Index case 1 reported a pressing sensation on the scalp, dizziness, weakness in the legs, difficulty with concentration and hoarseness of voice, dry throat, nausea, poor concentration and memory. Index case 2 reported fatigue, constant headaches, poor concentration and intracranial congestion. Both cases had multiple neurological symptoms. None of the workers had symptoms consistent with 'multiple atopic symptoms' or 'multiple respiratory symptoms'. Half of the controls experienced only one symptom, specifically intermittent headaches; however, they did not meet the criteria for MSMSs or MNs.

MOULD IDENTIFICATION OF AIR AND SURFACE SAMPLES

Mould was evident indoors (see Figure 1), and outer walls with broken gutters were observed during a walkabout. *Cladosporium* and *Aspergillus* species were found in all the air samples, including outdoor samples. *Aspergillus* spp was the most common fungal species isolated from wall scrapings in all the areas except one room (control office with no water damage) and was also detected in the outdoor sample. *A fumigatus* was detected in two of the six (33.0%) areas sampled. *Penicillium* spp were isolated from five areas, including the clinics, but not from the index case office and outdoors. No growth was detected from the wall swab sample collected from the reference office. Most indoor fungal counts were less than those for outdoors (590 cfu/m^3) except for the X-ray room (640 cfu/m^3) and the index case's office, which had the highest fungal concentration (too numerous to count).

TABLE II: SYMPTOMS REPORTED BY INDIVIDUALS SUSPECTED OF EXPOSURE TO MOULDS

SYMPTOMS		INDEX CASES		CONTROLS					
		1	2	1	2	3	4	5	6
MSBSs	Sore or dry throat	●	–	–	–	–	–	–	–
	Nasal congestion	–	–	–	–	–	–	–	–
	Unusual fatigue	–	●	–	–	–	–	–	–
	Irritated eyes	–	–	–	–	–	–	–	–
	Headache	–	●	–	●	●	●	–	–
MNs	Headache	–	●	–	●	●	●	–	–
	Concentration problems	●	●	–	–	–	–	–	–
	Dizziness	●	–	–	–	–	–	–	–

Key: MSBSs – Multiple sick building syndrome symptoms (at least three), MNs – Multiple neurologic symptoms (at least two), (–) did not report this symptom.

IMMUNOLOGICAL TEST RESULTS

Serum sIgE antibody results against the five moulds are shown in Table III. Phadiatop results were positive for 80% (4/5) of the sera tested and one worker was non-atopic. Specific IgE to mould allergens (m1, m2, m3, m6 and m228) was negative for all the workers except index case 2 (C2), who tested positive for *A alternata* (m6). Specific IgG was determined for potential screening of mould exposure. Two of the five employees (C2 and Cl3) had elevated sIgG antibody levels, significantly different from the other three, to all five tested moulds (see Table III). The calculator tool described by Raulf et al (2019; https://www.ipa-dguv.de/ipa/publik/litinfos/immunocap/index.jsp) was used to classify specific IgG values from patients with a suspicion of HP. Based on the calculation, C2 and Cl3's results for *P chrysogenum* (m1) and *A fumigatus* (m3) were not conspicuous. However, the sIgG to *C herbarum* (m2) was higher than 97% of the German controls for both C1 and Cl3. In addition, for *A alternata* (m6), Cl3's result is higher than 99.1%, and C2's result is higher than 100% of the German controls (Raulf et al, 2019).[13] The results for both C2 and Cl3 for *A flavus* were also higher than the reported cut-off value.[25]

The percentage inhibition of >70% or >75% is regarded as showing cross-reactivity.[27,28] In this study, the percentage inhibition for all four moulds was less than 10%. This result means that, most likely, there is no cross-reactivity between the antibodies against the four moulds and the anti-*A alternata* antibodies. This is not surprising, because the antibody levels to these moulds were very low, that is, *A flavus* (0.04 kUA/L), *P notatum* (0.03 kUA/L), *C herbarum* (0.16 kUA/L) and 0.27 kUA/L for *A fumigatus*.

DISCUSSION

This case investigation explored workers' exposure to mould in a non-industrial building, focusing on screening tools and the challenges of interpreting serological tests without standardised reference values. The index building is an aged structure with enduring signs of moisture damage on the internal and external wall surfaces, primarily due to leaks in the roof and plumbing and poor or inadequate drainage. The findings revealed that airborne viable moulds were present in the index and reference buildings. Identifying *Cladosporium* and *Aspergillus* species in all the air samples, including the reference sample, provides strong evidence in support of the possibility of an outdoor source of contamination. The presence of these commonly isolated species indoors and outdoors further suggests that there was no specific source of contamination within the indoor environment. However, the absence of these mould species (*Penicillium* sp and *A fumigatus*) in the outdoor samples, but which were found in the indoor samples, indicates the potential presence of an indoor source of contamination. This highlights the potential health risks to vulnerable occupants in the building. It is worth noting that *Cladosporium* spores are commonly found in both indoor and outdoor air, with *C herbarum* being one of the most extensively studied fungal species in allergy research along with *A fumigatus* and *A alternata*.[29,30] *A fumigatus*, in particular, is recognised as the most common allergenic strain of the *Aspergillus* genus.[2] Moulds such as *Penicillium*, *Cladosporium* and *Alternaria*, which require low to medium moisture, are considered relevant outdoor mould species.[3,4] The presence of water stains and visible mould on the walls due to water intrusion in the building was evident at the time of sampling; this supports the existence of an environmental problem that probably contributed to the mould growth. The fact that management was aware of employee complaints and initiated the investigation reinforces the seriousness of the matter. Various health effects, including allergy, infection, irritation and toxic reactions, can result from mould growth in water-damaged buildings.[4,8] Studies have consistently demonstrated the abundance of mould spores in the environment, making them underestimated sources of respiratory allergens and potential triggers of airway diseases.[2,4]

Since *A alternata* was not isolated during this investigation, we cannot conclusively link it to workplace exposure. Previous reports have suggested that although *A alternata* is a major environmental allergen, sensitisation is not always associated with occupational exposure.[31]

Sensitisation to *A alternata* may also trigger co-sensitisation to the allergen sources; therefore, understanding the immunological mechanism will help to improve allergy diagnostic methods.[32] Furthermore, *A alternata* allergens have homologues

TABLE III: SPECIFIC IGE AND IGG TEST RESULTS OF FIVE EMPLOYEES

Identification	Sex	Phadiatop mix	*Penicillium chrysogenum*		*Cladosporium herbarum*		*Aspergillus fumigatus*		*Alternaria alternata*		*Aspergillus flavus*	
		PAU/L	sIgE kUA/L	sIgG mgA/L	sIgE kUA/L	sIgG mgA/L	sIgE kUA/L	sIgG mgA/L	sIgE kUA/L	sIgG mgA/L	sIgE kUA/L	sIgG mgA/L
C1	Male	**2.57 +ve**	0.00 –ve	2.44 –ve	0.00 –ve	10.50 –ve	0.01 –ve	3.30 –ve	0.01 –ve	6.92 –ve	0.01 –ve	3.01 –ve
C2	Male	**1.59 +ve**	0.03 –ve	**37.20 +ve**	0.16 –ve	**51.70 +ve**	0.27 –ve	**46.00 +ve**	**8.00 +ve**	**21.40 +ve**	0.04 –ve	**55.40 +ve**
CI3	Female	**19.2 +ve**	0.06 –ve	**28.10 +ve**	0.01 –ve	**46.30 +ve**	0.02 –ve	**45.30 +ve**	0.02 –ve	**14.90 +ve**	0.02 –ve	**36.00 +ve**
CI4	Female	**2.36 +ve**	0.00 –ve	6.39 –ve	0.02 –ve	10.00 –ve	0.01 –ve	9.10 –ve	0.01 –ve	3.14 –ve	0.01 –ve	10.00 –ve
CI5	Male	0.11 –ve	0.06 –ve	13.30 –ve	0.05 –ve	7.36 –ve	0.07 –ve	16.90 –ve	0.10 –ve	3.93 –ve	0.09 –ve	6.32 –ve
Cut-off values		**0.35[a]**	**0.35[a]**	**27[b]**	**0.35[a]**	**37[b]**	**0.35[a]**	**39[b]**	**0.35[a]**	**12[b]**	**0.35[a]**	**22.1[c]**

Key: C – index case, CI – control, [a] Thermofischer cut-off values, [b] Raulf et al cut-off values, [c] Huang et al cut-off value. Note that the former name for *P chrysogenum* was *P notatum*.

with other important allergenic moulds, such as *Aspergillus*, *Penicillium* and *Cladosporium*,[3,5,13,32–34] which were detected in environmental samples in this study, making the interpretation of the results difficult. There is also evidence of cross-reactivity between *A alternata* antigenic proteins Alt a 3, Alt a 6 and Alt a 8, *Cladosporium*; however, further immunological tests such as specific component analysis and inhibition are needed for confirmation.[2] This study did not pursue component-resolved diagnosis, as the components are unavailable for ImmunoCAP testing. Makkonen et al[35] showed a significant correlation between several mould species, including *A fumigatus* and *Cladosporium cladosporioides* (0.669, $p < 0.001$), *A niger* (0.918, $p < 0.001$), *Penicillium* spp (0.849, $p < 0.001$); *C cladosporioides* and *A niger* (0.646, $p < 0.001$), *Penicillium* spp (0.588, $p < 0.001$); and, finally, between *A niger* and *Penicillium* spp (0.905, $p < 0.001$).[35]

The use of serology for diagnosing mould-related diseases is complex and different criteria must be fulfilled.[1,3,18] Considering the seasonal variation in the dissemination of mould and the possibility that the investigation was conducted outside the peak of *A alternata* presence, it is plausible that the species was not detected during sampling.[32] In addition, the time lapse between exposure and investigation might have resulted in missed detection, as complaints started in late January to early February of 2022 and sampling occurred only in April 2022. Given the dynamic nature of mould infestation, different species may vary at different periods, further complicating the detection process.[18] Consequently, sensitisation to *A alternata* in these highly sensitised individuals may not necessarily be attributed to workplace exposure.[3]

The findings from this investigation suggest that more workers experienced symptoms than just the initial case and that the symptoms improved when they were away from the building, indicating possible work-related exposure. This phenomenon aligns with previous studies where symptoms improved after leaving the building, resulting in the expression 'building-related symptoms'.[22] The reported intermittent smell or unidentifiable odour and volatile organic compounds (VOCs) or chemical vapour may be attributed to a by-product of moulds. Moulds are known to produce alcohols, aldehydes, sulfur and VOCs, which can cause a musty odour and possibly lead central nervous system (CNS) symptoms similar to those reported by index case 2 – for instance, the difficulty experienced with concentrating and headaches.[4]

Regarding the atopy and mould sensitisation investigation, 80% of the five workers, including both index cases, were positive, predisposing them to developing other allergies.[36] All the workers tested had a negative IgE to the mould allergens (m1, m2, m3, m6 and m228), except for the one highly sensitised to *A alternata*. This observation suggests that atopy and sIgE alone may not be sufficient for investigating occupational mould exposure; additional testing of IgG is therefore recommended as a marker of exposure. In addition, symptoms associated with mould exposure occur more often than IgE-mediated mould sensitisation and a type 1 reaction may not be the primary factor in building-related symptoms.[3,22]

Two workers (2/5) showed elevated levels of IgG antibodies to *C herbarum*, *P chrysogenum*, *A fumigatus*, *A flavus* and *A alternata*, indicating their ongoing exposure to these antigens. Serum IgG antibodies against environmental and occupational mould antigens may reflect the level and extent of antigen exposure, where higher levels are usually associated with more severe disease.[20] Given that the two workers live in different domestic settings, it is improbable that they would have shared the same exposure in those settings. In addition, whereas CI3 suffered from rhinitis, she was asymptomatic at the time of the investigation and had no work-related complaints despite having the highest Phadiatop (atopy) result. This suggests that C2 could be sensitive to the moulds identified in the work environment.

Previous occupational studies have shown high sIgG concentrations to various microbial agents, including moulds. But differentiating between HP and healthy individuals based on sIgG concentrations can be challenging due to variations

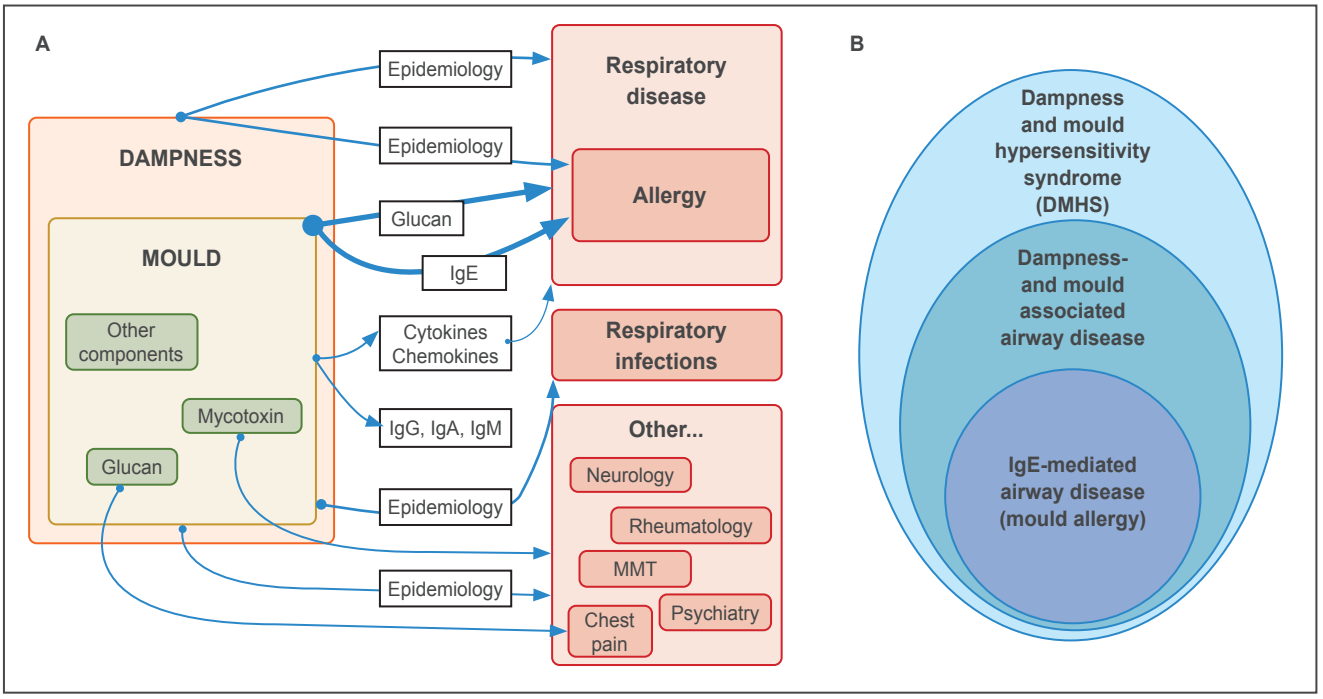

Figure 2: (a) Estimates of evidence for a causal relationship between dampness, mould exposure and disease, (b) shows a shift from diseases with accepted biomarkers and more defined nature of symptoms (eg respiratory allergy) to syndromes or symptoms, which are frequently subjective (eg cognitive difficulties, memory loss or fatigue) and complicate the evaluation of cause and effect relationships in DMHS. Note that the thickness of the arrows indicates the weight of the evidence in the figure. Key: MMT – mixed mould mycotoxicosis.[20]

depending on the antigen.[3,13,37] One of the challenges in interpreting IgG results is that South Africa does not have established reference ranges for moulds and therefore ranges from other countries were used of necessity.[13,25] Based on the German tool, irrespective of the sIgG results for *P chrysogenum* (m1) and *A fumigatus* (m3) in two workers being high, they were not conspicuous.[13] However, the sIgG to *C herbarum* (m2) is higher than 97% of the German controls in two workers, including index case 2. Furthermore, similar comparative results were found for *A alternata* (m6), where one worker's result was higher than 99.1%, and the other was higher than 100% of the German controls.[13]

The use of serum sIgG testing has shown promise as a tool for exposure assessment in occupational environments with high microbial concentrations.[38] Quantifying IgG antibodies against moulds in the workplace provided valuable information on exposure in workers, corroborating previous research findings.[20,35] However, standardised cut-off values are still lacking, which may have an impact on the diagnosis of diverse and immunocompromised working populations.[39] While the German reference ranges for sIgG may not be ideal for the South African population, given the demographic differences, they did reveal significant elevations of specific IgG antibodies to mould species identified in the workplace and are consistent with the symptoms reported in the questionnaire.

Another conspicuous challenge is that most individuals' clinical symptoms may be triggered by more than one allergen, making it difficult to identify the major allergen, especially in workplace environments with multiple exposures.[20] Sensitisation (19.2–22.5%) to at least one of the mould species *Alternaria*, *Aspergillus* or *Cladosporium* has been reported. This has indicated that the inhalation of fungal spores can induce sensitisation and respiratory allergic symptoms and HP.[2,15,16,35,40,41] The symptoms reported by the index case (C2) are more suggestive of DMHS than HP, including headaches, impaired cognition and an inability to concentrate, or 'brain fog'.[19] Specific criteria for DMHS are summarised in Table IV; and, based on the information gathered from the questionnaire, index cases 1 and 2 (C1 and C2) met at least three criteria (points 1, 3 and 5). Therefore, DMHS is possible, although it is not well accepted due to a constellation of symptoms without an obvious pathophysiological mechanism. However, the findings provide sufficient evidence to accept an association between mould exposure and allergies or hypersensitivity, but not sufficiently substantive to prove exposure to mould. Although C1 showed no positive IgE or IgG results, this does not conclusively rule out mould exposure, as the connection is not straightforward as described. Similarly, in the case of C2, the elevated IgG levels suggest only exposure without offering a definitive explanation for the symptoms. Additional testing is therefore necessary to determine the underlying immunological mechanism between exposure and symptoms. Figure 2 shows the causal relationship between dampness and mould exposure in buildings and that health effects are mainly associated with clinical and epidemiological data. Visible mould or dampness is often assessed as an environmental factor, with higher correlations to clinical health effects than specific exposure markers. The connection between mould exposure and allergic respiratory disease is evident within DMHS.[19] However, the immunological response to DMHS is multifactorial,which poses a challenge when applying serology in diagnosis.[42]

TABLE IV: CLINICAL CRITERIA FOR DAMPNESS AND MOULD HYPERSENSITIVITY SYNDROME (DMHS)[18]	
1	History of mould exposure in water-damaged buildings with or without any symptoms.
2	Increased morbidity due to infections. This is an early stage of the disease.
3	Suffering so-called SBS or BRS. That means the individual feels unwell when entering a water-damaged building, but the symptoms improve when away from the problematic building from 1 to 2 days.
4	Development of multiple chemical sensitivity.
5	Increased scent sensitivity compared to their healthy stage. The patient may report an ability to smell a mouldy odour – for example, from the clothes of a a person standing nearby.
Diagnostic criteria rating: If all the five criteria are met – very probably DMHS. If four to three criteria are met – DMHS is probable. If two criteria are met and typical clinical symptoms – DMHS is possible.	

Despite considerable overlapping between symptomatic and non-symptomatic individuals, the combined measurements of mould-specific IgGs and IgEs may be useful in confirming previous mould exposure in workers who show clinical symptoms of hypersensitivity to such antigens and who have evidence of mould growth in their work environments. It could be argued that other workers (CI3) also showed elevated IgG levels; however, specific IgG concentrations do not imply morbidity but that these individuals may have been exposed to moulds at some point. Makkonen et al reported that IgG titres remain intact for prolonged periods despite the apparent antigen exposure discontinuing: this suggests a long, steady level of IgG following the immune stimulus.[35] The time lapse between exposure and investigation affirms this. In this study, anti-IgE antibody inhibition was performed, but the anti-IgG antibody inhibition on the sera of the two patients with elevated anti-IgG antibodies could not be conducted due to limited serum availability. This missed opportunity could have possibly shed light on whether the high anti-IgG antibody concentration to *A alternata* (which was not isolated) resulted from cross-reacting antibodies present in the moulds that were isolated (*Cladosporium* species, *A fumigatus*, *A flavus*, *Penicillium* spp).

This investigation highlights that not all workers will react the same way because of varying inherent levels of susceptibility. Moreover, this investigation highlights the under-reporting of symptoms, as it was only when the first index case (C1) came forward that the investigation discovered other workers had been experiencing problems but were hesitant to report them. Identifying and removing the causative antigen is crucial to managing workers' health. This investigation is pivotal in preventing many workers from unknowingly developing more chronic conditions. In this instance, the workers were temporarily relocated to other offices while mould remediation work progressed. During this phase, their symptoms resolved, further emphasising their work-relatedness. A post-intervention assessment will be conducted after the remediation work is complete.

The complexity of bio-aerosol composition in the building environment makes accurate evaluation of mould exposure challenging, especially distinguishing between occupational and domestic exposure. Therefore, laboratory tests play a critical role in patient or worker management, and careful environmental and clinical history-taking is essential to establishing workplace association or confirming causal links to mould exposure.

Preventing the adverse health effects of mould exposure involves avoiding water intrusion and performing periodic building maintenance. Healthcare providers, occupational hygienists, building maintenance personnel and workers should work together to evaluate and manage the symptoms and diseases related to mould exposure.[34] Furthermore, there is a lack of sufficient data on exposure–disease and dose–response relationships for mould bio-aerosols, and this makes it difficult to establish safe limits.[43] NIOH has received several requests for health evaluations due to mould exposure in various occupational settings (laboratories, offices, biocontrol processing, university museum, etc) but more frequently in office environments. This case investigation specifically targeted an office setting, highlighting the need for proper building maintenance to prevent exposure and its associated challenges. Recognising and swiftly remedying potential bio-aerosol exposure can lead to a conducive and productive work environment for all workers, including medical professionals, as in this case, who are a valuable and scarce resource in the country.

Moreover, workers must be included in the risk-assessment process and trained to recognise the signs and symptoms of mould exposure, particularly if water-damaged or damp building structures are identified. Whereas the topic has been described in the literature, this article highlights the challenges arising from the poor reporting of symptoms, delays in initiating investigations and the complexity of testing and associating exposure with symptoms.

LIMITATIONS

One limitation of this case investigation is that some mould could not be identified at the species level due to multiple species within the same genus being involved. The symptoms reported by workers were non-specific, which could be attributed to other non-biological exposures (eg chemical sensitivity). This ambiguity in symptom presentation might lead to the possibility of diseases being overlooked and the risk of workers' conditions being mismanaged.

In addition, environmental and immunological assessments were done approximately two months after the onset of symptoms, and therefore some moulds may not have been present during the sampling. Although the cases may have been exposed to mould allergens outdoors, the improvement of symptoms when away from work, as reported by the complainants, supported the building-related nature of their symptoms. While efforts were made to assess the cross-reactivity of mould allergens, this may have been limiting as some mould species may not have been included.

Furthermore, the lack of standardised environmental sampling methods for airborne mould exposure and health assessment

presents challenges in interpreting the data. Therefore, and effort must be made to standardise investigations on occupational exposure–disease and dose–response relationships. It is important also to emphasise that the challenge of interpreting serological tests without standardised local IgG reference values could possibly have an impact on the diagnosis made by clinicians. The clinical aspect was not covered in this series but warrants consideration in future research. Finally, it is worth acknowledging that some of the articles cited are old and the limited literature on this topic might be attributed to the complexity of these investigations.

CONCLUSION

This investigation found a plausible association between health effects and mould exposure in a non-industrialised water-damaged occupational setting. Indoor air quality measurements and IgG antibodies were useful indicators of mould contamination exposure. Both sIgE and sIgG testing helped to identify potential occupational exposure to moulds and its association with symptoms among workers. However, the results should be interpreted with caution due to the variability of the individual responses. The study emphasises the importance of understanding mould sensitisation and selecting appropriate laboratory testing methods in order to diagnose and manage the health effects accurately.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

This article has been peer-reviewed.

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