

Aeroallergens and viable microbes in sandstorm dust

Potential triggers of allergic and nonallergic respiratory ailments

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Aeroallergens and antigens in sandstorm dust, extracts of which were skin prick test (SPT) positive in allergic patients, were detected by rocket immunoelectrophoresis and ELISA. Fungi and bacteria isolated by agar settle plates and soil dilution and soil washing methods were enumerated and identified. Cat dander, *Acacia*, *Alternaria*, *Aspergillus*, *Chenopodium*, *Cladosporium*, Bermuda grass, *Pithecellobium*, *Prosopis*, *Rumex*, cultivated rye, and *Washingtonia* palm allergens were detected by both methods. Viable microbes including 1892 ± 325 colony-forming units (cfu) of bacteria, and 869 ± 75 cfu of fungi were isolated per gram of dust by the soil dilution method. Randomly selected microbial colonies on streaking and subculture were found to consist of between two and seven mixed colonies. Fungi including *Alternaria*, *Aspergillus*, *Botrytis*, *Cladosporium*, *Mortierella*, *Mucor*, *Mycelia sterilia*, *Penicillium*, *Pythium*, *Ulocladium*, *Verticillium*, and some yeasts were isolated. *Actinomyces*, *Bacillus*, *Pseudomonas*, and mostly coagulase-negative *Staphylococcus* species were identified, but the bulk of unidentified bacterial isolates were mainly mixed colonies of rods, cocci, coccobacilli, and some filamentous types. Six-hour agar settle-plate counts during sandstorms were 100 and 40% higher for bacteria and fungi, respectively, than without sandstorms. The most abundant aeroallergens were those of *Acacia*, *Alternaria*, *Aspergillus*, Bermuda grass, *Cladosporium*, cultivated rye, *Prosopis*, and cat dander. *Pithecellobium dulce*, *Rumex crispus*, and *Washingtonia* palm allergens were detectable for the first time in Riyadh. IgE reactivities of the dust in man were demonstrated by ELISA using sera from atopic, exposed, and normal subjects. These results indicate that sandstorm dust is a prolific source of potential triggers of allergic and nonallergic respiratory ailments, and the methods mentioned here should be routinely used for quick sampling of the environment.

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A wide variety of airborne particles, including various allergens and organic vapors of the outdoor environment, farms, industries, and homes, are implicated in rhinitis, asthma, allergies, and a host of other upper respiratory tract infections (1). It is well established that almost any organic dust or volatile chemical that reacts with proteins may cause allergic rhinitis, asthma, or hypersensitivity pneumonitis (2). Crook et al. (3) have reported that viable microorganisms in dust cause most work-related upper respiratory tract infections. Therefore, it is of the utmost importance that allergens,

viable microbes, and other noxious agents that prevail in any particular environment, or are induced by changes in meteorologic conditions, be identified and studied. These measures will not only help to monitor the levels of these agents in the environment but also aid clinicians and allergists in advising and treating patients as well as those at risk before they are exposed and sensitized (4).

Respiratory tract infections are common throughout the world. In Saudi Arabia, sporadic sandstorms form dust clouds that may last for only

a few hours or persist for days. These sandstorms are so unpredictable that most outdoor workers perform their duties with their faces or nostrils covered in anticipation of the next storm. During these sandstorms, a variety of airborne particles remain suspended in the fine dust; some get blown over long distances and are forcibly introduced into human orifices such as the eyes, nostrils, and ears. Most local residents attribute the onset, recurrence, and exacerbation of the rhinitis, colds, sinusitis, asthma, and other respiratory disorders that they regularly suffer to the dust from these sandstorms.

Dust-borne particles from soybean were found to be the major cause of epidemics of asthma in Barcelona (5), and it is well documented that in some areas of the world where thunderstorms occur, there is an association with epidemics of asthma (6–8). In two such instances in Melbourne, Australia, there were five- to 10-fold increases in the number of patients with asthma admitted to hospital emergency departments (4). Similar observations have been made in the UK (7, 8). The importance of thunderstorms in respiratory ailments is thus well established. A similar role for sandstorms as an active propagator of offensive or potent “excitants” causing allergic and nonallergic respiratory reactions in man has not been investigated.

This preliminary study aimed to isolate, enumerate, and identify viable microbes in sandstorm dust; to assess the skin-test reactivity of sandstorm dust extracts; and to determine the aeroallergen components of these extracts. The results of such a study should serve as baseline information on sandstorm-borne allergens and potentially pathogenic microbial agents for further investigations. The study should also alert practitioners in countries where sporadic sandstorms or other meteorologic changes occur to the importance of the aeroallergens and viable microbes that are introduced by these storms. The methods applied in this study are inexpensive and rapid and could be routinely used in the monitoring of patients' work and living environments.

Material and methods

Dust collection and extraction

Dust samples were collected during eight of the sandstorms that occurred in Riyadh between May 1991 and December 1993. For this collection, sterile glass Petri dishes were placed outdoors on concrete bases and on walls at several specific sites of a large residential family compound in Riyadh, Saudi Arabia. Apart from slight modifications, dust samples were extracted as described by Price & Longbot-

tom (9) and by Kimura et al. (10). Briefly, all samples collected during each sandstorm were thoroughly mixed, sieved, and placed in sterile polythene bags. One gram of each sample was kept for microbial enumeration, and the rest was weighed and suspended in phosphate-buffered saline (PBS) containing 0.4% phenol at a ratio of 1/50 (w/v). The suspension was shaken for 12 h at 4°C, after which it was centrifuged (15 000 g, 15 min), filter sterilized (0.22- μ m filter, Millipore, Bedford, MA, USA), dialyzed against several changes of sterile, distilled deionized water, and then lyophilized. Part of each dust extract was resuspended in deionized water (1/1, w/v), glycerinated, and stored for further clinical evaluation or stored as powder at -20°C until needed.

Isolation, enumeration, and identification of viable fungi and bacteria

For the isolation and culture of microorganisms, the soil-dilution plate (11), the soil-washing method (12), and agar settle plates were used. For the settle-plate method, replicates of sterile, glass Petri dishes containing different culture media (Czapek-Dox, potato dextrose, and sheep blood agar) were placed at various sites during sandstorms for 6 h at any one time. The process was repeated at these sites when there were no sandstorms, and replicate plates were incubated at 25, 37, and 45°C. For the soil-washing and dilution-plate methods, 1-g dust samples were placed in a sterile conical flask, and 100 ml of sterile distilled water was added. Each sample was shaken at 100 rpm on an orbital shaker (Model 361, Fisher Scientific, USA) for 10 min and then allowed to stand for 5 min. After each wash, the supernatant was aseptically poured into a sterile container and saved. The process was repeated 15 times for each dust sample. After thorough agitation, aliquots of 0.15 ml were taken in replicates from each of the supernatants, serially diluted, and then mixed with 15 ml each of molten Czapek Dox agar, potato dextrose agar, and sheep blood agar by the soil-dilution-plate method. After the final wash, samples of each residual soil were evenly spread in sterile Petri dishes, and molten agar (cooled to 45°C) was added gently and thoroughly mixed. Plates of all three growth media were incubated in triplicate at the various temperatures and examined daily for microbial growth. Colony counts from the soil-dilution plates were calculated by weighted averages, and the mean of all counts was expressed as colony-forming units (cfu) per gram of dust. Colonies on settle plates were counted for several experiments and average counts taken for bacteria and fungal colonies. Fungal isolates were identified in most cases by

genera, and colonial morphology pigmentation and Gram staining were done on selected bacterial colonies (cfu). Some 500 colonies were randomly subcultured by streaking to establish the homogeneity of cfu, and counts of any subcolonies noted were made; otherwise, no further identifications were carried out.

Allergens

Cat-allergen extracts were purchased from ALK (Hørsholm, Denmark) and Dome Hollister-Stier. All the other commercial allergen extracts were purchased either from Greer Laboratories, Inc. (Lenoir, NC, USA) or from Dome Hollister-Stier (Miles, Inc., Elkhart, IN, USA). Most fungal and pollen extracts were prepared locally as indicated in the list (see Results under ELISA). The methods used to extract pollen and fungi have been previously described by Kwaasi et al. (13) and by Kim & Chapras (14), respectively. The protein content of all extracts was determined by the method of Lowry et al. (15).

Rabbit antisera to dust and other extracts

Apart from antisera to *Alternaria alternata* (Lot no. LJ 327530) and *Cladosporium herbarum* (Lot no. LJ 32750) that were purchased from Pharmacia AB (Uppsala, Sweden), all other hyperimmune antisera to dust and other allergen extracts were produced in our laboratory. For each extract, six home-bred New Zealand White rabbits were immunized according to the protocol previously described (13), with the following modifications. Immunizations were carried out with extracts containing increasing amounts of proteins starting with 0.2 mg/ml per kilogram of body weight of rabbit on day 1, followed by regular injections at 7- and 10-day intervals, to final doses of 2 ml containing 2 mg of protein per kilogram of body weight at weeks 12–15. Antibody production was carefully monitored by Ouchterlony double immunodiffusion with successive test bleeds, and the immunoglobulin G (IgG) fractions were prepared as previously described (13).

Ion-exchange fractionation, affinity purification, and biotinylation of antisera

All antisera were initially fractionated by ion-exchange chromatography on Sephadex DEAE A-50 (Pharmacia, Uppsala, Sweden). The use of this anion resin ensured that all serum protein components but IgG were bound to the column, leaving the eluate rich in IgG. Samples were concentrated by ammonium sulfate (45%) precipitation and

freeze-drying. Ion-exchange fractionated antisera were further purified by affinity chromatography on Affigel-10 (Bio-Rad, Hemel Hempstead, UK), coupled with the corresponding semipurified extract according to the manufacturer's directions. Briefly, each column was equilibrated in 20 mM Tris (pH 7.2), and then the ion-exchange purified IgG-rich antiserum was applied to the column in the same buffer. Elution of bound antibodies was carried out in 0.05 M citrate buffer (pH 2.6) containing 1 M NaCl. Fractions were quickly neutralized on elution by the addition of saturated sodium phosphate. For each specific antiserum, all fractions were pooled, repeatedly dialyzed at 4°C against several changes of PBS, and then lyophilized.

Purified IgG antisera were conjugated to biotin-NHS (Bio-Rad, Hemel Hempstead, UK) by the method of Bayer et al. (16) with minor modifications as follows: 1.7 mg of biotin was dissolved in dimethylformamide (Sigma, Poole, Dorset, UK), and 9.4 µl of this solution was then added per milligram of extract-specific IgG in PBS. The mixture was gently shaken at room temperature for 4 h and then dialyzed against sterile PBS at 4°C. It was then centrifuged, and the supernatant containing the biotinylated IgG was collected, divided into aliquots, and stored at -20°C until needed.

Enzyme-linked immunosorbent assay (ELISA)

For the antibody sandwich ELISA, 100 µl of semi-purified rabbit antidust (0.1 mg/ml) antiserum was dissolved in 0.05 M carbonate buffer (pH 9.6) and used to coat Nunc Immuno I microtiter plates in triplicates. After overnight incubation in a humid chamber at 4°C, all plates were washed (3×5 min), and the wells filled with 100 µl of PBS, pH 7.4, containing 0.05% Tween-20 and 3% bovine serum albumin for 2 h to block all nonspecific reactive sites. The plates were again washed, and 100 µl of replicate dilutions of dust and other extracts (pre-determined by checkerboard titrations for each extract) was added to all wells. The plates were incubated for 2 h at 37°C and washed, and 100 µl of biotinylated rabbit antidust IgG was added. This step was followed after further washes by the addition of avidin-streptavidin alkaline phosphatase complex (Amersham, UK). The plates were again washed (3×5 min each) and 100 µl of para-nitrophenol phosphate (1 mg/ml) in 1 M diethanolamine buffer, pH 9.8, was added to all wells. Plates were incubated, and the reaction was stopped when suitable chromogen development was attained. The ELISA plates were read at 405 nm, and the scores obtained from these assays were calculated by comparison with the mean and SD for all the various controls at a range of

dilutions by the Dynatech MR 600 automatic Microplate reader (Dynatech Laboratories, Inc.) interfaced with an IBM computer and specifically programmed to calculate the mean and SD, as we have previously reported (13, 17, 18). Results were considered positive only if an ELISA score was 2 SD or higher than the mean of all controls.

Microtiter plates were coated with sandstorm-dust extract to assess the immunogenic and allergenic potential of sandstorm dust in the local human population. Sera were tested from the following: 45 atopic patients who were skin prick test (SPT) reactive to sandstorm dust and three or more indoor and/or outdoor allergens, 15 gardeners who were not atopic but were sensitized and had demonstrable immunoblot reactivities to date-palm pollen (13) and later (unpublished data) *Washingtonia* palm and Bermuda grass pollen, and 10 apparently normal indoor workers. The mean atopic IgE levels (Phadezym IgE PRIST, Pharmacia Diagnostics AB, Uppsala, Sweden) were significantly higher than those of the gardeners ($P=0.004$) and the normal indoor workers ($P=0.002$). The mean total IgE levels of the gardeners were within the normal range for the local population (17). Due to the highly sensitive nature of the avidin-streptavidin method employed in these assays, in which biotinylated secondary antibodies were used, a high human serum background to signal ratio was observed during checkerboard titration experiments with serum dilutions of 1:5–1:40; therefore, a dilution of 1:50 was used with an extended chromogen development time. IgE ELISA was performed with biotinylated avidin-streptavidin secondary antibodies, and results were analyzed as previously reported (17).

For all assays, rigorous controls included replicates of test sera at several dilutions, diluent controls, controls of wells with and without conjugates, and wells with no coating antigens, and all ELISA experiments were repeated with preimmune sera pooled from all the rabbits that were immunized to produce the hyperimmune antisera. To ensure specificity of extracts, we carried out absorption experiments with the hyperimmune serum pool as follows: rabbit anti-sandstorm-dust serum was sequentially absorbed with predetermined excesses of each of the test extracts or allergens, and the absorbed serum was used in repeat ELISA. The rationale was that if antibodies to all the allergens precipitated out, the results obtained with absorbed antiserum would be negative (18, 19).

Rocket immunoelectrophoresis

To assess the relative levels of the various test allergens (extracts) in sandstorm dust, we fol-

lowed a modification of the original method of Laurell (20). Agarose in Tris-buffered (0.025 M, pH 8.6) tricine was warmed to 55°C and mixed with an equal volume of hyperimmune antiserum to a final concentration of 1% agarose. This was layered onto precoated (0.2% agarose) glass plates at 7.5 ml per plate measuring 650×100 mm. The different allergen extracts were adjusted to the same protein concentration (25 µg/ml), and 10 µl of each was electrophoresed (cathode to anode) in tricine buffer with cooling (10 V/cm of agarose, 4°C) until the bromophenol blue dye reached the end of the plate. Plates were pressed, washed, dried, stained, and then destained. Rockets were measured by a Bio-Rad peak-height estimator and then photographed. Control experiments were done with pooled preimmune rabbit serum and with absorbed hyperimmune serum, as described below in relation to ELISA.

Results

Sandstorm-dust extract contained 183.72 mg (± 12.6 mg) protein/g dust, and ordinary outdoor dust contained 56.8 mg (± 23.2 SD) protein/g dust. Ordinary Riyadh dust extract gave positive SPT in only 25% of patients tested as compared to 47% with sandstorm-dust extract. A wheal 6 mm or larger with erythema or pseudopods was regarded as a positive result.

Viable fungal and bacterial counts obtained from the soil-dilution-plate method, as calculated by weighted averages, were 869 (± 75) fungi, including yeasts, and 1892 (± 325) bacteria cfu/g of dust. Six-hour sampling of the environment by the agar settle-plates method revealed about 100% increase in bacterial and 40% increase in fungal counts during sandstorms. When colonies from the various methods were subcultured by streaking 500 randomly selected colonies, it became evident from extensive Gram staining of bacteria and studies of the resultant colonial characteristics and pigmentation that most cfu obtained on initial isolation were a conglomerate of between two and seven colonial types of fungi, bacteria, and yeasts, all of which appeared as one colony on initial isolation (Table 1). Apart from the miscellaneous species of unidentified bacteria and fungi from the *Mycelia sterilia* group, the most frequently isolated bacteria were species of *Actinomyces*, *Bacillus*, *Pseudomonas*, and some coagulase-negative *Staphylococci*. Fungal species belonging to the genera *Alternaria*, *Aspergillus*, *Cladosporium* (mainly *C. herbarum*), *Mucor*, *Penicillium*, and *Ulocladium* were the most frequent isolates (Fig. 1).

Rocket immunoelectrophoresis results of the extracts and allergens are presented in Fig. 2.

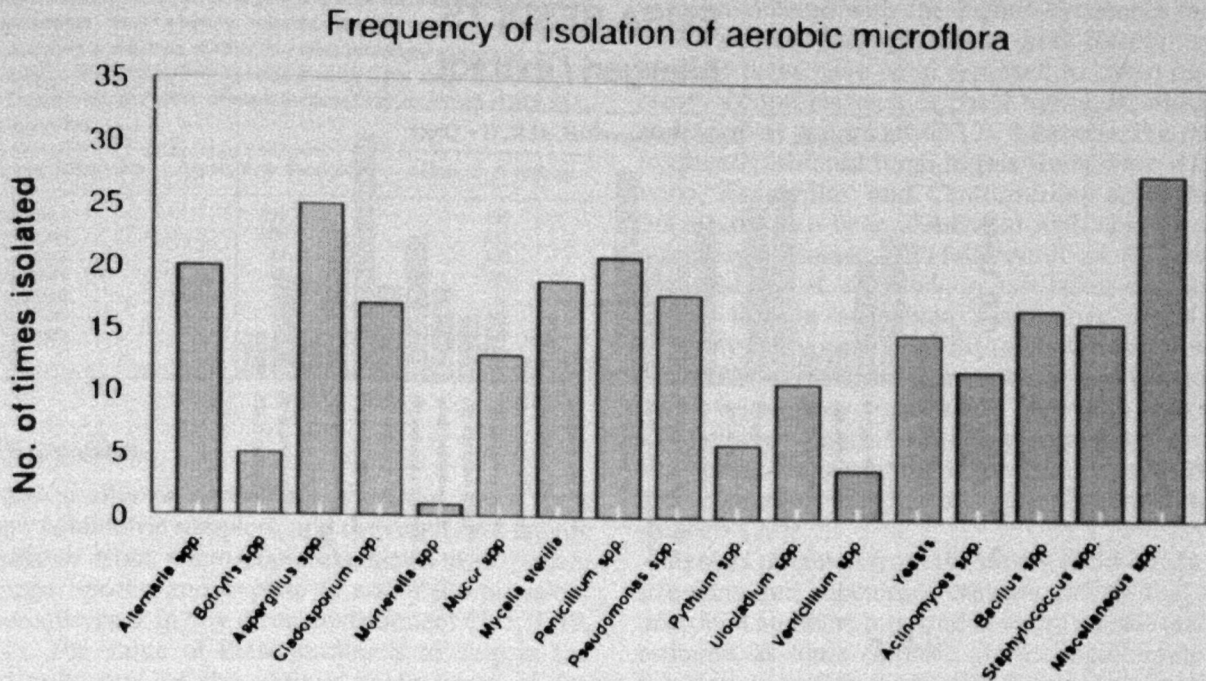


Fig. 1. Number of times that each microbe was isolated per sandstorm by any of three methods was scored and presented as histograms. Thus, each organism had 32 chances of being isolated for eight sandstorms sampled.

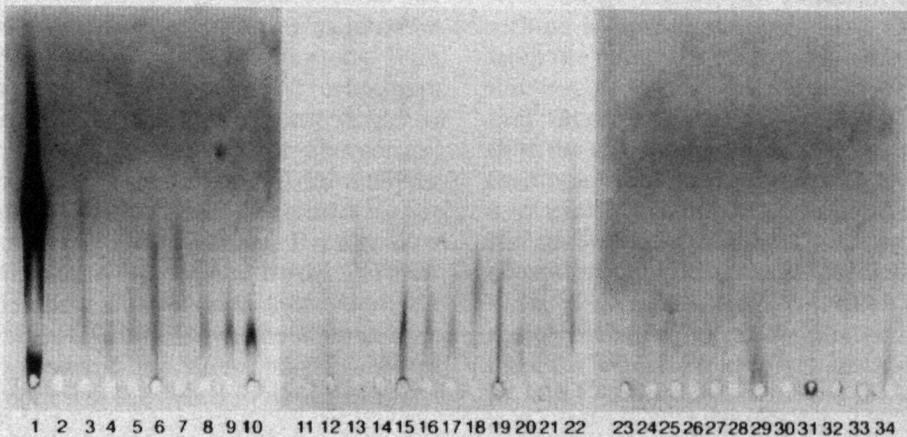


Fig. 2. Rocket immunoelectrophoresis results using rabbit antiserum to sandstorm dust are presented. All extracts (10 μ l each) were adjusted to same protein concentrations before electrophoresis. Extracts are as follows: 1) sandstorm dust, 2) buffer/dye, 3) *Prosopis* spp., 4) *Alternaria* sp., 5) *Cladosporium* sp., 6) Bermuda grass (R), 7) cat dander/hair (ALK), 8) *Aspergillus* spp. (H), 9) *Washingtonia* sp., 10) Riyadh dust (no sandstorms), 11) camel hair, 12) *Chenopodium* sp., 13) *Phoenix dactylifera* (date pollen), 14) bovine serum albumin control, 15) *Aspergillus* spp. (R), 16) Bermuda grass (H), 17) *Rumex crispus*, 18) *Prosopis* spp. (R), 19) cultivated rye (*Secale cereale*), 20) *Pithecellobium dulce*, 21) *Plantago lanceolatum*, 22) *Acacia* spp., 23) *Dermatophagoides pteronyssinus*, 24) *Amaranthus* spp., 25) HMU dust, 26) *Verticillium* sp., 27) *Artemisia* spp., 28) *Medicago sativa* (alfalfa), 29) *Phleum pratense* (timothy grass), 30) *Periplaneta americana/Blattella orientalis*, 31) *Atriplex canescens* (wing scale), 32) *Salsola pestifer* (Russian thistle), 33) *Dermatophagoides farinae*, 34) *Ulocladium* spp. In a few cases, local extracts and those from commercial sources were tested as different extracts.

Sandstorm dust, Bermuda grass, *Aspergillus* spp., cat, *Acacia* spp., *Prosopis*, *Alternaria*, *Cladosporium*, *Pithecellobium*, cultivated rye, *Washingtonia* palm, *Chenopodium*, and *Rumex* spp. were detected.

ELISA results using pre- and postabsorption hyperimmune rabbit antiserum are presented in Table 2 and Fig. 3A. Human IgE ELISA of sera from 45 atopic patients, 15 gardeners, and 10 normal subjects are presented in Fig. 3B. IgE

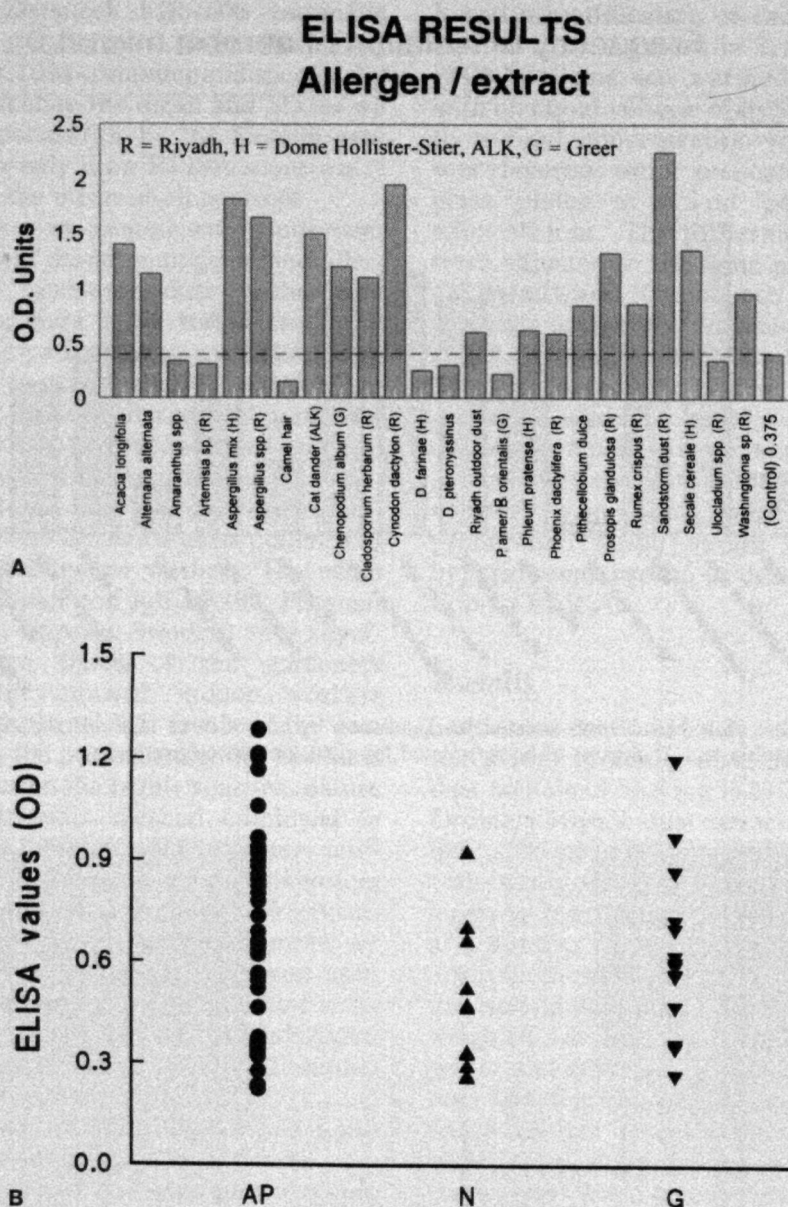


Fig. 3A. ELISA scores of allergens and extracts reactive to affinity-purified and biotinylated rabbit antiserum to sandstorm dust. Values ranging from 0 to 2.5 were calculated by comparison to mean and SD for all sera and reagent values, and results were analyzed as described in text.

Fig. 3B. Results of anti-IgE ELISA of sandstorm-dust reactivities in human sera. Totals of 45, 15, and 10 sera from atopic patients (AP) with multiple reactivities to at least three allergens, sensitized gardeners, and normal subjects, respectively, were assayed. Please note that, due to superimposition of similar, or merging of closely related values, only nine of normal (N) and 13 of gardeners' (G) sera could be counted.

reactivities (OD cutoff of 0.5) were detected in 31/45, 4/10, and 11/15 of atopic, normal, and gardeners' sera, respectively.

Timothy-grass, date-pollen, and *Ulocladium* allergens, which were barely detectable by rocket immunoelectrophoresis, were detected in all the ELISA experiments in which affinity-purified biotinylated rabbit antiserum was used. Apart from cat dander and cultivated rye (Table 2), antibody activity in the antiserum to sand-

storm dust could be abrogated by sequential absorption.

The results of ELISA and rocket immunoelectrophoresis indicate that the most abundant aero-allergens detectable in this study were *Acacia*, *Prosopis*, Bermuda grass, cultivated rye, *Alternaria*, *Aspergillus*, *Cladosporium*, and cat dander. Consistently detectable levels of *Pithecellobium dulce*, *Rumex crispus*, and *Washingtonia* palm aeroallergens were detected for the first time in Riyadh.

Table 1. Composition of 500 colony-forming units with mixed colonies. Some 500 colonies were randomly selected, streaked on agar plates, and investigated to ascertain whether they were pure single colonies or mixture. Putatively single colony-forming units grew as either single type or mixture of between two and seven different bacteria, fungi, or mixture of both types of organisms

Number possessing	Apparent no. of colonies	Actual no. of colonies
1 Colony	175	175
2 Colonies	206	412
3 Colonies	64	192
4 Colonies	49	196
5 Colonies	3	15
6 Colonies	2	12
7 Colonies	1	7
Total	500	1009

Discussion

Most studies on aeroallergens employ spore traps and volumetric samplers, and the results are mainly collated from microscopic examination of pollen, fungal spores, and hyphae to assess the prevalent aeroallergens in any given environment (3, 5, 9, 10, 21). The value of these studies is to help in the identification of the various components of the environments under study. Due to the unpredictable and sporadic nature of sandstorms in Saudi Arabia, these conventional aerobiologic methods mentioned above are not always suitable since any methods employed to study these storms must be rapid; hence, the methods used in this study. With the development of immunochemical techniques, antibodies can now be directed against single or multiple components, or even major allergens(s) contained in dust or any environment for analysis.

Extracts from sandstorm dust contained higher amounts of protein than ordinary Riyadh dust collected when there were no sandstorms. Furthermore, the sandstorm dust evoked more skin test responses in subjects tested. By virtue of its design, the study also assessed the viable microbial flora that may be trapped in the dust and its aerosol. The bacteria and fungi that were isolated indicate that the sandstorm microclimate actively supports the dissemination of large numbers of different microbes. Therefore, unsurprisingly, the protein content of sandstorm dust extract is appreciably higher than in ordinary Riyadh dust.

Further analyses of 500 randomly selected, apparently single colonies revealed that only 35% of these were pure isolates (a single colony on subculture) and that in some cases there were between two and seven mixed microbes dispersed within the same aerosol or propagule and growing as a single cfu of mixed organisms (Table 1). The clinical implication of this microbe-microbe con-sociation is that the deleterious effects of one pathogenic microbe could augment those of another or other members of the mixed colony. The

bacteria isolated with the highest frequency were *Pseudomonas*, *Staphylococcus*, and *Bacillus* spp. Most of these have been reported to cause respiratory symptoms such as chest tightness, wheeze, and nasal or eye irritation (3). Likewise, the most frequently isolated fungi in this study were *Alternaria*, *Aspergillus*, and *Cladosporium* spp.: these fungi have also been associated with allergic and nonallergic diseases. O'Hallaren et al. (22) have reported that *A. alternata*, in particular, can cause severe asthma and even respiratory arrest in children and young adult asthmatics. Some Ascomycetes fungi also can cause allergic and immunotoxic respiratory disorders. Members of the Dematiaceae family of fungi, to which *M. sterilia* and some *Alternaria* and *Aspergillus* spp. belong, are commonly implicated in allergic fungal sinusitis (23).

Results obtained from the purity check of cfu on streaking and subculture indicate clearly that the microbial numbers reported here are a gross underestimate, as some colonies are a conglomerate of between two and seven cfu (Table 1). Another factor that could have contributed to underestimation is cynereisis of agar settle plates in the heat. The numbers of viable microbes compare closely with those known to exacerbate allergies and asthma in household dusts (24). On extrapolation from the results in Table 1, which considers aerobic but not anaerobic microbes, it becomes obvious that sandstorm dust contains far more microbes than the number considered above the sensitization threshold levels for respiratory ailments. It contains a multiplicity of aeroallergens and is rich in opportunistic infectious agents, making it a complex ecosystem that needs to be studied in detail.

As a generalization, cat allergens have been rightly "labeled" as indoor allergens by workers all over the world. House pets are not normally kept in Saudi Arabia. Apart from a few expatriates who bring their pets from abroad or acquire them, all cats and dogs are stray or feral. There is great abundance of food for these animals to scavenge; consequently, there is a large population of cats outdoors. This situation is made even worse by the fact that the cats have tremendous fecundity and multiply very rapidly. Added to this is the hot weather, which makes the cats molt, desquamate, and shed their fur or dander. Their urine and excreta also dry up very quickly in the high temperatures. Even though cat hair and dander are well-characterized, potent indoor allergens, it must be emphasized here that, unlike other parts of the world, cats and dogs are not kept in houses and are the source of an outdoor allergen but are not likely to contribute to the indoor allergen load in Saudi Arabia. This sociocultural difference makes it come

Table 2. ELISA results of allergens and other extracts with hyperimmune antiserum to sandstorm dust before and after absorption. ELISA of all commercial allergens and local extracts to detect antigens in sandstorm dust using affinity-purified hyperimmune antiserum as described under Material and methods. Results of direct, indirect, double antibody sandwich ELISA, and absorption experiments were analyzed as described. Relevant controls with preimmune positive sera with sandstorm-dust extracts were also included. Sources of allergen or extracts are in parentheses. Extracts or allergens labeled R were obtained locally and extracted in our laboratories in Riyadh, those labeled H were from Dome Hollister-Stier, that labeled ALK was from ALK Laboratories, and those labeled G were from Greer Laboratories

Allergen/extract	ELISA values	
	Hyperimmune serum (before absorption)	Absorbed serum (after absorption)
<i>Acacia longifolia</i> (R): acacia, wattle	1.46	0.15
<i>Alternaria alternata</i> (R)	1.33	0.12
<i>Amaranthus</i> spp. (R): pigweed	0.35	0.09
<i>Artemisia</i> spp. (R)	0.32	0.10
<i>Aspergillus</i> mix (H): four species	1.82	0.08
<i>Aspergillus</i> spp. (R)	1.78	0.09
<i>Atriplex canescens</i> (G) wing scale	0.41	0.07
Camel hair (R)	0.35	0.06
Cat hair (ALK)	1.75	0.35*
Cat hair (H)	1.71	0.32*
<i>Chenopodium album</i> (G)	0.62	0.02
<i>Cladosporium herbarum</i> (R)	1.12	0.02
<i>Cynodon dactylon</i> (H): Bermuda grass	1.77	0.04
<i>C. dactylon</i> (R): Bermuda grass	1.95	0.02
<i>Dermatophagoides farinae</i> (H)	0.23	0.04
<i>D. pteronyssinus</i> (G)	0.21	0.03
HMU dust (G)	0.29	0.04
<i>Medicago sativa</i> (H): alfalfa	0.15	0.03
<i>Periplaneta americana/Blattella orientalis</i> (G)	0.26	0.03
<i>B. orientalis</i> (R)	0.28	0.02
<i>Phleum pratense</i> (H): timothy grass	0.59	0.03
<i>Phoenix dactylifera</i> (R): date pollen	0.67	0.01
<i>Pithecellobium dulce</i> (R): blackbead	0.88	0.02
<i>Plantago lanceolatum</i> (H): plantain	0.30	0.03
<i>Prosopis glandulosa</i> (H): mesquite	1.30	0.02
<i>P. glandulosa</i> (R)	1.45	0.03
<i>Rumex crispus</i> (R)	0.92	0.02
<i>Salsola pestifer</i> (G): Russian thistle	0.15	0.02
Sandstorm-dust pool (R)	2.20	0.05
<i>Secale cereale</i> (H): cultivated rye	1.35	0.33*
<i>Ulocladium</i> spp. (R)	0.62	0.05
<i>Washingtonia</i> palm (R)	0.91	0.06
Reagent, preimmune/other controls	0.29	NA**

*Antiserum activity could not be completely absorbed. **Not applicable.

H: Hollister Stier; G: Greer Laboratories; HMU dust: house-dust mix, mattress dust, upholstery dust.

as no surprise that cat dander was detected at high concentrations by both methods.

Absorption experiments are an acceptable indirect method of confirming the presence or absence of specific antibodies in sera (18, 19). The fact that it was not possible to absorb completely all of the cat-specific antibody activity from the antiserum to sandstorm dust with the two commercial cat allergens (Table 2) is also not surprising and can be explained in three ways. Firstly, the commercial allergens used in this study would have been purified to remove most of the crude natural proteins, resulting in an extract rich in the commercially preferred "major Fel d allergens" while cat-specific antibodies in anti-sandstorm-dust antiserum contain most or all natural cat allergenic products such as fur, dander, urine, excrement, and a host of other materials. Secondly, several cross-reactivities with cat allergens have been reported (25, 26), and it is also highly probable that some of these cross-reactive components, whether identified in this report or not, abound in the environment

and are incorporated in the sandstorm-dust extract and the antiserum produced to it. Thirdly, primary sensitization by potent allergens that can lead to multiple cross-reactivity with other dusts and pollen allergens (32) is a well-documented phenomenon. In a multicomponent extract such as sandstorm dust, these major contributory factors should not be overlooked. However, it has been previously reported that cat allergens are a major cause of perennial rhinitis in Saudi Arabia (28), and our data confirm this fact. High levels of this allergen or components cross-reactive with cat allergens prevail in the local dust; it is important that specific efforts be made to investigate cat allergy in a nationwide study to estimate its prevalence so that control measure may be instituted to combat its prevalence as a health hazard.

Although the aeropollens that were used or detected in this study represent neither all known allergenic pollen nor pollen from all plants that grow in this part of the world, the few that were detectable in the present test panel must be

regarded as important constituents of sandstorm dust in Riyadh, at least during the period of the study. Noteworthy also is the fact that *P. dulce*, *R. crispus*, and *Washingtonia* palm-pollen antigens have emerged as detectable novel aeroantigens, and their role in evoking IgE reactivities and contributing to allergies here and in other parts of the world should be studied. *Prosopis* and *Acacia* are well-known allergenic genera (28, 29) which should be essentially entomophilous in their dispersal, but, depending on the dryness of the environment, they are capable of anemophilous dispersal, shedding considerable pollen when dry (29), and have caused serious allergies in the southwestern and south-central regions of the USA (28). These plants are not only indigenous to the Riyadh region but are perennial, and several foreign (non-indigenous) varieties have been introduced due to their ease of growth. The topography in Riyadh is similar to those of the already mentioned regions of the USA; hence their abundance as aeroallergens. Bermuda grass, which is known to be one of the most common pollen allergens in Saudi Arabia (27), was as abundant as *Prosopis* and *Acacia* species.

ELISA of sandstorm-dust anti-IgE in human sera indicated that 40% (4/10) of normal subjects, 73% (11/15) of gardeners, and 68.8% (31/45) of known allergy patients reacted positively. These high percentages obtained in all three groups may be due to the multifactorial nature of sandstorm dust and the consequent cross-reactive components in dust binding additively to microwells. Patient sera were from atopic patients who had known multiple allergies to at least three or more indoor and/or outdoor allergens. Over 30% of sera from allergy patients were ELISA negative, but patients known to be allergic to at least three allergens other than sandstorm-dust extracts may be seasonal and not perennial rhinitis/allergy sufferers, or those that are allergic to components that are absent, that are not present in large enough amounts, or that do not cross-react with components in sandstorm dust extracts.

Positive ELISA and positive SPT were positively correlated. Sera from the 47% atopic patients that were SPT positive to sandstorm dust were all ELISA positive in varying degrees. The fact that there was an additional population of ELISA-positive but otherwise SPT-negative atopic patients confirms our previous observation (17) that ELISA in which biotinylated and avidin-streptavidin reagents are used can detect a further population of low SPT responders, and these assays are more sensitive in our setting than the SPT in detecting reaginic antibodies in human sera. The gardeners were not skin tested with sandstorm-dust extracts. Their sera were included as a putatively nonatopic

group but one known to be sensitized and with positive IgG immunoblot reactivities to date palm (*Phoenix dactylifera* L.) (13) and recently (unpublished data) to Bermuda grass and *Washingtonia* palm pollen.

The positive reactivities in apparently normal subjects and the gardeners could be explained by the fact that they may be sensitized to a number of cross-reacting peptides or components in sandstorm dust that evoke the production of low levels of reaginic antibodies which act additively. Detailed studies of allergen-specific anti-IgE, and the anti-IgG isotype repertoire and other parameters are required to define clearly the allergic status of these subjects.

Previous immunoblot studies (17) in which sera from these gardeners were used indicated that there were no differences between their date pollen-specific bands and those of exposed atopic patients. Even though the sample of normal sera investigated in the present study ($n=10$) was relatively small, the fact that four normal people and 11 of the gardeners reacted positively to sandstorm-dust anti-IgE ELISA clearly indicates the need for a more detailed study. Prevalence studies of sensitization and allergies to all the agents identified in sandstorm dust in a larger sample of atopic and nonatopic subjects throughout the region will throw more light on the role sandstorm dust and its components play in allergies and other respiratory tract affections. Attempts should be made in further studies to catalog the periodicity of these sandstorms over a long period of time so as to make them more predictive.

Various epidemics of asthma and allergies attributable to various dusts of plant origin that have been blown several miles across countries and US states have been reported (30–32). A review of the IgG ELISA results with purified rabbit antisera indicates that cultivated rye, like cat antibodies, persisted even after repeated and sequential absorption (Table 2). Cereal grains are staple in the Arabian peninsula, the Arabian Sea, and its environs. It is probable that large amounts of rye, rye-like, or other unknown cross-reacting graminaceous dusts have been blown in several miles from within or outside the country (30). One typical example of this cross-reactivity was a study with dust of *Ricinus communis* (castor bean). This potent allergen was shown to cross-react with house dust, pollens, or molds (31); and sera from castor-allergic subjects were shown to possess interspecific and intergeneric cross-reactivity (32). Other possible sources are allergens from all of the other cross-reacting plants of the Gramineae family (33), or as grain dust from some of the numerous grain mills throughout Riyadh.

The results of this study strongly indicate that sandstorms are active propagators of a wide variety of microbes and potent anti-IgE-evoking aeroallergens which are potentially pathogenic to susceptible subjects. Dust from any surrounding can be extracted and used as an antigen or to produce antiserum, as has been demonstrated here and elsewhere (9, 10). Physicians, patients, and the general public should be adequately educated about the health implications of exposure to sandstorm dust so that those at risk can avoid its deleterious effects or receive prophylactic treatment.

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