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The microbiology of actinomycetes organisms known to cause farmer's lung disease and their occurrence from coal mines of Telangana

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Abstract

Farmer's Lung Disease (FLD) is a severe, often chronic, hypersensitivity pneumonitis (a type of allergic reaction) that causes swelling of the alveoli in the lungs. It is triggered by the breathing of thermophilic actinomycetes, a cluster of Gram-positive, branching bacteria that thrive in high-humidity, high-temperature environments, such as moldy silage, grain, and manure. While first and foremost linked with farming, these organisms are ubiquitous in soil and environmental dust, potentially leading to respiratory issues in other dusty environments, including industrial settings. This study aims to isolate and identify actinomycetes and fungal species from coal mine dust in Telangana to understand their potential role in occupational extrinsic allergic alveolitis. Ecological samples were collected from Telangana coal mines operated by SCCL, focusing on core and buffer zones to assess thermophilic actinomycetes and fungi. A total of coal mine samples were collected from various locations in Telangana and analyzed for the presence of actinomycetes using culture dependent and culture independent methods. As a result of this study, there are significant implications for the prevention and control of FLD in coal miners and surrounding communities, emphasizing the need for regular monitoring of actinomycetes in coal mines, as well as the implementation of effective management programs. The application of efficient control strategies to lessen exposure to these respiratory risks. This study enhances our comprehension of actinomycetes microbiology in coal mines and highlights the necessity of tackling the health hazards linked to FLD in high-risk jobs.

Keywords: farmer, FLD, SCCL, actinomycetes, thermophilic, fungal, telangana, coal mines, microbiology

Introduction

FARMER'S LUNG is an occupational disease which was registered in 1965 under the National Insurance Act (Industrial Injuries Act) of 1946. For the purpose of the Act, it is defined as "pulmonary disease due to the inhalation of the dust of mouldy hay or of other mouldy vegetable produce, and characterized by symptoms and signs attributable to a reaction in the peripheral part of the broncho pulmonary system and giving rise to a defect in gas exchange".

The disease is allergic in character, allergy now being taken to imply a greatly enhanced ability of an individual to react immunologically to a specific antigen following the development of hypersensitivity. Particles which carry one or more antigens implicated as causative of an allergy are said to be allergens. The inhalation of particulate allergens in the form of spores of actinomycetes, fungi and the proteins of animals is responsible for a number of diseases akin to and including farmer's lung, of which those listed in Table 1 are well known. The organisms concerned, although clearly eliciting an allergic pulmonary response, do not cause infection by colonization of the respiratory airways or lung parenchyma (Blyth, 1970) [7]. *Aspergillus fumigatus*, known to effect such colonization, can also cause pulmonary allergy when inhaled in large quantities. Allergic disorders such as farmer's lung disease have been variously classified according to the emphasis placed on details of their pathology. In the U.S.A. they are commonly categorized as hypersensitivity pneumonitis, in this country they are commonly described as examples of allergic alveolitis, or extrinsic allergic alveolitis to emphasize the external source of the allergens. Cattle are also susceptible to Extrinsic allergic alveolitis. The first confirmed case of farmer's lung in a

Cow was described by Pirie et al. (1971a) ^[31] in spite of worldwide recognition of Farmer's Lung, there is limited microbiological data from Indian coal mine environments. This

study aims to isolate and identify actinomycetes and fungal species from coal mine dust in Telangana to understand their potential role in occupational extrinsic allergic alveolitis.

Some examples of extrinsic allergic alveolitis due to actinomycetes and fungi**

Disease	Source of allergens	Precipitating antibodies identified against
Farmer's lung (Pepys & Jenkins, 1965)	Mouldy hay, grain and straw	<i>Micropolyspora faeni</i> , <i>Thermoactinomyces vulgaris</i>
Mushroom worker's lung (Sakula, 1967)	Mushroom compost	<i>M. faeni</i> and <i>T. vulgaris</i>
Bagassosis (Salvaggio et al., 1967; Lacey, 1971)	Mouldy sugar cane	<i>T. vulgaris</i> , <i>T. sacchari</i>
Maple bark disease (Emanuel et al., 1966) ^[19]	Mouldy maple bark	<i>Cryptostroma corticale</i>
Sequoiosis (Cohen et al., 1967)	Mouldy redwood sawdust	Species of <i>Graphium</i> and <i>Aureobasidium</i>
Malt worker's lung (Riddle et al., 1968)	Mouldy malt	<i>Aspergillus clavatus</i>

Material and Methodology

Sample collection

Ecological sample were collect from Telangana coal mines operated by The Singareni Collieries Company Limited (SCCL). Sampling targeted both core zones (mining lease areas) and buffer zones (within a 10 km radius) to assess occupational exposure and environmental distribution of the thermophilic actinomycetes and fungi.

Samples included coal dust, soil, and air particulates, collected aseptically in sterile containers and transported to the laboratory under controlled conditions. The collection followed systematic monitoring protocols in line with environmental, social, and governance (ESG) standards and Ministry of Environment, Forest and Climate Change (MoEF & CC) guidelines, often involving third-party audits to ensure compliance.

Collected samples were processed for microbiological isolation, including culture on Actinomycete Isolation Agar and incubation at 45–55 °C to favor growth of thermophilic actinomycetes.

Sputum culture

Sputum consists of mucus and enmeshed material expectorated following -coughing. When sputum is cultured directly or after digestion by pancreatin, the =viable population of micro-organisms can be selectively grown over a wide range of temperatures, and it yields a wide variety of filamentous and yeastlike fungi (Kahanpaa, 1971) ^[22]. *Asp. fumigatus*, *Penicillium* spp., *Geotrichum candidum*, *Candida albicans* and *Torulopsis* spp. are common examples of dominant and subdominant species. Less information is available on the occurrence of thermophilic and other actinomycetes although these have been found to be of general occurrence in smears and cultures of sputum from farmers in Orkney (Blyth & Wardrop, unpublished). *Micropolyspora faeni* has been recovered by Moore (Lacey & Lacey, 1964).

Serological evidence

(a) Mouldy hay and thermophilic actinomycetes

(1) Extraction of dominant antigens by freeze-thaw and trichloroacetic acid

Pepys, Riddell, Citron & Clayton (1962) showed that extracts of hays known to have produced respiratory disease contained antigens which precipitated with serum immunoglobulins of sensitized farmers. Extracts from a number of micro-organisms derived from the hay samples did not, however, yield significant antigens. A further examination of the serological reactivity of patients by Pepys et al. (1963) was made with regard to the thermophilic actinomycetes known to be common in

disease-inducing hays. They found that Farmer's Lung Hay (FLH) antigen developed in hay after incubating sterilized samples at 40 or 60° after (a) inoculating with mixed suspensions of micro-organisms from antigenically active hay, (b) inoculating with mixtures of pure cultures of thermophilic actinomycetes after treating the hay with ammonia vapour or fungal inoculation to achieve a pH value of 7-0 or (c) inoculating at pH 7-0 with pure cultures of *M. faeni* or *T. vulgaris*. *Micropolyspora faeni* provided the richest source of FLH antigen recoverable by freeze-thaw methods from pure cultures grown on V-8 vegetable juice, Czapek-Dox and nutrient agars. Filaments and spores of the organism were rich in the same antigens recoverable from agar. Using the extracts from variously treated hay samples and pure cultures at strength of 10 mg/ml, arcs of precipitation were shown to develop in double diffusion tests with positive sera in agar (Ouchterlony, 1953) and at 30 mg/ml in immune Electrophoresis tests modified from the method of Scheidegger (1955). Immuno electrophoresis showed that 3 major antigens were identifiable, each negatively charged but separable by their rates of migration. As a result, 3 precipitin arcs (A, B and C) occurred in positive tests. Inhibition

analyses conducted by absorbing positive sera with known antigens prior to testing showed that extracts of *M. faeni* inhibited the reaction of the sera towards FLH antigen recovered from mouldy hay. Pepys & Jenkins (1965) partially separated the 3 major antigens of *M. faeni* by extraction with trichloroacetic acid (TCA). Freeze-thaw extracts from pure cultures of the organism were treated with 1/10 vol of 40% TCA solution and allowed to precipitate in the cold. Both the precipitate recovered by centrifugation and the supernatant fluid gave antigenic components active in gel diffusion and immune electrophoresis tests. Antigens a and b (i.e. precipitating in the A and B areas) occurred in the precipitate, whereas antigen c was recovered from the supernatant. Although more than one precipitin arc occurred in each of the 3 areas, each area was recorded individually in order to assess degree of reaction of a test serum. Grade 3 reactions showed precipitation in all 3 areas, grade 2 reactions in 2, grade 1 reactions in one area and grade O reactions were negative.

The gradings were clinically significant, those patients showing grade 3 reactions having a history of more frequent and severe attacks than those in lower gradings.

Kobayashi, Stahmann, Rankin & Dickie (1963) also claimed an enhanced specificity for TCA extracts from mouldy hay when compared with extracts from carbol-saline treated materials. As a rapid method for extracting a, b and c antigens from cultures of *M. faeni*, Wenzel, Emanuel & Zygowicz (1968) have advocated a simple extraction

procedure using 0-2 M pyridine acetate buffer as vehicle. (in) Fractionation and chemical characterization of major TCA antigens Antigens from mouldy hay and *M. faeni* were examined in detail by LaBerge & Stahmann (1966a, b) [25]. Trichloroacetic acid extracts from mouldy hay were progressively precipitated with 50-60%, 60-70% and 70-90% of ethanol. Immunoelectrophoresis of the original extracts showed the presence of A, B and C precipitation areas when reacted against positive sera. Antigen a was fractionated by ethanol into 3 components, a^1 , a^2 and a^3 and antigen b into 2, b^1 and b^2 . Cultures of *M. faeni* recovered from the mouldy hay sample gave identical TCA-soluble extracts. Extracts obtained in phosphate buffer after ultrasonic treatment of the organism, followed by TCA in the cold, yielded 5 antigens, 3 of which were acid soluble. In addition, the medium in which the organism had been grown gave a TCA-soluble antigen not isolated from mouldy hay or *M. faeni*. Ethanol precipitated antigens, chromatographed after purification by passing through a column of DEAE (diethylaminoethyl) cellulose, gave evidence of a glycopeptide constitution (LaBerge & Stahmann, 1966b) [25]. The polysaccharide moiety was found to be composed largely of arabinose, galactose and glucosamine, all known to occur widely as cell wall components of Gram positive organisms.

(m) Serological diagnosis in farmer's lung disease

Much essentially basic work has been done on the development of generally acceptable techniques for the conduct of serological tests to diagnose and prognose farmer's lung disease. A working party of the Public Health Laboratory Service (Murray, 1967) [29] reported that gel diffusion was marginally superior to complement fixation in the diagnosis of mild forms of the disease, but that the 2 techniques gave identical results with moderate and severe cases. Murray, Pepys & Crichton (1967) [30] showed that latex particles could readily be coated with antigens from *M. faeni* and used for agglutination (flocculation) tests with sera. The titres recorded gave some indication of severity of the disease and the test compared favourably with gel diffusion, but was more rapidly and easily conducted. In both latex agglutination and complement fixation tests, antigens used were prepared from 45° broth cultures of *M. faeni* by extraction with glycine after homogenization and ultrasonic treatment. Doubtfully positive reactions may be enhanced by increasing the relative concentration of antibody, either by concentrating serum by diffusion against polyethylene glycol (Carbo wax) or by reconstituting it to a predetermined volume in saline after lyophilization. Jameson (1968) has, however, described a rapid and very sensitive precipitin test using immuno-osmophoresis to eliminate losses due to dilution and undirected migration of reactants in gel diffusion. In a group of 12 cases negative in gel diffusion, 11 became positive after concentration of sera and immuno-osmophoresis. Gray, Wenzel & Emanuel (1969) [19] have described an immunofluorescence test for the detection of *M. faeni*: Parratt (in Grant et al., 1972) has used a similar method to identify cases in Scotland.

(iv) The antigenic potency of extra- and intra-cellular antigens of *M. faeni* in test systems

A detailed serological analysis of the extracellular antigens of *M. faeni* recovered by concentrating supernatant culture fluids by dialysis against 40% polyethylene glycol was

made by Fletcher et al. (1970). Two batches of broth tested against highly reactive sera from 2 farmer's lung patients showed the presence of 29 antigenic components: antigens 1-5 were identified as components of antigen c of Pepys & Jenkins (1965). In a series of cases of farmer's lung disease investigated, 75% of sera were positive; 20% of cases suffering from other respiratory disorders, but exposed to the same environment, were also positive. Severity of disease did not influence the distribution of the zones of precipitation, although their numbers were greater in severe or moderate clinically established cases. Gibson (1971) has shown that most antigens occur during exponential growth in broth cultures, and Edwards (1971) [18] has used a double diffusion method for separating antigens from culture fluids as a means of purifying antigenic components of purifying antigenic components.

Jameson (1968, 1969) reported that not only broths but also the concentrated dialysates from broth cultures were instrumental in reacting with positive sera by immuno-osmophoresis. From the work described in 1969, it was evident that the use of broths containing extracellular exudates resulted in more reactions with test sera than were obtainable using mycelial extracts. Despite this, concentrated extracts of *M. faeni* which were run through a column of Sephadex 150 gave 3 fractions rich in antigen a of Pepys & Jenkins (1965) when subjected to conventional electrophoresis. Among other tests, the Sephadex fraction in immuno-osmophoresis was selective in distinguishing between 2 groups in a series of farmer's lung cases, those possessing antibody to antigen a and those deficient in this antibody. This finding may be aetiologically significant and indicate that sources of sensitization other than *M. faeni* need to be sought, especially as in this series of sera deficient in A antibody, only one of six reacted to *T. vulgaris*, the other accredited allergen.

(b) Fungal antigens

Although the evidence that the thermophilic actinomycetes are the organisms carrying the antigens essential for provoking farmer's lung disease is convincing, sera from patients may react to a number of extracts from other sources. Pepys et al. (1962) showed that 60 of 71 patients with the disease and who had precipitins to mouldy hay also possessed them to *Asp. fumigatus* in 25% of cases, *Cladosporium herbarum* in 16%, *P. notatum* in 6%, *Mucor* spp. in 52% and *Humicola lanuginosa* in 11%. Inhibition tests showed, however, that only extracts of mouldy hay when added to positive sera caused loss of reactivity in gel diffusion. Pepys et al. (1963) stated that extracts from hays inoculated individually with *M. pusillus*, *Asp. fumigatus*, *H. lanuginosa*, *H. stellata* or *Paecilomyces* spp. reacted with more farmer's lung sera than did untreated hay. Hapke et al. (1968) have forwarded the suggestion that another antigen or antigens besides those carried by *M. faeni* may be implicated in the disease. This followed from their observations that if chronic patients were tested against 12 hay extracts additional to the individual hay samples implicated in their own environments, a sharp rise in positivity was observed. It may also be significant that Williams (1963) reported an attack of farmer's lung disease when testing the coarse crepitations, but not the fine, fraction of mouldy hay dust where elements of the actinomycetes would be most likely to occur. Grant et al. (1972) commenting on the seronegative, clinically established cases, stated that the courses of their sensitization remained obscure. In cattle, Pepys & Jenkins

(1965) reported that precipitins to *M. faeni* were identifiable whether clinical respiratory disease was present or absent, and tended to correlate positive results with 'fog fever'. By contrasting groups of cattle showing fog fever with those suffering from indoor respiratory disease, Pirie et al. (1971b) [31] found that reactivity to *M. faeni* and *Asp. fumigatus* occurred solely in the latter

Result

The microbiological study of coal mine samples collected from Telangana exposed the presence of diverse fungal species and thermophilic actinomycetes. These microbes were isolated from sputum samples, soil, air, and coal dust, representative extensive environmental distribution and potential occupational exposure.

Table 1: Microbial Occurrence in Telangana Coal Mine Samples

S. No.	Sample Type	Microorganisms Identified	Type	Significance in FLD
1.	Coal Dust	<i>Micropolyspora faeni</i>	Actinomycete	Major causative agent
2.	Sputum Samples	Mixed actinomycetes & fungi	Mixed	Indicates exposure
3.	Air Samples	<i>Candida albicans</i>	Yeast	Respiratory colonizer
4.	Coal Dust	Thermophilic actinomycetes	Bacteria group	High FLD risk
5.	Soil	<i>Penicillium spp.</i>	Fungus	Secondary allergen
6.	Coal Dust	<i>Thermoactinomyces vulgaris</i>	Actinomycete	Known allergen
7.	Soil	<i>Aspergillus fumigatus</i>	Fungus	Causes allergic reactions
8.	Air Samples	<i>Geotrichum candidum</i>	Fungus	Opportunistic allergen

The outcome signify that coal dust samples showed the highest presence of thermophilic actinomycetes, mainly *Micropolyspora faeni* and *Thermoactinomyces vulgaris*, which are well-known causative agents of Farmer's Lung Disease. Fungal species such as *Aspergillus fumigatus* and *Penicillium spp.* were predominantly found in soil and air samples. The presence of mixed microbial populations in sputum samples confirms occupational exposure among

individuals working in coal mine environments. Overall, the findings suggest a significant risk of hypersensitivity pneumonitis in coal miners due to continuous inhalation of contaminated dust particles. Photograph showing the antimicrobial activity of three strains of actinomycetes against *Bacillus subtilis* ATCC 6633 and *Bacillus stearothermophilus* ATCC 2328, relevant to respiratory/occupational exposure in farmer's lung disease."



Plate 1: Photograph showing the antimicrobial activity of three strains of actinomycetes against *Bacillus subtilis* ATCC 6633

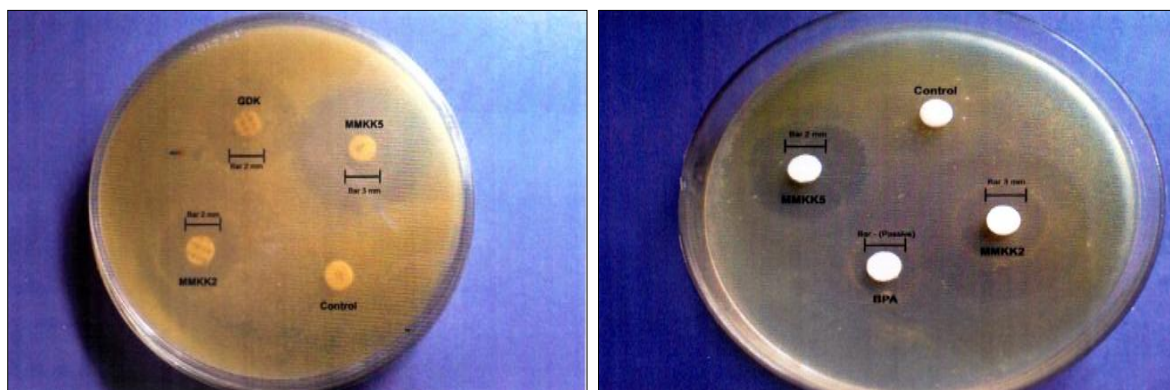


Plate 2: Photograph showing the antimicrobial activity of three strains of actinomycetes against *Bacillus cereus* ATCC 11778 & actinomycetes against *Bacillus megaterium* ATCC 9885



Plate 3: Photograph showing the antimicrobial activity of three strains of actinomycetes against *Micrococcus luteus* ATCC 2170 & *Actinomycetes* against *Escherichia coli* ATCC 2343

Sputum consists of mucus and enmeshed material expectorated following coughing. When sputum is cultured directly or after digestion by pancreatin, the viable population of micro-organisms can be selectively grown over a wide range of temperatures, and it yields a wide variety of filamentous and yeastlike fungi (Kahanpaa, 1971)^[22]. *Asp. fumigatus*, *Penicillium* spp., *Geotrichum candidum*, *Candida albicans* and *Torulopsis* spp. are common examples of dominant and subdominant species. Less information is available on the occurrence of thermophilic and other actinomycetes although these have been found to be of general occurrence in smears and cultures of sputum from farmers in Orkney (Blyth & Wardrop, unpublished). *Micropolyspora faeni* has been recovered by Moore.

Extracts made from *M. faeni*, administered in carbol-saline aerosols to 15 farmer's lung patients, produced characteristic symptoms after 5-7 h (Pepys et al., 1963). More extensive experiments were reported by Pepys & Jenkins (1965), and positive results in 6 cases treated with TCA extracts of defatted mouldy hay were obtained by Barbee, Dickie & Rankin (1965)^[5]. Fink, Hensley & Barboriak (1970) have created an experimental allergic alveolitis in the rat which may act as a model for further investigations.

Freeze-thaw and ultrasonic treatment are procedures commonly regarded as releasing large amounts of intracellular components from micro-organisms. Trichloroacetic acid extraction is likely to produce a wider overall spectrum of substances by adding some cell wall components to those leached from the cytoplasm. Much of the serological evidence has suggested that the antigens derived by these methods are more restricted in type and number than those of extracellular origin extracted from growth media and their dialysates. These observations might be construed to indicate that sensitization to antigens may follow retention of actinomycete spores in lung parenchyma sufficiently long to allow diffusion into the alveolar environment. Spore germination may also occur (Gibson, 1971). Points in the respiratory airways where branching takes place are areas provoking an interruption in air flow conducive to an accumulation of particles (Casarett, 1958)^[9], and retention may equally occur on isolated patches of squamous epithelium (Casarett, 1960)^[10]. Druett (1967)^[17] has suggested that 2/3 of a deposited insoluble dust passes rapidly to the gastrointestinal tract, 1/6 more reaching the same location during the first day. The remaining 1/6 may show prolonged retention with a half life of c. 120 days. Channell et al. (1969)^[11] reported that spores of *Asp. clavatus*, the cause of maltworkers lung, could be recovered

up to a month from volunteers who had been exposed only once. Particle clearance by alveolar phagocytes adds innumerable potential sites of sensitization to those already possible in the respiratory and gastrointestinal tracts. Based on these results, serological study was performed to assess the immune response against microbial antigens present in showing individuals.

Table 2: Serological Reaction to Antigens

Reaction Grade	Number of Antigen Bands	Clinical Severity
Grade 0	No reaction	No disease
Grade 1	1 band (A/B/C)	Mild
Grade 2	2 bands	Moderate

It is well established that positive serological tests are most common in acute cases of disease, but become less numerous with increase in time from the date of the last attack. recorded that 82% of their patients were positive for up to 3 years from an acute episode, whereas only 22% were positive beyond this period. Negative serological findings do not therefore prejudice a clinical diagnosis and should not affect unduly claims for compensation under the Industrial Injuries Act. This is especially so as widely varying results have been obtained by the use of different antigens and test systems. The highest percentages of positive reactors to *M. faeni* and also to *Asp. Fumigatus* were obtained by Jameson (1969) who attributed them to the use of more highly concentrated antigens than had been studied by others, notably Pepys et al. (1962). Significantly he found antibodies to some antigens of both organisms in a high proportion of non-farmer's lung (normal) groups of sera. The groups included antenatal, tuberculous and asthmatic patients. Observations such as these indicate a need for assessing farmer's lung patients on all available criteria, and for standardizing serological tests and the reagents employed (Mackenzie & Austwick, 1965)^[27]. The environmental distribution of microbial load was assessed across different zones of the coal mining area, including core and buffer zones.

Table 3: Environmental Distribution of Samples

Zone Type	Location Description	Microbial Load	Risk Level
Buffer Zone	Within 10 km radius	Moderate	Moderate
Core Zone	Active mining area	High	High

It has always been difficult for the microbiologist to give an objective opinion as to the potential safety or otherwise of a particular mouldy hay or grain crop, either with a view to mycotoxin content or respiratory hazard. An account of the occurrence of precipitins to *M. faeni* in cattle herds of south Scotland and northwest England (Pirie et al., 1972)^[31] has shown that in a farm where a farmer complained of a recurrence of disease, the incidence of precipitins in his cows had increased from 9 to 76%. None of 75 animals on a farm feeding good hay and only 1 of 58 animals fed silage were seropositive. A high incidence of precipitins in a herd would seem to indicate a hazardous situation for the farmer and his stock. It is interesting to speculate on the utilization of this evidence as a means of preventing disease.

Discussion

Incidence

Farmer's lung disease is probably the commonest form of extrinsic allergic alveolitis in Britain. Staines & Forman (1961) reported figures of 11.5/100,000 for East Anglia, 73.1/100,000 for southwest England and 193.1/100,000 for Wales. Recording only the more severe cases referred to hospital, Boyd (1971) reported a prevalence of 110/100,000 for Caithness, presumably a conservative estimate in view of the restricted nature of the sample. The recent information from a pilot survey by Grant et al. (1972) in Scotland has, however, shown a very different picture, the figures for the combined Orkney Islands of Stronsay and Sanday being 86/1000, Ayrshire 86/1000 and East Lothian 23/1000. When cases with negative serology are removed from the survey, the amended figures of 43 and 36/1000 for Orkney and Ayrshire, respectively, still represent 20 times more cases than have been reported from any other location in Britain.

Organisms known to cause Farmer's Lung and their Occurrence

Two thermophilic actinomycetes are known to cause the disease, the more important being originally known as *Thermopolyspora polyspora*. The species was given a detailed examination by Cross, Maciver & Lacey (1968)^[13], who allocated it to the genus *Micropolyspora* and described it as a new species, *M. faeni*. Sensitized individuals who inhale the organism or its antigenic fractions develop clinical symptoms (Pepys et al., 1963; Pepys & Jenkins, 1965). The second causative organism is *Thermoactinomyces vulgaris* (syn. *Micromono-spora vulgaris*), which produces resistant endospores capable of retained viability in dried soils for c. 100 years (Cross, Walker & Gould, 1968; Dorokhova, Agre, Kalakoutskii & Krassilnikov, 1968; Cross, Davies & Walker, 1971; Cross & Johnston, 1971)^[12, 14, 15, 16].

Both actinomycetes are of wide distribution in natural habitats and occur as strains varying in substrate and temperature ranges. The strains implicated in farmer's lung disease are primarily encountered in overheated hay and grain. Overheating may result in temperatures of 50-70° when produce is stored with moisture contents >35% (Gregory, Lacey, Festenstein & Skinner, 1963; Gregory & Lacey, 1963a, b). A range of fungal species including *Asp. fumigatus*, *Absidia ramosa* and *Mucor pusillus* is also encountered.

It is generally assumed that sensitization of a susceptible subject follows the inhalation of the high concentrations of

spores likely to be found in the enclosed, ill ventilated conditions of farm stock buildings when feeding stuffs are being worked. Lacey & Lacey (1964) estimated the concentrations of airborne spores before, during and after the shaking of mouldy hay in cowsheds. Using a cascade impactor (May, 1945) and an Andersen sampler (Andersen, 1958)^[3], they showed that concentrations of up to $1-6 \times 10^5$ spores/m³ occurred in the air whilst the material was being shaken and that 98% of the spores were of actinomycetes. Of this peak concentration 90% was deposited from the air within 20 min. The comparatively modern practice of moist grain storage in silos is most adequate and economic when efficient sealing restricts air supplies, and under these conditions mould growth is arrested. If, however, the seal is broken, moulding is rapid and during subsequent unloading procedures, aerial spore concentrations as high as 3×10^9 /m³ have been recorded (Lacey, 1969). Fifty % of the cases recorded by Grant et al. (1972) were due to mouldy hay and 27% were due to mouldy grain; mouldy straw was also a source of allergens.

Inhalation, Retention and Elimination of Respiratory Allergens

The organic dusts inhaled in enclosed buildings consist of a wide range of particles, amongst the insoluble representatives of which are pollens, propagules of algae, fragments of higher plant tissues, spores and mycelia of fungi and actinomycetes, bacteria and parts of the exoskeletal system of insects, along with an inorganic fraction rich in silica. Austwick (1966)^[4], in a review of respiratory fungal disease, illustrated diagrammatically the limits of penetration of different spore types in relation to air inhaled at an initial velocity of 150 cm/sec declining finally to 1 cm/sec in the alveolar ducts and sacs. The largest spore forms tend to become deposited in the nasopharynx and trachea, the smallest may penetrate to the alveolar spaces. As the spores of the allergenic actinomycetes measure 0.5-1.3 µm diam, penetration deep into the pulmonary spaces is assured. The results of the examination of traces from volumetric spore traps has shown that the lung normally copes with an intake of 12-15,000 fungal spores/m³ of air inspired during June-September in Britain (Gregory, 1961). Estimated figures of this sort contrast with those which may be compiled from the data of Lacey & Lacey (1964). Assuming a spore concentration of 16×10^6 /m³, in enclosed conditions it may be computed that an exposure of 30 min would result in the inhalation of 480×10^6 spores and retention of perhaps 200×10^6 in the lower respiratory tract. The fate of inhaled particles depends largely on the area of deposition. With the exception of the alveoli, their sacs and ducts, the respiratory airways are shrouded in mucus. Mucus is composed of 95-97% of water, 1-2% of salt and 2.5-3.5% of mucin, a mucopolysaccharide complex containing sialic acid and mucoprotein constituents. It is produced by glands and goblet cells of the mucosa (Kilburn, 1967)^[23], and is regarded as a blanket of two layers, the outer nearest the lumen of the airway being a stiff gel transport layer of high tensile strength and the inner a more fluid (sol) layer in contact with underlying mucosal cells. The outer gel layer is kept in motion by the beating of cilia from mucosal cells which are bathed in the sol layer. Particulate material adhering to the gel layer is passed upwards towards the hypopharynx and enters the gastrointestinal tract after being swallowed. Speed of movement of the gel layer mucus has

been estimated at 0-15-0-30 cm/min in the bronchioles, 0-25-1-0 cm/min in the bronchi and 3-0-4-0 cm/min in the trachea (Hilding, 1957). Apart from its protective influence against the penetration of mucosal cells by particles, mucus necessarily prevents the transudation of fluid and preserves equilibrium in water relations.

As both mucus and cilia are absent from alveoli many of the inhaled spores of actinomycetes will impinge on an area in which alveolar cells and their processes are covered solely by a surfactant layer (Pattle, 1967). This layer is composed of an outer insoluble lipoprotein area c. 5 nm thick, adjoining the alveolar lumen and overlying an inner slime or jelly of similar constitution. The surfactant, by lowering surface tension in the alveoli (James, 1965), acts as a stabilizer in which inhaled particles become coated. The coating renders them hydrophobic and so they remain unaggregated and suitable for phagocytosis. Phagocytic alveolar macrophages are assumed to swim in or on the surfactant. One of the primary responses of the lung to inhaled particles is to increase the number of macrophages proportionally to the total dust exposure (Morrow, 1967)^[28]. Macrophages attempt to digest ingested material and it is thought that those containing indigestible debris enter the mucus blanket and are eliminated by the gastrointestinal tract. If, however, the ingested particles are of pathogenic significance, i.e. capable of eliciting an inflammatory response, the macrophages involved may migrate to the lymphatics. It is estimated by Davis (1963) that 10% of alveolar dusts pass to the lymphatics and the rest are moved in the mucus blanket.

The efficiency with which particulate matter is eliminated from the lung depends largely on ciliary action and phagocytosis. These activities may be impaired by a number of factors including pre-existing respiratory disease, tobacco smoking, alcoholic intoxication and anoxia. Some factors affect ciliary function; others, secretion of mucus. Although most of the particles are eliminated during the first few hours after deposition, some may take weeks or years to be eliminated (Rylander, 1968; Channel et al., 1969)^[11].

Common types of respiratory hypersensitivity

In a review of the immunological basis of allergy, Lessof (1972) summarized the principal allergic diseases of the respiratory system. Inhaled allergens such as pollens, house dust, feathers, moulds and animal danders sensitize some 10% of the population which has a heritable predisposition to allergy, and is known as a topic. In atopic individuals, allergic rhinitis (including hay fever) and allergic asthma predominate. The reaction (type 1) is mediated by reaginic antibodies which are cell bound and belong to immunoglobulin antibodies of the E class (Stanworth, 1971). The reaction may be summarized as follows:

Antigen + cell-bound reagentin mast cells release of histamine, Slow Reacting Substance (SRS), etc.

If the vasoactive substances released act on the upper respiratory tract, dilation of capillaries together with an increase in their permeability give increased secretions and tissue fluid, and the symptoms of hay fever are felt. If, however, the reaction occurs lower in the respiratory tract, it may result in bronchospasm, increased secretions and the development of an asthmatic attack. Identification of the antigen responsible may be made by intradermal skin testing. A weal and flare are seen at the test site after a short time if the result is positive.

In contrast to the above reaction, which is essentially of immediate anaphylactic type, allergic alveolitis is usually, but not exclusively, found in nonatopic subjects who show a type III Arthus reaction mediated by circulating, cell free antibodies of the classes IgA, IgG or IgM, which can be made to precipitate with antigen in serological investigations. The pathological features of the reaction are thought to be due to the formation of immune complexes between antigen and antibody in the lung capillaries. In farmer's lung disease, these complexes, which also combine with some of the enzymes of serum complement, may disappear slowly from circulation. The very solubility of the complexes may be responsible for provoking symptomatology and be controlled by antigen excess. Patients with more antibodies may have milder symptoms because of the removal of insoluble complexes by the reticuloendothelial system.

Clinical features

The syndrome is described by Grant et al. (1972) and Stretton (1972) as one having an acute or insidious onset, possibly determined by the degree of exposure to antigen. Acute illness results several hours after exposure and is characterized by malaise, sweating, chills, anorexia, breathlessness and a sensation of tightness in the chest. Cough is usually distressing and often nonproductive. With more gradual onset, progressively increasing breathlessness may be accompanied by weight loss.

On X-ray examination there is a micronodular pulmonary shadowing. There is also restrictive ventilatory defect due to reduced distensibility and reduced diffusing capacity. In consequence Forced Vital Capacity (FVC), carbon monoxide transfer factor (TCO), and partial pressure of oxygen in the blood (Pa.O₂) are reduced. Resolution of all the abnormalities follows withdrawal from and subsequent avoidance of antigens. Irreversible chronic disease is marked by dyspnoea and cough.

Histopathological features

Lung biopsies from patients suffering acute attacks of the disease show a number of characteristic changes (Emanuel, Wenzel, Bowerman & Lawton, 1964, Hapke et al, 1968, Seal et al., 1968)^[19]. Inter-alveolar septa are usually infiltrated by large histiocytic and small round mononuclear cells, and a diffuse interstitial inflammatory infiltrate results. Noncaseating sarcoid-like granulomata with epithelioid and giant cell elements are common. They appear within 3 weeks and resolve over some 12 months. Neither bacterial nor fungal elements are visualized in these lesions, although Wenzel & Emanuel (1965) recovered *M. faeni* from biopsy material incubated at 60°. Granulomata together with intra-alveolar exudate account for X-ray changes.

In chronic disease, a diffuse, fine interstitial fibrosis is common, although focal areas of peribronchial distribution may be frequent. Cystic change, resulting in cavities several cm in diam can arise in heavily fibrosed areas (honeycomb lung). In the lung parenchyma from both acute and chronic stages of the disease, foreign body giant cells, some of which contain birefringent inclusions are not uncommon. Their significance has not been fully assessed, although considerable diagnostic value has been attached to them by Emanuel et al. (1964)^[19]. Except in the fatal, early, acute case reported by Barrowcliff & Arblaster (1968)^[6], surprisingly little severe vasculitis, a dominant feature of

Arthus reactions, has been reported for farmer's lung disease. Some workers have therefore questioned the resemblance between the disease and the classic Arthus reaction in which inflammatory change and necrosis of the vascular system result in haemorrhage. Considerable intra-alveolar haemorrhage was reported by Pirie et al. (1971a)^[31] in bovine farmer's lung and also vascular infiltration by polymorphonuclear leukocytes in intradermal skin tests. The mechanism is said to involve the liberation of lysosomal proteolytic enzymes (cathepsins) which effect subsequent vascular changes (Kabat, 1967)^[21]. That immune complexes, using Bovine Serum Albumin (BSA) and anti-BSA serum at the region of antigen-antibody equivalence may cause lesions in rat skin similar to those of farmer's lung has recently been demonstrated by Spector & Heesom (1969) in their studies of granulomata. Cases of the disease in which radiological evidence indicates an increase in fibrosis despite avoidance of exposure to antigen, pose greater problems for diagnosis than acute episodes. Decrease in antibody renders such cases unreactive serologically and symptoms resemble those met in diffuse idiopathic pulmonary fibrosis, pulmonary tuberculosis and other pneumoconioses (Fletcher, Rondle & Murray, 1970).

Conclusion

Treatment of severely ill patients in the acute stage of farmer's lung disease is by the administration of corticosteroids which probably stabilize the lysosomal membranes of polymorphonuclear leukocytes thus obviating the effects of tissue damage from proteolytic enzymes. The hypoxic patient may require 28-35% of oxygen. Rest and supportive treatment are adjuncts (Stretton, 1972).

Grant et al. (1972) suggested a number of measures regarded as necessary to reduce the prevalence of the disease. These included (1) an educational programme to familiarize workers with information on the disease and its prevention; (2) financial assistance to enable farmers of smaller properties to buy equipment for drying hay and cereal crops; (3) official propaganda to encourage silage production; (4) encouragements to aid in the construction of well ventilated and mechanically equipped stock buildings; (5) personal protection by the use of efficient respirators (Gourley & Braidwood, 1971). Nash & Eason (1972) commented on the proposals and suggested that a new attitude towards moulding of stored crops was necessary especially in view of the popularity of hay as opposed to silage production. They advocated a reappraisal of current standards of field management even where artificial drying is practised. The use of organic acid additives, particularly those containing propionic acid has had considerable success in inhibiting mould growth in stored cereal grain, but technical difficulties of evenly applying the additives to hay while baling are considerable.

References:

1. Wild LG, Mosenifar Z. Farmer's Lung (Hypersensitivity Pneumonitis). Medscape. 2023.
2. Sarika K, Sampath G, Govindarajan R, et al. Isolation and characterization of antimicrobial actinomycetes from coal mine soils of Telangana, India. Saudi Journal of Biological Sciences. 2021;28(6):3553–3558.
3. Andersen AA. New sampler for the collection, sizing and enumeration of viable airborne particles. J Bacteriol. 1958;76:471–476.
4. Austwick PKC. The role of spores in the allergies and mycoses of man and animals. Colston Papers, Butterworth. 1966;18:321–330.
5. Barbee RA, Dickie HA, Rankin J. Pathogenicity of specific glycopeptide antigen in farmer's lung. Proc Soc Exp Biol Med. 1965;118:546–552.
6. Barrowcliff DE, Arblaster PG. Farmer's lung; a study of an early acute fatal case. Thorax. 1968;23:490–495.
7. Blyth W. Fungi in relation to allergy and infection. Pharm J. 1970;204:599–605.
8. Boyd DHA. The incidence of farmer's lung in Caithness. Scott Med J. 1971;16:261–267.
9. Casarett LJ. Ph.D. Thesis. Rochester, N.Y.; 1958.
10. Casarett LJ. Some physical and physiological factors controlling the fate of inhaled particles. II. Retention. Hith Phys. 1960;2:379–386.
11. Channell S, Blyth W, Lloyd MM, Weir DM, Amos WMG, Littlewood AD, Riddle HFV, Grant IW. Allergic alveolitis in maltworkers: a clinical, mycological and immunological study. Q J Med. 1969;38:351–364.
12. Cross T, Johnston DW. Thermoactinomyces vulgaris. II. Distribution in natural habitats. In: Barker AN, Gould GW, Wolf J, editors. Spore Research. London: Academic Press; 1971. p.101–110.
13. Cross T, Maciver AM, Lacey J. The thermophilic actinomycetes in mouldy hay: *Micropolyspora faeni*, sp. nov. J Gen Microbiol. 1968;50:75–85.
14. Cross T, Walker DW, Gould GW. Thermophilic actinomycetes producing resistant endospores. Nature. 1968;220:352–355.
15. Davies CN. Handling of particles by human lungs. Br Med Bull. 1963;10:49–55.
16. Dorokhova LA, Agre NS, Kalakoutsii LV, Krassilnikov NA. Fine structure of spores in a thermophilic actinomycete, *Micromonospora vulgaris*. J Gen Appl Microbiol. 1968;14:295–302.
17. Druett HA. The inhalation and retention of particles in the human respiratory system. Symp Soc Gen Microbiol. 1967;17:165–175.
18. Edwards JH. The production of farmer's lung antigens. Med Lab Tech. 1971;28:172–178.
19. Emanuel DA, Wenzel FJ, Bowerman CI, Lawton BR. Farmer's lung: clinical, pathologic and immunologic study of twenty-four patients. Am J Med. 1964;37:392–405.
20. Hiding AC. Ciliary streaming in the bronchial tree and the time element in carcinogenesis. N Engl J Med. 1957;256:634–641.
21. Kabat EA. Structural Concepts in Immunology and Immunochemistry. New York: Holt, Rinehart & Winston; 1967.
22. Kahanpaa A. Bronchopulmonary occurrence of fungi in adults. Acta Pathol Microbiol Scand Suppl. 1971;227:227–235.
23. Kilburn KH. Cilia and mucus transport as determinants of the response of lung to air pollutants. Arch Environ Health. 1967;14:77–84.
24. Kobayashi M, Stahmann MA, Rankin J, Dickie H. Antigens in mouldy hay as the cause of farmer's lung. Proc Soc Exp Biol Med. 1963;113:372–380.

25. LaBerge DE, Stahmann MA. Antigens from mouldy hay involved in farmer's lung. *Proc Soc Exp Biol Med*. 1964;121:458–466.
26. Lacey J, Lacey ME. Spore concentrations in the air of farm buildings. *Trans Br Mycol Soc*. 1964;47:547–554.
27. Mackenzie DWR, Austwick PKC. The study of mycotic allergens in Britain. *Prog Immunobiol Stands*. 1965;164:164–172. Basel: Karger.
28. Morrow FE. Adaptations of the respiratory tract to air pollutants. *Arch Environ Health*. 1967;14:127–136.
29. Murray IG. The complement fixation test in farmer's lung: a preliminary study. *Mon Bull Min Hlth Lab Serv*. 1967;26:167–172.
30. Murray LG, Pepys J, Crighton WD. The latex agglutination test in farmer's lung. *Mon Bull Min Hlth Lab Serv*. 1967;26:96–103.
31. Pirie HM, Dawson CO, Breeze RG, Selman IE, Wiseman A. Precipitins to *Micropolyspora faeni* in the adult cattle of selected herds in Scotland and North West England. *Clin Allergy*. 1972;2:181–189.