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OF POULTRY WORKERS AND SCREENING OF THEIR
SERA FOR ANTIFUNGAL PRECIPITINS**

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ABSTRACT

401 poultry workers from different locations in Egypt were examined for mycoflora in sputum, laryngeal swabs and fungal precipitins in serum, 79 sera were examined by double immunodiffusion and counterimmunoelectrophoresis. 47.4% had fungi in their sputum. 368 fungal isolates were recovered both in pure and mixed cultures. The most predominant fungi were species of *Aspergillus*, *Penicillium*, *Mucor* and *Rhizopus*. More than half of *Aspergilli* were *A. niger*. Precipitating antibodies against various fungi were detected in 17 out of 79 serum samples. The relation of the host and fungi and significance of results are discussed.

INTRODUCTION

Fungi are ubiquitous in nature and their spores are found in the atmosphere in large numbers. Both man and animal can be subjected, accordingly to continuous contact with one or more of such fungi. Although the majority of fungi are not known to cause harm in man or animals, yet some fungi have been frequently reported to be able to cause infection (mycosis),

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intoxications (mycotoxicosis) or allergic reactions (hay fever, allergic bronchopulmonary aspergillosis; ABPA).

Although some fungus infections are known to be zoonotic, i.e. are transmitted from animals to man, however, mycotic affections of the respiratory tract by cross infection from man to man has not been yet substantiated. Nevertheless, animals can contribute to the infection indirectly. This may be expected in poultry farms, where *Aspergillus* species e.g. *Aspergillus fumigatus* can cause outbreaks of pulmonary aspergillosis in chickens (Refai and Rieth, 1966), whereby the *A. fumigatus* is released in the atmosphere of such a farm and the chance of inhalation of large numbers of spores in man is increased.

The aim of this study is to search for fungi in sputum or laryngeal swabs of poultry workers and to screen their sera for the presence of antifungal precipitins.

MATERIAL AND METHODS

Four hundred and one poultry workers in different locations in Egypt were examined clinically. Of these, 25 had chronic bronchitis, 6 bronchial asthma, 185 with smokers cough with morning sputum but without clinical chest findings and 185 were asymptomatic workers.

Sputum was collected from 215 cases, laryngeal swabs from the 185 asymptomatic workers and blood from 79 cases. The sputum samples were taken, early in the morning before breakfast and after washing the mouth with an antiseptic solution, in sterile screw-capped bottles and the swabs in sterile test tubes. Blood samples were collected from 79 cases, 48 of them were labourers having smokers cough. All samples were transported immediately after collection in an ice-box to Ain Shams Mycology laboratory.

Sputum was liquified and homogenized with pancreatin and swabs were shaken for 10 minutes in sterile distilled water. Both sputum and swab-wash were cultured on brain-heart infusion agar and sabouraud dextrose agar as well as on modified

Waksman's medium. The cultured tubes and plates of the first two media were incubated at 37°C and the third medium at 50°C. Plates of sabouraud dextrose agar, which were exposed in the atmosphere of the various poultry houses for 15 minutes were incubated at room temperature. The isolated organisms were identified according to their morphological and biochemical characteristics.

All sera were examined by double immunodiffusion (DID) and couter immunoelectrophoresis (CIE). The antigens used were prepared locally from *Aspergillus fumigatus*, *A. flavus*, *A. niger*, *Penicillium*, *Mucor* and *Rhizopus* species as well as *Candida albicans* and *Geotrichum candidum*.

RESULTS

Of the 401 poultry workers 190 (47.4%) were found to be positive mycologically, i.e. harbour fungi in their sputum. The highest incidence was recorded among smokers (89.2%). In case of the asymptomatic workers fungi in sputum were found in 10.8%. Only one out of 6 cases with bronchial asthma (16.6%) and 4 out of 25 cases of chronic bronchitis (16%) had fungi in their sputum (Table I).

368 fungal isolates were recovered, both in pure and in mixed cultures. The most predominant fungi were species of *Aspergillus*, *Penicillium*, *Mucor* and *Rhizopus*. Other fungi such as *Cladosporium*, *Hormodendrum*, *Geotrichum*, *Alternaria*, *Fusarium* and *Paecilomyces* were rarely isolated from the sputum. Four species of the genus *Aspergillus*, namely, *A. niger*, *A. flavus*, *A. fumigatus* and *A. nidulans* constituted 37% of the total isolates. More than half of the *Aspergilli* were *A. niger*. *A. fumigatus* isolates amounted to 10.9% of the *aspergilli* recovered (Table II). *Penicillium* species (Table II) represented the second major group (20.6%), followed by *Mucor* (17.1%) and *Rhizopus* (7.3%).

The fungi isolated from the 4 cases with chronic bronchitis were mostly mixed cultures of the three species of *Aspergillus*. In one case pure culture of *Penicillium spinulosum* was recovered. One out of the 6 cases with bronchial asthma had *A. niger* and *A. flavus* in his sputum.

Table I
Clinical, mycological and serological results of
poultry workers.

Clinical findings	Mycological results					
	No. examined	No. positive	%	No. examined	No. positive	%
Chronic bronchitis	25	4	16.0	25	3*	12.0
Bronchial asthma	6	1	16.6	6	1**	16.6
Smoker's cough	185	165	89.2	48	13***	27.0
Asymptomatic workers	185	20	10.8	—		

The positive sputum samples originating from workers with smoker's cough but without clinical chest findings and from laryngeal swabs of asymptomatic workers were mostly mixed cultures with *A. niger* the most commonly isolated fungus. The fungi isolated from the atmosphere of poultry houses were *A. niger*, *A. flavus*, *A. fumigatus*, *P. cyclopium*, *P. spinulosum*, *P. chryogenum*, *Mucor*, *racemosus*, *Rhizopus* and *Alternaria* species. Precipitating antibodies (Table I) against various fungi were detected in 17 out of 79 serum samples examined (21.5%). Antibodies to *A. fumigatus* alone were detected in one sample, and to *A. fumigatus* and other fungi as well in 8 cases. The comparison of the sputum mycological results (table I) in cases of chronic bronchitis reveals no conformity. The two cases positive serologically to *A. fumigatus* and *Geotrichum candidum* were

* Sputum culture was positive only in one case and was not conforming to the results of the serology.

** Sputum and serum were positive to *A. fumigatus*, *A. flavus* and *A. niger*.

*** All cases were negative in culture.

Table II
Fungi isolated from the sputum of
poultry workers

Fungi isolated	% in relation to the genus	% in relation to the total isolates
Aspergillus niger	52.9	
A. flavus	36.1	37
A. fumigatus	10.9	
A. nidulans	1.1	
Penicillium sp. nulosum	28.9	
P. cyclopium	26.3	20.6
P. chrysogenum	15.3	
P. italicum	15.7	
P. oxalicum	10.3	
Mucor racemosus	6.4	17.7
M. pusillus	36.6	
Rhizopus stonifer	14.3	7.3
Candida albicans	64.3	3.8
C. species	35.7	
Other fungi*	—	14.2

negative mycologically. On the other hand, the two other cases with fungi in the sputum, showed no corresponding antibodies in the sera. It is however, interesting to mention that one out of the 6 cases of bronchial asthma showed antibodies to *A. fumigatus*, *A. flavus*, and *A. niger* and the 3 aspergilli were recovered from the sputum. Of the 48 labourers with smokers cough examined serologically precipitins were demonstrated in 13 serum samples to various types of aspergilli and penicilli. Each sample was positive, at least to 3 types of fungi. However, no fungi could be isolated from laryngeal swabs taken from these cases (Table III).

* *Cladosporium*, *Hormodendrum*, *Geotrichum*, *Alternaria*, *Fusarium*, *Paecilomyces* and *Torulopsis*.

Table III
Comparison of sputum and serum results

clinical group	No. of cases	Sputum	Serum
chronic	1	—	A. fumigatus
bronchitis	1	—	Geotrichum
	1	A. niger, penicillium, Cladosporium, Fusarium,	A. fumigatus + A. flavus
	4	A. fumigatus, A. niger, A. flavus, penicillium	—
	18	—	—
B. onchal asthma	1	A. flavus, A. niger,	
	1	A. fumigatus, A. niger, A. flavus.	A. fumigatus, A. niger, A. flavus
	4	—	—
Smoker's cough	48	—	13 positive to one or more of the 8 fungi used as anti- gen.

* 3 with three types of Aspergilli, 1 with Aspergilli & Penicillium, 2 with A. fumigatus & A. flavus, 1 with A. niger, 2 with Penicillium, 2 with all antigens, 1 with Penicillium, Mucor, Candida and 1 with Penicillium & Rhizopus & Mucor & Candida.

DISCUSSION

From the results obtained in this work it can be concluded that the predominant species of fungi recovered from the sputum of poultry workers are more or less similar to those isolated from the atmosphere of poultry houses. It was not surprising to find *A. niger* on the top of the isolated fungi, as this type has been proved in several studies to be the most prevalent in Egypt (Refai and El-Bahay, 1963). El-Sarangawy (1977) reported also that *A. niger* was the most common fungi recovered from the sputum of asthmatics. On the contrary Sarangway (1966), who failed to isolate *A. fumigatus* from the sputum of his cases, this fungus was isolated 15 times from the sputum of poultry workers in this study (10.9%). However, this incidence seems to be much low in comparison with other parts of the world, where *A. fumigatus* is frequently isolated from the sputum. Our results agrees with Sarangawy (1977) that in many cases more than one fungus was isolated from the same patient. Hansen in 1957 showed that *aspergillus* is one of the atmospheric moulds which is most frequently responsible for asthma, to the same extent as *Cladosporium*, *Alternaria* or *penicillium*.

It is important to remember that presence of fungi alone in sputum is not diagnostic of bronchopulmonary mycosis. The combination of presence of the fungus in sputum and it's precipitins in the blood is suggestive of it's invasive nature. The non conformity of sputum culture and serological results denotes the non pathogenicity of the fungus present in the sputum but indicated the previous exposure to the fungus for which it's precipitins are present in the serum. The detection of fungal antibodies in the serum does not certainly mean presence of the disease to this fungus, but it difinitely suggests previous exposure to this fungus. Positive precipitin tests are also found among persons with no evidence of pulmonary disease who have been exposed to organic dusts containing fungal antigens (Nicholson 1972 and link et al. 1968). Positive precipitin reactions have even been found occasionally in persons who gave no known history of exposure to antigenic organic dusts. These persons included office workers, patients at chest ciinics, hospitalized patients and volunteers (Phanuphak et al. 1975 and Wenzel et al. 1974). Some

Researchers believe that a positive precipitins test reflects exposure to tested antigen (doPico et al. 1976) and does not certainly mean immunologic pulmonary disease (Russel et al. 1978). Other clinical and laboratory data are required to diagnose presence of the disease state. Aspergilli may cause allergic bronchopulmonary disease manifested by bronchial asthma, blood or sputum eosinophilia, recurrent pulmonary eosinophilic infiltrate, elevated IgE, aspergillus precipitins in serum, aspergilli in sputum plugs and central bronchiectasis which may proceed to pulmonary fibrosis and disability if the condition is not diagnosed early and treated with corticosteroids.

The spore of Aspergilli are 2-4 micron in diameter and when carried by air can be inhaled and reach periphery of bronchial tree. Three varieties of clinical manifestations are provoked by aspergillus i.e. Aspergilloma, allergic bronchopulmonary aspergillosis and septicaemic manifestations with the aspergillus behaving like an invasive parasite (Molina 1976). Allergic aspergillosis can show itself clinically in three ways. Sometimes by allergic alveolitis comparable to farmer's lung, by aspergillus asthma and lastly by mixed picture of allergic bronchopulmonary aspergillosis (Hinson et al. 1952, Pepys et al. 1959, Campbell and Clayton 1964, Simon 1971, Edge et al. 1971, Jordan et al. 1971 and Chomy et al. 1970). It is known that aspergillus can be present as a saprophyte in the respiratory tract and it can become pathogenic in patients who are debilitated, together with the use of large doses of antibiotics, corticosteroids or immunosuppressive agents (Molina 1976). Thus we can see the diversity of the relationship between the host and the fungus and the need to consider in diagnosis and evaluation of the results; the saprophytic nature of the fungus, the immunological state of the host such as whether atopic or not or whether immunodeficient and the state of lung and bronchi (Molina 1976). It is worth following the 17 workers who showed fungal precipitins in their blood. This with special reference to the worker with sputum and serum positive to *A. fumigatus*, *A. flavus*, and *A. niger*. It is interesting that from 185 asymptomatic workers none showed precipitins in their serum while 20 of them (10.8%) had fungi cultured from the sputum. This finding confirms the opinion that precipitins are present in the serum when the fungus induce immunological reaction as it penetrates and invades tissues.

As counterimmunoelectrophoresis (CIE) gives results nearly similar to those of double immunodiffusion test (DID) and because it needs shorter time to read, it is recommended to use CIE for preliminary examination specially if large number of samples are to be examined. CIE gives results after 1-24 hours while DID gives results after 3-8 days.

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