The Medical Journal of Malaya
Vol. XVIII, No. 3, March 1964.

THE ISOLATION OF HISTOPLASMA CAPSULATUM FROM A PATIENT IN A CHEST HOSPITAL

By J. T. Ponnampalam, Department of Bacteriology,
Institute for Medical Research, Kuala Lumpur.

The isolation of Histoplasma capsulatum from the soil of a cave infested with bats in Malaya is the first report of the presence of the fungus in Malayan soil (Ponnampalam, American Journal of Tropical Medicine & Hygiene, in publication).

Marsden (1953) in the Annual Report of the Institute for Medical Research, Kuala Lumpur, mentioned a case of histoplasmosis on histopathological examination. Kunaratnam *et. al.* (1960) described a case of histoplasmosis in Singapore.

Histoplasmin skin testing and serological examination of patients admitted to the Lady Templer Hospital, Kuala Lumpur, have indicated that a certain percentage show evidence of infection (Ponnampalam, unpublished data). In one of these cases Histoplasma capsulatum was isolated from the sputum.

The case history and laboratory examination are herewith described.

Case Report.

J. A., a Malay girl aged 13 was first admitted to the Lady Templer Hospital on the 23rd July, 1962 with a history of cough productive of yellow sputum and fever off and on for a year, with recurrent episodes of haemoptysis during this period. Previously she was admitted to a General Hospital in May the same year for two weeks where X-ray examination showed fibrous markings and cystic disease of the right lung adjacent to the mediastinum (Fig. 1). At the end of this period she was referred to a chest clinic where a bronchogram (Fig. 2) revealed the extent of the cystio disease affecting the right lower lobe. She was referred to Lady Templer Hospital with a view to surgery as the lung leision was not responding to medical treatment.

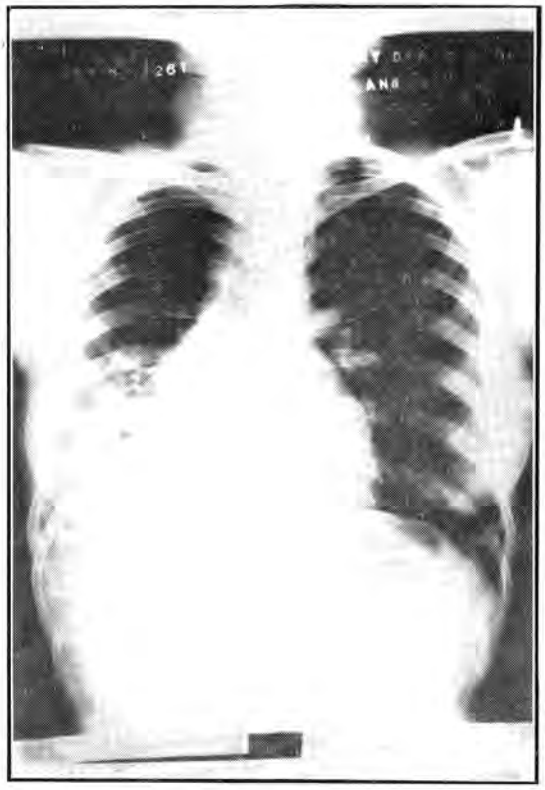

Figure 1.
Radiological Examination on 16.5.1962. showing fibrous markings and cystic spaces in the right lower lobe.

Figure 2. (Right).
Bronchogram on 17.6.62 showing the extent of the cystic disease.

Figure 3.
Radiological examination of chest on 20.7.62.

At the time of admission on 27.7.62 she had a chronic discharge from the left ear. Temperature was 98.4°F. The result of the radiological examination (Fig. 3) was similar to that of 16.5.62. The left lung was normal. ESR = 13 mm; Haemoglobin = 80%; white cell count = 8000, with polymorphonuclears = 52, lymphocytes = 44, and eosinophils = 4, the eosinophil count rising to 12 four months later and remaining at this level on discharge on 10.1.63. Pus from the left ear grew Pseudomonas pyocynaeus. Direct examination and culture of the sputum at intervals of a month during hospitalisation failed to demonstrate Mycobacterium tuberculosis. The tuberculin reaction on 23.7.62 was 12 mm. induration.

She received no anti-tuberculosis therapy in view of the negative sputum cultures. During her stay of 6 months in hospital the temperature remained fairly constant at 98.4°F, except on a few occasions when it was 99.4°F. A right pneumonectomy was done on 4.9.62, and histopathology revealed a non-tuberculous granuloma of the lung. Culture of the lung removed at operation, and sputum cultures for histoplasmosis were not carried out as the patient was not being investigated with histoplasmosis in view. It was only a month later that she was included in the survey of patients for histoplasmosis at the Lady Templer Hospital, and it was incidentally found that she had a positive complement fixation test for histoplasmosis.

The Mantoux test, histoplasmin skin test, and the complement fixation test for histoplasmosis carried out between October, 1962 and January, 1963 prior to her discharge home were: —

24.10.62. Mantoux test — 24 mm. induration. Histoplasmin skin test — negative. Complement fixation test with histoplasmin and other antigens — Histoplasmin 1:16; Yeast 1:16; Blastomycin 1:8.

The complement fixation test for histoplasmosis was repeated on 10.12.62 and was as follows: -

Histoplasmin — negative; Yeast — 1:8; Blastomycin — negative.

In view of an elevated complement fixation test for histoplasmosis following operation, six specimens of sputum collected on alternate days over a period of two weeks were examined, commencing 7.1.63. Each specimen was injected into 5 Swiss white mice, the animals not receiving any antibiotic cover. Each specimen of sputum was diluted with an equal amount of sterile physiological saline prior to inoculation. At the end of 8 weeks 8 mice were found alive out of a total of 30, the rest having succumbed to bacterial infection due to a Gram negative organism. When the surviving mice were sacrificed peritoneal adhesions, exudation, enlargement of liver and spleen were noted. These organs were removed, pooled and ground in a mortar with sterile sand and inoculated on to Sabouraud dextrose agar and blood brain heart infusion agar. A month later Histoplasma capsulatum (with its characteristic tuberculate chlamydospores on microscopical examination) was isolated from the pooled livers and spleens. The yeast phase was demonstrated in mice.

Direct examination of the Giemsa stained smears of the sputum prior to mouse inoculation did not reveal the presence of Histoplasma capsulatum. The patient was discharged home on 22.1.63.

While at home after discharge she had

pyrexia for 3 weeks associated with an exacerbation of her cough with increased amounts of purulent sputum. She was re-admitted for review on 18.7.63 still complaining of cough with a fair amount of sputum. Serological and skin tests carried out a day after readmission were as follows: -

19.7.63. Mantoux test — 19 mm. induration.
Histoplasmin skin test — Negative.
Complement fixation test — Histoplasmin — negative Yeast — 1: 8.

Six specimens of sputum were collected and examined as described above, following readmission, and found to be negative for Histoplasma capsulatum.

DISCUSSION.

Histoplasmosis may occur as an acute self-limiting catarrhal condition which is a primary infection or may progress to the chronic cavitary state. The signs, symptoms, and prognosis vary greatly with the clinical type of infection. Mortality in disseminated cases of the disease is 83 per cent while in the chronic pulmonary type it is 32% (U.S. Public Health Service Co-operative Mycoses Study 1961). Infection occurs via the respiratory tract with primary lesions in the lungs as a result of inhalation of spores of Histoplasma capsulatum from the soil. As this patient lived at a distance of many miles from Kuala Lumpur, examination of soil from the neighbourhood of her home was not carried out. Serological examination of patients at Lady Templer Hospital pointed to a high incidence (19.8%) of histoplasmosis in these patients (Ponnampalam, unpublished data). Sputum studies in patients with a positive serology have confirmed the findings of Furcolow (personal communication) that the fungus is recovered infrequently and with great difficulty from their sputum. The histoplasmin skin test is of limited value, being negative on two occasions in this patient. The serological test is a useful screening test to select those patients on whom sputum studies are to be carried out. If emphasis were placed only on the skin test this patient would have been missed. From a survey of over 250 patients at Lady Templer Hospital it was found that only 10.5% had a positive histoplasmin skin test, while 19.8% had a positive serology. It appears that the histoplasmin skin test is depressed in illness, and that patients suffering from histoplasmosis would be missed if only skin testing were done without serological examination

Complement-fixing antibodies appear after a few weeks of infection. The titres may be as low as 1: 2 to 1: 8 with histoplasmin antigen, while they may vary from 1: 40 to 1: 160 with the yeast phase antigen.. A small percentage of normal individuals have antibodies in low dilution. A single specimen of serum would be of little value in interpreting antibody response in either proven or unproven infections. Serial tests should be done throughout the infection. Such tests in proven histoplasmosis have shown a rise in antibody titre with a peak during the second and third week of the disease and a gradual falling off at 4 to 8 months until a baseline is reached.

Cross-reactions with Blastomyces antigens sometimes occur in the complement-fixation test from patients with histoplasmosis. In almost all instances, however, the titre is greatest with the homologous antigen.

Hypersensitivity. Intradermal injection of 1: 000 dilution of standardised histoplasmin shows a positive reaction a few weeks following infection. The test is read after 48 hours, and the reaction must show at least 0.5 cm. of induration to be considered positive. The skin test is sometimes depressed in illness. A positive histoplasmin skin test means past or present infection.

Patients sensitive to histoplasmin also may have cross reactions to blastomycin and coccidioidin. Histoplasmin injected into a site where a previous Mantoux test was carried out may cause a bigger induration than that caused at a site where no previous Mantoux test was carried out.

A single injection of histoplasmin in known negative and positive reactors does not affect humoral antibody formation. Multiple injections, however, may cause a rise in antibodies in positive reactors but not in negative reactors.

As Histoplasma capsulatum was isolated from the Sputum of this patient after pneumonectomy, and as the complement fixation tests have been repeatedly positive, and in view of the previous findings of a sputum repeatedly negative for Mycobacterium tuberculosis, it may be concluded that this was a case of chronic cavitary histoplasmosis. Radiological findings provide supporting evidence for this diagnosis, although per se radiological examination is not diagnostic. She will be reviewed periodically with a view to treatment with amphotericin B should the disease become progressive.

SUMMARY.

The first case of histoplasmosis in Malaya diagnosed on a positive culture and serology is described. The isolation of Histoplasma capsulatum from Malayan soil and the presence of positive reactors to the histoplasmin skin test suggests the possibility of histoplasmosis being present in the country to a significant extent. It presents an important differential diagnosis in the treatment of chronic chest conditions.

ACKNOWLEDGEMENT.

I am indebted to Dr. M. J. R. Snelling and Mr. H. M. Mcgladdery for allowing me access to their case; to Dr. I. G. Murray of the London School of Hygiene and Tropical Medicine, for confirming the identity of the fungus and to Professor H. B. Maitland, Head of the Department of Bacteriology, Institute for Medical Research, for much valuable advice and criticism.

REFERENCES.

Conant, N. F., *et al*, 1959. Manual of Clinical Mycology, 2nd edition. W. B. Saunders, Philadelphia and London.

Kunaratnam, N., *et al*, 1960. A case of histoplasmosis in Singapore. Singapore Medical Journal, Vol. 1, No. 3, p. 99.

Marsden, A. T. H., 1953. Annual Report of the Institute for Medical Research, Kuala Lumpur.

Mashburn, J. D., Dawson, D. F., and Young, J. M., Pulmonary Calcification in Histoplasmosis. Amer. Rev. of Resp. Diseases, Vol. 84, No. 2, p. 108.

Ponnampalam, J. T., 1963. Isolation of Histoplasma capsulatum from the soil of a cave in Central Malaya. Amer. Jour. of Trop. Med. & Hyg. (in publication).

United States Public Health Service, Co-operative Mycoses Study 1961. Vol. 177, No. 5, page 292. Course and prognosis of untreated histoplasmosis.