

3. Title

Study of the Dermatophytes Indigenous to Thailand

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This study was prompted by the major medical problems the dermatophytic fungi present to the military in times of stress. The project was initiated in November of 1965 in collaboration with Dr. Renoo, dermatologist at Women's Hospital in Bangkok. One morning each week, two members of this laboratory go to the clinic to collect material from patients with dermatologic problems. Late in July 1966, a similar collaborative effort was established with Dr. Vinitta, Laboratory Service Department of Medical Science, to obtain cultures from two additional hospitals in the Bangkok area. This source of material will be of value in that it will increase the number of male patients and provide patients from a lower economic stratum.

Patients presenting themselves to these clinics (Women's, Tobacco Monopoly, Bangrak Hospitals) were cultured irrespective of the clinical diagnosis. Fungi were isolated from approximately 40 per cent. Cultures were prepared by first cleansing the area of the lesion with 70 per cent alcohol and transferring material (skin, hair, nail) directly to two plates of Sabouraud-Cycloheximide-Chloramphenicol medium. The plates were sealed with paper tape, to prevent contamination, and periodically examined during a 21 day incubation at 25°C. Blood agar plates were also inoculated and incubated at 37°C when clinical appearance of the lesion indicated either a possible primary or secondary bacterial infection.

The most frequently isolated organism was Candida albicans, 38.56%, followed by Trichophyton rubrum, 34.22%; Trichophyton mentagrophytes, 15.12% Epidermophyton floccosum, 6.62%; Microsporium gypseum, 1.89%; Trichophyton tonsurans, 0.94%, Microsporium audouinii, 0.76% and Trichophyton concentricum, 0.19%.

Frequency of various fungi isolated from lesions on the following areas of the body.

A. Body (trunk, face, arms & legs) (190/529) 35.92%

| | |
|---------------------------------------|--------|
| 1. <u>Trichophyton rubrum</u> | 50.53% |
| 2. <u>Trichophyton mentagrophytes</u> | 17.37% |
| 3. <u>Candida albicans</u> | 13.16% |
| 4. <u>Epidermophyton floccosum</u> | 9.47% |
| 5. <u>Microsporium gypseum</u> | 4.74% |
| 6. <u>Microsporium canis</u> | 3.68% |
| 7. <u>Trichophyton tonsurans</u> | 1.05% |

B. Feet (149/529) 28.17%

| | |
|---------------------------------------|--------|
| 1. <u>Candida albicans</u> | 53.69% |
| 2. <u>Trichophyton mentagrophytes</u> | 22.15% |
| 3. <u>Trichophyton rubrum</u> | 19.46% |
| 4. <u>Epidermophyton floccosum</u> | 2.68% |
| 5. <u>Microsporium audouinii</u> | 1.34% |
| 6. <u>Microsporium canis</u> | 0.67% |

| | |
|--|--------|
| C. Groin (70/529) 13.23% | |
| 1. <u>Candida albicans</u> | 44.28% |
| 2. <u>Trichophyton rubrum</u> | 35.71% |
| 3. <u>Epidermophyton floccosum</u> | 14.29% |
| 4. <u>Trichophyton mentagrophytes</u> | 5.71% |
| D. Nails (52/529) 9.83% | |
| 1. <u>Candida albicans</u> | 90.38% |
| 2. <u>Trichophyton rubrum</u> | 9.62% |
| E. Hands (48/529) 9.07% | |
| 1. <u>Trichophyton rubrum</u> | 45.83% |
| 2. <u>Candida albicans</u> | 33.33% |
| 3. <u>Trichophyton mentagrophytes</u> | 12.50% |
| 4. <u>Epidermophyton floccosum</u> | 4.17% |
| 5. <u>Microsporum audouinii</u> | 4.17% |
| F. Scalp (11/529) 2.08% | |
| 1. <u>Trichophyton rubrum</u> | 36.36% |
| 2. <u>Trichophyton tonsurans</u> | 27.27% |
| 3. <u>Trichophyton mentagrophytes</u> | 18.18% |
| 4. <u>Microsporum canis</u> | 9.09% |
| 5. <u>Microsporum gypseum</u> | 9.09% |
| G. Oral (lips and tongue) 9/529) 1.70% | |
| 1. <u>Candida albicans</u> | 66.67% |
| 2. <u>Trichophyton mentagrophytes</u> | 22.22% |
| 3. <u>Microsporum canis</u> | 11.11% |

The incidence of fungi isolated from men (250) and women (279).

| | <u>Males</u> | <u>Females</u> |
|---------------------------------|--------------|----------------|
| Body (trunk, face, arms & legs) | 30.00% | 40.86% |
| Feet | 39.20% | 18.28% |
| Groin | 15.60% | 11.83% |
| Nails | 3.20% | 15.41% |
| Hands | 8.00% | 10.03% |
| Scalp | 2.00% | 2.15% |
| Oral | 1.60% | 1.79% |

The results of this survey clearly indicate the importance of fungi in dermatologic lesions in the tropics. It should be stressed that the distribution of etiologic agents among this population may not be identical to those which should be expected among U.S. personnel under field conditions. Some factors influencing the results are age, sex, occupation, socio-economic level and the influence of different geographic

areas. For example, children are more susceptible to scalp infections, and infants and older people more likely to have oral disease. Women were found to have more nail infections, and men more tinea pedis. This may be due to the fact that women are more apt to have their hands immersed in water for long periods of time, whereas men in the business world are required to wear shoes for longer periods than required of women. The influence of socio-economic level, hygienic habits and local areas of endemicity are self evident.

The species and prevalence of dermatophytes encountered is comparable to those seen in many parts of the world including the United States. However, several unusual organisms were encountered. Many of the isolates of Trichophyton rubrum were unlike those seen in the United States. They were characterized by a zone of bright yellow pigment at the periphery of the colony, with the typical deep red pigment restricted to the center of the colony. Colonial and microscopic morphology of these isolates were otherwise typical. An unusual Microsporum species, with characteristics of both M. canis and M. audouinii, was isolated from gibbons in the SMRL gibbon colony. Subsequently, similar organisms were isolated from human disease. A more complete description of this organism will be included in a discussion of the dermatologic disease in gibbons. The single isolate of Trichophyton concentricum serves as a reminder that this "exotic" organism does occur in Thailand and cases of tinea imbricata could occur among U.S. personnel. The single isolate is not an indication of the prevalence of this organism since the disease is endemic to several locations in Thailand where many clinical cases occur. Bangkok is not an area of high endemicity for tinea imbricata.

Tinea versicolor, a superficial mycosis, was frequently noted among out-patients seen in the dermatology clinics, among inmates in Thai prisons and among U.S. personnel. Cases have been confirmed by microscopic examination and by isolation of a yeast organism (Pityrosporum orbicularis) thought by some workers to be the etiologic agent. Dr. Renoo has expressed an interest in this disease and a limited effort has been made to obtain serum from patients and to isolate organism from clinical lesions. Antibodies to organisms identified as P. orbicularis have been detected using an indirect fluorescent antibody procedure. Reports from U.S. medical personnel in central Thailand indicate troops in this area have a high incidence of tinea versicolor, however, the infection does not create a military problem due to the extremely superficial nature of the disease. No major effort is being expended to study this disease.

The most striking finding revealed by the analysis of cultures from dermatologic patients was the major role Candida albicans plays in the etiology of skin diseases. These findings substantiated our earlier impressions and supported the additional investigations that have been conducted with this organism. Two studies have been initiated.

The comparative merits of four media, albumin from chicken and duck eggs, and human serum for the identification of C. albicans was determined. More than 100 strains of recently isolated Candida have been studied and the conclusions are that rice agar medium with 1% tween 80 is superior to corn meal agar with tween 80, Czapek's agar and commercial chlamydo-spore agar. Human serum is the best of the rapid methods (2 hours vs 48 hours required for conventional media) but not as sensitive as rice with tween 80. A brief report authored by the assistant investigators is planned for publication in the Thai medical literature.

A preliminary study was also initiated to evaluate the embryonated chicken egg as a rapid, inexpensive method for determining the pathogenicity of Candida strains. Fifty three strains of Candida albicans and 17 strains of C. stellatoideae, C. krusei, C. parakrusei, C. parapsilosis, C. quilliermondi, C. pseudotropicalis and C. tropicalis were used to determine the route of inoculation yielding the most reproducible lethality in embryonated eggs. Intravenous inoculation was selected as the route for determining the relative virulence of Candida species in subsequent studies.

Relative virulence has been determined using four strains of C. albicans and one strain each of C. tropicalis, C. krusei, C. stellatoideae, C. parapsilosis and C. pseudotropicalis. All determinations were made in parallel with a standard strain of C. albicans to minimize the variation among eggs from week to week,

Seven dilutions of inoculum (standardized by viable colony counts) were inoculated intravenously into each of 10 embryonated eggs. Death of embryos was determined at 24 hours. Relative virulence determinations showed all four C. albicans strains to be similar in virulence with the other Candida strains exhibiting a much lesser virulence. A simple, practical method for separating C. albicans from the other species was sought. It was found that 24 hour cultures of Candida species washed from the slant with 5 ml. of saline, and a 1:8 dilution of this suspension inoculated I.V. into embryonated eggs would provide this separation. C. albicans produced death in approximately 50 per cent of the embryos while other Candida species were not lethal. These results indicate the embryonated chicken egg to be a less costly and more rapid substitute for the classical adult rabbit pathogenicity test. No additional studies are planned with Candida species.

In January of 1966 an unusual dermatophyte was isolated from four gibbons suffering from skin lesions. Subsequently, 72 gibbons in the SMRL colony in Bangkok were cultured irrespective of lesions, and the organism recovered from 66.7 per cent (48/72). The organism is unusual in that it is a Microsporum species exhibiting gross and microscopic characteristics as well as nutritional requirements of both M. canis and M. audouinii. The organism was referred to Dr. L. Ajello, Communicable Disease Center, Atlanta Georgia, and Dr. Irene Weitzman, Columbia University for species determination. The isolates were identified as a distinct variety but within the limits used to define M. canis. A similar organism was isolated in 1962 from a gibbon imported into Germany from Thailand, and reported in the literature as M. audouinii. Our data clearly indicate this to be a common organism among Thai gibbons and an important disease agent in the gibbon colony. The hyperkeratotic dermatologic lesions in the gibbons produced by this organism have resisted treatment and present a therapeutic problem. This aspect will be covered more thoroughly in the Laboratory Animals Section of this report. Further characterization of these isolates is in progress including the range of pathogenicity and clinical response to various therapeutic agents. Infection was established on the backs of ten quinea pigs which subsequently responded to either 10 or 20 mg/kg/day of oral griseofulvin. The clinical response with 20 mg/kg/day was better than with the lower dose, however, the gibbons have failed to respond with doses of griseofulvin as high as 40 mg/kg/day.

Experimental infection was established on the forearm of an adult male producing an inflammatory reaction (10 X 10 mm) which slowly resolved to a more characteristic "ringworm" lesion. The area remained active for more than two months as evidenced by periodic recovery of the organism from the lesion. No treatment was used and all clinical evidence of disease slowly disappeared. This organism (a dysgonic M. canis) has been isolated from four human lesions. One from a 4 year old girl (ear lesion) who owned a gibbon and again from an 18 year old girl with wrist and arm lesions. Unfortunately, the gibbon died a few days before the cause of the lesion was known and was therefore not available for culture. The 18 year old girl denied any association with animals and gibbons in particular. The other two cases were SMRL veterinarians who had ample opportunity to contract the disease from infected gibbons. The first two cases responded to the usual topical applications used for dermatophytoses, whereas the two veterinarians responded dramatically to applications of TINACTIN. The organism was shown to be sensitive (in vitro) to 10 mcg of griseofulvin. The importance of this organism in man is still questionable, but the significance in present and future gibbon colonies is apparent.