Chapter VII

DISCUSSION

Some of the conclusions are as follows:

The diseases are caused by different fungi resulting in losses to animals, particularly those causing (i) dermatomycoses and (ii) systemic mycoses. Dermatomycoses is more widely prevalent than suspected, because of the environmental influences favouring occurrence and spread; meteorological data for 1975 revealed monthly average temperature variations from 7.6°C to 20°C, humidity from 48% to 94% and monthly total of rainfall from 1.4 mm to 300.0 mm. Fig. 6 shows incidence of disease in relation to climatic conditions. Ainsworth and Austwick (1955), Pepin and Austwick (1968) and Bora (1966) also found close relationship between seasonal variations and incidences of mycotic infections.

The fungi that cause the more important systemic mycoses of farm livestock are also ubiquitous. They grow on damp, decomposing vegetable matter, and liberate vast number of air-borne spores which constitute the inoculum potential. Isolation of the pathogens, estimation of spore concentrations in the atmosphere of animal shed, grazing field and silo and determination of fungus flora of animal feedstuffs and biological effects of the contaminants are thus of considerable importance.
The occurrence of fungi on different animals as dermatophytes has been recorded as follows:

**Cattle:** Out of 236 samples, 41 samples are infected with dermatophytic fungi (17.37%). Kaplan et al. (1959) as cited by Gupta (1967) reported that in a survey during 1956-57 by the Communicable Disease Centre, U.S.A., 27.2% of 2361 domestic animals were positive for fungi. Gupta (1969) found an incidence of 2.46% in cattle in Nissar district. Six different species, *M. canis* (1), *M. gypseum* (1), *T. mentagrophytes* (3), *T. rubrum* (1), *T. verrucosum* (34), and *T. violaceum* (1) are recorded. Vinokurov (1975) recorded an infection rate of 35% in a group of 771 animals in USSR. Abu-Samara (1975) recorded the rare occurrence of *M. canis* infection in calves.

**Goat:** Of 113 samples, 9 dermatophytic isolates (7.96%) belonging to three species, *T. verrucosum* (5), *T. mentagrophytes* (3) and *M. canis* (1) are recorded. Pepin and Austwick (1968) also described these three fungi recorded from goats detected on three occasions out of 15 samples.

**Buffalo:** Of 99 samples, 6.06% are infected with dermatophytic fungi. The fungi involved are - *M. gypseum* (2), *T. mentagrophytes* (3) and *T. violaceum* (1). Abou-Gabal et al. (1976) found ringworm infection in 47% of 480 animals in Egypt. Isolates were commonly *T. verrucosum* and *T. mentagrophytes.*
Pig: 128 lesion materials on examination gives 13 dermatophytic isolates (10.15%) belonging to four species, *T. verrucosum* (6), *T. mentagrophytes* (3), *M. gypseum* (2) and *M. canis* (3). Ek (1965) found *T. verrucosum*, Mc Pherson (1956) and Ginther et al. (1964) found *T. mentagrophytes* and Stankushev et al. (1976) found infection of *M. gypseum* in Pigs.

Horse: Out of 79 lesion samples, 10.12% are infected with dermatophytic fungi, *T. equinum* being the most common (6 out of 8 isolates) and *T. mentagrophytes* (1) and *T. tonsurans* (1) comes next. This is the only occasion when *T. tonsurans* is isolated out of a total 876 samples common to all animals. In India three species *T. equinum, T. mentagrophytes var granulare* and *M. gypseum* causing equine ringworm have been recorded by Kulkarni and Choudhuri (1969), Tewari (1963) and Padhya et al. (1966). Of 196 isolations Petrovich (1975) recorded 89% *T. equinum, 8.4% M. equinum* and 3.9% *T. gypseum*.

Dog: Dog is the second important host for dermatophytic fungi. Of 133 samples, 12.78% are dermatophytes. Ainsworth and Austwick (1955) reported much higher incidence of infection in dogs, 28%. Six different species, *M. canis, M. gypseum, T. mentagrophytes, T. rubrum, T. verrucosum* and *T. violaceum* are recorded. Infection by *Trichophyton* species is less frequent than that by *Microsporum* species. Keplan and Gump (1958) have also reported *T. rubrum* infecting dog. Hajsig et al. (1975) isolated *M. gypseum* from dog in Yugoslavia. There is no report
of isolation of *T. rubrum* from dog so far in India.

**Monkey:** A total of 88 specimens are obtained from different sources. In majority of cases the animals are from Zoological Parks. Of 31 specimens received in 1974, 5 are from animals killed during hunting. It is of interest to note that dermatophytic infection is prevalent when the animals are free living also. Of 9 species isolated, 6 are *Trichophyton simii*, 2 are *T. mentagrophytes* and 1 is *Microsporum gypseum*. Emmons (1940) recorded occurrence of *T. mentagrophytes* and Pepin and Austwick (1968) recorded *T. simii* and *M. gypseum* respectively.

It is noted that of all 7 mammalian hosts cattle harbour most of the fungi, 17.37% dermatophytic and 46.18% of other fungi. The second important host is dog with 12.78% dermatophytes and 19.54% other fungi. Other animals supporting higher incidence of fungi are pigs and horses.

The clinical appearance of lesions on different hosts appears not to be very informative except that in most cases (70%) crust formation is the general rule. No clear difference is observed between lesions caused by *Trichophyton* and *Microsporum* species.

In regard to the age influencing susceptibility of animals it appears to be more common in young animals below 1 year of age. No conclusive data, however, on incidence of infection in relation to age are recorded. McPherson (1957) recorded over all incidence in calves being 7.34% and in adults
Kamyszek (1975) also observed greater incidence of the disease in young animals.

The analysis of figures on month-wise distribution of infected materials collected in the four-year period is also of interest. The highest number of skin materials are received and collected in the month of December, January and February. From March onward number of samples gradually decreases to a minimum of 8 in July but from August there is increase in incidence of infection. It is probable that ringworm in animals is predominantly a disease of winter months although prevalent throughout the year (Fig. 6). Kamyszek (1975), while studying the epidemiology of ringworm in cattle, reported similar observations. The factors for this seasonal fluctuation could not be clearly ascertained. Less humid and periods with lesser rain and low temperature seem to encourage skin infections. Another cause for higher winter incidence is the yarding together of animals where a high rate of infection due to direct contact is possible.

It thus appears that dermatophytic infections in animals in Nagaland are of a high order with large number of fungi involved in the aetiology of skin infections. Vinokurov et al. (1975) showed that economic losses were great due to ringworm in cattle by way of reduction in weight gain and damage to hides.

The mycological aspects of the above findings are of importance in relation to the source of inoculum, saprophytic behavior of the pathogens during some stage, method of
transmission of the disease. The inoculum potential of the pathogens is attributed to varied sources such as farm house, silos, hay, house fly, mice and air.

The air borne route for transmission of ringworm appears to be of minimum importance because a very low percentage of dermatophytic fungi, *T. verrucosum* (1.39% to 3.23%) and *T. mentagrophytes* (1.54% to 1.61%) is isolated from the air of animal houses. High air counts of a particular pathogen may act as a marker of dissemination and thus potential cross-infection, but low air counts may not indicate an absence of cross-infection. If this is so, infections are effected by direct fall-out into exposed tissues but there is no evidence to support this.

Furthermore, lesions in ringworm infection progress if suitable conditions for mycelial growth exist, including a warm atmosphere and slightly alkaline pH of the skin (Blood and Henderson, 1968). pH is also important as it affects rate of multiplication of a microorganism. The more rapid it is, the more the likelihood of establishing the infection against the activity of the host defense mechanism. Regarding the pH range, dermatophytes show a remarkable wide pH range (4 to 10), the optimum being 6.5; the growth decreases as the pH increases above 7. Stockdale (1953, 1977) recorded optimum pH level at 6.6 for most dermatophytes and a wide pH range as observed in the present findings. The susceptibility of humans to ringworm infection is much greater before puberty than afterwards when the skin pH
falls from about 6.5 to about 4.0 (Blood & Henderson, 1968). A similar relationship in animal pathogenicity can also be assumed with the observation of higher ringworm incidence in calves.

The growth of all dermatophytic isolates is also effected by temperature, optimum being 25°C, except for the isolate *T. verrucosum*, which grows best at 37°C. There is very little growth at 15°C, while growth is nil at 40°C. Stockdale (1953) found that dermatophytes grow best in culture at 25°C-35°C, but optimum have not been reported.

The growth of dermatophytes in vitro varies considerably with the composition of the media, SGA being best (SGA, PSA and CMA). The change in the composition of the media with the addition of urine (cow), sodium chloride and hot-spring water at different concentrations show that urine and NaCl are inhibitory to the growth of all isolates, while addition of hot-spring water favours growth of the isolates except *T. verrucosum*.

Dermatophytes also attack keratinized tissues, the stratum corneum and hair fibres, resulting in autolysis of the fibre structure. Inoculation experiments with hairs of different animals and embryonated eggs suggest that all dermatophytes are not equally virulent in establishing an infection. *M. canis* and *T. verrucosum* are pathogenic to all hairs (cattle, dog, horse and man), while *T. simii*, *T. tonsurans* and *T. violaceum* are pathogenic only to human hair. Except for *T. rubrum*, *T. tonsurans* and *T. violaceum*, all other isolates produce lesions on chorioallantoic membrane of embryonated chicken eggs suggesting
that they are weak pathogens.

The environment may also be a possible source of infection in animals. The sampling of air of animal shed and field throughout the year (1975) reveal that air spores are likely to be concerned in the incidence of disease. It is found that Cladosporium, Aspergillus and Penicillium are the most prevalent types of spores in air of animal sheds and field. Cladosporium occur more during May-July, while Aspergillus and Penicillium types occur abundantly during March-November. The changes in the spore concentration as well as frequency of types occurring are noted with the variations in the climate (Fig. 7). Cold dry period (November-March) and hot humid period (May-September) are characterised by changes in temperature, humidity and rainfall from month to month. The presence of such types of fungal spores as Helminthosporium and Pithomyces whose metabolites are known to be pathogenic to animals is of importance (Roberts et al., 1963; Hall, 1965; Shotwell & Ellis, 1976; Forgacs & Carll, 1962; Dodd, 1965; Keogh, 1975; Caple et al., 1976; Kirton et al., 1976).

Furthermore, spore concentrations of Aspergillus and Penicillium and actinomycetes are greater in the cowshed than in the field causing probably other mycotic diseases like abortion in farm cattle. This is confirmed by the data (Table 33) obtained from the State Cattle Breeding Farm, Larie, Kohima, showing 10 abortions (9.09%) against 28 new births (25.45%) in a total population of 110 animals in 1977. There appears to be
a relationship between fungal spoilage of stored feed and spore concentrations within cowshed with that of abortion incidence. Baruah (1961) has also shown that cattle are maximally exposed to Aspergillus-type spores while being fed hay, which is also the common source of Aspergillus and mucoraceous infection.

Another important aspect is the abundant presence of actinomycetes spores in animal sheds, some of which may be pathogenic to animals as allergens. Isolation of Thermoactinomyces vulgaris (9.0%), Micropolyspora faeni (11.9%) and Nocardia sp. (4.5%) from the ensilage and also subsequent isolation of M. faeni (16.6%) from oropharyngeal samples from cattle with respiratory disease symptoms indicate a relationship, emphasising the importance of silo microflora as a contributor to the airspora and respiratory diseases of unknown or uncertain etiology. These observations lend weight to the suggestions by Pirie et al. (1971), Omar & Kinch (1966), Macleod (1966) and others that airborne organisms were the source of pulmonary distress in cattle. Harzog (1970) found the condition prevalent at an altitude of 1,000-1,200 m above sea level. Kurup et al. (1976) presented evidence of prevalence of thermophilic actinomyte, M. faeni in hay, maize, wood dust and house dust samples. Pelhate (1975) while studying the microflora of ensilaged fodder, recorded similar findings.

Foodstuff are subject to spoilage by several fungi. Christensen (1965) has described a wide variety of fungi that invade cereal grains and their products. The total counts of aerobic bacteria, actinomycetes and fungi on the feed samples
FIG. 7. RAINFALL, % RH AND AVERAGE TEMP. AND INCIDENCE OF DIFFERENT FUNGI IN THE YEAR 1975
Table 33: Breeding data of State Farm, Larie, Kohima, 1977*

<table>
<thead>
<tr>
<th>Name of affected animal</th>
<th>Total No. of Animal</th>
<th>Total No. of New birth</th>
<th>Total No. of abortion</th>
<th>Date of abortion</th>
<th>Pregnancy stage (months)</th>
</tr>
</thead>
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<tr>
<td>Habili</td>
<td>110</td>
<td>28</td>
<td>10</td>
<td>23.3.77</td>
<td>6-7</td>
</tr>
<tr>
<td>Lanu</td>
<td></td>
<td></td>
<td></td>
<td>1.4.77</td>
<td>6-7</td>
</tr>
<tr>
<td>Sharmila</td>
<td></td>
<td></td>
<td></td>
<td>29.6.77</td>
<td>6-7</td>
</tr>
<tr>
<td>Rukma</td>
<td></td>
<td></td>
<td></td>
<td>1.10.77</td>
<td>3-4</td>
</tr>
<tr>
<td>Rambha</td>
<td></td>
<td></td>
<td></td>
<td>9.10.77</td>
<td>6-7</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>11.10.77</td>
<td>6-7</td>
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<tr>
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<td></td>
<td></td>
<td>3.11.77</td>
<td>3-4</td>
</tr>
<tr>
<td>Subha</td>
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<td></td>
<td>6.11.77</td>
<td>6-7</td>
</tr>
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<td></td>
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<td>3-4</td>
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<td>23.11.77</td>
<td>6-7</td>
</tr>
</tbody>
</table>

*Source: Directorate of Veterinary & Animal Husbandry, Nagaland
examined ranged from 8 to 2891 micro-organisms per 100 gms. The frequency of infection by fungi is the highest and no pattern is established for different months, except the highest distribution recorded in September. It appears that there is a relationship trend for Penicillium to decrease in population whereas there is increase in Aspergillus and Mucor. The conditions favourable for growth of Aspergillus and Mucor may not be conducive for Penicillium during that period (Fig. 8).

The incidence of different Aspergillus species in feed samples on being analysed shows that A. flavus Link ex Fr. is the most common occurring in all samples. Bryden et al. (1975), in a survey of Australian feedstuffs recorded 83 isolates from 109 samples. Of these 40 isolates were shown to be capable of aflatoxin production. Zapletal et al. (1976) also isolated a rapidly growing strain of A. flavus from a feed mixture implicated in an outbreak of aflatoxicosis in pigs. A. terreus is the next common species with 16% incidence. The percentage of occurrence of A. fumigatus, A. niger and A. candidus are 9.5, 14.5 and 7.5% respectively. Brown (1975), in animal health 1974, record A. terreus as the most common fungus found in grains associated in a fall in milk production and cattle deaths. Hesseltine et al. (1975) suggest that A. niger may play an important role in aflatoxin production by A. flavus. In this study also in all but one feed sample (B-1), where aflatoxin was present, A. flavus and A. niger are both present. This interaction is however, not clarified. Lillehoj and Zuber (1975) reported that low-grade grain heavily
contaminated with fungi represents a source of fungal propagules; this primary inoculum potential can be particularly hazardous when lots of corn are blended for commercial purpose.

Similar results have been reported by Lillehoj and Fennell (1975) who observed *A. terreus* commonly in badly deteriorated maize samples but did not find in less damaged maize samples; this indicates a relationship between extensive deterioration and development of the fungus. Goodman and Christensen (1952) also identified occurrence of *A. flavus* and *A. terreus* in mouldy maize.

Of the four samples of grade-B feed mixture obtained from separate farms and apparently with different storage conditions, three samples are positive for aflatoxin. Kiermeier et al. (1977) examined 105 feed samples from cattle farms and found that 45 were positive for aflatoxin, mostly aflatoxin B₁. Maggon et al. (1969) reported that of 9 strains of *A. flavus* isolated from soil, 5 formed large amount of toxin (15-19.5 mg/50 ml), two gave small amounts (0.6-1.4 mg/50 ml medium), and two were negative. According to Mehan and Chohan (1973), 21 strains of *A. flavus* isolated from cotton, maize and wheat seeds, 16 isolates formed aflatoxin B₁ with levels at 0.85 to 1.72 mg/50 ml of medium.

The results of the present study do not appear to be alarming because of the low incidence and low levels of aflatoxin detected which is within the guide line level of 20 ppb aflatoxin in feed. However, according to Newberne (1973), low-level long
Figure 8. Seasonal incidence of Penicillium, Aspergillus & Mucor in feed.
term exposure can result in failure to grow with development of lesions most marked in the liver. So, low-level aflatoxin poisoning to the livestock is thus not insignificant.

A possible correlation has, however, been observed between the source of the substrate situated at different altitudes and levels of aflatoxin production. Feed from farm at Dimapur at an altitude of 200 meter showed toxin levels at zero and 1.1 ppb. The feed from Ghaspani farm (800 meter) showed 6.5 ppb of aflatoxin while feed from Kohima farm (1500 meter) showed higher aflatoxin level at 20 ppb. This finding indicates that altitude may have some effect on aflatoxin production, other conditions not varying to great extent. However, there is no evidence to establish a relationship between altitude and aflatoxin formation.

Probably moisture is the most important single factor controlling fungal growth. Christensen (1957) grouped fungi that occur in seed into groups that occur in the field and those that occur primarily in storage. The three samples with 1.1, 6.5 and 20 ppb of aflatoxin $B_1$ are at moisture levels of 16.2%, 16.3% and 16.4% favourable for toxin formation, and the fourth sample (B 1) with 14.5% moisture exhibited no aflatoxin formation. This agrees with the findings of Shotwell (1977) who found that moisture levels must be above 16% if toxin is to form.

The evidence that some isolates of *Aspergillus flavus* produce aflatoxin in animal feed is based on: (1) when feed extracts were spotted in TLC plates with aflatoxin standards, the
material was identified as aflatoxin B₁ and (2) when the extracts were injected into chicken eggs, the embryos died at certain doses.

The other side effects include splayed foot, diminution in growth and short legs in surviving birds. The average weight ratio of surviving chicks from non-incubated eggs to controls is 1:1.2, while the ratio between surviving chicks of 7-day incubated eggs to controls is 1:1.4. Choudhury and Manjrekar (1967) have reported severe growth retardation and short legs in sublethal doses of aflatoxin. Adekunle and Bassir (1975) in their studies with aflatoxin B₁ in chicken embryo also reported decreased body and organ weight in hatched birds. In embryos injected with 0.023 µg/egg, toxins were detectable in the spleen, liver, kidneys, muscle, brain and bone of day-old chicks as unchanged compounds at 1-6.8 ppb. The mutagenic, teratogenic and carcinogenic effects of aflatoxins in animals were discussed by Zawirska (1975). A possible etiological relationship between aflatoxins and tumours in man has also been advanced.

The result on the whole, indicate the occurrence of mycoses in animals and also the effects of mycotoxicoses likely to constitute the health hazards of man.