STUDIES ON THE DIAGNOSIS, PATHOGENESIS AND TREATMENT OF **TRICHOPHYTON VERRUCOSUM** BODIN, 1902 INFECTION IN CATTLE

By

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This is to certify that the thesis "STUDIES ON THE DIAGNOSIS, PATHOGENESIS AND TREATMENT OF TRICHOPHYTON VERRUCOSUM BODIN, 1902 INFECTION IN CATTLE" submitted for the degree of Master of Veterinary Science (Medicine) of the Orissa University of Agriculture and Technology is a faithful record of bonafide and original research work carried out by Surendra Kumar Ray, under my guidance and supervision. No part of the thesis has been submitted for any degree or diploma.

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(S. K. MISRA)

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CONTENTS

LIST OF TABLES

***

LIST OF ILLUSTRATIONS

ILLUSTRATIONS

***

CHAPTER I

INTRODUCTION

Mycoparasitic infection in man and animals is of utmost importance at the present. Since modern therapeutics have reduced the incidence of various bacterioses and rickettsioses, and some viral diseases have been controlled by successful vaccination, increased interest has been taken in fungal diseases. Dermatophytoses or ringworm is a clinical entity caused by members of the closely related group of fungi collectively termed as dermatophytes. These fungi parasitize the keratinized tissues of the body, such as the epidermis, the hair and the nails and they produce alterations of the structures invaded. These alterations, together with attendant immunological reactions, are clinically classified as ringworm (Kaplan et al., 1958). Dermatophytoses are therefore true mycoses of the keratinized structures of homoiothermous animals including man (Dvorak and Otcenasek, 1969). Dermatophytoses of animals, especially those of domestic animals, represent a serious economic problem. In addition, animals are a frequent source of human dermatophytoses.

Bovine ringworm is a well recognised disease which has generally been considered by the farmers to be a natural hazard of stock keeping. This disease may assume economic importance when the valuable and pedigreed animals destined for sale and export are involved. Severely affected animals may be unthrifty and the young-stocks suffer a retarded growth (McPherson, 1957). The damage to the skin profoundly affects its texture

and consequently the market value (Blank, 1955).

Ringworm in cattle is world-wide in distribution and is caused mainly by Trichophyton verrucosum Bodin, 1902 (Dawson, 1963). This fungus causes the vast majority (93 to 100 per cent in some areas) of ringworm infection in cattle (McPherson, 1957; Mahajan and Mohapatra, 1963). Sabouraud's species T. album, 1908, T. onchraceum, 1910 and T. discoides, 1910 have been reduced to varietal status or cultural types of T. verrucosum by Ainsworth and Georg (1954) and Cantanei and Schoesboe (1953). At the International Congress of Dermatology in Stockholm (1957), Georg submitted a system which includes T. verrucosum as a valid species. Dvorak and Otcenasek (1964) have reported this dermatophyte in various other hosts such as canary, chickens, cat, dog, donkey, drome-dary, goat, horse, mule, sheep and swines.

Age of the host appears to have a significant effect on the incidence of T. verrucosum infection in cattle. Calves under one year of age are much more susceptible than the adult ones (McPherson, 1957; Misra, 1971) and as such adult cattle are seldom affected unless they are malnourished or otherwise poor in condition. Animals when kept together in large groups and in close proximity to each other for long periods are highly susceptible. In addition to the spread of infection through direct contact from one animals to the other, indirect contamination through brushes, harness, fences, wood works and walls which allow the fungus to survive for years, thus increase the incidence of this disease. Seasonal effect on the overall

incidence of bovine ringworm is not significantly related but the incidence has been reported to be more in winter and subsequently decreasing in spring in Great Britain and U.S.A. (Blood and Henderson, 1963). The nature of herd keeping and the poor hygienic condition as imposed by extreme climates are the important factors to aggravate this infection.

T. verrucosum parasitizes the non-living keratinized layers of the skin and its appendages but is normally incapable of invasion of underlying living tissues. In cattle the typical lesion is a heavy grey-white crust raised perceptibly above the skin. The lesions are roughly circular and about one inch in diameter. In the early stages the surface below the crust is moist, in older lesions the scab becomes detached and pityriasis and alopecia may be the only obvious abnormalities. Lesions are most commonly found on the neck, head and perineum but a general distribution over the entire body may occur, particularly in calves; and in severe cases the lesions may coalesce (Blood and Henderson, 1963). The experimental infection of laboratory animals (mainly rabbits) with different strains of T. verrucosum follows a similar pattern of development of natural ringworm in calves but the natural disease often assumes a chronic form and is of longer duration than in experimentally infected rabbits (Cox and Moore, 1963). The period after experimental infection before distinct lesions appear is about four weeks in calves. A resistance to reinfection occurs after recovery from experimental infection but the importance and duration of this immunity in field cases is unknown (Blood and Henderson, 1963).

A pre-requisite for effective prevention and treatment of this disease is a correct laboratory diagnosis of the species and differentiating it from other variety of agent(s) responsible for such type of infections. This is only possible by mycological methods. In the infected hairs, T. verrucosum is recognised by the sheath of chains of rather large arthrospores and by the hyphae which are often clearly seen to be tunnelling the hair shaft. The isolation of T. verrucosum from cattle, by culture, has always rendered more difficulty in comparison to other dermatophytes because of its slow growth and more liable to be overgrown by other saprophytic moulds in the primary cultures and for the requirement of enriched medium (Ainsworth and Austwick, 1955). The colonies are heaped and deeply folded in appearance. The micromorphology of the culture is often poor and the most frequent elements found are large number of chlamydospores. The macroconidia and microconidia are normally absent, only some strains produce them in limited numbers (Dvorak and Otcenasek, 1969).

Different kinds of treatment have been tried against T. verrucosum infection in cattle with varying degree of success. The most effective treatment is the antibiotic griseofulvin but, unfortunately, cost precludes its routine use in large animals. Other chemotherapeutic agents and indigenous plant extracts are screened for their antifungal activities in vitro, to find out cheaper and more potent substances against various dermatophytes including T. verrucosum (McPherson, 1969; Misra, 1971). Milks (1949) has remarked "the fungicidal property of a preparation

may be high *in vitro* and yet of little value in therapeutics because of its inability to penetrate the stratum corneum, a hair follicle to come in contact with the fungus". Hence substances having *in vitro* antifungal activity against *T.verrucosum* have to be assessed *in vivo* for their therapeutic value.

Ringworm in cattle imposes a great public health importance. One of the earliest record was made in ninteenth century by Earnst, Veterinary Surgeon from Zurich, stating a case of an young girl who was infected with ringworm from a cow. Although cattle are the preferred hosts for *T. verrucosum*, this dermatophyte is also infectious for man (Fowle and Georg, 1947; Rook and Frain-Bell, 1954; Shome, 1969; Misra, 1971). Human infections due to *T. verrucosum* are confined almost entirely to the rural population. The most common type of lesion described in human infections with this dermatophyte is of a suppurative character. Frequently, human contract this disease by direct contact with infected cattle but may do so by exposure to contaminated fomities. However, it is not known whether human transmits this infection to cattle or other animals.

In India, where the geographical and climatic conditions are very condusive and favourable for the growth and spread of ringworm infection by *T. verrucosum* in cattle causing economic and public health hazard to the farmers and as a whole to the community, investigations on diagnostic procedure and for cheaper and convenient method of treatment are very insufficient during past years. Considering the importance of the infection and the magnitude of the problem both in man and animals, the

author envisages to undertake the following investigations to evolve a better diagnostic procedure and clinical approach.

1. To study and compare different methods under field and laboratory conditions for evolving correct and effective diagnostic method(s) for T. verrucosum infection in cattle.
2. To investigate the pathogenesis of T. verrucosum in natural cases and in experimentally infected calves and laboratory animals.
3. To evaluate the in vivo therapeutic values of certain chemicals and plant extract known to have in vitro antifungal action against T. verrucosum.

It is fervently hoped that the above studies will elucidate further information on the diagnosis, pathogenesis and treatment of T. verrucosum infection in cattle, a continuing problem to the veterinary clinicians and mycologists.

CHAPTER II

REVIEW OF LITERATURE

Incidence of Dermatophytoses in Cattle

Investigations in this country and other parts of the world on the incidence of dermatophytoses in cattle have revealed that T. verrucosum is the principal cause of bovine ringworm.

Moerlein (1946) studied the clinical materials from several herds of cattle and isolated seven strains of T. album (T. verrucosum).

During 1955 Ainsworth and Austwick published their report on survey of animal mycoses, sponsored by Agricultural Research Council, Britain and observed that the dermatophytes isolated from all the 71 clinical cases of ringworm in cattle belonged to one species, T. verrucosum. Out of 71 cases 41 were culturally positive for T. verrucosum. T. mentagrophytes which had been occasionally recorded from cattle in Britain, was not met during the survey and they confirmed the view that T. mentagrophytes rarely attacked cattle in that country.

Mortimer (1956) reported that 15 per cent of cattle were infected with ringworm, out of approximately 2,000 cattle examined in East Anglia and the organism involved was T. verrucosum.

Gentles and O'Sullivan (1957) isolated T. verrucosum from 99 bovines and T. mentagrophytes from one only in four

year survey of cattle ringworm in Scotland.

McPherson (1957) in a survey of 513 herds in North Britain with 30,766 cattle found that 133 (25.7 per cent) herds and 333 (2.39 per cent) cattle were affected with ringworm caused by T. verrucosum.

Menges and Georg (1957) conducted a survey of animal ringworm in the United States. Speciments of hairs from 105 cattle were cultured and T. verrucosum was isolated from 21 (20 per cent). T. verrucosum was the only dermatophyte isolated from cattle. The 21 cases were from 10 states and represented 14 herds of cattle. The number of isolations reported did not represent the total number of cases of ringworm in the 14 herds.

OzeGovic and Grin (1957) in an examination of 2,672 adult cattle, 391 heifers and 343 calves for ringworm reported that in about 93 per cent of cases infection was caused by T. verrucosum. T. mentagrophytes was less frequent and T. violaceum isolated from one calf only.

Kaplan et al. (1963) in a study on recent developments of animal ringworm and their public health implications, examined 53 skin scrapings from cattle and isolated only 29 strains of T. verrucosum (50 per cent).

Tiwari (1961) isolated 42 strains of T. verrucosum from cattle in Uttar Pradesh. The other dermatophytes recovered from ringworm lesions in cattle in his study included T. mentagrophytes var granular (15), T. rubrum (6) and T. violaceum (2).

Monthly surveys of 3,174 cattle in 165 herds revealed ringworm infection in 2.5 per cent cattle in 27 herds (16 per cent). The only pathogen was T. verrucosum (Klokke and Kamp, 1962).

In general, T. mentagrophytes rarely causes cattle ringworm but Krentel and Kuhne (1962) isolated it from 37 cases in contrast to T. verrucosum from only 3 cases.

Cannole (1963) reviewed on dermatomycoses of animals in Australia and stated that in 1959, two strains of T.verrucosum were isolated from bovine skin scraping at their institute and during 1960-62, 32 strains of T. verrucosum had been isolated from field specimens. In addition,14 strains had been isolated from stock at their institute.

Klokke (1964) reported infection of cattle by T. verrucosum from North India.

Padhye et al. (1966) found T. verrucosum infection in seven calves near Poona.

Klokke and Durairaj (1967) while investigating the causal agents of superficial mycoses in South India isolated three strains of T. verrucosum from cattle.

According to Dawson (1963) the various dermatophytes recorded from cattle were T. verrucosum, T. mentagrophytes, T. rubrum, T. violaceum, M. gypseum and M. canis.

A subsiding epizootic of cattle ringworm was attended by Mahajan and Mohapatra (1963) at various farms of Hissar (Haryana) where T. verrucosum was the only pathogen isolated.

Samples from cattle on 433 premises were studied by Pepin and Austwick (1963). Two hundred and twentythree were found to be infected with ringworm and T. verrucosum was isolated from 199 of those. No dermatophyte could be grown from the other 24 infected samples but they believed that any of these were not infected by species other than T. verrucosum. Herd out breaks were reported on 13 occasions with estimated infection rates of 53 and 70 per cent in two dairy herds.

Kechnic and Thacik (1969) examined 333 cattle from all parts of Czechoslovakia, only 191 yielded T. verrucosum alone and three T. mentagrophytes and two having both the dermatophytes.

Of 228 cultures isolated from calves 3-12 months old in Valdimir region, U.S.S.R., 153 were identified as T. faviforme var discoides, 32 as T. faviforme var album, 26 as T. gypseum var granulosum, eight as T. gypseum var asteroides and four as T. crateriforme (Baranov, 1970).

Gupta et al. (1970) examined 6,062 cattle of different age groups and observed clinical lesions of ringworm in 275 of them. On cultural examination the over all incidence was found to be 2.45 per cent. The various dermatophytes recovered from this study were T. verrucosum (131), T. mentagrophytes (9), T. violaceum (2), T. terrestre (1) and Microsporum gypseum (2). However, T. verrucosum was found to be the common etiological agent of ringworm in cattle.

The survey carried out by Singh and Singh (1970) included a total number of five hundred and eightythree cattle,

out of which skin scrapings were collected from fiftyeight cases having ringworm lesions. Only 14 strains of T. mentagrophytes var granular were isolated and none revealed T. verrucosum infection.

In Romania ringworm in cattle was caused by T.verrucosum, no other species was detected (Alteras, 1970).

In Orissa, Misra (1971) carried out an extensive survey on the incidence of ringworm in man and animals and examined 4,840 herds of cattle. He observed ringworm lesions in 243 cases (5.02 per cent), out of which 137 (56.38%) were culturally positive. The dermatophytes isolated were 123 strains of T. verrucosum (93.4 per cent) and 9 strains of T. mentagrophytes var granular (6.6 per cent).

Satija and Gautam (1972) investigated an outbreak of ringworm infection in the Government Livestock Farm, Hissar and found that the outbreak was due to T. verrucosum infection.

Predisposing Factors

(i) Age :

Cattle of all ages were susceptible to ringworm infection caused by T. album (T. verrucosum) but the disease was probably more common in calves than adults (Hoerlein, 1945).

The percentage of T. verrucosum infection in cattle in relation to age as observed by Menges and Georg (1957) were 40 per cent, 4 per cent, 5 per cent and 0 per cent in cattle of below two years, 3- 4 years, 5- 6 years and seven years and

above respectively.

McPherson (1957) stated that young-stock were affected more frequently than mature cattle, the overall incidence in calves being 7.34 per cent and in adults 0.43 per cent. In rearing herds the incidence was higher than in dairy or beef herds.

According to Connole (1963) the infection occurred chiefly in young animals but adults were also affected. Both dairy and beef cattle were susceptible.

Gupta (1967) observed that age had a definite effect on the incidence of ringworm in cattle. They found that the incidence in calves was 9.4 per cent whereas in heifers and adult cattle it was 2.01 and 0.19 per cent respectively.

Kaplan (1967) remarked that in general T.verrucosum infection was observed more frequently in cattle and was more extensive in calves than in older calves.

Mahajan and Mohapatra (1963) stated that only cow-calves below the age of one year were found to be infected and no adult milch or stray cattle were met with ringworm infection.

The study of Misra (1971) revealed that out of 1123 calves under one year of age examined, 217 calves (19.32 per cent) and out of 3,717 adult cases examined, 26 (0.69 per cent) were found to be clinically positive for ringworm infection. On examination of materials from the above clinical cases dermatophytes were isolated from 131 (60.36 per cent) of the 217 calves and 6 (23.07 per cent) of the adult ones respectively.

Satija and Gautam (1972) observed that calves below one year of age were mainly affected with T. verrucosum infection. They recorded a high incidence (24 per cent) in suckling dairy calves.

(ii) Environment :

T. verrucosum infection was very common in cattle especially during winter months (Hoerlein, 1946).

Mortimer (1955) remarked that cattle ringworm caused by T. verrucosum was far more prevalent in the winter months. The most probable cause of the high winter incidence was yarding together of animals where direct contact was inevitable. He further believed that lack of direct sunlight might be a factor in the high winter incidence.

McPherson (1957) observed that the incidence did not appear to be influenced by season of the year, location of the herd, nutrition, the lighting ofthe cattle sheds and on the presence of lice on the cattle.

Blood and Henderson (1963) described that the incidence of ringworm in cattle was increased in winter when the animals were housed in close proximity to each other for a long time.

An outbreak of cattle ringworm in Hissar investigated by Mahajan and Mohapatra (1968) was started since May and till July the cases were many in number. On questioning to the officials working at such farms, that was the period when maximum number of animals were affected with T. verrucosum

infection every year. In all instances the calves were stabled together in one shed.

Analysis of the data on ringworm infection in cattle by Pepin and Austwick (1963) on a seasonal basis showed that 34.5 per cent of all samples and 39.9 per cent of infected samples were received during the months of December, January, and February. The proportion of specimens found positive was almost always highest in December averaging 74.6 per cent, the other figure in other months lying between 30 per cent (March) and 62.5 per cent (August), the overall average was 50 per cent. The above figures lended some support to the opinion that cattle ringworm was predominantly a winter disease but also indicated that it was not uncommon throughout the year.

Misra (1971) recorded the incidence of ringworm in cattle mainly due to T. verrucosum on monthwise basis during the study period from December, 1963 to November, 1970. The highest incidence of ringworm observed by him was during the months of June, July and August.

Satija and Gautam (1972) reported that the spread of T. verrucosum infection mainly occurred during winter months and the incidence was reduced by immediately segregating the affected calves from the herd.

Clinical Lesions

Moerlein (1945) described that the lesions encountered in the field varied from the squamous type in which only scali-

ness and partial loss of hair were seen which had thick crusty scabs of asbestos-like material. There might be pus formation under the scabs and if pruritus was present raw areas were noted. Often the lesions resembled those of parasitic skin diseases. In general the lesions were circular, discrete showing little tendency to coalesce. This together with the formation of heavy crusts was often enough to distinguish the lesions from those caused by the parasitic mites.

La Touche (1952) reported that the sites of the ringworm lesions due to T. discoides in calves to be the skin and hair of the face, above the muzzle, around the eyes and at the base of the ears and that other parts were rarely involved.

Georg (1954) while discussing the diagnosis of ringworm in animals stated that lesions of ringworm due to T. verrucosum in cattle were usually found on the head but might be scattered on body, legs and tail (in calves lesions might be very extensive) and were coin-sized or distinct plaques with heavy greyish white crusts. When crusts were removed, moist bleeding areas were seen. Old lesions lost the heavy crusts showing scaliness and broken off hair stumps.

Connole (1963) described that the lesions occurred as discrete, roughly circular areas which might extend to extensive areas of greyish white crusts with tendency to become thickened. The lesions were most common on the head (chiefly around the eyes) and neck, often extending along the shoulders, hind quarters and limbs.

Mahajan and Mohapatra (1963) stated that lesions in almost all cases were confined to face, neck, ears and rarely extending posteriorly towards the tail. Infrequently, lesions were also seen on the forelegs. They were typically circumscribed, circular of varying size, raised slightly from the body surface which when scraped, left a hyperaemic or sometimes a bleeding base.

Pepin and Austwick (1963) reported that crust formation on lesions was found in 34.6 per cent of cases and the average maximum diameter of lesions were 49.4 mm (54 cases). Most infected cattle had more than ten lesions and six of 37 cases became generalised. Ringworm lesions in cattle were heavy grey white asbestos-like crusts raised perceptibly above the skin, roughly circular and about 1- 3 cm in diameter. In early cases lesions were small while in advance cases they were larger, detached with pityriasis and alopecia. The lesions were most commonly seen on the head, around the eyes, neck and forelegs. A general distribution over the entire body was not uncommon among the calves three to five months of age (Gupta et al., 1970).

Misra (1971) observed that the lesions in cattle as discrete nearly circular with raised boarders having thick, greyish crusts viz. asbestos-like appearance. The lesions in early stages were small and later they become larger and confluent. The lesions were mostly seen on head, around the eyes, neck and ears often extending to dewlap, shoulders, hindquarters and limbs.

(1) Characteristics of the Spores and Hyphae of T.verrucosum in the Skin Scrapings :

Hoerlein (1945) observed that the spores of T. verrucosum were rather large, 4-6.5 microns in diameter and were found in sheaths around the hairs. Spores and hyphae also occurred within the hairshaft. When the spores sheath was well developed the spores were noted to be in chains.

Georg (1954) suggested that in case of T. verrucosum infection, there would be sheath or isolated chains of large spores (5-8 microns) lying on surface of hair and mycelium invading interior of hairs. Mycelium and chains of spores would also be seen in skin scrapings.

Austwick (1955) remarked that the arthrospores around the bases of the infected hairs were very small (2 to 4 microns in diameter) and infection could probably originate from even one spore lodging in a hair follicle. It was estimated that each infected hair might carry over 30,000 arthrospores in its spore sheath.

Beneke (1966) included T. verrucosum in the group of trichophytons where ectothrix hair invasion was the characteristic diagnostic feature.

Gupta (1967) reported that in case of T. verrucosum infection in cattle there were isolated chains of spores within and outside the hair fibre (ectoendothrix type) and the skin scrapings also revealed the presence of mycelium and chains of arthrospores.

Mahajan and Mohapatra (1963) recorded presence of spores of T. verrucosum in hairs in 45 cases out of 48 ringworm cases. The majority of them showed typical large spored endothrix infection of the hair, however, in a few instances ectoendothrix hair invasion was also encountered. No ectothrix hair infection was noticeable.

Dvorak and Otcenasek (1969) in their recent book "Mycological diagnosis of animal dermatophytoses" have stated that in case of T. verrucosum infection the invasion of hair of cattle was nearly always of the endoectothrix type with large spores (3.5 - 10 microns). In skin scales branched septate hyphae, more or less arthrosporulated, were found.

Misra (1971) observed that in cattle out of 243 cases examined by him, 203 (83.53 per cent) were showing invasion of hairs by fungal elements. Ectoendothrix invasion of hair with spores were commonly seen in T. verrucosum infection. However, ectothrix invasion of hairs with spores forming a sheath was also seen in 2% of cases of T. verrucosum infection.

(ii) Cultivation and Cultural Characteristics :

(a) Primary Isolation : The medium used by Moorlein (1945) for primary isolation of T. album was Sabouraud's dextrose agar or maltose agar (dextrose or maltose 4 per cent, peptone 1 per cent, agar 1.8 per cent). He noted that growth of T. album at a pH of 8 to 9 was almost as good as at the optimum of pH 6-7. When media with a pH of 3 to 10 were used

for isolation, it was noted that many saprophytic moulds (Mucors and Aspergilli) were inhibited to such an extent that contamination was less of a problem. He recommended an incubation temperature of 30°C in the isolation of T. album because of its slow growth.

Fowle and Georg (1947) successfully isolated the faviform trichophyta (T. album and T. discoides) on blood agar base and they indicated that blood agar base medium supplied the necessary factors for growth which were absent in Sabouraud's maltose agar.

Ox bile agar was suggested for primary isolation of T. verrucosum (Littman, 1947).

Georg (1954) stated that a thiamine enriched medium containing antibiotics to inhibit the growth of bacteria and saprophytic fungi and an incubation temperature of 37°C were required for the isolation of T. verrucosum from clinical materials. He effectively demonstrated the use of a selective medium containing 0.1 mg per ml cycloheximide (actidione) for reducing the growth of mould contaminants when isolating T. verrucosum from cattle hairs. By this method he obtained three times the number of isolations made from other media using the same sample.

Austwick (1954) advocated the physical separation of the spores and hyphae of contaminants from those of the dermatophyte in the skin scraping for the isolation of T. discoides from cattle. He observed that nutrient agar

(beef extract 10.0 g, peptone 10.0 g, sodium chloride 5 g, agar 15 g, distilled water 1000 ml, penicillin 20 i.u. and streptomycin 40 i.u. per ml) with a pH of 7.5 was most suitable for the isolation of T. discoides because, it gave a higher growth rate than Sabouraud's beerwort, Littaman, or malt agar. The addition of antibiotics to suppress bacteria did not effect the growth of the fungus.

Georg (1954) suggested that Sabouraud's dextrose agar at pH 5.5 was the standard medium for the primary isolation of ringworm fungi from clinical materials. Several antibiotics, penicillin (20 units per ml), streptomycin (40 units per ml) and cycloheximide (0.5 mg per ml) had to be added to inhibit the growth of contaminating bacteria and saprophytic fungi.

Haley and Stonerod (1954) remarked that the growth of T. faviforme on Sabouraud's dextrose agar was slow and scanty. They suggested to use heart infusion tryptose thiamine agar (beef heart infusion from 500 g, bactotryptose 10 g, sodium chloride 5 g, bacto-agar 15 g, thiamine hydrochloride 10 g, distilled water 1000 ml) with a pH of 6.8 or Difco Blood Ager Base to which thiamine (0.1 mg thiamine added to 100 ml melted, partially cooled agar) for primary isolation of faviform trichophyta. Streptomycin (40 units per ml) and penicillin (20 units per ml) were added to get rid of the bacterial invaders.

T. verrucosum was grown from skin and hair of 95 of 159 cattle, using Sabouraud's glucose agar containing 0.5 mg

per ml of actidione (cylohexamide), 20 units penicillin and 40 units streptomycin. On Sabouraud's medium without antibiotic supplements only 12 cultures of T. verrucosum were obtained (Thacik and Kachnic, 1962).

A comparative study of the value of various media and their combinations for primary isolation of T. verrucosum from animal material was undertaken by Mahajan and Mohapatra (1968). They found that brain heart infusion agar alone gave the maximum number of isolations (33.61 per cent). It was further noted that brain heart infusion agar in combination with lablemco agar or Sabouraud's medium with thiamine gave the highest yield (54.55 per cent). Sabouraud's agar with thiamine in combination either with lablemco agar or Sabouaurd's agar and thiamine plus inositol gave the minimum number of isolations (33.61 per cent).

Dvorak and Otcenasek (1969) recommended that the basic medium for primary isolation was Sabouraud's dextrose agar with cycloheximide and chloramphenicol. The above media consisted of bacto-tryptose 10.0 g, bacto-beef extract 3.0 g, sodium chloride 5.0 g, agar 10.0 g, distilled water 1000 ml (dissolved slowly by heating to boiling point and added) dextrose 20 g, yeast extract 3.0 g, thiamine 0.05 g, chloramphenicol 0.05 g and actidione 0.5 g.

Gupta and Singh (1970) made a comparative evaluation of three culture media for the primary isolation of dermatophytes from clinical materials. On an average, Sabouraud's

glucose agar with chloramphenicol, actidione and thiamine gave the maximum isolates in comparison to Sabouraud's glucose agar with chloramphenicol and Littman oxgall agar respectively. They recommended the simultaneous use of the above three media in order to have maximum number of isolations for the cultivation of dermatophytes from animals heavily contaminated with a number of bacteria, yeasts, and saprophytic fungi.

(b) Macro-and Micromorphology of T. verrucosum :

Hoerlein (1945) observed that the growth of T. album in the media was almost submerged and radiated from the centre in bundles of hyphae. By the end of two weeks the size would vary from 5 to 20 mm and while mostly submerged might have a small amount of white powdery aerial mycelium in the centre. At four weeks the colony was from 20 to 40 mm and might show a considerable amount of white powdery mycelium over the central half. The colony was usually flat, but might show some elevation and early formation of covolutions. By the end of eight weeks the colonies developed an almost velvety duvet over most of the surface which might become very convoluted. Some colonies, however, remained moist through out their development. Many microconidia were produced in the aerial mycelium and occurred singly, sessile, and lateral on undifferentiated hyphae as a rule, but occasionally the spores were seen in bunches. A few macroconidia were noted. They were rare and about six microns wide by 55 microns long. Many of the hyphae had swollen tips.

According to Fowle and Georg (1947), T. album typically produced a glabrous spongy colony with waxy colour. This might

become dark tan to brown after several weeks and some white powdery growth might appear at the base of the acuminate colony. T. discoides produced a colony which was also habitually glabrous, but had a fairly regular disc shape and a small raised centre which might or might not be present. The colony frequently had a greyish tan to greyish yellow pigment and had a powdery or velvety surface of short fine white down. Microscopic examination of the glabrous colonies revealed irregular mycelia with an abundance of irregularly formed intercalary and terminal chlamydospores. Spore-bearing structures were characteristically absent.

Georg (1954) described the cultural characteristics of T. verrucosum. The growth was very slow and might not appear until 10 to 14 days. Colony was usually small, heaped and folded with white to greyish moist and smooth or velvety surface. Thin irregular mycelium with swellings (Chlamydospores) which occurred in large numbers often in chains and the presence of a few single-celled spores (microconidia) were the characteristic features. Microconidia might be numerous on thiamine enriched medium.

Ainsworth (1959) stated that cultures of T. verrucosum attained a colony diameter of 5-20 mm in 2 weeks. The growth was often moist and largely submerged in the medium. Aerial mycelium might develop. Chlamydospores were usually numerous, while microconidia were produced less frequently and macroconidia were rare.

Beneke (1966) described that the colonies of T. verrucosum were slow growing, heaped, deeply folded, glabrous and waxy or with a fine white velvety surface. Colours varied in isolations from white to bright yellow. Microscopically only chlamydospores were seen in the hyphae on Sabouraud's dextrose agar. On media enriched with thiamine microconidia were usually produced and on rare occasions 3- 5 celled macroconidia might be seen varying in size and shape.

Growth of T. verrucosum was extremely sluggish and hardly a few mm in diameter. The colonies were heaped, deeply folded, glabrous and waxy in majority, however, a small proportion of them did not show any folding and colonies in such cases were disc shaped. Some were yellowish white and others brilliant yellow onchre in colour. Microscopically, only chlamydospores were seen which were more numerous in subsurface growth of the colony. Mycelium was irregular. None of the isolates tested produced macro-or microconidia on rice grain medium enriched with thiamine or yeast extract (Mahajan and Mohapatra, 1968).

Dvorak and Oteenasek (1969) described the characteristic macro-and micromorphology of T. verrucosum elaborately in their recent book. The rate of the growth of the colony of T. verrucosum was slow, it attained 5- 15 mm in diameter after 10 days. The mature colony (after 20 days) was mostly irregular, asteroid, polygonal or lobulate only sometimes its ground plan was regular, circular to asteroid. Very often a large zone of irregular submerged mycelium developed. The aerial part

of the colony can remain underdeveloped for a long time, sometimes it was almost visible. The colonies of some strains were membranous, more or less powdery or velvety. They might be flat or raised with a marked tendency to wrinkle. The surface colour could be grey, salmon, apricot or pale lemon yellow. The reverse could be unpigmented or salmon. The micromorphology was poor. The most frequent elements found were chlamydospores often arranged in more or less long chains. The macroconidia were normally absent, only some strains produced them in limited numbers. They were irregular, smooth, with thin walls, 4-7 cells, measuring 4- 9 by 16- 60 μ m. The microconidia were mostly absent, only some strains produced them in limited numbers. They were ovoid, clavate to pyriform, 2- 3 by 3- 4 μ m. Favic chandeliers were often present and pectinate hyphae only occasionally observed.

(c) Nutritional Requirements : Investigation on the requirements for the growth of T. discoides by Robbins et al. (1942) had shown that the dermatophyte suffered from complete deficiencies for pyridoxine, inactive inositol and molecular thiamine and partial deficiencies for unidentified substances present in peptone, casein hydrolyzate, hydrolysed egg albumin, malt extract, gelatin and filtrate from white patatoes. The authors demonstrated that vigorous growth could be obtained on thiamine and peptone medium.

The effect of thiamine, pyridoxine and inositol, singly and in all possible combinations on the growth of several strains of T. discoides and T. onchraceum were studied on a synthetic

basal medium with glucose and asparagine (Mackinnonand Allende, 1948). Their study revealed that four strains had complete deficiency for inositol, one strain for thiamine, another strain for thiamine and inositol and one strain had complete deficiency for inositol, pyridoxine and thiamine.

Georg et al. (1956) remarked that T. verrucosum had absolute nutritional requirements for certain vitamins. About 90 per cent of the strains studied required thiamine and inositol for growth about 10 per cent required only thiamine and one strain had been found which required thiamine, inositol and pyridoxine.

Georg and Camp (1957) studied the nutritional pattern of 100 strains of T. verrucosum and reported that none of the strains grew on either ammonium nitrate or casein vitamin-free agar media. Eighty four strains were shown to require thiamine and inositol for growth and sixteen strains only thiamine. Additions of other water soluble vitamins singly or in combination with the required vitamins produced no effect on the growth.

Beneke (1966) while describing the nutritional pattern for Trichophyton species stated that 84 per cent of T. verrucosum required both thiamine and inositol while the rest 16 per cent could grow well in thiamine alone.

Klokke and Durairaj (1967) found that growth of all the three strains of T. verrucosum was stimulated on casein-thiamine-inositol media while thiamine alone did not produce any marked response.

The study of Mahajan and Mohapatra (1963) revealed that 90 per cent of the strains tested required additives like thiamine and inositol and showed partial need for thiamine alone and in 10 per cent there was slight stimulation by thiamine and inositol both.

Most of the strains of T. verrucosum required thiamine and inositol and some thiamine only (Dvorak and Otcenasek, 1969).

(d) Keratinolysis of Hair in vitro : Lu (1962) advocated slide culture method in the study of hair digestion by dermatophytes. In his study it was observed that only two of the four strains of T. verrucosum showed keranolytic activity, each producing only a few wedge-shaped perforations on an average of 12.1 days. When cultured on 0.1 per cent yeast extract agar, however, three of the four strains produced numerous wedge-shaped perforations on an average of six days.

Mahajan and Mohapatra (1963) studied the ability of five isolates of T. verrucosum to perforate human adult, human child, horse, cow and dog hairs and found that none of the strains tested perforated any hair.

Dvorak and Otcenasek (1969) remarked that the hairs of cattle, dog, goat, horse, mouse and man were attacked by T. verrucosum coxially.

Pathogenesis of T. verrucosum

(1) Survival of Spores of T. verrucosum :

Solmons (1954) recorded substantial evidence of the

survival of T. verrucosum for 2 years and one year 6 months on cattle enclosures.

Walker (1955) had shown that T. verrucosum remained viable on infected hairs in the laboratory for 15 months and she had also demonstrated the presence of T. verrucosum infected hairs on a scratching post used by cattle infected with ringworm.

McPherson (1957) observed that in the dark at room temperature arthrospores of T. verrucosum were viable in skin scrapings even after 4½ years and in skin scab 1.6 mm thick, arthrospores of T. verrucosum were protected from the action of ultraviolet light equivalent to 437 hours of mid-day, mid-summer, and mid-altitude sunshine. He further experimentally demonstrated that while ultra-violet light was lethal in a few minutes to T. verrucosum in vitro, hair, skin and lesion scabs individually and collectively protected the dermatophyte.

While reviewing on dermatomycoses of animals in Australia, Connole (1963) stated that in their institute, T. verrucosum remained viable on skin scrapings and hair samples which had been stored in the dark for 15 months.

T. verrucosum has been isolated from walls, fences and a cleaning machine in a cattle farm in India (Klokke, 1963).

Kachnic and Thacik (1969) reported that T. verrucosum survived five months in hair and crusts from cattles burried 5 cm beneath the surface of cbwhouse bedding. They further observed that of 800 hairs obtained from T. verrucosum lesions in cattle,

only one hair yielded the agent after 72 hours in a cow's stomach.

(ii) Natural Infection-Related Clinical Lesions and Histopathological Changes :

The clinical lesions produced by natural infection of T. verrucosum in cattle have already been reviewed in a former chapter.

Ringworm lesions in calves due to T. verrucosum infection fully developed in 3- 4 weeks of infection (Rook and Frain-Bell, 1954).

Mortimer (1955) performed a histopathological examination of natural ringworm lesions in cattle and demonstrated large number of leucocytes in the corium around the follicles and also in the stratum corneum. Occasionally micro-abscesses were present and rarely there was any macroscopic evidence of pustular folliculities. This mild reaction suggested that the true host of T. verrucosum were cattle which exhibited a more stable host-parasite relationship. He also reported spontaneous recovery of cattle ringworm due to T. verrucosum.

In summer outbreak of T. verrucosum infection in cattle, the mean persistence of the lesions was 17 weeks and in the winter outbreak it was 10 weeks (Ford, 1956).

Sellers et al. (1956) made a preliminary observation on natural ringworm due to T. verrucosum in cattle. Healthy calves were put into the pen which had previously housed the ringworm infected calves. These calves developed natural lesions

within 23 days. It was not possible to detect the lesions until infection was well established. Biopsies were only of value in observing the lesions at its height of activity and during the subsequent healing process which was complete after approximately three months.

Misra (1971) observed secondary lesions due to T. verrucosum in experimentally infected calves which persisted beyond 90 days. In a histo-pathological examination of natural skin biopsies collected from ringworm (T. verrucosum) infected calves, he further observed that the shaft of the hair both in the epidermis and dermis were invaded with large number of filamentous septate hyphae as a result of which there was autolysis ofthe hair. Majority of the follicles were splintered off ending in autolysis of hair as a result of which many of them were either empty or the cortex and medulla of the hair were replaced by homogenous pinkish mass. The inner and outer epithelial root sheath of the hair was not involved in the degenerative process. The epidermis had mild hyperkeratosis and acanthosis of the malphigean layer. The lesions in the dermis consisted of perivascular and perifollicular infiltration of lymphocytes.

(iii) Experimental Infection-Related Gross Lesions and Histopathological Changes :-

Artificial inoculation of cattle of various ages with T. verrucosum as demonstrated by Hoerlein (1945) showed that the disease might be easily spread with either infected hair and scales or cultures. The first evidence of lesions appeared on

on about the 18th days and the lesions were usually well established by the 24th day. At that time, much of the hair over the area of inoculation had fallen. The skin was scaly. The hairs were usually infected on the 13th day. Some of the lesions showed the thick asbestos-like crust in about two and a half months. Natural spread to an uninoculated animal was noted, lesions being present about two and a half months after lesions appeared on two inoculated calves. In one experimental heifer which was artificially infected and which had recovered immunity to several types of known infective material was noted a year after recovery. Guinea pigs, dogs and cats were susceptible to artificial infection.

Fowle and Georg (1947) showed that inoculations with T. album and T. discoides were unsuccessful in guinea pigs, but produced typical ringworm lesions in rabbits.

Dey and Kakoti (1956) could infect guinea pigs with T. verrucosum.

Sellers et al. (1956) stated that after experimental transmission with natural material or culture of T. verrucosum lesions usually became evident at 14- 21 days and were well developed, often with greyish-white plaques of crusty material with broken-off hairs by 28 days. The lesions healed in 3 to 4 months, the crusts falling off and leaving a scaly bald patch on which hair grew. Recovered animals were resistant to reinfection.

The above authors made a detailed histopathological study of skin biopsy collected from these experimental animals

and observed that there was extensive proliferation of mycelium in the stratum corneum which lined the infundibulum of the hair follicles. Thick weft of mycelia were formed at the mouth of the follicle. Gradually, the mycelia infiltrated into the inter-follicular stratum, corneum and penetrated down into the inner epithelial sheath. Hyphae which branched down wards were present on the cortex and medulla of the hair shaft. Many shedding hairs were surrounded and penetrated by the mycelia. The mycelia in due course were transformed into spores. The early localised lesions became widespread in later stages with inflammatory exudates in the stratum corneum. The interdermal and intradermal micro-abscesses around the follicles usually ruptured resulting in the liberation ofpools of exudates and leucocytes.

McPherson (1959) made some experimental studies for assessing antimycotic agents against T. verrucosum. He observed that guinea pigs experimentally infected with T. verrucosum were spontaneously cured within 26 days of experimental inoculation. Multiple lesions could be produced in calves by intradermal injection of spore suspensions of T. verrucosum. The lesions in his winter group of calves existed for 10 weeks while on his summer group the average was 17 weeks giving an overall average of 15.5 weeks.

Cox and Moore (1963) demonstrated that experimental infection of rabbits with different strains of T. verrucosum followed a similar pattern. The marked initial inflammatory phase was followed by the formation of an indurated crusty lesion with infected hair follicles. They remarked that the above

patterns followed in the development of ringworm in calves but the natural disease often assumed a chronic form and was of longer duration than the experimentally infected rabbits. It was further seen that recovery from infection with T. verrucosum resulted in an apparent immunity to reinfection, not only with the homologous strains but also strains of different origin and to infection with T. mentagrophytes. They could not summerise the factors affecting immunity to reinfection but there was no apparent relationship between circulating antibody titre and susceptibility to reinfection. In experimentally infected calves with T. verrucosum typical lesions were produced on the body and head and disappeared from the head after 8 weeks. No circulating antibody as observed in case rabbits was detected during or after infection. Hence they concluded that no evidence existed for any direct relationship between antibody and immunity although resistance of cattle to reinfection with T. verrucosum following recovery from infection was reported like rabbits. They were unsuccessful in experimentally infecting guinea pigs with six strains of T. verrucosum including culture of bovine, equine and ovine origin.

Mahajan and Mohapatra (1968) failed to produce any lesion in guinea pigs with their isolates of T. verrucosum however, the number tested was extremely meagre to draw any conclusion.

The experimental investigation carried out by Misra (1971) revealed that all animals (sheet, goat, pig, dog, cat and cattle) used for reproduction of T. verrucosum infection

manifested the first appearance of the lesions by 1- 2 weeks which continued to persist upto 3- 6 weeks in them except cattle. In guinea pigs the lesions disappeared by second week. In cattle, however, the primary lesions healed up by 40th day. The essential gross changes in all experimental animals were characterised by the formation of greyish white granular, powdery or crustaceous lesions. In cattle the lesions were more pronounced on the skin of head, neck, and ears rather than flank and chest. In this study pup showed early papular and pustular lesions which later developed into scales.

Misra (1971) further stated that the common microscopical alterations in his experimental studies were characterised by the invasion of hair by spores or hyphae in addition to hyperkeratosis and perivascular cuffing in the dermis by mononuclear cells.

Treatment

Various kinds of treatment against ringworm due to T. verrucosum in cattle have been advocated.

Baker and Davis (1954) used 'Captan' (N-trichloromethylmercapto-4-Cyclohexene-1, 2-dicarboxamide) in a concentration of 1 in 300 to 1 in 500 successfully in the control of ringworm in 700 cattle when applied as a spray at the rate of 1.0 to 1.5 gallons per animal twice two weeks apart.

Nazarov (1954) treated ringworm in cattle by rubbing powdered sulphur into the lesions and by dusting it over the rest of the body. This was repeated after 3 days, thereafter

only the visible lesions were treated. An alternative powder for the initial dressing contained three parts sulphur to one part of copper sulphate. At the sametime byres were disinfected with a mixture of 10 parts of 4 per cent solution of formaldehyde, five parts creolin and 10 parts kerosene in 30 parts of water.

Brander (1955) in reporting the treatment of an outbreak of ringworm in a Guernsey herd with a solution of dichlorphen in spirit (strength not given) recorded that two months after the initial treatment the ringworm lesions were well under control.

The treatment of ringworm in cattle depended on the stage, on the size of the lesions and on the thickness of the crusts. When the lesions were in the beginning stage 5 per cent iodine ointment or lotion could be used. In advance cases, the external treatment was supported by systemic treatment with sodium iodide applied either orally or intravenously (Kral, 1955).

Ford (1956) reported on the treatment of two outbreaks of ringworm in a group of cattle. Two methods were used, one being the twice weekly application of phenyl-mercuric acetate ointment (strength not given) and the other, the weekly intravenous injection of sodium iodide in 33 per cent w/v solution at a rate of 3 g per cwt of body weight. The above treatment greatly affected the course of the disease.

Foster (1957) conducted a clinical trial using hexadecamethylene-I : 16-bis (isoquinolinium chloride) as a topical antifungal on a series of over 100 cases of bovine ringworm presenting the characteristic lesions of T. verrucosum. The result of the above trial was very encouraging. Rapid resolution of the lesions at all stages of development was observed including those that had previously been treated unsuccessfully with other antifungal compounds. No signs of toxicity or irritation were seen.

Gold and Jones (1953) described rapid cure of ringworm in cattle due to T. discoides after treatment with 0.25 per cent of hexadecamethylene-I : 16-bis (isoquinolinium chloride).

An antifungal agent 'Hexetidine' (bis-I, 3 beta-ethylhexyl-5-methyl-5-aminohexa-hydropyrimidine) was reputed to be highly successfull in ringworm infected young calves following one treatment (Kalisz, 1953).

Lauder and O'Sullivan (1953) claimed that daily administration of griseofulvin orally at the rate of 60 mg per kg for 8 weeks prevented the establishment of artificial infection of T. verrucosum in calves. Further, they could also cure the established lesions of the experimental T. verrucosum infected calves with griseofulvin.

Noskov (1953) reported complete eradication of ringworm in cattle by treatment with alkaline solution of formaldehyde. On six farms, 200 calves and heifers with ringworm were sprayed with a solution of 0.4 per cent formaline plus 0.5 per cent caustic soda at 2.5 lb atmospheric pressure and the spray was repeated

at weekly intervals. The above treatment proved quite successful in the control and eradication of ringworm.

O'Brien and Sellers (1953) tried four antifungal agents : 0.2 per cent solution of Hexadecamethylene-bis-isoquinolium chloride, alcoholic solution of 10 per cent iodine and 2 per cent phenol, 0.1 per cent alcoholic solution of phenyle mercuric dinapthalmethane disulphonate and 2 per cent alcoholic solution of dichlorphen in a group of yarded cattle with extensive lesions on head and neck due to T. verrucosum but could not assess their efficacy as both the treated and controlled animals recovered spontaneously.

Ivashkov (1959) treated 43 ringworm infected calves successfully by rubbing into the lesions a 5, 10 or 15 per cent solution of chlorphos (Diptrex).

McPherson (1959) reported that chemotherapeutic agents like benzalokonium chloride and one per cent centrimide prevented the radial extension of experimental ringworm lesions in guinea pigs (infected with T. verrucosum). Further, he screened a series of chemicals in vitro to assess the antimycotic action against T. verrucosum and reported that Captan, monosulphiram, sodium hypochlorite, 5 quaternary amonium compounds and 7 surface active anionic detergents were effective in ten minutes while during the same period only three surface active agents proved fungicidal.

Forenbacher et al. (1960) obtained a complete cure of ringworm in 125 cattle infected with T. verrucosum with topical

application of a 0.25 per cent solution of Tecquil (Hexadecamethylene-1 : 16-bis-isoquinolinium chloride) marketed under the name of Tinevet. The infected areas were painted with the drug two times at 48 hours intervals. The drug was also found efficacious against experimental infection of T. verrucosum in guinea pigs.

Polyakov and Spesivseva (1960) found that a 2 per cent formaldehyde and 1 per cent sodium hydroxide could kill dermatophytes in three hours at 65 to 75 of temperature. They also used 2 per cent slaked lime, iodine monochloride, formalin liniment emulsion, griseofulvin and sodium or potassium iodide as fungicidal agents for the control of dermatomycoses of farm animals.

Successful treatment of ringworm could be achieved by an average daily doses of 40 mg of griseofulvin per kilogram body weight and also by topical application of different antifungal agents like Tinavet, Captan, Seleen, Xydermo and Vioform (Uvarov, 1961).

Pearson and Rankin (1962) claimed success in the treatment of ringworm in calves with griseofulvin in daily doses of 2.5 g in case of large calves and 2 g in smaller ones for 10- 12 days. Two calves when given an inter-rupted treatment with griseofulvin also responded satisfactorily.

Torda and Pacenovsky (1963) treated 22 ringworm calves and heifers aged 4- 18 months within 14- 19 days with a single dermal application of 5- 10 per cent solution of bromophenyle

isothiocyanate in diethylene glycol.

Blood and Henderson (1963) stated that the only systemic treatment of ringworm in common use in farm animal was intravenous injection of sodium iodide (1 g per 30 lb body weight) as a 10 per cent solution. More than oneinjection was often required along with topical application of fungistatic agents.

Cobb et al. (1963) observed considerable degree of clinical improvement in naturally infected (ringworm) calves when treated with griseofulvin. The minimum effective dose was 10 mg per kilogram daily for seven days as a dreneh or mixed with the feed or milk.

Application of a 5 per cent solution of Neguvon on the T. verrucosum lesions of 27 cattle effected improvement in eight animals after a week (Drezancic et al., 1963). The thick scaly deposits were removed from 33 cattle and those areas were rubbed with 5 per cent Neguvon emulsion. In seven days, the skin was clean and hair growth was resumed in 93.9 per cent of the cases. Of 74 cattle similarly treated by the above workers with a 3 per cent Asuntol emulsion, 95.9 per cent were cured.

Tordo and Pacenovsky (1963) sprayed four groups of ringworm infected calves with 5 or 10 per cent suspension of parabromonphenylisocyanate in either triethylene glycol or paraffin. Best result was obtained by a single spray of 10 per cent suspension in triethylene glycol. The drug was well tolerated and lesions healed completely within 3- 5 weeks.

Anderson and Compell (1964) successfully treated cattle ringworm due to T. verrucosum in four to eight weeks with 15,000 to 300,000 i.u. of vitamin A.

Johnson (1964) demonstrated the clinical recovery of ringworm lesions on adult cattle and young calves by oral supplimentation with vitamin A palmitate. Recoveries were obtained without use of any other medication externally or internally while lesions persisted on untreated animals. Clinical response occurred in about two weeks.

Treatment of calves infected with T. verrucosum with 30 per cent aquous acetic acid and after 10 days with sulphur powder plus 5 per cent salicylic acid and 10 per cent copper sulphate was found to be effective (Baranov, 1965).

Grunder et al. (1967) performed clinical trials by spraying tThiadiazine preparations i.e. Hoe-252 and Hoe-297 on 467 cattle suffering from Trichophyton infections alongwith the local application of 0.5 to 1.0 per cent of the above solutions for 4-7 weeks and observed that Hoe-252 treated animals were completely cured whereas Hoe-297 could bring about 53.53 per cent clinical cure.

Fortyone ringworm infected beef cattle were divided into three groups which received griseofulvin at the rate of 14, 10, 6 mgs per kg respectively for 30 days. In five animals T. verrucosum was still demonstrable by culture after 21 days but all were negative by the end of treatment (Kielstein and Hulring, 1967). They also suggested that 10 mg of griseofulvin

per kg body weight for 30 days might be given as feed additive to cattle suffering from ringworm.

Lax et al. (1967) treated 473 calves suffering from ringworm with a preparation containing 10 gms of sulphur, 16 gms of potassium hydroxide, 6 gms of sodium salicylate, 20 ml of distilled water and 15 ml of soap spirit (spirit saponatus) and observed that with one application on the skin, the condition cleared up in 447 calves and the remaining 26 calves needed second application for the cure.

Gupta et al. (1963) suggested the use of the active principle of Cassia tora seeds for treatment of ringworm in cattle. Chrysophenic acid was found to be an active ingredient of the Cassia tora seeds. According to these workers, a 2 per cent solution of this active principle in castor oil base was an optimum and a safe concentration for the treatment of ringworm in cattle caused by the genus Trichophyton.

Neuman and Platzer (1963) published their findings on a clinical trial involving 43 calves infected with T. verrucosum. Thiabendazole used as an antifungal agent was compared with a variety of other treatments. It was found that 2- 4 per cent thiabendazole in an ointment base (Lanolin and vaseline) applied twice 3- 5 days apart produced good results with full clinical recovery. The addition of dimethyl sulphoxide to the ointment had no significant effect. Spraying with thiabendazole in a watery emulsion was not found to be effective, neither was oral treatment.

Nikiforov (1963) observed that T. faviforme was sensitive in vitro to 0.56 - 2.06 microgram per ml griseofulvin and

in calves the infection was prevented by 10 - 20 mg per kg over 35 days. Ointment containing 1 - 2 per cent of trichothecin controlled infection in calves within 7 - 30 days with no relapse after 4 - 8 months.

In vitro tests and experiments using mice, guinea pigs, cattle, and horses proved 'Bay Va 5337' (3-ethylamino-1, 2-benzisothiazole hydrochloride) to be an effective broad spectrum fungicide. Used topically it was effective against various species of *Trichophyton* including *T. verrucosum* (Plempel and Boshagen, 1968).

Becker (1969) applied Defungit (bensuldazic acid) topically by means of different models of spray apparatus in 242 *Trichophyton* infected cattle. He observed that 0.25 per cent and 0.3 per cent solutions were effective but 0.5 per cent solution was considered optimum.

In a preliminary *in vitro* trial, Cuturic and Hajsig (1969) attempted to grow mycelia of *T. verrucosum* in media containing various concentrations of six different fungicides including trichlorphen and centrimide (cetavlon). Of the fungicides which proved effective *in vitro* and which were then tested in treatment of eight herds of cattle affected with ringworm, only trichlorphen and centrimide and also the later associated with a locally produced liniment gave satisfactory results.

Gupta and Singh (1969) studied the comparative effect of salicylic benzoic acid ointment, acetic acid (30 per cent), Asuntol (5 per cent), Neguvon (5 per cent), formaline sodium hydroxide solution, copper sulphate unslaked lime spray and

Grisovin F. P. tablets and recommended the use of copper sulphate-unslaked lime spray as the most effective treatment.

Sulphur (2 g) and methionine (2 g) added daily to the diet of calves had a curative effect within 26-30 days in recent cases of ringworm. More than 60 days were however necessary in neglected cases where deeper layers of skin were involved with no cure in some cases. Sulphur and methionine were easy to administer, improved the condition of the animals and also had a preventive effect (Uzuev, 1969).

Viola and Stefnon (1969) suggested the use of trichlorphen (Neguvon) for the treatment of ringworm in calves. They treated three groups of cattle showing the lesions of ringworm daily for seven days with solutions conteining 4 per cent, 6 per cent or 8 per cent trichlorphen, rubbed into the lesions. With the highest concentration complete cure was obtained in 5 days but toxic symptoms, salivation and dyspnoea, were observed in some calves. The 6 per cent ointment gave complete cure in 7 days with insignificant toxic symptoms, the 4 per cent concentration caused no toxic symptoms but a week after treatment six of the 25 calves were not completely free of ringworm.

Misra (1971) made an in vitro screening of 46 test agents consisting of 36 extracts of plant materials, two oils and eight chemicals to asses their antifungal activity against T. verrucosum. Out of which 35 test agents were found to possess antimycotic activity. Chloroform extracts of Carcuma longa, Thibendole, Difolatan and griseofulvin were found to have satisfactory antifungal activity against T. verrucosum. In a clinical

trial, he further demonstrated that griseofulvin @ 20 mg per kg of body weight given orally every day for 15 days cured T. verrucosum infection in calves. Daily application of Ephytol lotion and Multifungin ointment for 15 days gave variable results.

Satija and Gautam (1972) treated an outbreak of cattle ringworm due to T. verrucosum and reported the effectiveness of four drugs viz. iodine ointment (5 per cent), acetic acid (40 per cent and 30 per cent), Alugan (0.2 per cent) and copper sulphate-unslaked lime spray. Out of these, copper sulphate unslaked lime spray was found to be very effective for treating ringworm in herd problems. Alugan 0.2 per cent was also equally good. Iodine ointment and 40 per cent acetic acid were found good in individual cases but 30 per cent acetic acid was irritant and caused wounds.

CHAPTER III

MATERIALS AND METHODS

The present study was undertaken in the Department of Veterinary Medicine, Orissa University of Agriculture and Technology, Bhubaneswar, during the period from May 1971 to April 1972. Cattle of various age groups, breed, sex and localities were studied during the course of investigation. The sources included were Cuttack Goshala, District Livestock Breeding Farm (Cuttack), State Livestock Breeding Farm (Chiplima), University Research Dairy Farm (Bhubaneswar), Central Clinic (Orissa Veterinary College) and Private herds in Bhubaneswar N. A. C. Area.

Collection of Clinical Materials

On careful clinical examination cattle showing characteristic ringworm lesions were selected. Active ringworm lesions were located on each affected animal. The lesions were sponged with 70 per cent alcohol to remove surface contaminants and medications. After the alcohol was dried, the active edges of a few lesions were scraped slowly and gently with a flame-sterilised Bard Parker knife. The scrapings thus collected included hairs and scales, packed in a piece of white paper and kept in a small white envelope. The skin scrapings were collected from each ringworm infected cattle separately in an envelope with an information sheet containing date and source of collection, age, sex, breed, site of lesions, description of the lesions etc. These were then brought to the laboratory for examination.

Microscopical Examination of Clinical Materials

A small portion of the clinical material (hairs and scales) was placed in one or two drops of 10 per cent potassium hydroxide (KOH) on a clean glass slide and was covered with a thin coverslip. The preparation was heated gently over a flame for a few seconds without boiling. Then it was examined under the low and high powers of the microscope for the presence of fungal spores and mycelium and their arrangements. When the primary KOH mount examination did not reveal fungal elements, were preserved in a Koplin's jar for overnight and was again examined.

Cultivation, Isolation and Identification of Dermatophytes

(1) Primary Isolation in Different Media :

All skin scrapings collected were examined culturally. Each sample was screened in four different media viz. (1) Sabouraud's dextrose agar (dextrose 40 g, peptone 10 g, agar 20 g and distilled water 1000 ml, pH adjusted to 5.6), (2) Sabouraud's dextrose agar with thiamine (0.05 per cent), (3) Sabouraud's dextrose agar with 0.5 per cent Lablemco and Brain Heart infusion agar (4 per cent in distilled water). Each of the above media was fortified with actidione (Cycloheximide), penicillin G sodium and streptomycin at the rate of 0.5 mg, 20 units and 40 units respectively. For every sample one tube from each media was inoculated with the skin scrapings (hairs and scales) by

heat sterilized and cooled nichrome needle. Skin scrapings were inoculated at two different places on the media. The inoculated tubes were incubated at room temperature (25-30°C) for a period of four weeks. If no growth was observed after the fourth week, the tubes were discarded. The dermatophyte colonies (suspected colonies) appeared during incubation were transferred to another tube containing Sabouraud's dextrose agar and thiamine to avoid contaminants. Then each isolate was studied for identification.

(ii) Macroscopic Examination of Cultures :

The gross morphology of the colonies (rate of growth, general topography, texture, surface pigmentation and pigmentation on reverse) of each isolate was observed and noted.

(iii) Microscopic Examination of Cultures :

(a) Examination in Mounting Fluid : A small portion of the culture was removed with a flamesterilised nichrome needle, placed on a slide, torn apart with two sterile needles in a drop of lactophenol or lactophenol cotton blue* and covered with a coverslip. The portion of the culture was always taken from the edge of the colony in older cultures and preferably from the centre in younger cultures. The preparation was then viewed under low and high powers of a microscope. All cultures

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* Lactophenol cotton blue was prepared by mixing phenol 20 g, lactic acid 20 g, glycerin 40 g, cotton blue 0.05 g and distilled water 20 ml.

were studied in this manner at various intervals of incubation for a month and the characteristic micromorphology was noted.

(b) Slide Culture Technique : About 15 ml of Sabouraud's dextrose agar was poured into a sterilised petri-dish and was allowed to solidify. Agar blocks about one cm^2 and 2- 3 mm deep were obtained from this by using a flame sterilised knife. A slide was placed on a bend glass rod in the bottom of a petri dish, covered and were sterilised. A block of the agar square was placed on the slide in petri dish using sterile technique. The centre of the four sides of the agar block were inoculated with the study fungus and a sterile coverslip was placed centrally over the inoculated agar block. Then about 3 ml of sterilised distilled water was added to the petri dish to prevent desiccation of the culture. This was incubated at room temperature (25- 30°C) and the slides with the growing cultures were removed from the dishes and viewed under a microscope periodically for 20 days. After sporulation was marked, the coverslip was lifted carefully and placed on a drop of lactophenol cotton blue over a clean slide with the mycelial surface down. The agar block on the slide was also lifted and discarded. A drop of lactophenol cotton blue was placed on this slide and covered with a clean coverslip. The excess mounting fluid was blotted away from the sides of coverslips of the two preparations. The edges were sealed with nail polish and both were examined under a microscope. By this technique the undisturbed sporulation characteristics were studied.

(c) Examination of Microphorphology of Cultures Grown on Special Meida : Each strain of the isolation which were

not identified through the earlier examinations were inoculated into the following special media viz. Rice grain medium (Ajello et al., 1963), Sabouraud's dextrose agar with 1 per cent yeast extract and corn meal agar (Difco) to induce sporulation. The cultures of each strain from all the three media were examined in lactophenol cotton blue and studied for the presence of microconidia and macroconidia.

Other Diagnostic Tests for T. verrucosum

(i) Nutritional Tests :

Casein agar was used as the basal medium for nutritional studies and various vitamins incorporated were thiamine, inositol and nicotinic acid.

Stock casein agar consisting of casein 10 per cent acid hydrolyzed vitamin free 20.0 ml, glucose 40 g, Mg SO_4 0.1 g, KH_2 PO_4 1.8 g, agar 20 g and distilled water, q.s. 1000 ml was prepared. After dissolving the medium by heating, it was distributed into Erlenmeyer flasks (100 ml per flask) and autoclaved for 15 minutes at 120°C. Part of the medium was dispensed into test tubes and used as control (vitamin free). The rest of the medium was used for incorporating various vitamins.

Similarly stock vitamin solutions viz. Thiamine solution (thiamine hydrochloride 1.0 mg + distilled water 100 ml), Inositol solution (inositol 250.0 mg + distilled water 100 ml) and Nicotinic acid solution (nicotinic acid 10.0 mg + distilled

water 100 ml) were prepared. These vitamin solutions were autoclaved at 120°C per 10 minutes and stored in refregerator.

Thiamine-casein agar, inositol-casein agar and nicotinic acid-casein agar were prepared by adding 2 ml of stock vitamin solution to 100 ml melted casein agar. In the preparation of thiamine-inositol-casein agar solutions of both vitamins (2 ml each) were added to 100 ml melted casein agar. Each medium was distributed into test, tubes sterilised and slanted.

Twenty strains of T. verrucosum grown on Sabouraud's dextrose agar were taken to study their nutritional requirements. Two test tubes from each of the different vitamin incorporated media were inoculated with only one small particle of the investigated culture (the size of the pin head) to avoid transferring the not vitamin free medium. Another two casein agar tubes (without any vitamin) were also inoculated with the culture and were taken as control. These inoculated test tubes were incubated at 37°C for 7 - 10 days. After incubation the comparative growth of colony of each strain in different vitamin incorporated casein agar tubes and control tubes were studied and recorded.

(11) Hair Perforation Test :

Keratinolytic activity of 20 strains of T. verrucosum which were used during nutritional test was studied on hair culture in vitro by modified slide culture technique (Lu, 1962). The hairs of human adult, cattle, goat, dog and rabbit were used

in this study. Two kinds of culture media were used for slide cultures: (1) 2 per cent plain agar without additional nutrient and; (2) 0.1 per cent yeast extract was added to the plain agar to observe the effect of nutrition on the fungus growth and hair digestion. To obtain a thin layer of agar, 15 ml of autoclaved medium was poured into sterile petri dish and cut into small blocks (one cm in length and 0.6 cm in width). The hairs obtained were thoroughly washed with a shampoo, rinsed in distilled water and was allowed to dry in room temperature. After drying the hairs were cut into short strands of 1.5 to 2 cm in length. All hairs were sterilised by autoclaving at 120°C for ten minutes.

A block of the above culture medium, one cm in length and 0.6 cm in width was placed on the glass slide lying on a U-shaped glass rod at the bottom of a petri dish. These agar blocks were inoculated at centre for their entire length with inoculum obtained from the margin of T. verrucosum culture to be tested. With sterile forceeps the hair strands were placed at a right angle to the line of inoculum. These preparations were covered with a sterilised cover glass gently pressed to cause adherance to the agar block. Sterile distilled water was added to the petri dishes and the slide cultures were maintained at room temperature for a month. Periodically, strands of hairs were removed from the petri dish, placed on a slide and cleared in a drop of lactophenol. Then they were examined under a microscope and the mode of perforation or destruction of hair caused by T. verrucosum was noted.

Natural Infection of T. verrucosum Related Clinical Lesions and Histopathology

The natural infection of T. verrucosum in cattle was investigated in Cuttack Goshala, University Research Dairy Farm, Bhubaneswar and District Livestock Farm, Cuttack and observations were recorded for one year. A detail account of the outbreak during the first investigation in May 1971 and history of previous outbreaks were collected. Informations on the number of animals affected with T. verrucosum infection during the study period, their age, seasonal variation of the disease and nutritional status of the affected animals were collected.

Clinical Lesions :

The clinical lesions produced in cattle at various stages of the disease were observed. The type of clinical lesions and their site of predilection were recorded.

(ii) Histopathology :

Skin biopsies were collected from active lesions of naturally infected (T. verrucosum) calves and preserved in 10 per cent neutral formal saline. The skin tissues thus preserved were processed, sections at 4-6 microns were cut and strained by Haematoxylen-Eosin and PAS staining methods for studying the various tissue changes and presence of fungal elements.

Experimental Inoculation Related Gross Lesions and Histopathology

Experimental inoculation studies were carried out on

two healthy male calves (about one year of age) obtained from the University Research Dairy Farm and on six rabbits of both sexes. The calves were free from any skin disease. During the experiment each calf was kept in separate pen and maintained on a balanced diet and green grass. Rabbits weighing about 500 g to 1 kg were utilised for the experimental studies. Each rabbit was kept in a separate cage during the experiment. Wet bengal gram was given as feed to the rabbits with supplementation of green grass.

(i) Preparation of Inoculum :

Various strains of T. verrucosum isolated were maintained on Sabouraud's dextrose agar tubes at room temperature and subcultured monthly. Standard suspensions were made from the above cultures after verifying their purity under a microscope. About one cm^2 of surface grown fungal culture was removed by a long nichrome needle aseptically and grinded by sterilised glass homogeniser in 5 ml of Sabouraud's broth (dextrose 40 g, peptone 10 g and distilled water 1000 ml). The number of viable spores in suspensions was determined retrospect, in certain experiments by plating out on Sabouraud's agar plate and counting the number of colonies developed after 7-10 days incubation at 28- 30° C.

Portions of skin scrapings (heavily infected with arthrospores of T. verrucosum) collected from natural T. verrucosum infected calves were grinded by a sterilised glass mortar and pestle in 5 ml of Sabouraud's broth.

(ii) Experimental Inoculation :

On each calf 24 sites (each 2 cm^2) randomly distributed all over the neck and body were selected for experimental inoculation. The sites were shaved, cleaned with 70 per cent alcohol by swabbing and allowed to dry. The areas were then scarified by a scalped blade gently and infected by placing a drop of fungal suspension on each scarified area. One calf received the clinical material suspension while the other received the culture suspension.

Rabbits were close shaved on both flanks and scarified with a scalped blade in three selected areas on each side. With culture suspension of T. verrucosum rabbits were infected by placing a drop of chlamydospore and mycelial suspension in infusion broth on scarified skin. One rabbit was infected with six different strains of T. verrucosum maintained in the laboratory for different periods. One strain was received from All India Institute of Medical Sciences, New Delhi. Another five strains of T. verrucosum were used to experimentally infect five rabbits separately. These five rabbits after recovery from above experimental infection were reinoculated with homologus or different strains of T. verrucosum. On each occasion the virulence of the strain was confirmed using an uninfected rabbit.

(iii) Record of Gross Lesions :

The experimental calves were observed daily for 3 months to study the development of lesions. Skin scrapings

were collected periodically from these experimental lesions and examined microscopically. The experimentally infected rabbits were followed for 1½ to 2 months and the observations were recorded.

(iv) Histopathology :

Skin biopsies from these experimentally infected calves and rabbits were collected and preserved in 10 per cent neutral formal saline. The tissues were processed, stained and examined in the similar way as that of natural infection.

Survival of Spores of T. verrucosum in Dried Skin Scrapings

Twentytwo skin scrapings* from ringworm (T. verrucosum) infected calves collected during the period from 7th April 1970 to 7th June 1970 were preserved in the laboratory at room temperature to study the survival period of spores of T. verrucosum. All the skin scrapings were KOH positive and culturally positive for T. verrucosum when previously examined during 1970 (Misra, 1971). On 10th April 1972 all the skin scrapings along with their envelopes were placed on a thin layer of thymol crystals covering the bottom of a tin container and were tightly closed for 48 hours. After 5 days each skin scraping was examined by 10 per cent KOH for the presence of spores and hyphae of T. verrucosum. Simultaneous cultures were also made from each sample on Sabouraud's dextrose agar with thiamine, chlorampheni-

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* Samples were received for study by courtesy of Dr. S.K.Misra, Dean, Faculty of Veterinary Science & Animal Husbandry, Bhubaneswar - 3, Orissa.

col and actidione. The findings of KOH examination and cultural examination were recorded.

Treatment of Experimental and Natural Cases of T. verrucosum in Cattle

The therapeutic efficacy of two chemicals viz. Thibendole[1] and Difolatan[2] and one plant extract viz. chlorform extract of Curcuma longa L. (Haladi)[3] having in vitro antifungal activity against T. verrucosum (Misra, 1971) were assessed in vivo. They were tried both in experimentally and naturally infected (T. verrucosum) calves. Skin toxicity test was also conducted in all cases. The therapeutic efficacy of each was assessed on the basis of three tests (Gupta, 1967) viz. clinical improvement, microscopical examination of skin scrapings (KOH test) and cultural examination. The term 'effective or cure' was used when the experimental or clinical lesions disappeared, microscopical examination and cultural examination failed to reveal the organism.

(i) Skin Toxicity Test :-

Rabbits and guinea pigs were used for skin toxicity test. The test agents were applied (topically) repeatedly on

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1. Thibendole : A product of M/s Merck Sharp and Dhome of India Limited, Bombay. Each 20 g of Thibendole contains 15 gm of thiabendazole.
2. Difolatan : A product of M/s Chevron Chemical and Co. Limited, California, U.S.A. Difolatan or sulfenide (N-(1,1, 2, 2-tetrachlorethyl hyprophthalimide) is an organic chlorinated hydrocarbon used as a follicular fungicide or seed protectant in agriculture.
3. Chloroform extract of Curcuma longa L. - Extracted by soxhalation with chloroform.

the shaved skin of both rabbits and guinea pigs for four months. Gross skin reactions and histopathological changes in the skin biopsies collected from these rabbits and guinea pigs were studied.

(11) Evaluation in Experimentally Infected Calf :

The calf which was experimentally infected with culture suspension of T. verrucosum during pathogenesis study was utilised in this trial. Out of the 24 inoculated sites 13 sites having distinct lesions of T. verrucosum were selected. Ointments of Thibendole (5 per cent and 7 per cent), Difolatan (1 per cent and 3 per cent) and Chloroform extract of Curcuma longa L. (10 per cent) were prepared in vaseline. Each ointment was applied topically on three different lesions thrice weekly for 3 weeks. The lesions were selected randomly for application of each ointment. The rest three lesions on the same calf were kept as control. After 15th day and 20th day of initial treatment the treated and control lesions were exposed to the three tests described earlier. After 20th day of examination the various concentrations of test agents which satisfied the three tests (i.e. experimental lesions disappeared, microscopical and cultural examination became negative) were taken as 'effective'.

(11) Clinical Trial :

During the course of this investigation a clinical trial was conducted in calves suffering from natural infection of T. verrucosum in Cuttack Goshala. The calves were a mixed population of Hariana, Godabari and nondescript breed and were

under one year of age. Thirtyfive calves having active ringworm lesions (positive for T. verrucosum both microscopically and culturally) were divided into five groups and kept in separate enclosures. Group I, Group II and Group III received the topical application of 5 per cent Thibendole, 3 per cent Difolatan and 10 per cent Chloroform extract of Curcuma longa L. ointments (in vaseline) respectively thrice weekly for 2- 3 weeks. While Group IV received oral treatment of Thibendole (@ 55 mg of thiabendazole per kg body weight per day) for 15 days. Group V remained untreated throughout the treatment period. In the beginning of initial treatment, the crusts of the lesions were scraped and then the ointments were applied.

Calves in each group were observed for their clinical improvement on every 2- 3 days interval. Simultaneous microscopical and cultural examination of the skin scraping from these calves were also made on the same day of observation. The results of these three tests were noted.

CHAPTER IV

RESULTS

Details of Cattle Examined, Their Source and Incidence of Clinical Cases of Ringworm

The present investigation included 4910 cattle of different age groups and were from various sources (Table 1). The sources were dairy farms and Goshala where the animals were kept in groups, the Central Clinic of the College where animals were presented from small number of cattle (1- 4) maintained by Government servants in Bhubaneswar city and by farmers from its nearby villages and small herds of cattle (10- 15) maintained by Gowalas in Bhubaneswar N. A. C. area. The later group of cattle had a poor nutritional status and were always kept in a poor hygienic environment. Out of 4910 cattle, 756 were under one year of age, 651 were between 1- 2 years of age and 3503 were above two years of age.

On clinical examination it was observed that 174 cases (3.54 per cent) were exhibiting characteristic ringworm lesions (Fig. 1, 2, 3 and Table 1). Among calves under one year of age 156 cases (20.63 per cent) and between 1- 2 years of age 18 cases (2.76 per cent) had clinical lesions of ringworm. Adults were free from infection. The percentage of incidence in relation to nature of sources is tabulated in Table 1. In farms and Goshala the incidence varied from 0 per cent to 32.65 per cent and the percentage of cases from other sources were 2.32 to 2.75.

TABLE 1. -- Details of Cattle, Their Source and Incidence of Clinical Cases of Ringworm.

Sl. No.	Source	Total No.of cattle examined	Age groups						Total No.of cattle clinically positive.	Percentage
			Under one year		Between 1-2 years		Above 2 years			
			No.of animals examined.	No.of animals clinically positive.	No.of animals examined.	No.of animals clinically positive	No.of animals examined.	No. of animals clinically positive		
1.	Cuttack Goshala.	721	65	41	32	5	574	-	46	6.38
2.	District Livestock Breeding Farm, Cuttack.	136	36	18	25	-	125	-	13	9.67
3.	State Livestock Breeding Farm, Chiplima.	43	29	14	14	-	-	-	14	32.55
4.	University Research Dairy Farm, O.U.A.T., Bhubaneswar.	63	13	-	15	-	35	-	-	0
5.	Central Clinic, Orissa Veterinary College, Bhubaneswar.	1270	133	23	160	7	927	-	35	2.75
6.	Private herds in Bhubaneswar N.A.C.area	2622	425	55	355	6	1342	-	61	2.32
	TOTAL :	4910	756	156 (20.63%)	651	18 (2.76%)	3503	-	174	3.54

Microscopical Examination of Clinical Materials (KOH Examination)

Clinical materials (scales and hairs) examined in 10 per cent KOH mount showed the presence of fungal elements in 146 samples (33.80 per cent) whereas the rest 23 samples (16.10 per cent) were negative. The hairs were seen to be invaded with large number of fungal spores measuring 4- 10 microns in diameter and hyphae in 133 samples (94.52 per cent) out of 146 samples. In these samples the spores were marked to be arranged in sheath or in isolated chains lying on the surface of hair and also the interior of hair was invaded by arthrospores and hyphae (ectoendothrix hair invasion Fig. 4). Eight samples (5.48 per cent) showed only pure endothrix invasion of hair (Fig. 5). Among these 146 samples the skin scales of 25 samples revealed the presence of branched septate hyphae (more or less arthrosporulated).

Cultivation, Isolation and Identification

(1) Primary Isolation in Different Media :

It can be seen from table II that cultural examination of the clinical materials (both KOH positive and negative) in four different media viz. Sabouraud's dextrose agar, Sabouraud's dextrose agar with thiamine, Sabouraud's dextrose agar with 0.5 per cent Lablemco and brain heart infusion agar yielded 21, 67, 23 and 61 isolates of dermatophytes respectively (Fig.6). Brain heart infusion agar gave the maximum number of isolations (35.03 per cent) which was followed by Sabouraud's dextrose agar

TABLE II. -- Results of Cultural Examination in Different Media.

Sl. No.	Name of the media	KOH Positive (146)		KOH Negative (29)		Total number of samples : 174
		Culture positive	Culture Negative	Culture positive	Culture negative	Total No. of isolations in different media
1.	Sabouraud's dextrose agar.	21 (14.33%)	125 (85.62%)	-	29 (100%)	21 (12.06%)
2.	Sabouraud's dextrose agar with thiamine.	53 (36.30%)	93 (63.70%)	4 (14.23%)	24 (85.72%)	57 (32.75%)
3.	Sabouraud's dextrose agar with 0.5% Leblemco.	28 (19.17%)	118 (80.83%)	-	28 (100%)	28 (16.09%)
4.	Brain heart infusion agar.	53 (39.72%)	93 (60.28%)	3 (10.71%)	25 (89.29%)	61 (35.05%)

with thiamine (32.75 per cent).

TABLE III. -- Consolidated Result of KOH Examination and Cultural Examination.

Clinically suspected cases	KOH positive	KOH Negative
174	146 (83.90%)	28 (16.10%)
Culture positive	60 (34.48%)	5 (2.87%)
Culture negative	86 (49.42%)	23 (13.23%)

It can be further noted from table III that 65 samples of clinical materials were culturally positive although Sabouraud's dextrose agar with thiamine and brain heart infusion agar yielded only 57 and 61 isolates respectively. More number of clinical materials became culturally positive when cultivated simultaneously in both the media in comparison to their cultivation in individual medium. However the total number of isolations did not exceed 38 per cent.

Table III shows a clear contrast between two positive figures viz. KOH positive and culture positive.

(ii) Macro-and Microscopic Examination of the Isolates and Their Identification :

All the 65 isolates of dermatophytes were identified as T. verrucosum, on the basis of macro and microscopic examination of the cultures. Three clinical samples positive for T. verrucosum also revealed the presence of another pure culture of fungus

(not a dermatophyte) which was identified as Scopulariopsis brevicaulis (Fig. 7).

The rate of growth of T. verrucosum was extremely slow and the colonies attained a diameter of 5- 15 mm after a fortnight. The colonies were usually heaped and deeply folded or lobulate (Fig. 8). The texture was moist and glabrous or powdery or velvety. The surface colour was white tan or yellow. The reverse colour of the colony was yellow or absent.

Microscopical examination of all the isolates of T. verrucosum revealed large number of chlamydospores and thin irregular mycelia (Fig. 9). The chlamydospores were at times in chains. No microconidia or macroconidia were detected in any of the cultures. Special media viz. rice grain medium, corn meal agar and Sabouraud's dextrose agar enriched with one per cent yeast extract did not help in the production of micro- or macroconidia in any of the isolates.

Other Diagnostic Tests for T. verrucosum

(i) Nutritional Requirements :

The vitamin requirements of 20 isolates of T. verrucosum have been mentioned in table IV. None of the isolates grew in vitamin free casein agar or in casein agar fortified with nicotinic acid media. The growth of all 20 strains of T. verrucosum was stimulated on casein-thiamine-inositol media with varying degree of growth (+++ to ++++). Eighty per cent

of the strains showed some growth into subsurface of casein-inositol media while growth in the rest 20 per cent were in casein-thiamine media alone.

TABLE IV. -- Nutritional Requirements of T. verrucosum.

No. of strains Total-20	Casein agar	Casein agar + thiamine	Casein agar + inositol	Casein agar + thiamine + inositol	Casein agar + Nicotinic acid
9	-	-	±	++++	-
6	-	-	±	+++	-
4	-	++++	-	++++	-
1	-	+++	-	+++	-

- = No growth.
± = Some growth into the subsurface of the media.
1- 4 + = Growth evaluated by comparison.

(11) Keratinolysis of Hairs in vitro :

In studying the keratinolytic activity of twenty strains of T. verrucosum it was observed that none of the strains perforated the hairs of cattle, dog, goat and rabbit. But five strains perforated human adult hairs coxially (Fig.10) within 11- 17 days with an average of 14.4 days only when cultured on agar media enriched with yeast extract (Table V). No perforation was observed when tested in plain agar medium. The number of perforations on each strand varied from 5- 16.

Fig. 1. Calf showing characteristic ringworm (due to T. verrucosum) lesion around the eye.

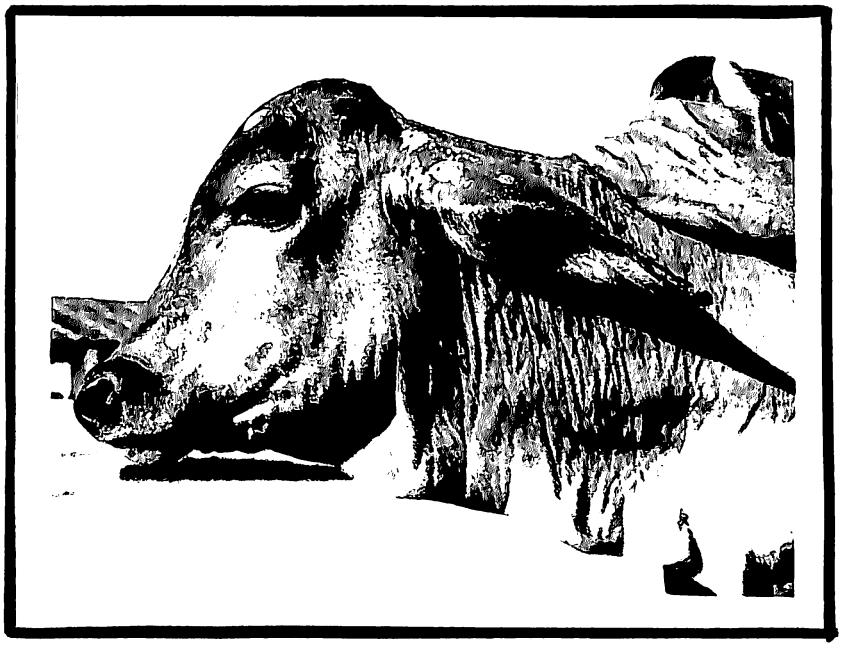


Fig. 2. Calf showing characteristic ringworm (due to T. verrucosum) lesions on the forehead and base of the ear.

Fig. 3. Calf above one year of age showing characteristic early ringworm (due to T. verrucosum) lesions on the dewlap.

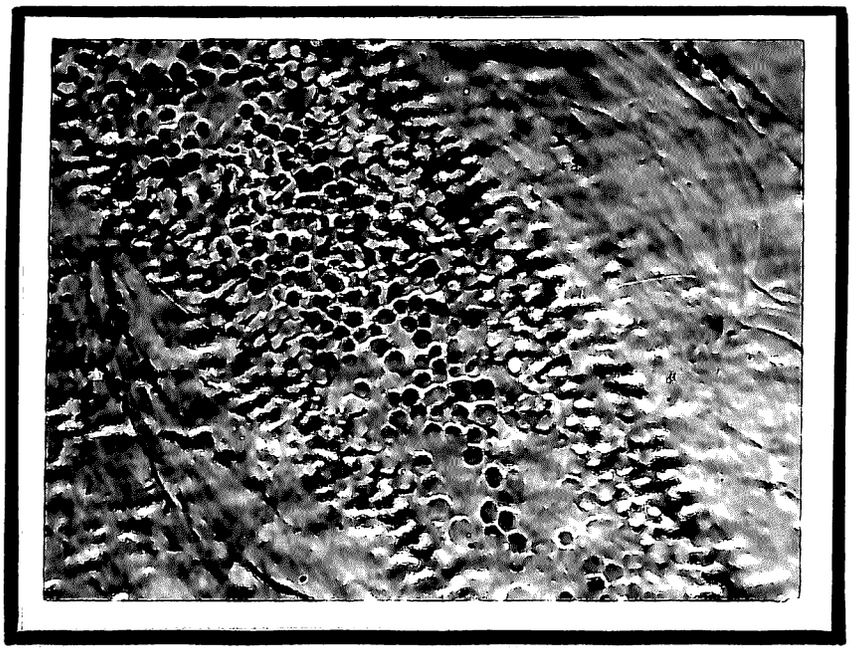


Fig. 4. Hair showing endoectothrix invasion of spores of T. verrucosum in KOH mount x 450.

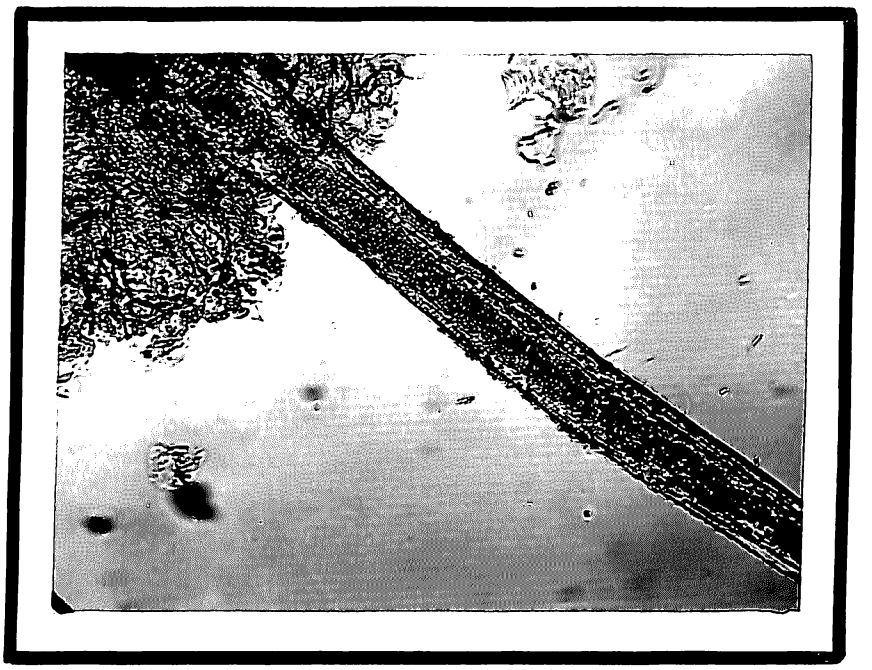


Fig. 5. Hair showing endothrix invasion of spores of T.verrucosum in KOH mount x 100.

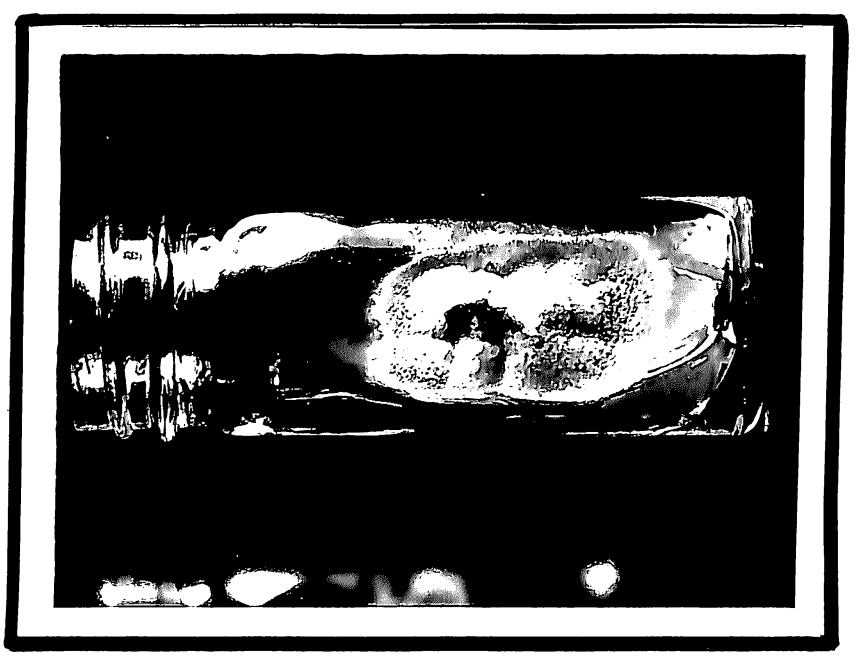
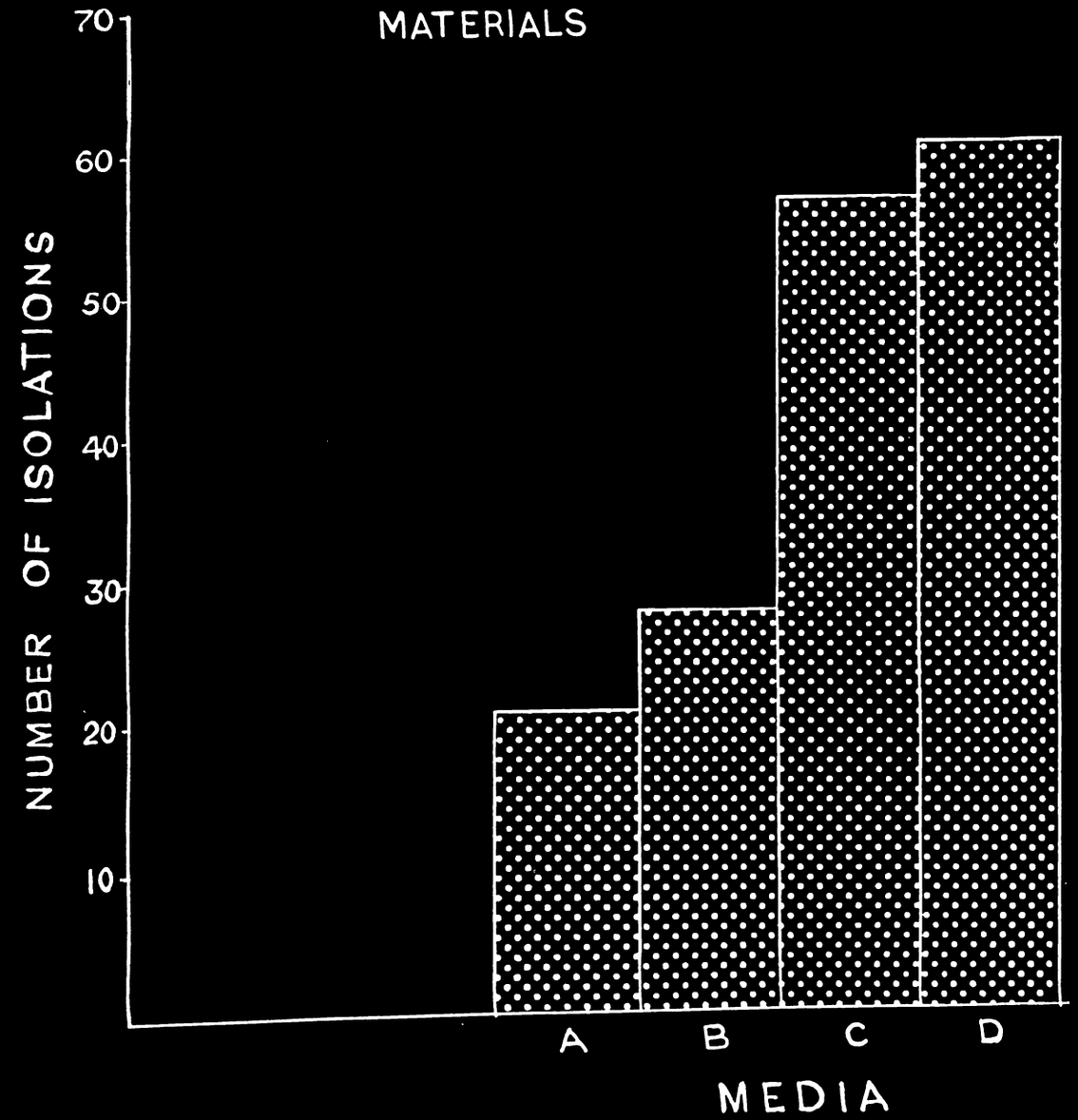


Fig. 7. Twenty days old colony of Scopulariopsis brevicaulis grown on Sabouraud's dextrose agar.

Fig.6 BAR DIAGRAM SHOWING COMPARATIVE VALUE OF DIFFERENT MEDIA FOR PRIMARY ISOLATION OF T. VERRUCOSUM FROM CLINICAL MATERIALS



MEDIA

A = Sabouraud's dextrose agar

B = Sabouraud's dextrose agar + 0·5% Lable mco

C = Sabouraud's dextrose agar + thiamine

D = Brain heart infusion agar

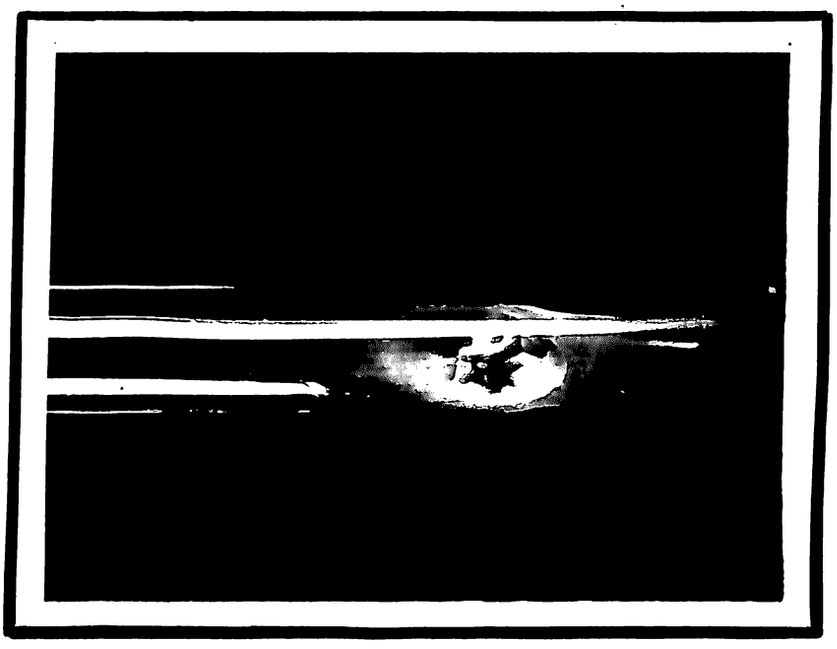


Fig. 8. Twenty days old colony of T. verrucosum grown on thiamine enriched Sabouraud's dextrose agar.

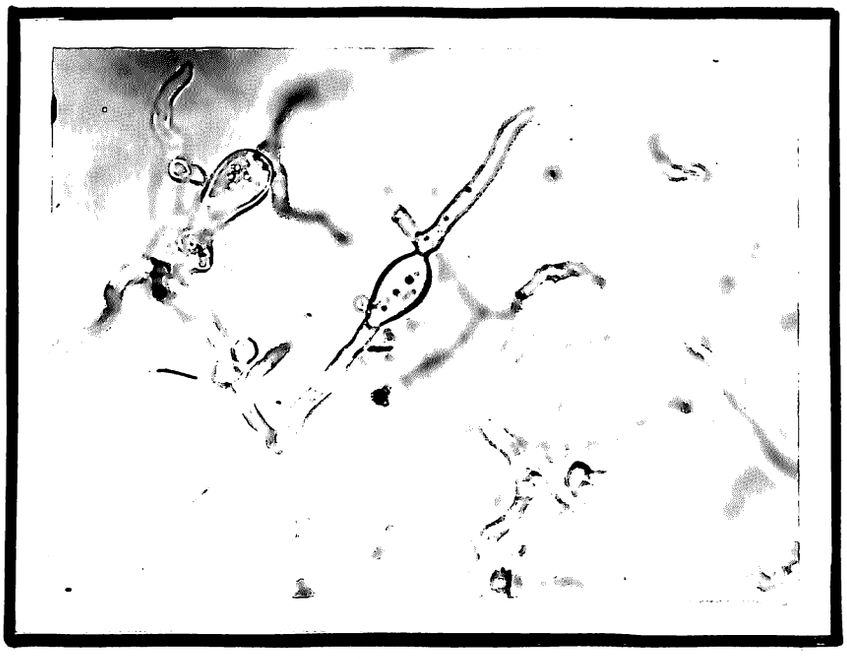


Fig. 9. Photomicrograph showing irregular mycelia and chlamydospores of T. verrucosum.

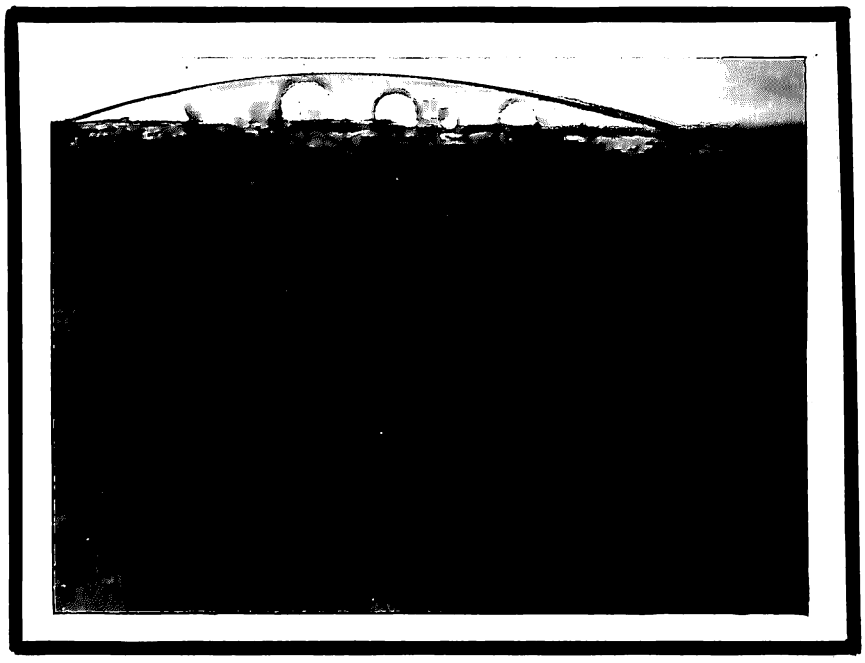


Fig. 10. Photomicrograph showing coxial perforation of human adult hair by the spores of T. verrucosum x 450.

TABLE V. -- Hair (Human Adult Hair) Digestion of T.verrucosum.

Sl. No.	Strain No.	Day on which perforation began.	Form of perforation.	No. of perforations observed on each hair in 20 days
1.	V-5/71	15th	Coxial	6 - 15
2.	V-9/71	15th	-do-	9 - 16
3.	V-20/71	11th	-do-	6 - 16
4.	V-22/71	17th	-do-	9 - 15
5.	V-10/72	14th	-do-	10 - 12

Incidence of Natural Infection of T. verrucosum-Related Clinical Lesions and Histopathological Changes

Analysis of data collected by Misra (1971) and the present study carried out in Cuttack Goshala, District Livestock Farm, (Cuttack) and University Research Dairy Farm, Bhubaneswar, revealed that T. verrucosum infection occurred as outbreaks and the duration of persistence of infection was variable. In Cuttack Goshala the infection was present during early part of 1970 and continued till 1972 affecting new calves which were included in the group from time to time. But in the District Livestock Farm, Cuttack, the outbreak was present during 1970 and 1971 and during 1972 not a single case was detected. Similarly in University Research Dairy Farm, there was an outbreak of T. verrucosum infection during 1969-70 but during 1971-72 the farm was completely free from infection.

In summarizing the information collected during collection of clinical materials from 68 samples yielding T.verrucosum cultures and follow up of natural cases, the following

facts were observed :

(1) Age Distribution :

Table VI illustrates the age distribution of the 65 calves culturally positive for T. verrucosum. Almost all cases viz. 61 (93.84 per cent) were under one year of age.

TABLE VI. -- Age Distribution of T. verrucosum Infected Cattle.

Total No. of cases	Age groups		
	Under one year	Between 1- 2 years	Above 2 years
65	61 (93.84%)	4 (6.16%)	-

(ii) Seasonal Distribution :

Table VII depicts that larger number of cases (64.61%) of T. verrucosum infected calves were detected during May to August and the incidence was low during January to April.

TABLE VII. -- Seasonal Distribution of T. verrucosum Infected Cattle.

Month and Year		No. of cases observed (Total - 65)		Percentage (%)
May	'71	8		
June	'71	20	42	64.61
July	'71	10		
August	'71	4		
September	'71	2		
October	'71	2	14	21.64
November	'71	4		
December	'71	6		
January	'72	3		
February	'72	3	9	13.85
March	'72	2		
April	'72	1		

(iii) Clinical Lesions :

The lesions in the beginning were small nodules which were covered by hairs and were detected only on careful clinical examination. These small nodules gradually became flat, rounded, sharply defined and enlarged in size. These rounded areas were first covered with scales and later with heavy grayish white crusts viz. asbestos-like crusts. Sometimes these lesions coalesced. The boarders of the lesions were slightly raised and the lesions measured about 1- 4 cm in diameter. A raw bleeding surface was left when the scales and crusts were scraped off from the lesions. In the early infective stage slight itching, rubbing the affected area against wall, woodworks etc., was marked.

The usual sites of lesions were the face, around the eyes (Fig. 1), ears, neck (Fig. 2) and dewlap (Fig. 3). In some cases lesions all over the body were also marked. The location of the lesions in the above 65 cases have been summarized in table VIII. The lesions in majority of the cases (43.08 per cent) were located on head, neck and dewlap. Claves examined at Chiplima showed generalised lesions and the skin scrapings collected from these generalised lesions in three calves revealed the presence of T. *brevicaulis*.

TABLE VIII. -- Location of *T. verrucosum* Lesions in 65 Culture Positive Calves.

Sl. No.	Location of Lesions	No. of cattle affected.	Percentage (%)
1.	Head (Forehead, face around the eyes and ear)	15	23.08
2.	Neck and dewlap	16	24.61
3.	Head, neck and dewlap	28	43.08
4.	All over the body	6	9.23

(iv) Course of Infection :

Individual cases followed during the study were seen to persist the infection for a period of about four months. Fresh lesions were observed on the affected calves even after the appearance of initial clinical lesions. Spontaneous recovery was marked in majority of the cases when left untreated. Calves once recovered from infection when left amongst T. verrucosum infected calves for 6 months did not take up the infection. But healthy calves when housed in the affected pens developed clinical lesions during the period.

(v) Histopathological Changes of Skin in Natural Infected Calves :

The cornified layer of the epidermis had extensive proliferation of mycelia and spores. These were more commonly present in the infundibulum of the hair follicles and the shaft of the hairs (Fig. 11). The mycelia had tendency of growing downwards and occasionally invaded the bulb of the hairs. Mycelia were also present in the deeper layer of the epidermis.

The stratum corneum showed marked thickening due to parakeratosis and hyperkeratinisation. However, focal but large areas of degeneration and desquamation of the layer were also evident. The desquamated materials were adherent to the surface and contained some shedding hairs, mycelia and spores. The cells of the stratum granulosum and germinativum showed hyperplasia and hyperchromatism of their nuclei. In these layers microabscesses could also be seen (Fig. 12). The entire

epidermis had marked infiltration of macrophages, lymphocytes and occasional eosinophils. The changes were more extensive in the perifollicular areas. However, the dermis (Fig. 13) and the pilosebaceous glands were also involved in this process. In addition to the above changes the dermal blood vessels showed perivascular cuffing with mononuclear cells. The cuffs had 3 to 4 layers of cellular accumulation.

Experimental Infection - Related Gross Lesions and Histopathological Changes

(i) Experimental Infection in Calves and Rabbits, and Gross Lesions :

Successful lesions were produced in both the calves experimentally infected with culture and clinical material suspensions of T. verrucosum. The first gross lesions appeared on 16th day of postinoculation in the calf which received the culture suspension whereas the lesions on the other calf appeared on 17th day of postinoculation. The first appearance of the lesions was characterised by the presence of white, granular crusts on the inoculated sites. Gradually these white granular crusts were deposited more and more and the lesions became prominent by the 28th day of postinoculation (Fig. 14). The lesions in the calf which received clinical material suspension looked more or less like natural ringworm lesions. The scales and hairs collected from these experimental lesions in both the calves were found to be infected with spores and hyphae from 16th day onwards. The infection persisted both clinically and microscopically till 56 days of postinoculation but was in a stage of gradual disappearance. By 70th day, all the lesions healed up and microscopical examination became negative.

A single rabbit was inoculated with six different strains of T. verrucosum maintained in the laboratory at room temperature (25- 30°C) for a period of 1- 6 months. The susceptibility of this rabbit to T. verrucosum and the degree of infectivity of different strains of T. verrucosum have been showed in table IX. The rabbit did not accept the strain V-20/71 which was maintained for about six months in the laboratory but by using fresh isolates lesions were produced successfully in the rabbit.

The results of experimental infection of five rabbits with five different strains of T. verrucosum can be seen from table X. The type of lesions produced in all cases with different strains were of similar nature. Within 2- 3 days of inoculation, a inflammatory phase was observed. The inoculated areas became prominent with raised inflammatory boarders. Gradually, this inflammatory phase was stubsided and indurated crusty lesions were produced within 7- 10 days of postinoculation (Fig. 15). The crusts and hairs showed the presence of spores and hyphae by the 10th day. The indurated crusts started falling off by 13th day and by 26th day lesions healed off completely. When some of these recovered rabbits were inoculated with a homologous strain (H) or a different strain of T. verrucosum small erythematous papules were observed on the inoculated sites within 2 days and these papules disappeared spontaneously by 5th to 7th day of reinoculation.

TABLE IX. -- Results of Experimental Infection of Six Strains of *T. verrucosum* in a Rabbit.

Sl. No.	Strain	Approximate age of the strain (months)	Cource of infection (Days after inoculation.								
			5	10	15	20	25	30	35	40	45
1.	V-20/71	6	-	-	-	-	-	-	-	-	-
2.	V-29/71	5	-	+	+++	XX	XX	X	-	-	-
3.	V-41/71	3	+	+++	+++	XX	X	-	-	-	-
4.	V-13/72	1	++	+++	+++	XX	XX	X	-	-	-
5.	V-19/72	1	++	+++	+++	XX	XX	X	-	-	-
6.	V-AIMS/71	Not known	-	-	-	-	-	-	-	-	-

\+ = Small erythematous nodules.

++ = Thin indurated crusts.

+++ = Skin lesions with elevated thick crusts.

XX = Loosening of the crusts.

X = Exposure of raw healed surface.

TABLE X. -- Infectivity of Single Strain of T. verrucosum in Rabbits.

Sl. No.	Sex of the rabbit.	Strain	Course of initial infection and effect of reinoculation (Days after inoculation)											
			5	10	15	20	25	30	35	40	45	50	55	60
1.	Male	V-3/72	-	+	+++	XX	X	=	-	-	R (H)	-	-	-
2.	Male	V-3/72	++	+++	+++	XX	X	-	-	-	R(V-13/72)	+	-	-
3.	Female	V-11/72	++	+++	+++	+++	XX	X	-	-	R (H)	-	-	-
4.	Female	V-13/72	++	+++	+++	XX	XX	X	-	-	-	R(V-11/72)	+	-
5.	Female	V-19/72	++	+++	+++	XX	XX	X	-	-	-	R (H)	+	-

\+ = Small erythematous papules.
++ = Thin indurated crusts.
+++ = Skin lesions with elevated thick crusts.
XX = Loosening of the crusts.
X = Exposure of raw healed tissue.

(ii) Histopathological changes of the skin of the Experimentally Infected Calves and Rabbits :

In calves large number of spores and mycelia invaded the shaft of the hairs, deeper layers of the epidermis and the sebaceous glands. Thickening of the cornified layer due to hyperkeratinisation, acanthosis and degeneration of the granulosa cell layers were also evident. The epidermis and the dermis revealed moderate infiltration of mononuclear cells such as lymphocytes and macrophages with occasional presence of eosinophils (Fig.16).

The superficial layer of the epidermis including the shaft of the hairs in rabbits were invaded by the hyphae and spores. The tissue reactions in different layers of the epidermis and the dermis were minimal.

Survival of Spores of T. verrucosum in Dried Skin Scrapings.

Altogether 22 samples of skin scrapings collected during 1970 were re-examined during 1972. Out of 22 samples, 10 samples (45.46%) were habouring arthrospores and hyphae of T. verrucosum detected on microscopical examination whereas the rest 12 samples (55.55%) were negative. On cultural examination of 22 samples, only 7 (31.82%) yielded colonies of T. verrucosum. These cultural positive seven samples were from the 10 microscopical positive samples. It can be seen in Table XI that the spores of T. verrucosum survived in the dried skin scrapings at room temperature from 1 year 8 months 3 days to 2 years 3 days.

Fig. 11. The shaft of the hair showing invasion of mycelia and spores of T. verrucosum in natural cases. PAS x 450.

Fig. 12. Epidermis showing microabscess in natural T. verrucosum infection in calf. PAS x 100.

Fig. 13. Photomicrograph showing mononuclear cell infiltration in dermal layer of the skin naturally infected with T. verrucosum. H.E. x 450.

Fig. 14. Experimental T. verrucosum infection in calf showing lesions having whitish granular crusts on the neck.

Fig. 15. Experimental T. verrucosum infection in rabbit showing indurated crusty lesions.

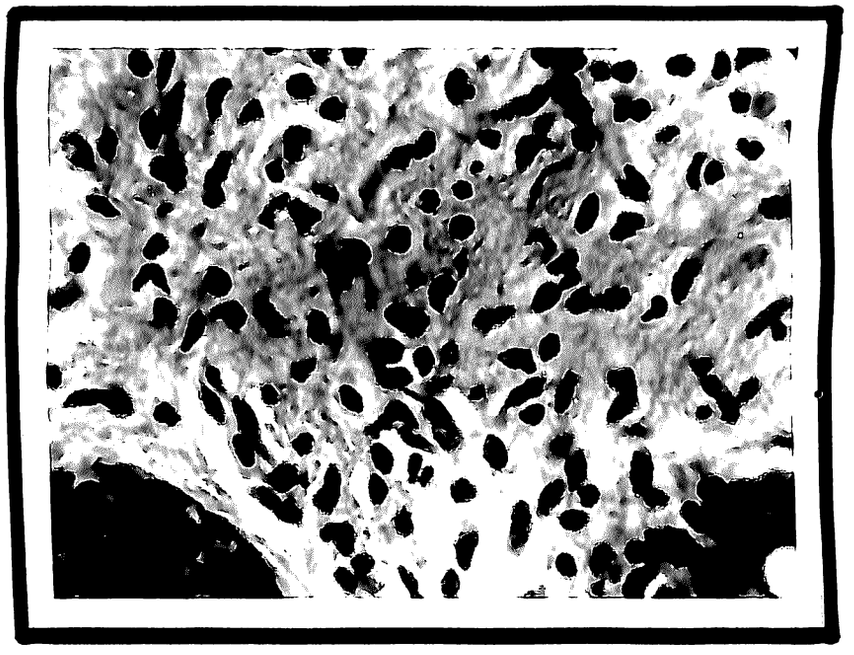


Fig. 16. Experimental T. verrucosum infection in calf showing mononuclear cell infiltration in the dermis. H.E. x 450.

TABLE XI. -- Survival of Spores of T. verrucosum in Dried Skin Scrapings.

Sl. No.	Sample No.	Date of collection.	Date of re-examination	Results of cultural examination.	Period of survival
1.	B-33/70	7.4.70	10.4.72	+	2 yrs. 3 days
2.	B-36/70	10.4.70	-do-	+	2 yrs.
3.	B-39/70	-do-	-do-	+	-do-
4.	B-40/70	-do-	-do-	+	-do-
5.	B-43/70	-do-	-do-	+	-do-
6.	B-48/70	6.6.70	-do-	-	1 yr. 8 months 4 days
7.	B-49/70	7.6.70	-do-	-	1 yr. 8 months 3 days
8.	B-50/70	-do-	-do-	+	-do-
9.	B-51/70	-do-	-do-	+	-do-
10.	B-52/70	-do-	-do-	-	-do-

+ = Positive.
- = Negative.

Treatment of Experimental and Natural Cases of T. verrucosum

(i) Skin Toxicity Test :

No gross skin reactions or tissue changes on histopathological examination were observed in the rabbits and guinea pigs used in this test.

(ii) Treatment in Experimentally Infected Calf :

The experimental lesions which received Thibendole

5 per cent and 7 per cent, Difolatan 3 per cent and chloroform extract of Curcuma longa L. 10 per cent satisfied the three tests viz. experimental lesions disappeared, microscopical and cultural examination became negative when examined 20 days after the initiation of treatment. Microscopical and cultural examination of materials from the experimental lesions which received Difolatan 1 per cent became positive although the experimental lesions had regressed. The control sites were still exhibiting the experimental lesions and were microscopically and culturally positive for T. verrucosum. No side effects or evidence of local or systemic toxicity were observed.

(iii) Clinical Trial :-

It can be seen from table XII that Thibendole 5 per cent, Difolatan 3 per cent, chloroform extract of Curcuma longa L. 10 per cent and Thibendole oral were effective against natural infection of T. verrucosum (Fig. 17, 18, 19, 20, 21, 22, 23 and 24) and the calves were cured in 14.6 ± 3.7, 13.8 ± 5.4, 19.1 ± 3.2 and 15.6 ± 3.2 days respectively (Fig. 25). The clinical lesions in the control group were still persisting even after 20 days of the initiation of treatment given to the other groups. Calves in Group I, Group II and Group III showed a similar pattern of healing of the clinical lesions, although the time taken for complete cure were different in each group. When the medicaments were applied on the clinical lesions after removing the crusts the healing was quicker.

After 5 days of administration of Thibendole orally,

Fig. 17. Calf A showing clinical lesions of T. verrucosum infection before treatment with thibendole 5% ointment.

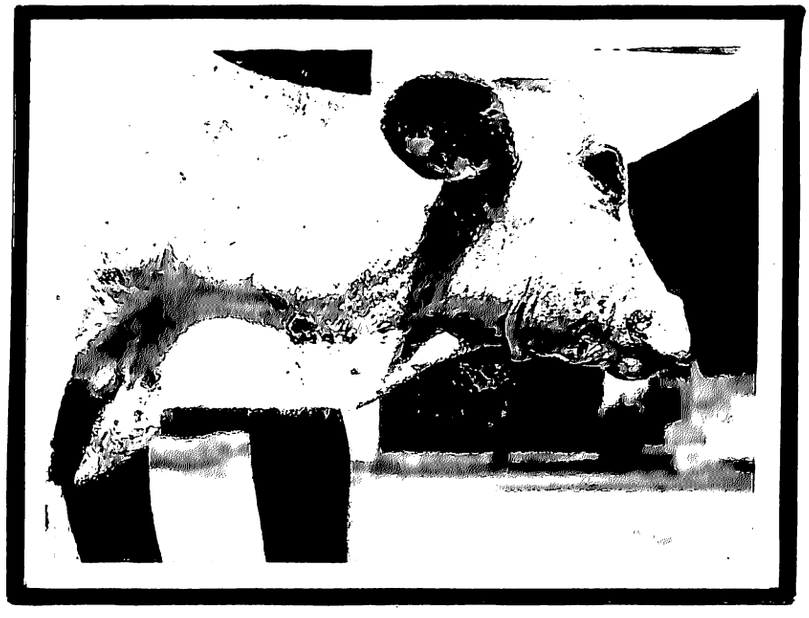


Fig. 18. Calf A showing disappearance of clinical lesions of T. verrucosum infection after 10 days of treatment with thibendole 5% ointment.

Fig. 19. Calf B showing clinical lesions of T. verrucosum infection before treatment with Difolatan 3% ointment.

Fig. 20. Calf B showing disappearance clinical lesions of T. verrucosum infection after 10 days treatment with Difolatan 3% ointment.

Fig. 21. Calf C showing clinical lesions of T. verrucosum infection before treatment with chloroform extract of Curcuma longa L. 10% ointment.

Fig. 22. Calf C showing disappearance of clinical lesions of T. verrucosum infection after 10 days treatment with chloroform extract of Curcuma longa L. 10% ointment.

Fig. 23. Calf showing clinical lesions of T.verrucosum infection before treatment with thibendole (oral).

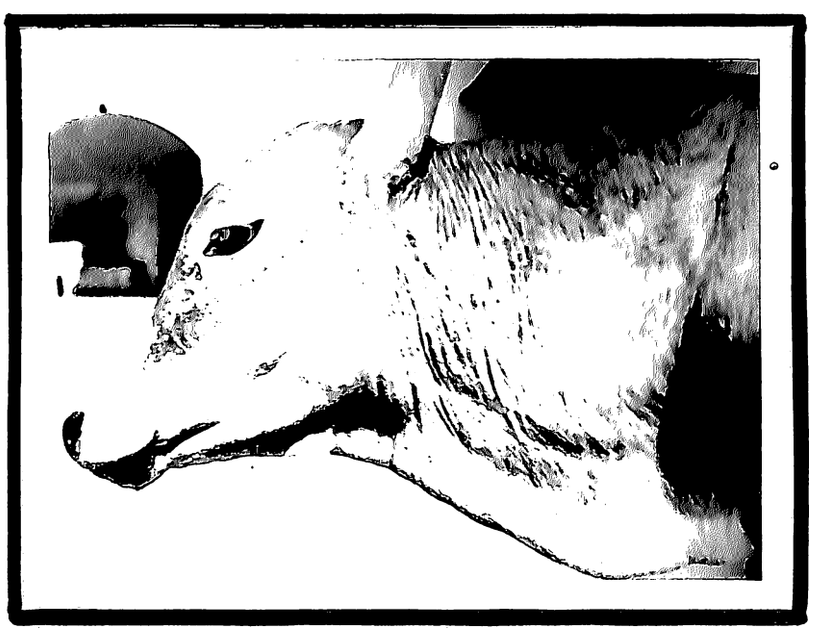
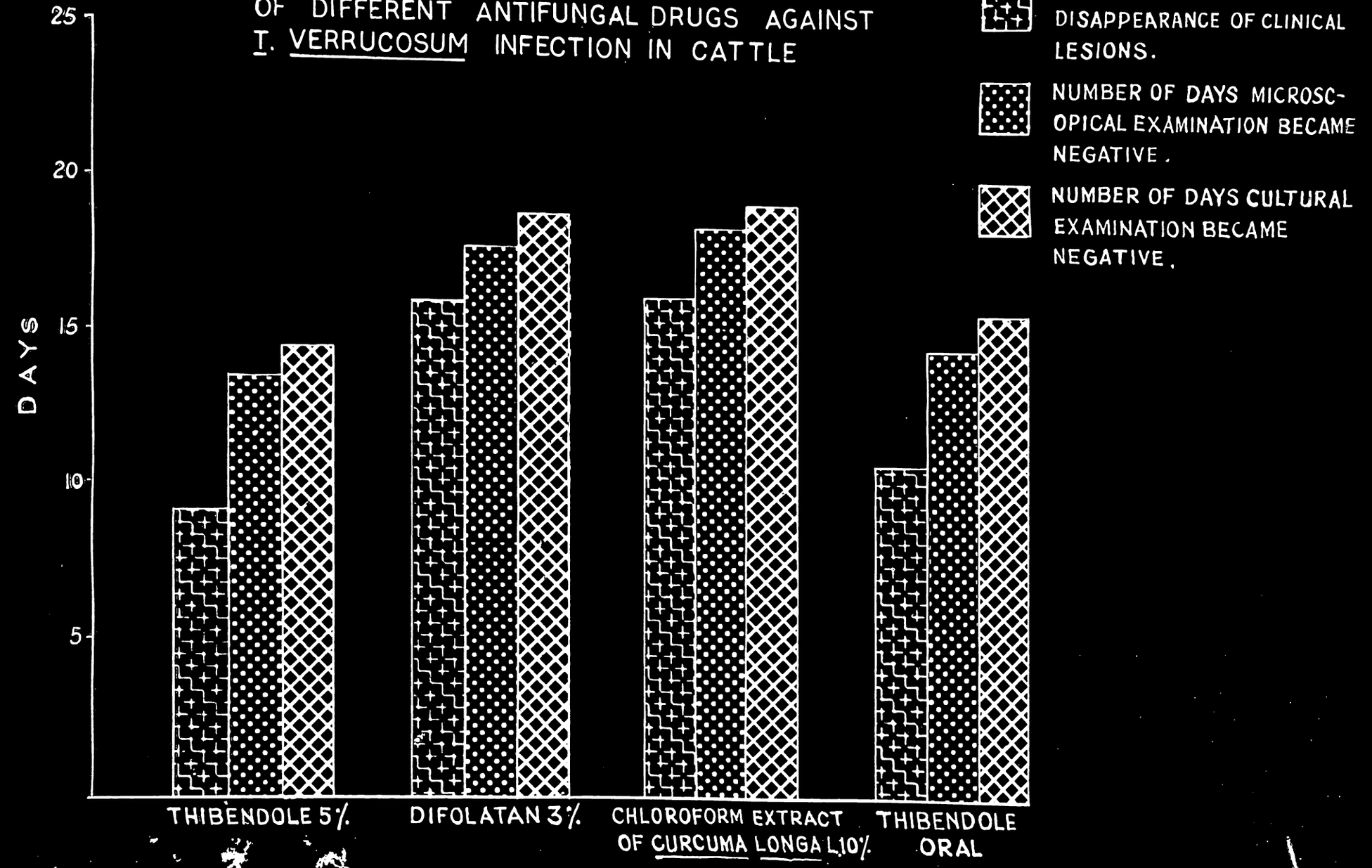


Fig. 24. Calf D showing disappearance of clinical lesions of T. verrucosum infection after 10 days treatment with thibendole (oral).

Fig.25 BAR DIAGRAM SHOWING COMPARATIVE EFFICACY OF DIFFERENT ANTIFUNGAL DRUGS AGAINST T. VERRUCOSUM INFECTION IN CATTLE



CHAPTER V

DISCUSSION

During the present investigation, a total number of 4910 cattle were examined and out of which 174 cases (3.54 per cent) were found to be clinically positive for ringworm. The investigations undertaken by McPherson (1957), Klokke and Kamp (1962), Gupta et al. (1970) and Misra (1971) revealed that the overall incidence of clinical cases of ringworm in cattle were 2.89, 2.5, 4.37 and 5.02 per cent respectively. Pepin and Austwick (1963) observed that the infection rate of cattle ringworm in two herds varied from 68-70 per cent. The work conducted presently showed that the highest rate of infection in a herd (Goshala) was 32.55 per cent.

On cultural examination of clinical materials from the above 174 clinical cases 65 isolates (37.35 per cent) of dermatophytes were obtained. These isolates were later identified as T. verrucosum. Similar reports on the exclusive isolation of T. verrucosum from ringworm cases of cattle are also available (Hoerlein, 1945; Mortimer, 1955; McPherson, 1959; Mahajan and Mohapatra, 1968; Satija and Gautam, 1972). Other species of zoophilic, geophilic and anthropophilic dermatophytes viz. T. mentagrophytes, T. violaceum, T. rubrum, T. terrestre, Microsporum canis and M. gypseum which have also been isolated from cattle (Tiwari, 1961; Dawson, 1968; Gupta et al., 1970 and Misra, 1971) were not encountered

during the present investigation. However, Misra (1971) reported very low incidence (6.6 per cent) of T. mentagrophytes infection in cattle from this area.

In the present study three ringworm cases revealed concurrent infection of T. verrucosum and S. brevicaulis. Kinter and Blender (1961) reported a case of mycotic dermatitis due to S. brevicaulis in cattle. However, further work is necessary to find out the role of S. brevicaulis in causing skin lesions in cattle.

The majority of the clinical materials showed endo-ectothrix hair invasion on KOH examination with large number of spores of 4- 10 microns in diameter and hyphae. Endoectothrix hair infection of T. verrucosum has been reported by Gupta (1967), Dvorak and Otcenasek (1969) and Misra (1971). Beneke (1966) stated that ectothrix hair invasion was the characteristic diagnostic feature of T. verrucosum. The present observation does not correspond to the statement of Beneke (1966) and also to the findings of Mahajan and Mohapatra (1963) whose majority of samples had endoth-rix hair infection. This variability in the hair invasion by spores and hyphae of T. verrucosum has been explained by Mahajan and Mohapatra (1963). According to them hair invasion at its root is of typical ectothrix character but as infection spreads upwards, it is more of endothrix nature. It is quite likely that in the same preparation, one may encounter both endo-and ectothrix type of infection depending upon the portion of hair under examination.

Isolation of cattle ringworm fungi is always rendered difficult or even precluded by the growth of saprophytic moulds in primary culture (Austwick, 1954) and since then or before, the various means to overcome this difficulty have been evolved in the form of many modifications in media or technique employed (Mahajan and Mohapatra, 1963). In the present investigation a comparative study of four different media for primary isolation of T. verrucosum was made. Brain heart infusion agar alone gave the maximum number of isolates of T. verrucosum which was followed by Sabouraud's dextrose agar with thiamine. This agrees with the findings of Mahajan and Mohapatra (1963). It is interesting to note that the number of isolates of T. verrucosum increased when simultaneous use of brain heart infusion agar and Sabouraud's dextrose agar with thiamine was made. Gupta and Singh (1970) have rightly recommended the simultaneous use of three different media (Littman oxgall agar, Sabouraud's dextrose agar with chloramphenicol, actidione and thiamine and Sabouraud's dextrose agar with Chloramphenicol) in order to obtain maximum number of isolations for cultivation of dermatophytes from animals heavily contaminated with a number of bacteria, yeasts and saprophytic fungi. The increase in the number of isolations is due to the fact that a sample which is culturally negative in one media may be culturally positive in another and vice-versa. Further, it was observed in the present study that Sabouraud's dextrose agar with thiamine increased the number of isolations of T. verrucosum when compared to

Sabouraud's dextrose agar alone. The incorporation of thiamine in the media increased the number of isolations. Gupta (1967) remarked that vitamin media were indispensible for maximum growth of T. verrucosum. The total number of isolations did not exceed 33 per cent in any of the media used alone or in combinations. Mahajan and Mohapatra (1963) obtained similar results but Misra (1971) recorded a increased number of isolations (56.33 per cent).

The rate of growth of T. verrucosum was extremely sluggish and the macromorphological character of the colonies agrees to the descriptions of Beneke (1966), Mahajan and Mohapatra (1963) and Dvorak and Otcenasek (1969). Some colonies without folding appearance or disc shaped as observed by Mahajan and Mohapatra (1963) could not be detected presently. The examination of cultures in lactophenol cotton blue revealed the presence of irregular mycelia and large number of chlamydospores. Absence of microconidia and macroconidia even in vitamin enriched media was observed although many workers (Hoerlein, 1945; Beneke, 1966 and Dvorak and Otcenasek, 1969) have described the presence of these reproductive structures. An attempt to stimulate the production of macro-and microconidia of T. verrucosum in special media like corn meal agar, rice grain medium and Sabouraud's dextrose agar enriched with yeast extract was proved unsuccessful. In the past, Gupta (1967) and Mahajan and Mohapatra (1963) have also failed to observe any micro-and macroconidia of T. verrucosum in special media like wort agar and rice grain medium and media enriched

with thiamine or yeast extract.

The nutritional requirements of various strains of T. verrucosum have been studied by several workers. The present nutritional study of 20 strains of T. verrucosum revealed that 80 per cent of strains had an absolute requirement of thiamine and inositol while the rest 20 per cent required thiamine alone. This nearly corresponds with the observations of Georg and Camp (1957), Beneke (1966) and Dvorak and Otcenasek (1969).

Human adult hair was perforated coxially by the spores of some strains of T. verrucosum. The perforations occurred only in yeast extract enriched medium. The average time taken for perforation was 14.4 days with a number of perforations (5- 16) on each strand. Lu (1962) demonstrated similar results except some deviation in the average time taken for perforation which was six days and 12 days in agar medium enriched with yeast extract and in plain agar medium respectively. However, none of the strains of T. verrucosum studied by Mahajan and Mohapatra (1963) could perforate human adult, human child, horse, cow and dog hairs.

In investigating the occurrence of natural infection of T. verrucosum in dairy farms and Goshala, it was observed that the disease was continued to be a problem in Cuttack Goshala for continuously three years. But in the dairy farms under observation, the infection disappeared within a year or two after the first or second outbreak. The management and

hygienic conditions in the dairy farms and the nutritional status of the animals maintained there, were far superior to that of the Goshala. It was also observed that due to shortage of calf pens in the Goshala, almost all the calves (about 100) were kept in one pen which was not the practice in the dairy farms. Ainsworth and Austwick (1955) stated that there were several cases in which cattle ringworm recurred in association with a particular farm and the disease was observed annually in young housed stock. The factors responsible for such occurrence were not discussed by them. La Touche (1956) remarked that the structure and condition of the cattle sheds of many small farms which precluded the effective application of even the most elementary principles of hygiene, predisposed the prevalence of T. verrucosum infection in cattle.

The clinical lesions of T. verrucosum is very characteristic and the present observations agree with the descriptions of Hoerlein (1946), Georg (1954), Mahajan and Mohapatra (1963) and Misra (1971). In the present investigation the appearance of the lesion was traced from the beginning when the lesions were in the form of small nodules which were covered by hairs and could not be detected on naked eye unless otherwise examined carefully. However, none of the above workers have included this interesting feature in their description. The usual sitesof lesions were the face, around the eyes, ears, neck and dewlap. Similar were the observations of Hoerlein (1946), McPherson (1957), Mahajan and Mohapatra (1963) and Austwick (1963) and Misra (1971). Pepin and Austwick (1963)

reported that lesions in six of 37 cases were generalised where as in the present study six of 65 cases were generalised. Out of the present six cases, three had concurrent infection of S. breviçaulis. The association of S. breviçaulis with cattle dermatitis has been reported by Kinter and Blender (1961).

Calves under one year of age were found more susceptible to T. verrucosum infection. This agrees with the findings of Hoerlein (1946), McPherson (1957), Mahajan and Mohapatra (1963), Misra (1971) and Satija and Gautam (1972). Adults were free from infection although McPherson (1957) has reported the infection in 0.43 per cent of adult cattle.

Seasonal occurrence of T. verrucosum has been observed by several workers and a high incidence is generally observed in winter months (Ainsworth and Austwick, 1955; Mortimer, 1956; Satija and Gautam, 1972). According to the reports of Mahajan and Mohapatra (1963) and Misra (1971) in our country the infection is prevalent throughout the year having the peak during rainy season. In the present study larger number of cases were also observed during rainy season (May 1971 to August 1971). Misra (1971) suggested that the deviation of seasonal incidence of cattle ringworm of this region from Western countries was most likely due to the fact that cattle, especially calves and milking cows were confined to the byres which were usually damp due to heavy rains.

The healing of clinical lesions of T. verrucosum in certain calves was completed inabout four months. Spontaneous recovery from the infection, refusal to reinfection and

transmission of infection from clinical cases to healthy calves were also observed. According to Ford (1956), the mean persistence of T. verrucosum lesions were 17 and 10 weeks in summer and winter outbreaks respectively. Sellers et al. (1956) stated that healthy calves put into the stable which had previously housed T. verrucosum infected calves developed natural lesions within 28 days and the healing process was completed in approximately three months. They also observed spontaneous recovery of lesions in naturally infected calves within four months of initial infection. Spontaneous healing of lesions after 150 days of initial infection has also been reported by O'Brien and Sellers (1953). Hence, the present observations are in agreement with the findings of the above workers.

The histopathological studies of the biopsy specimens carried out in naturally infected calves revealed that the fungal elements were most commonly seen in the cornified layer of the epidermis. According to Sellers et al. (1956), the dermatophytes have the affinity to proliferate in the keratinised layers. The observations made in the present study as regards to the presence of the fungi were in agreement to the above authors. Further evidence of mycelia and spores in the infundibulum, shaft of the hairs and the hair follicles were recorded during the present study. Invasion of these structures with mycelia and spores has been described in a similar manner by Mortimer (1955), Sellers et al. (1956) and Misra (1971). The invasion of the hair shafts and subsequent downward growths

of the fungi resulted in the involvement of the hair follicles and the deeper structures.

Parakeratosis, hyperkeratinization and hyperplasia of the epidermal layers were due to the hyperactivity as a result of tissue reactions against the infective materials. Such observations have also been made by Sellers et al. (1956) and Misra (1971). The inflammatory reactions comprised of infiltration of macrophages and lymphocytes with occasional eosinophils and microabscesses in the epidermal layers were similar to those observed by earlier workers. However, extensive perifollicular inflammatory changes observed during the present study were due to the infection of the follicles with the infective materials and subsequent tissue reactions in the nearby tissues.

In the present experimental investigation, both culture and clinical material suspensions of T. verrucosum were used for establishing infection in calves and were found equally infective. The first gross lesions appeared on the 15th day of postinoculation, became distinct on 23th day and persisted about nine weeks. Moerlein (1945) observed that lesions were first seen on 13th day and were well established on 24th day of experimental inoculation. According to Sellers et al. (1956) in experimental transmission of T. verrucosum lesions usually became evident at 14- 21 days and persisted for about 3- 4 months. McPherson (1969) recorded that the existence of experimental lesions in calves was for 10- 17 weeks with an average of 15 weeks. Misra (1971) reported

that in calves the lesions began within 1- 2 weeks of experimental inoculation and healed off by 40th day. Natural spread from these experimentally infected calves to other healthy calves or secondary lesions on the inoculated calf as have been observed by Moerlein (1945) and Misra (1971) respectively were not encountered in the present experimental studies. Experimental lesions produced by clinical material suspensions resembled the natural ringworm lesions. This observation is similar to that of O'Brien and Sellers (1953).

In the present study, older culture of T. verrucosum failed to establish the infection in rabbit while experimental lesions were produced in rabbit by using different strains of fresh cultures of T. verrucosum. The experimental lesions thus produced in different rabbits with different strains of T. verrucosum followed a similar pattern. The marked initial inflammatory phase was followed by the formation of indurated crusts. The lesions healed off by 25th day. Cox and Moore (1963) observed that the pattern of development of experimental lesions in rabbits was similar to the pattern of development of natural T. verrucosum lesions in calves but natural ringworm lesion was chronic in nature and of longer duration than experimental lesions in rabbits. The present observation agrees to their findings also.

It was interesting to note that rabbits when reinoculated with homologous or different strains of T. verrucosum after their recovery from experimental lesions of T. verrucosum

did not accept the challenge. Cox and Moore (1963) suggested that recovery from infection with T. verrucosum resulted in an apparent immunity to reinfection not only with homologous strain but also strains of different origin. They were of the opinion that the findings of Sellers et al. (1956) in respect of reported resistance of cattle to reinfection with T. verrucosum following recovery was in the line with that of rabbit.

In the experimental animals inoculated with culture suspension of T. verrucosum, the nature of the presence of the organisms and the tissue reactions were more or less similar except the intensity of the lesions. The inflammatory reactions were less severe in experimental animals as compared to the naturally infected animals. However, the presence of microabscesses in the epidermal layer as observed by Misra (1971) were not observed during the present study. The reason may be that the above author used the intradermal method of inoculation whereas in the present study theinoculation method was mostly by scarification. As regards to the pathological aspects of T. verrucosum in rabbits no detail studies have been made so far except Cox and Moore (1963). They stated that the mycelia and the spores invaded the shaft of the hairs and the tissue reactions were very mild. In the present study the nature of invasion of the fungi and the tissue changes were almost similar as indicated above.

The spores of T. verrucosum were found to survive in dried skin scrapings preserved at room temperature for more

than 2 years. However, in the past McPherson (1959) could demonstrate the survival of the spores of T. verrucosum in the dark at room temperature even after 4½ years. The skin scrapings used in the present investigation if also preserved for a longer period may able to demonstrate the exact survival period of the spores of T. verrucosum.

Over the years a large number of antifungal drugs against T. verrucosum infection in cattle have been formulated and made available for clinical use but none of them are proved to be very effective. In the present study only three antifungal agents viz. Thibendole, Difolatan and chloroform extract of Curcuma longa L. have been selected by virtue of their marked in vitro antifungal activity against T. verrucosum (Robinson et al., 1964; Misra, 1971). It was found that there was no evidence of skin toxicity by continuous application of these antifungal agents on the shaved skin of laboratory animals (rabbits and guinea pigs) over a period of four months.

A single calf with 24 experimental lesions produced by application of chlamydospores and hyphal suspensions of T. verrucosum on the scarified skin was found suitable and to provide satisfactory test condition for topical evaluation of antimycotic agents. In the past a few drug possessing antimycotic activity against T. verrucosum have been assessed successfully in a similar way by McPherson (1959) in calves with multiple experimental lesions produced by intradermal injection of spore suspensions of T. verrucosum. The superio-

rity of this type of biological evaluations under control condition for assessing topical antimycotic agents over the clinical trials on natural field infections of T. verrucosum has been stressed by O'Brien and Sellers (1953) and McPherson (1959). They have been of similar opinion that the variation in the duration of lesions in natural cases on different individuals probably reflects the development of immunity. This variant together with the unknown age of the lesions and other uncontrolled and perhaps unknown factors inseparable from clinical trials on natural field infections, renders assessment of antimycotic agents by this means unsatisfactory.

The present clinical trial was conducted with equal number of natural cases of T. verrucosum in each group including the control group maintained in a similar environment. The term complete cure was used when the clinical lesions disappeared, the microscopical and cultural examination became negative for the organisms. In addition to effecting a clinical cure, the object of ringworm therapy would be the elimination of the causative agent from the skin and its appendages, otherwise the patient would remain a potential source of infection for others. Among the topical preparations used in this trial Thibendole 5 per cent ointment was found to bring about clinical cure in 9.1 days by two applications weekly. This is in agreement with the findings of Neuman and Platzer (1963) who could bring about clinical recovery of forty eight calves suffering from T. verrucosum infection with local application of a 4 per cent thiabendazole ointment, two applications 3- 5

days apart. However, they have not mentioned whether the causative agent (T. verrucosum) was completely eliminated from the patients during this period. In the present study Thibendole 5 per cent brought about complete cure in 14.5 days. The duration between the clinical recovery and the elimination of causative agent was 5.4 days. This suggests that the drug should be applied about a week more even after the clinical recovery in order to make the patient completely free from infection.

The therapeutic efficacy of two other new antifungal agents, Difolatan and chloroform extract of Curcuma longa L., against T. verrucosum infection in calves was demonstrated in this study. They brought about complete cure in 13.8 and 19.1 days respectively. Difolatan is a known agricultural fungicide and now it can be used safely in veterinary medicine in view of its successful use against natural infection of T. verrucosum. This has stimulated the idea of screening more and more number of agricultural fungicides for their suitability against T. verrucosum infection in cattle. Curcumine is a major constituent of Curcuma longa L. which is reported to possess local as well as systemic anti-inflammatory property. The other fractionates and essential oil of Curcuma longa L. and the major constituent curcumine have also been found to possess antibacterial activity (Prasad and Sirsi, 1956; Srimal et al., 1971). The proved in vitro antifungal activity of chloroform extract Curcuma longa L. (Misra, 1971) and its present successful therapeutic use against clinical cases of

T. verrucosum has sufficiently established this commonly available medicinal plant as a good antifungal drug. Hence, this antifungal property of Curcuma longa L. is a new addition to its pharmacological properties along with its established antibacterial and anti-inflammatory properties.

Thibendole (thiabendazole @ 55 mg per kg body weight daily) given orally brought clinical recovery of natural cases of T. verrucosum in 10.3 days although cultural examination became negative in 15.6 days. No toxic symptoms were observed during or after the treatment. Robinson et al. (1964) also reported that thiabendazole administered orally to a variety of animal species over a 2 years period had no adverse effect. Neuman and Platzer (1968) could not demonstrate cure of a natural T. verrucosum infected calf by oral administration of thiabendazole @ 50 mg per kg body weight. This variability may be due to the fact that the drug was screened only in a single calf and also it was not continued for a minimum period resulting in underdosing. The present systemic use of Thibendole is of value as the results are comparable to that of Staron et al. (1964) who could cure ringworm cases in dogs by incorporating thiabendazole in their feed. The later authors are of the opinion that when thiabendazole given orally is absorbed into the blood stream and interferes with the protein metabolism of the fungus. Thibendole is less costly than griseofulvin and hence its systemic use against T. verrucosum infection in cattle can be recommended.

As suggested by Gupta (1967), the treatment with any of the drugs mentioned above should be continued for some days more (3- 6 days) after the macroscopic lesions disappears. The obvious reason is that it takes 3- 6 days for the lesions to be culturally negative after the macroscopic lesions have disappeared. The topical application of drugs in an ointment form may not be convenient when the lesions are extensive and a large number of calves are affected at a time. In such instances oral administration of Thibendole is advisable.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The present study was undertaken to evolve a better diagnostic procedure to investigate the pathogenicity and to find out a suitable chemotherapeutic agent(s) against T.verrucosum infection in cattle. Out of 4910 cattle examined, 174 cases (3.54 per cent) showed the clinical lesions suggestive of ringworm. On KOH examination, 146 skin scrapings (83.90 per cent) were positive for the presence of fungal spores and hyphae. The cultural examination of the 174 samples yielded 65 strains of T. verrucosum. No other dermatophytes were encountered.

Microscopic examination of KOH positive skin scrapings revealed the presence of large number of fungal spores measuring 4- 10 microns in diameter and hyphae. Endoectothrix and endothrix hair invasions were observed in 94.62 per cent and 5.43 per cent of KOH positive samples respectively.

Cultural examination in four different media viz. Sabouraud's dextrose agar, Sabouraud's dextrose agar with 0.5 per cent Lablemco, Sabouraud's dextrose agar with thiamine and brain heart infusion agar yielded 21, 23, 57 and 61 isolates of T. verrucosum respectively. Brain heart infusion agar gave the maximum number of isolations (35.05 per cent). Simultaneous use of brain heart infusion agar and Sabouraud's dextrose agar with thiamine gave an increased number of isolates than the isolations made in individual media.

The rate of growth of T. verrucosum was found to be extremely slow and the colonies attained a diameter of 5- 15 mm after a fortnight. The colonies were usually heaped and deeply folded or lobulated. The texture was moist and glabrous or powdery or velvety. The surface colour was white, tan or yellow. The reverse colour of the colony was yellow or absent. The common micromorphology revealed large number of chlamydospores and irregular mycelia. Macro-and microconidia were absent.

It was found that 80 per cent strains of T. verrucosum had an absolute requirements of thiamine and inositol. The rest 20 per cent required thiamine alone.

Adult human hair was found to be perforated by the spores of T. verrucosum coxially within 11- 17 days with an average of 14.4 days. The number of perforations on each strand of hair varied from 6- 16. None of the strains perforated the cattle, dog, goat and rabbit hairs.

In a Goshala the natural outbreak of T. verrucosum infection in calves persisted for over a 3 year period by spreading from one calf to the other. Calves under one year of age were found more susceptible to T. verrucosum infection than the adults. The incidence of the disease was observed alround the year with a high incidence in the rainy season (May to August). The common sites of lesions were face, around the eyes, ear, neck and dewlap. The lesions were characterised by the appearance of small nodules gradually becoming flat, rounded, sharply defined and enlarged in size. These were

first covered by scales and later with heavy greyish white asbestos-like crusts. The size of lesions were 1- 4 cm in diameter. Lesions were found to persist in individual cases for about four months. Spontaneous recovery from the infection, refusal to reinfection after recovery and transmission of the infection from natural cases to the healthy calves were observed.

In natural cases mycelia and spores were observed in different layers of the epidermis especially in the cornified layers and the infundibulum of the hair follicles. The tissue reactions were mainly hyperkeratinization, hyperplasia and degeneration of the epidermal layers. Mononuclear cell infiltration, microabscesses and perivascular infiltrations were the chief inflammatory changes.

Calves were successfully infected with both culture and clinical material suspensions of T. verrucosum. The first gross lesions in experimental calves appeared on 15th to 17th day of postinoculation, which became distinct on 23th day and healed off by 70th day. The gross lesions were characterised by the presence of white granular crusts.

Older culture of T. verrucosum lost its infectivity to rabbit and fresh cultures produced experimental lesions in rabbits successfully. The experimental lesions produced by different strains of T. verrucosum in different rabbits followed a similar pattern viz. the marked inflammatory phase followed by the formation of an indurated crust. Rabbits recovered from

experimental lesions of T. verrucosum were resistant to reinfection with either homologous or different strains of T. verrucosum.

The histopathological changes of the experimental lesions were similar to that of the natural lesions except that the inflammatory reactions were less severe. In experimentally infected rabbits, the superficial layer of the epidermis including the shaft of the hairs were invaded by spores and hyphae. The tissue reactions were negligible.

The in vivo therapeutic efficacy of Thibendole 5 per cent and 7 per cent, Difolatan 3 per cent and chloroform extract of Curcuma longa L. 10 per cent against experimental T. verrucosum infection in a calf was assessed and found effective.

The topical application of Thibendole 5 per cent, Difolatan 3 per cent, chloroform extract of Curcuma longa L. 10 per cent ointments twice weekly and oral administration of Thibendole (@ 55 mg of thiabendazole per kg body weight daily) effected complete cure of calves with T. verrucosum infection in 14.6 ± 3.7, 13.3 ± 5.4, 19.1 ± 3.2 and 15.6 ± 3.2 days respectively.

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Th-62