STUDIES ON DIAGNOSIS AND THERAPEUTICS OF DERMAL MYCOSIS IN CAMEL CALVES

ऊँट के बछड़ों में त्वचीय फंफूद रोग का नैदानिक और चिकित्सीय अध्ययन

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B.V.Sc & A.H.

THESIS

MASTER OF VETERINARY SCIENCE

(Veterinary Epidemiology and Preventive Medicine)

।पशुधनं नित्यं सर्वलोकोपकारकम्।

2018

Department of Epidemiology and Preventive Veterinary Medicine
College of Veterinary and Animal Science, Bikaner
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THESIS

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By

KARUN KANT KAMAL

B.V.Sc. & A.H.

2018

Rajasthan University of Veterinary and Animal Sciences
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Date..15-01-2018

This is to certify that the thesis entitled "Studies on Diagnosis and Therapeutics of Dermal Mycoses in camel calves" submitted for the degree of Master of Veterinary Science in the subject of Veterinary Epidemiology and Preventive Medicine of the Rajasthan University of Veterinary and Animal Sciences, Bikaner, embodies bonafide research work carried out by Mr. Karun Kant Kamal under my guidance and supervision and that no part of this thesis has been submitted for any other degree. The assistance and help received during the course of investigation have been fully acknowledged. The draft of the thesis was also approved by the advisory committee on 22-12-2017

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This is to certify that Mr. KARUN KANT KAMAL, of the Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary and Animal Science, Bikaner has made all corrections/modifications in the thesis entitled "Studies on Diagnosis and Therapeutics of Dermal Mycoses in camel calves", which were suggested by the external examiner and the advisory committee in the oral examination held on..22/02/2018.. The final copies of the thesis duly bound and corrected were submitted on...23/02/2018...are forwardwd herewith for approval.

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Karun Kant Kamal

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LIST OF ABBREVATIONS

%	Per cent
gm	Gram
Hb	Haemoglobin
PCV	Packed cell volume
TLC	Total leukocytes count
TES	Total erythrocytes count
RBC	Red blood cell
DLC	Differential leucocytes count
ml	Milliliter
@	At the rate of
i.e.	That is
S.No.	Serial Number
et al.	et alii (and co-workers)
mg/dl	milligram per deciliter
Cu.mm	cubic millimeter
N	Neutrophil
L	Lymphocyte
B	Basophil
M	Monocyte
E	Eosinophil
PLT	Platelets
AST	Aspartate aminotransferase
ALT	Alanine aminotranferase
ALKP	Alkaline phosphatase
U/L	Units per liter
Mmol/l	millimole per liter
NRCC	National research centre on camel
EDTA	Ethylene diamine tetra acetic acid
SDCA	Sabouraud's dextrose chloramphenicol agar
Cu	Copper
Co	Cobalt
Zn	Zinc
Se	Selenium
ICAR	Indian Council of Agricultural Research
NRCC	National Research Centre on Camel
PPM	Part per million

INTRODUCTION

1. INTRODUCTION

Camel belongs to genus Camelus in the family camelidae under the suborder Tylopoda and order Artiodactyla. There are two types of camels, the one humped or dromedary camel (*Camelus dromedarius*), and the two humped or bactrian camel (*Camelus bactrianus*). *Camelus dromedaries* mean "running camel". Camelus is latin word for camel and Dromeus is Greek word for runner. The *Camelus dromedarius* also has many common names such as camel, one-humped camel, dromedary camel and Arabian camel.

Indian camel population is mostly confined to the North Western part of the country, especially in the state of Rajasthan. According to State Animal Husbandry 19th livestock census (2012), the camel population in Rajasthan is 325713, out of which, Bikaner possesses 46209 camels. Camel is the state animal of the Rajasthan.

Camel is an important component of the desert ecosystem where the flora of usually marginal land can meet the need of human food and energy. Rearing for drought purpose in arid and semiarid areas has decimated many livestock species. However, dromedary is still surviving in large numbers due to its outstanding tolerance to the high temperature, water deprivation, endurance for hunger and feed scarcity. It is referred, the excellent means for carrying loads, transportation, agriculture and defense services in conditions where other animals are scared and failure. Besides these, it's hide, hair and even milk are important products which contribute the passive role towards the rural economy.

The skin surface acts as an anatomical and physiological barrier between the animal and environment. The diseases of camels are closely related to their natural environment and the type of husbandry. Camelids like other livestock are exposed to a range of skin affections caused by bacteria, viruses, parasites and fungi. Among all type of skin

infections, fungal infections are more prevalent. Young animals are more prone to fungal infections than adult's ones, which could be related to the development of a stronger immunity in older animals due to the multiplicity of contacts with the fungus rather than an intrinsic role of age of animal (Descamps *et al.,* 2003 and Moriello *et al.,* 2003). This may be due to their weak immunity and the high pH of the skin as the pH of skin decreases with age (Radostits *et al.,* 1997).

The specific requirements, enzyme, genes responsible for pathogencity of dermatophyte species, the various defense mechanisms of the hosts may also affect incidence and severity of dermatophyte among host species and the particular distribution of lesions on the host. Gitao *et al.* (1998) reported higher infection rates of camel dermatophilosis from Kenya, Sudan and Saudi Arabia in the wet season as compared to the dry season. Identification and characterization of camel skin fungi along with type of the lesions has been described in India (Tuteja *et al.*, 2010a; Tuteja *et al.*, 2010b; Tuteja *et al.*, 2012; Tuteja *et al.*, 2013a; Tuteja *et al.*, 2013b; Tuteja *et al.*, 2014a; Tuteja *et al.*, 2014b and Ganguly *et al.*, 2017).

Camel is the most suitable mammal for use in extreme climatic conditions (Wilson, 1984 and Yagil, 1985). The skin infections causing contagious skin necrosis, dermatitis, wounds, abscesses or similar lesions pose constant problems in camel. These infections are chronic in nature. Though the diseases are not always fatal but an indirect great economical loss is incurred due to reduction in the working efficiency of the animals. At many occasions the skin lesions spread rapidly over the body surface and it is very difficult to manage these lesions. Consequently, the camel becomes useless for any purpose. The inability to work or death of the animal results in severe monetary loss which shatters the socio-economic status of camel owner. The people most closely associated with the camel in Rajasthan are the

Raika's. The Raika camel keepers have developed their own ways to treat diseases in camels, based on folk believes traditional knowledge, skills, methods and practices.

Several published reports from different countries (Mohamed and Hussein, 1996; Agab, 1998; Nomanda, 1998 and Muhammad *et al.*, 2005) have described the traditional practices used by the camel owners. Camel owners living in remote areas of the desert remain cut off from the comparatively progressive areas. The local environment specially the range land flora governs the needs of the resource poor farmers. So the local therapies especially the plant based therapies varied considerably from one region to other. Many of the present day remedies were borrowed from folk medicine. There is paramount need to document and validate this indigenous system of treatment and collate with the scientifically validated knowledge, so as to have better understanding about the rationale behind such practices.

Medicinal plants represent a rich source of antimicrobial agents (Mahesh and Satish, 2008). Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper rates as compared to modern pharmaceutical drugs. Plants generally produce many secondary metabolites, which constitute an important source of bactericides, fungicides, pesticides and many pharmaceutical drugs

Looking to the severity of dermal mycoses in this area, the present study was undertaken with following objectives:

1. To record gross lesions and clinical signs in camel calves affected with dermal mycosis.
2. To isolate and identify of fungi causing dermal mycoses in camel calves.

3. To find out changes in hematological and biochemical profile in camel caves infected with dermal mycoses.
4. To find out relationship between serum minerals (Zn, Co, Se and Cu), for maintaining skin integrity in camel calves infected with dermal mycoses.
5. To find out histopathological and pH changes of the skin in camel calves infected with dermal mycoses.
6. To determine therapeutic efficacy of herbal formulation for the treatment of dermal mycosis in camel calves.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 Clinical signs, lesions and age of the camel calves

Khamiev (1981) reported camel calves were more susceptible to ring worm, had similar lesions to those seen in cattle on head, neck and shoulders, with a possible extension to the flanks and legs, leading sometimes to pyoderma and emaciation.

McGrane and Higgens (1985) mentioned that camel owners were familiar with ringworm, which occurred most commonly in camels under three years of age. The condition characterized by circumscribed, crusty, hairless lesions, 1 to 2 cm in diameter, distributed over the head, neck, shoulder, limbs and flanks.

Singh (1988) reported that the lesions of ringworm in affected cattle-calves were grayish white asbestos like crusts raised perceptively above the skin. The lesions were roughly circular about 1-3 cm in diameter.

Abdurahman and Bornstein (1991) reported that ringworm usually occurred in young camels under three years of age and characterized by localized thickening of skin. Lesions appeared as circumscribed, crusty and hairless, distributed over the head, neck, shoulders and limbs.

Manefield and Tinson (1996) reported two types of ringworm lesions in camels. The first showed typical lesions that were gray-white in colour and characterized by small, round alopecic areas, which coalesce and mainly occur on the legs, neck and head of young animals. The second was a more generalized infection on head, neck, limbs and flanks.

Gitao *et al.* (1998) from Saudi Arabia observed that the lesions with mixed infection of *D. congolensis* and *M. gypseum* were discrete, circumscribed, crusty, hairless and were particularly found on the neck and forelegs in camel calves of less than one year of age, whereas other age group animals had extensive hair matting with crusty, hairless lesions, especially on the flanks.

Al-Ani *et al.* (2002) observed that calves aged 3 to 7 months developed clinical signs of ringworm. The skin of affected calves showed circular, circumscribed, grayish-white, crusty raised lesions. Focal pityriasis and alopecia were also observed. The lesions were most commonly found on the head, neck, dewlap and the chest area. In horses the lesions started as patches of raised hair and soreness. Several days later, the hair detached leaving bald, gray, shining areas approximately 3 centimeter in diameter.

Wernery *et al.* (2002) reported skin lesion caused by C. *albicans*. The 6-week-old camel calf had developed thick crusts near the hump and scapular regions.

Tuteja *et al.* (2010b) reported cutaneous alternariosis in camel calves approximately one year of age and incidence decreased with advancement of age. Lesions were observed throughout the body including the lips and udder

Wisal and Salim (2010) reported that ringworm occurred in camels less than 3 years age and was characterized by circumscribed crusty hairless lesion, 1-2 cm in diameter distributed over the head, neck, shoulder, limbs and flanks.

Tuteja *et al.* (2012) reported that skin candidiasis is an acute and contagious fungal skin infection of camel calves of less than one year of age, which causes morbidity in terms of reduced weight gain.

Lesions were observed initially on the back near the hump; later on the lesions extend towards the abdomen and whole body. Lesions were initially round in shape and measure less than one centimeter in size which might enlarge to more than 10 centimeter in size and may coalesce. The lesions were hard and fibrous crusts with papules accompanying alopecia. In prolonged cases it caused itching, uneasiness, bleeding and ulceration of skin and result in weakness and debility of camel calves.

Tuteja *et al.* (2013a) observed that *Microsporum spp.* had ability to degrade keratin and thus can reside on skin and its appendages and remain non-invasive. These fungi could grow to create distinctive lesion associated with ring worm. These lesions were small disc-shaped marking and found anywhere on the body.

Abdulaziz *et al.* (2016) from Saudi Arabia reported clinical signs of ringworm caused by *Trichophyton verrucosum* were non-pruriginous, dry circumscribed discrete, crusty hairless lesion distributed over the head, neck, shoulder, limbs and flanks.

2.2 Etiology of camel dermal mycoses

Georg (1954) proposed classification for dermatophytes based on their habitat. He divided them into three groups: 1. Zoophylic found mainly in animals, but transmitted to other animals and humans as well. 2. anthropophylic-found mainly in humans, transmitted amongst humans, but very seldom to animals; 3. geophylic- dermatophytes found mainly in soil that infect both humans and animals.

Boever and Rush (1975) reported the isolation of *Microsporum gypseum* from ring worm lesions of a zoo dromedary camel.

Chittawar and Rao (1982) reported *Microsporum canis* infection in dogs, main causative agent of mycotic dermatitis. Pal and Singh (1983) reported *Microsporum canis* infection in cattle.

Thakur *et al.* (1983) reported *Trichophyton verrucosum* as the primary causative agent of dermatomycosis in water buffalo in Punjab.

El-Kader (1985) screened 100 camels having clinical picture of skin diseases and found 34 camels positive for mange and 16 positive for dermatomycosis. The cultural examination of skin scraping revealed *Trichophyton verrucosum*, *Microsporum canis* and *Microsporum gypseum* infections.

Kuttin *et al.* (1986) made survey for ringworm infection in camels and reported more than 25% of young animals suffering from *T. verrucosum* infection, and less than 0.5% of the camels having *T. mentagrophytes* infection.

Mancianti *et al.* (1988) described dermatophytosis caused by *M. gypseum* in a camel (*Camelus bactrianus*) from a zoological garden (Pistoia, Italy).

Abdel-Gewad (1989) reported frequency of fungi in 120 claws samples of buffaloes and cows from different localities at Assiut governorate. The most common genera were *Chrysosporium*, *Aspergillus* and *Scopulariopsis in* addition to other dermatophytes namely such as *Trichophyton terrestre*, *T. rubrum*, *Histoplasma capsulatum*, *Phialophora gougerotii* and *Microsporum distortum*.

Abdurahman and Bornstein (1991) in Somalia reported that the most common dermatophytes found in camels were *Trichophyton mentagraphytes*, *Microsporum gypseum* and mixed infection of skin pathogens (eg. *Trichophyton spp.* and *Sarcoptic scabiei*).

Lewis *et al.* (1991) reported that *Microsporum and Trichophyton* were most frequently found dermatophytes in animals while the *Epidermatophyton* causes problems mainly in humans.

Fadlelmula *et al.* (1993) conducted study on camel ringworm at Eastern Sudan. Ringworm was diagnosed in 217 out of 498 camels (43.5%). The peak incidence of the disease was found in autumn and winter. The disease was observed more frequently among young growing calves (1-2 years) than older animals but the prevalence among male and female animals was found to be similar. *Trichophyton verrucosum* was isolated in pure culture for the first time from camel ringworm in the Sudan.

Mahmoud (1993) reported sixteen species of dermatophytes belonging to nine genera of keratinophilic and cyclohexamide resistant fungi from diseased camels. *Trichophyton, Microsporum* and *Chrysosporium* were the most common genera identified. The younger individuals were more susceptible than adults.

Rezabek *et al.* (1993) diagnosed histoplasmosis in nine horses during 1986-1990. The infection with *Histoplasma capsulatum* caused granulomatous placentitis and abortion. Four newborn foals died from severe granulomatous pneumonia within a few days of birth; and a weaning thorough-bred developed granulomatous pneumonia and lymphadenitis at 5 months of age.

Chandel and Kher (1994) reported *H. capsulatum* from miliar necroses of the lungs in dromedary camel.

Gitao *et al.* (1998) reported *Dermatophilus congolensis* and *Microsporum gypseum* causing skin infections in camels of a dairy farm in Saudi Arabia.

Kushwaha and Guarro (2000) reported *Trichophyton verrucosum* as the major causative agent of ringworm in bovine, ovine and caprine. Other species such as *M. canis, M. gypseum, T. mentagrophytes* and *T. equinum* have been isolated from some of these ruminants.

Lopez *et al.* (2000) recorded a case of cutaneous mucormycosis in a Spanish Thoroughbred mare. Animal was infected with *Absidia corymbifera*.

Al-Ani *et al.* (2002) examined 375 calves and 316 horses by direct microscopic examination for ringworm, the prevalence of ringworms in calves and horses were 30.6% (115/375) and 18% (57/316), respectively. *Trichophyton* and *Microsporum* species were common important pathogens capable of causing dermatophytosis.

Al-Ani and Roberson (2005) reported that *Epidermophyton, Microsporum, Trichophyton, Sporotrichum, Candida, Cladosporium, Coccidiodes, Blastomyces dermatitidis, Histoplasma capsulatum* and *Aspergillus fumigatus* were responsible for causing various diseases in camelids. Fungi in the genera *Trichophyton* and *Sporotrichum* were most prevalent fungi in camelids in USA.

Ebrahimi *et al.* (2007) reported *Trichophyton verrucosum* and *Trichophyton tonsurans* from healthy skin coat of camels from Iran.

Wernery *et al.* (2007) reported *Candida albicans* from skin scrapings of very young camel calves. The physical condition of dromedary calves was affected; they were smaller and weighed less than calves in the same age group.

Chermette *et al.* (2008) isolated wide variety of dermatophytes from animals, but a few zoophilic species were responsible for the majority of the cases, viz. *Microsporum canis, Trichophyton*

mentagrophytes, Trichophyton equinum, Trichophyton verrucosum and the geophilic species-*Microsporum gypseum.*

Khosravi *et al.* (2009) reported that *Cladosporium* and *Candida* species were the most frequent fungal isolates obtained from eye and nose of healthy dromedary camels in Iran.

Wisal and Salim (2010) found young camels of less than 3 years were more susceptible to dermatophytes than adults. They examined 136 skin scraping samples for dermatophytes which comprises of 90 from Alshowak and 46 from Alobied. *Trichophyton verrucosum, Trichophyton mentagrophytes, Trichophyton schoenlenii* and *Trichophyton tonsurans* were isolated from 77, 47, 9 and 3 samples, respectively.

Tuteja *et al.* (2010a) reported *Candida albicans* as the main etiological agent causing skin infections of the camel calves of less than one year of age.

Tuteja *et al.* (2010b) reported *cutaneous alternariosis* in camel calves of approximately one year of age in Rajasthan state of India. The disease occurs more frequently in semi-arid than arid region of the state.

Colin *et al.* (2013) discussed the various fungal agents which are pathogenic to human and animals. He described morphology and microscopic character of different pathogenic fungi.

Tuteja *et al.* (2013a) isolated *Microsporum* and *Trichophyton species* from camel skin lesions including *M. audouinii, M. canis, M. nanum, M. ferrugineum, T. verrucosum, T. mentagrophytes, T. schoenleinii, T. equinum, T. concentricum, T. tonsurans, T. violaceum, T. soudanense* and *T. rubrum.* These fungi caused sporadic

cases of skin infections in individually maintained camels as well as in herd. These fungi create distinctive lesions of ring worm. The lesions of ring worm observed with *Trichophyton* spp. were comparatively dry, hard, crusty, granulomatous and larger in size.

Tuteja *et al.* (2013b) reported skin infection in camel, caused by soil-born species *Absidia corymbifera. A.corymbifera* often causes food spoilage and only species known to cause disease in man and animals.

Tuteja *et al.* (2013c) reported *Aspergillus* as a causative agent of opportunistic infection in camels. Among these *A. fumigatus* was the most commonly isolated species, followed by *A. flavus, A. niger*, *A. terreus*, and *A. versicolor.*

Tuteja *et al.* (2014a) isolated dimorphic fungi viz. *Sporothrix schenckii, Coccidioides immitis* and *Penicillium marneffei* which causing skin infections in camels.

Tuteja *et al.* (2014b) reported that primarily the human pathogenic fungi *Epidermophyton floccosum* and *Scopulariopsis brevicaulis* also cause skin infections in camel.

Abdulaziz *et al.* (2016) carried out study at Qassim Region, Central of Saudi Arabia, to study dermatophytosis in a private farm of dromedary camels. Mycological examination of clinically diseased camels revealed the presence of *Trichophyton verrucosum* among them.

Baghza *et al.* (2016) conducted study among 165 suspected camels in Dhamar area, Yemen. The infection was significantly higher among young animals of less than 12 months. The frequency of *Trichophyton* and *Microsporum* genera were 89.4 and 10.6 per cent of the isolated genera, respectively. The identified species were *T.*

schoenlenii T. verrucosum, T. mentagrophytes, T. tonsurans, M. audouinii and *M. canis*. Almost half of the animals were found infected with *T. schoenlenii.*

Brilhante *et al.* (2016) reported that coccidioidomycosis and histoplasmosis are systemic mycoses caused by the dimorphic fungi *Coccidioides spp.* and *Histoplasma capsulatum*, respectively, which affect humans and a variety of other animals, including equines.

Shokri and khosravi (2016) found *Trichophyton verrucosum in* camels suspected of having dermatomycoses in Iran.

Ganguly *et al.* (2017) isolated and identified *Trichophyton verrucosum* from a case of dermal mycoses in dromedary camel.

Dewal (2017) identified *T. rubrum, T. verrucosum, T. schoenleinii, M. Canis, Candida albicans, Clodophialophora bantiana, Absidia corymbifera, coccidioides immitis, Penicillium spp., Aspergillus terreus, A. niger, A. versicolor* and previously unidentified species *Histoplasma capsulatum* in camels by culture examination.

2.3 Hematology and serum biochemical

Hussein *et al.* (1992) described that hematology of growing camel(1-12 months) of age and also reported that lymphocytes count are highest at 1 month of age and fell progressively during 6 months and stabilized after and nutrophills were also high at the age of one months and decreased slowly with advancing age.

Mwanzia and Mung'athia, (1997) observed that blood hematology and biochemical values were within the range in free ranging wildlife in outbreak of dermatophytosis in Kenya.

Rotstein *et al.* (1999) reported that hematology and serum biochemistry analysis were found normal except for an elevated globulin fraction (8.6 mg/dl; range, 6.4-8.2 mg/dl) in free ranging panthers who suffered by dermatophytes infections in Florida.

Atakisi *et al.* (2006) examined liver function tests in dermatophytic cattle which showed increased enzymes activities in dermatophytic calves compared to those of healthy group. This increase was highly significant in the case of ADA, LDH, ALT, and AST. While dermatophytic calves showed positive results in microscopic examination, healthy ones were negative.

Fatma and Ismail (2008) reported an increase in gamma glutamyle transferase (GGT), Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) in 80 one humped camels suffering from skin disease in Sinai.

Mathur *et al.* (2011) found decrease in lymphocyte count and increase in hemoglobin, TLC, MCHC, neutrophil and eosinophil count in cases of dermatomycosis in camels.

Farroq *et al.* (2011) reported normal reference heamatological values of one humped camel (*Camel dromedarius*) kept in cholistan desert. The mean values for hemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC) for males were 12.00±0.63 g/dl, 37.21±2.48 %, 6.83±0.38 $X10^6$/µl, 12.38±0.97 $X10^3$/µl and for females were 11.34±0.95 g/dl, 32.83±3.76 %, 7.31±0.58 $X10^6$/µl, 12.97±0.99 $X10^3$/µl, respectively. The difference in values were statistically non significant for males and females.

Foutah *et al.* (2012) reported a significant reduction of erythrocytic count, haemoglobin content and packedcell volume, beside leukocytosis, neutrophilia, lymphocytosis and insignificant

decrease in monocytes, eosinophils and basophiles were present in camels infected with ringworm. He also concluded that ringworm in camels induce some adverse effect on haemato-biochemical parameters.

Sindha *et al.* (2015) reported neutrophil, Hb, PCV, and TEC significantly lower in cases of fungal skin infections in canines.

Nagarajan *et al.* (2016) observed that there is no any abrupt derivation in haemoglobin, PCV but differential cell count revealed mild elevation of eosinophils in Hariana breed of cattle in Tamilnadu which suffered from chronic mycotic dermatoses.

Dewal V.S. (2017) reported that decrease in RBC, Hb, and lymphocyte and increase in TLC and neutrophils in hematological parameter and also increases in AST and ALT in camel affected with fungal infection.

2.4 Skin surface pH examination and histopathology

Thakur and Verma (1984) reported a clinical case of ringworm caused by *Trichophyton rubrum* in calf. He observed marked hyperkeratosis in the skin along with mononuclear cells infiltration in retipegs and presence of fungal spores forming sheath around hair follicles.

Singh (1988) found hyperkeratosis, hyperplasia, disintegration, acanthosis of epidermis, spongiosis, leucocytic infiltration and presence of fungal hyphae in dermis in cattle-calves by histopathological examination.

Foutah *et al.* (2012) found hyperkeratosis and acanthosis of epidermis and aggregations of neutrophils and lymphocyte in dermal layer were observed in camel.

Al-Salihi *et al.* (2014) found acanthosis, hyperkeratosis and infiltration with eosinophils, lymphocytes, macrophages and neutrophils and large numbers of fungal arthrospores and hyphae were present in histopathlogy of affected skin scraping of fungal lesions.

Gil Yosipovitch and Howard I. Maibach (1996) found significance of skin pH as possible promoting host susceptibility to candidal skin infection and increase pH is importing contributing factor in dermatitis.

Hanaa *et al.* (2011) found hyperkeratosis, hyperplasia, fungal hyphae and acanthosis in epidermal layer in affected teat from fungal infection, mainly *C. albicance* and *A.niger* in cattle.

Lambers *et al.* (2006) reported that skin layer has pH of about 4.0-4.5and help to protect the skin both physical and chemically, it natural acidity inhibits the growth of harmful bacteria and fungus growth.

2.5 Serum minerals responsible for maintaining skin integrity in animals

Van den Broek and Stafford (1988) reported the concentrations of zinc in serum, leucocytes and hair of normal dogs. The dogs with zinc-responsive dermatosis and the dogs with dermatitis not associated with zinc deficiency. The mean concentration of zinc in serum and hair in dogs with zinc-responsive dermatosis was significantly lower than in other dogs but the range of zinc concentrations overlapped that of the other dogs. The mean leucocyte zinc concentration was similar for each group of dogs. It is concluded that low zinc concentrations in serum and hair have only a corroborative value in the diagnosis of zinc-responsive dermatosis in dogs.

Thoday (1989) reported that diet deficient in zinc cause the many skin diseases in dog including zinc-responsive dermatosis. The plasma zinc concentration below the mean of the reference range supports the diagnosis.

Al-Qudah *et al.* (1994) reported that dermatologic lesions in heifer caused by dermatophytosis are sometimes associated with selenium deficiency. There was no response observed to other treatments that had been used prior to the administration of selenium.

Krametter *et al.* (2005) reported in two cases of zinc deficiency in dairy goats from different flocks and not associated with a zinc-deficient diet. Hard, dry, hyperkeratotic skin, hair loss and pruritus especially prominent on the back, legs, udder, face and ears were the most common clinical signs. On initial examination, serum zinc concentrations were low in both goats. Although mild skin lesions persisted during the early stages of zinc supplementation, skin lesions completely resolved after prolonged oral zinc supplementation. Withdrawal of zinc supplementation resulted in re-appearance of lesions in both animals.

Nisbet *et al.* (2006) reported the importance of zinc in calves with trichophyton infection. Serum zinc levels were lower in diseased calves than healthy ones.

Faye *et al.* (2008) determined trace elements in 240 Arabian camels from Emirates. The values of copper and zinc in serum samples were found 60.1 µg/100 ml and 20.0 µg/100 ml, respectively.

Pasa and Kiral, (2009) observed that the serum zinc concentration were found to be significant lower in camel calves with dermatophytosis tan those of healthy controls. Results shown that serum zinc were altered in calves with dermatophytosis.

Kojouri *et al.* (2009) reported in a humid area of Iran on 35 healthy and 35 infected cows that serum concentration of selenium and zinc in cattle with dermatophytosis were significantly lower than the healthy ones. In conclusion, it seems that zinc and selenium have a determinant role in immune status and the response of animal's immunity system to dermatophytosis.

Al-Qudah *et al.* (2010) reported the levels of trace minerals Zn, Cu, and Se in calves with dermatophytosis. The levels of Zn, Cu and Se were found significantly low in diseased calves than healthy calves.

Kachhawaha *et al.* (2011) reported mineral status in 30 cattle-calves infected with *T. verrucosum.* Mean plasma concentrations of zinc, selenium and copper in ringworm infected calves were 0.81±0.08, 0.10±0.008 and 0.48±0.03 mg/litre, respectively. The corresponding values for healthy animals were 1.64±0.20, 0.72±0.006 and 0.82±0.04 mg/litre, respectively. Results showed that plasma concentration of zinc and selenium with dermatophytosis were significantly lower ($p \leq 0.05$) than the healthy ones. It seemed that zinc, selenium and copper have a determinant role in immune status and the response of animal immunity system to dermatophytosis.

Kuria *et al.* (2013) reported normal status of minerals in camels in north-eastern Kenya, which evaluated from the blood plasma. The average normal level of zink, copper, cobalt and selenium in blood plasma are 0.91, 0.82, 0.08 and 0.23, respectively in part per million.

2.5 Therapeutics of camel dermal mycoses

Ainsworth and Austwick (1973) advocated use of Captan as fungicidal. Captan is a fungicide and its use causes lot of irritation in the affected camels.

Sharma and Dwivedi (1990) prepaired a herbal preparation containing onion (*Allium cepa*), garlic (*A. sativum*) and lemon (*Citrus limon*) extracts and powders of turmeric (*Curcuma longa*) and camphor in Karanj oil (*Pongamia glabra* [*P. pinnata*]). They used that prepration to treat ringworm due to *Trichophyton verrucosum* in 12 cattle and *T. verrucosum* and *Microsporum canis* in 21 dogs. Daily application of the drug preparation resulted in complete cure within 12-15 days of treatment. There was no sign of toxicity in animals treated with the preparation.

Schwartz and Dioli (1992) used of griseofulvin in camels and reported to have side effects such as nausea and diarrhoea.

Sharma *et al.* (1993) diagnosed ringworm in 13 calves, which were treated by daily application of an aqueous extract of garlic (Allium sativum Linn.) in petroleum jelly (1:10), and observed that complete resolution of all lesions was achieved in 12-14 days. The treatment induced initial signs of local irritation, followed by an inflammatory reaction.

Augusti (1996) reported that Onion and garlic contain many sulfur containing active principles mainly in the form of cysteine derivatives, viz. S-alkyl cysteine sulfoxides which decompose into a variety of thiosulfinates and polysulfides by the action of an enzyme allinase on extraction. Decomposed products are volatile and present in the oils of onion and garlic. These possess antidiabetic, antibiotic, hypocholesterolaemic, fibrinolytic and various other biological actions.

Pattnaik *et al.* (1996) observed that five aromatic constituents of essential oils (cineole, citral, geraniol, linalool and menthol) were tested for antimicrobial activity against eighteen bacteria (including Gram-positive cocci and rods, and Gram-negative rods) and twelve fungi (three yeast-like and nine filamentous). Against fungi the citral and

geraniol oils were found the most effective (inhibiting all twelve fungi), followed by linalool (inhibiting ten fungi), cineole and menthol (each of which inhibited seven fungi) compounds.

Paterson, S. (1997) used a combination of miconazole and chlorhexidine, which applied topically twice weekly was proved to be efficient for the treatment of dermatophytosis in horses

Joshi *et al.* (1998) reported a cysteine protease inhibitor exhibiting antifungal activity from pearl millet seeds which have been purified to homogeneity by ammonium sulphate precipitation and chromatographic procedures involving CM- sephadex and SP-sepharose cation exchange columns. The inhibitor exhibits potent antifungal activity against *Trichoderma reesei,* a dead wood fungus with minimum inhibitory dose to inhibit mycelial growth or spore germination is as low as 1µg / ml (250 ng/disc). In addition to *Trichoderma reesei,* the antifungal activity is observed against some important phytopathogenic fungi, namely, *Claviceps, Helminthosporium, Curvularia, Alternaria* and *Fusarium species.*

Wernery and Kaaden (2002) reported various hit and trial methods being adopted for treatment of dermal mycoses in camel. A variety of common fungicidal and fungistatic agents such as iodine, 5% sulphur in sesame oil, 5% salicylic acid, coal tar phenols (3.25%) with copper acetate (0.58%) and hydroxyquinolines may be applied topically as ringworm ointments onto the affected areas. Drugs used have included thiabendazole, flucytosine, and amphotericin B, but very little is known about their effect in camels.

Singh *et al.* (2004) observed that GC and GC-MS analysis of volatile oil obtained from *Piper nigrum* L resulted in the identification of 49 components accounting for 99.39% of the total amount, and the major components were β-caryophyllene (24.24%), limonene (16.88%),

sabinene (13.01%), β-bisabolene (7.69%) and α-copaene (6.3%). The acetone extract of pepper showed the presence of 18 components accounting for 75.59% of the total amount. Piperine (33.53%), piperolein B (13.73%), piperamide (3.43%) and guineensine (3.23%) were the major components. The oil was found to be 100% effective in controlling the mycelial growth of Fusarium graminearum in inverted petriplate technique. The acetone extract retarded 100% mycelial growth of Penicillium viridcatum and Aspergillus ochraceus in food-poisoning technique. Volatile oil and acetone extract were identified as a better antioxidant for linseed oil, in comparison with butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT).

Hassan *et al.* (2004) reported that calves with ring worm (*Trichophyton verrucosum*) were employed to use the efficacy of herbal medical therapy by once rubbing Aloe Vera gel leaves topically daily on the affected skin lesions compared by traditional topically iodine ointment 10% twice application in a daily base for continuous twenty days. Three weeks treatment course and two weeks post- treatment, notable rapid, effective and curative result with subside of all skin lesions and return to the accepted clinical picture were recorded in Aloe vera gel leaves treated calves comparing by iodine ointment treated ones.

Al-Ani and Roberson, (2005) reported that Amphotericin-B is the main stay of therapy for serious fungal infections and remains the most broad-spectrum antifungal agent available, but it may causes nephrotoxicosis. Prolong use of Griseofulvin is also helpful but causes diarrhoea in camels.

Erturk, (2006) studied therapeutic efficacy of eleven ethanolic extracts from spices of *Melissa officinalis, Mentha piperita, Laurus nobilis, Rhus coriaria, Dianthus coryophyllum, Piper nigrum, Capsicum annum, Juniperus oxycedrus, Erica arborea, Colutea arborescens*, and

Cuminum cyminum collected from various regions of Turkey and local markets. These were assayed for the in vitro antibacterial activity against 3 Gram-positive and 2 Gram-negative bacteria, using agar dilution methods. In addition, their possible toxicity to *Candida albicans* and *Aspergillus niger* was determined, using both agar dilution and disc-diffusion methods.

Singh *et al.* (2006) studied the antifungal activities of coriander oil and its oleoresin against eight fungi using the inverted petri plate and food poison techniques. Using the inverted petri plate method, the essential oil was found to be highly active against *Curvularia palliscens*, *Fusarium oxysporum*, *Fusarium moniliforme* and *Aspergillus terreus*. In the case of the oleoresin, more than 50% mycelial zone inhibition was obtained for the fungi *Fusarium oxysporum, Aspergillus niger* and *Aspergillus terreus*. Using the food poison technique, the essential oil showed 100% inhibition on the growth of *A. terreus, A. niger, F. graminearum* and *F. oxysporum,* whereas its oleoresin showed weaker fungitoxic activity, exhibiting 100% inhibition on the growth of *F. oxysporum* only.

Chaieb *et al.* (2007) reported that many essential oils are known to possess an antioxidant activity and antifungal properties and therefore they potentially act as antimycotic agents. Essential oil of clove (*Eugenia caryophyllata*) was isolated by hydrodistillation. It is observed that clove oil shows powerful antifungal activity.

Antoine-Moussiaux *et al.* (2007) in Niger area found that cow's milk butter being very effective in dermatophytosis.

Newbury *et al.* (2007) suggested that lime sulphur may be used in dogs and cats in combination with griseofulvin or itraconazole, but it has an offensive odour and may stain light-coloured hair.

Jeung and Choi (2007) carried out study to investigate the potential of using plant oils derived from *Leptospermum petersonii* Bailey and *Syzygium aromaticum* L. Merr. Et Perry as natural antifungal agents. The antifungal effects of essential oils at dermatophytes *Microsporum canis*, *Trichophyton mentagrophytes, Trichophyton rubrum*, *Epidermophyton floccosum*, and *Microsporum gypseum* were evaluated using the agar diffusion method.

Tuteja *et al.* (2011) standardized treatment for skin infection other than skin candidiasis. They used Sulphur in mustard (*Brassica* spp.) oil (1:10), for topical application at alternate days for 1-2 week.

Tuteja *et al.* (2012) standardized treatment protocol for skin candidiasis by incorporating the ethno veterinary knowledge used by the farmers. Therapeutic potential of three formulations consisting of 2% potassium iodide; 6% sulphur in mustard oil; and 6% sulphur and 3% salicylic acid in mustard oil were evaluated topically in naturally occurring cases of skin candidiasis in camel calves. All the three treatments were found effective with almost similar application schedule but with variable duration of treatment. This is long term treatment schedule with a minimum of eight applications is effective only against skin candidiasis.

Bhikane *et al.* (2015) reported Vetomax spray- a polyherbal preparation to be effective in treatment of dermatomycosis in cattle.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present study was conducted as per following headings:

3.1 Animals

3.2 Screening criteria for camel to be included in the study

3.3 Direct microscopic examination

3.4 Collection of skin scrapings

3.5 Surface skin pH examination

3.6 Culture and microscopic examination

3.7 Storage of fungal isolates

3.8 Collection of skin biopsy samples

3.9 Histopathological examination

3.10 Collection of blood samples

3.11 Hematological examination

(i) Hemoglobin (Hb) estimation

(ii) Determination of packed cell volume (PCV)

(iii) Total leucocytes count (TLC)

(iv) Total erythrocyte count (TEC)

(v) Differential leucocytes count (DLC)

3.12 Serum Biochemical estimation

(i) Total serum protein

(ii) Alkaline phosphates (ALKP)

(iii) Serum aspartate aminotransferase (SGOT)

(iv) Serum albumin

(v) Serum alanine aminotransferase (SGPT)

(vi) Serum globulin

3.13 Estimation of serum minerals (Zn, Co, Cu, Se)

(i) Digestion of serum samples.

(ii) Estimation of digested serum minerals by Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES)

3.14 Treatment trial of camel dermal mycosis (at 0, 3rd 7th and 11th day).

3.15 Statistical analyses

3.1 Animals

A total of 40 camel calves approximately one year of age irrespective of sex and breed were taken for the study from the organized herd of Indian Council of Agriculture Research-National Research Centre on camel, Bikaner, Rajasthan. Out of which sixteen camel-calves were found positive for dermal mycoses based on skin scrapings examination in 10% KOH solution.

3.2 Screening criteria for camel calves to be included in the study:

The camel calves having small disc-shaped, dry, hard, crusty, granulomatous skin lesions present anywhere on the body along with symptoms of pruritis and alopecia were included in the study.

3.3 Direct microscopic examination:

It was performed by placing the scrapings on a glass slide with two or three drops of 10 % potassium hydroxide and placing a cover slip over it. The sample was warmed for five minutes over a flame and was then carefully examined microscopically for the presence of hyphae and/or arthroconidia (Fig.-4).

3.4 Collection of skin scraping for mycological examination:

From affected camel calves with clear skin lesions, skin scrapings were collected with a blunt scalpel, particularly from the advancing border. Most recent lesions were chosen for scraping. These samples were collected in sterile vials meant for sterile collection of the samples. Post treatment skin scrapping samples were

collected from the sites initially having active lesions and were processed for mycological culture (Fig.-2).

3.5 Surface skin pH examination:

Skin surface pH of the affected camel calves both pre and post treatment was done by shaving healthy skin over the neck with a shaving razor. Then a fine thin layer of distilled water was made over the skin with a very fine sprayer. Over this portion skin pH was recorded with the pH papers (Hi-Media laboratories, Bombay) (Fig.-1).

3.6 Culture and microscopic examination:

Samples were first mixed with Sabouraud's dextrose chloramphenicol broth and were incubated at 28° C for 24 hours. Then, these samples were inoculated onto Sabouraud's dextrose chloramphenicol agar (SDCA) plates and were incubated at 28°C for 3-4 weeks. In case the growth appeared to be of dimorphic fungi, another plate was subculture and incubated at 37°C for 2-weeks for confirming the yeast stage of the isolate. These plates were examined daily for the growth of the fungi. The resultant growth was examined for the colony morphology. Microscopic examination was carried out using either lacto phenol cotton blue or calcoflour white stains using wet mount method (Halley and Standard, 1973).

3.7 Storage of isolates:

All these isolates were stored on SDCA slants for four months at refrigerator temperature and then were again subcultured at four month intervals, specially those isolates where identification of the isolate could not be made immediately.

Fig.1: Skin surface pH estimation

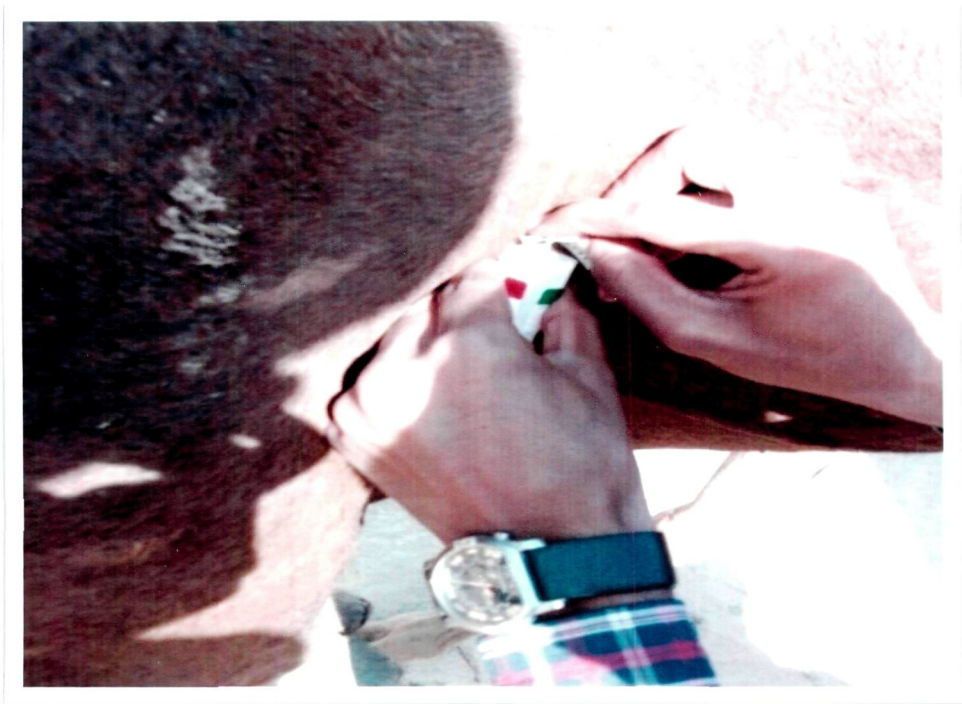

Fig.2: Skin scraping sample collection

3.8 Collection of skin biopsy samples:

From clear cut lesions biopsy samples were collected by biopsy punch designed for collecting animal skin samples (Fig.-3). Immediately these biopsy samples were stored in 10% formalin for histpathological examination. Post treatment biopsy samples were collected from the sites initially having active lesions.

3.9 Histopathological examination:

The biopsy skin tissue pieces were collected and preserved in 10% formol saline for histopathology. Prior to dehydration procedure the tissues were washed in running tap water for 4-6 hr. The sections were dehydrated in ascending grades of ethanol i.e. 70% (overnight), two changes each 80%, 90%, and 100% (1 hr), followed by 3 changes of xylene (1/2 hr each) and 2 changes of paraffin wax kept at 58°C (2 hr each). Finally the tissues were embedded in to paraffin blocks (overnight) and kept at 0°C until section cutting. Sections of 4-5 µm thickness were prepared and stained by routine haematoxylene and eosin (HE) staining protocol. Briefly, sections were kept in xylene (I and II) for 2 min each for clearing and rehydrated with descending grades of ethanol (100%, 90%, 80%, 70%) for 1 min each and in water for 2 min. These sections were stained with haematoxylene for 4-5 min and rinsed in water and acid alcohol (1 dip) to remove excess stain followed by 3-4 dips in ammonia water. Before commencing next step, sections were kept in water (1 min) and 95% alcohol (1 min) and then stained with eosin for 1 min. Sections were dehydrated using ascending grades of alcohol (70% to 100%) for 1 min each, finally cleared in xylene and mounted with DPX mounting medium.

Fig. 3: Skin biopsy sample collection

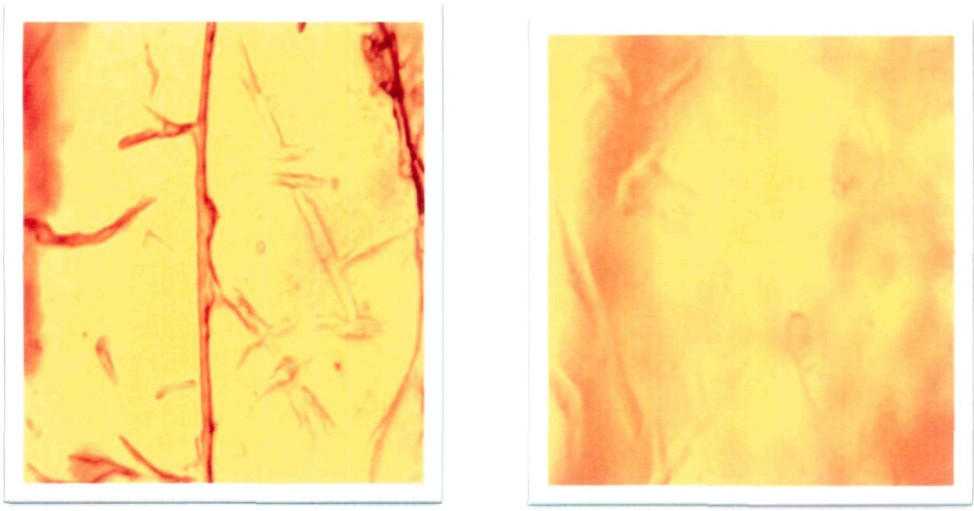

Fig. 4: Presence of fungal hyphae and arthocondia by microscopy examination (KOH mount)

3.10 Collection of blood samples:

For hematological examination blood samples from all these 16 cases of dermal mycoses were collected by jugular vein in sterile vacutainers having ethylene diamine tetra acetic acid (EDTA) disodium salt as an anticoagulant added at the rate of 1mg/ml of blood of as recommended by Jain (1986).

For biochemical studies, blood was simultaneously collected in another sterile vacutainers having no anticoagulant. These vacutainer tubes were kept in slanting position for one hour at 37^0C. Blood clots of these slants were broken and tubes were centrifuged at 3,000 rpm for 15 min. The serum was harvested in small Pyrex tubes and was stored in the deep freeze at -20^0C till analysis.

Blood samples were collected from all these cases on, 30^{th} and 60^{th} days of the start of the treatment.

3.11 Hematological examination:

For hematological examination blood samples were analysed for haemoglobin, packed cell volume, total erythrocyte count, total leucocyte count and differential leucocyte count as described by Jain (1986).

(i) Haemoglobin (Hb) estimation:

Hemoglobin was determined by Sahli-Hellige haemoglobinometer. Blood was drawn in Sahli's pipette up to 20 cubic millimeter mark. It was then transferred to haemoglobinometer tube containing 4-5 drops of 0.1N hydrochloric acid and mixed well. The tube was then kept for 5 minutes for the haemoglobin to change into acid haematin. The fluid was diluted with distilled water drop by drop and mixing after each drop until it matched to the colour of the standard comparison tubes. The

haemoglobinometer tube was read to give the amount of haemoglobin in g/dl of the blood.

(ii) Packed cell volume (PCV):

Packed cell volume estimation was made by microhaematocrit method. Non-heparinised capillary tubes were filled with blood up to three- fourth of total length. The blood adhered over the end of capillary tubes was wiped off with the help of a moist filter paper. The opposite ends of tubes were sealed over the spirit lamp by rotating between the thumb and the index finger for 2-3 seconds over the flame near its base. After perfect sealing of the end, the tubes were centrifuged for 5 minutes at 12,000 rpm in microhaematocrit centrifuge machine.

After centrifugation, packed cell volume was determined with the help of a special microhaematocrit reader scale. The bottom of the red column of capillary tube was adjusted with the zero line and the plasma level was matched with the hundred lines and top of red column excluding Buffy coat layer was read in per cent.

(iii) Total erythrocyte count (TEC):

The RBC pipette was filled up to 0.5 marks with the blood. The diluting fluid (Hayem's fluid) was drawn up to 101 marks. After shaking the pipette for three minutes, the fluid in its stem was discarded. The counting chambers of the haemocytometer were carefully charged with the diluted blood after placing cover slip. It was ensured that blood cells were evenly distributed over the counting chamber and overloading was avoided. The red blood corpuscles present in the four corner small squares and one small central square of the large central square were counted under high power of the microscope (40X).

Calculations

Numbers of red blood cells per cubic millimeter were calculated after multiplying the number of cells counted by 10,000 according to the following formula:

Total erythrocytes = Cells counted x 200 x 10 x 5 per cubic mm

Where:

200 stands for dilution

10 stands for depth in mm

5 stands for the $1/5^{th}$ of square millimeter counted

(iv) Total leucocytes count (TLC):

The WBC pipette was filled up to 0.5 marks with blood and the WBC diluting fluid was drawn up to 11 marks. After shaking the pipette for three minutes, the fluid in its stem was discarded. Counting chamber of the haemocytometer was carefully charged with diluted blood after placing cover slip. The cells were counted under low power objective of the microscope in the large four corner squares of the haemocytometer.

Calculations

The numbers of leucocytes in one cubic millimeter of blood were calculated by multiplying the total leucocytes counted by factor 50, according to the following formula:

Total leucocytes per cubic mm = 4 cells counted x 20 x 10

Where:

20 stands for dilution.

10 stands for depth in mm.

4 stands for the number of square millimeters counted

(v) Differential leucocytes count (DLC):

DLC was performed by staining the slides with Geimsa stain as follows (1).The dried blood film was flooded with methyl alcohol for 10

min.(2).The slides were washed gently with tap water to remove alcohol.(3). Fixed slide was stained in working dilution of Geimsa's stain (1:10) for 30 minutes.(4). The slide was washed with tap water and dried in air. (5). Different leukocytes were counted under oil immersion objective by using cedar wood oil.(6) Neutrophils, eosinophils, basophils, lymphocytes and monocytes were finally expressed into percentage.

3.12 Biochemical estimation:

Biochemical analysis of serum samples was made to ascertain liver function for by estimating serum total protein, alkaline phosphatase (ALKP), serum aspartate aminotransferase (SGOT), serum albumin, serum alanine aminotransferase (SGPT), serum globulin. These were determined by the Vet Test Chemistry Analyzer using kit supplied by Idexx laboratories. Procedure described for vet test chemistry analyzer were followed. Samples were kept at room temperature before feeding and were inserted as per manufacturer's manual in vet test analyzer using automatic pipettor, connected with the analyzer. All the necessary instructions given by analyzer were followed.

3.13 Estimation of serum minerals (Zn, Co, Cu, Se):

Serum minerals (Zn, Co, Cu and Se) were estimated by digesting the samples with triple acid mixture using standard procedures and the estimation was done by using ICP.

(i) Serum digestion procedure:

0.5 ml of serum sample was digested (by Kjeldahl method) with 10 ml of triple acid mixture (perchloric acid, nitric acid, hydrochloric acid in the ratio of 9:3:1 volume/ volume) in Kjeldahl tube for period of 4-5

hours till the solution became clear. Digested serum sample was transferred in to volumetric flask and volume was made 250 ml.

(ii) Mineral estimation by ICP

Estimation of minerals in digested serum samples was done by Inductively Coupled Plasma–Optical Emission Spectrometry (ICP-OES) as per the manufacturer's instructions (Thermo Scientific Ltd. USA). Instrument was allowed to warm up for 10-15 minutes and then the required sample details were feeded in the instrument and the diluted sample was subjected to estimation as per the described procedure by following instrument instructions (Fig.5).

3.14 Treatment trial of camel dermal mycosis:

Treatment of the dermal mycoses cases was done with herbal drug formulation developed by I.C.A.R.-National Research Centre on Camel (NRCC), Bikaner (Provisional Patent Filed). For smooth application for animal uses the formulation was diluted with clean tap water in the ratio of 1:1. (Fig. 6).

The herbal formulation was thoroughly mixed before application to the animals. After thoroughly cleaning the lesions with the scrotch pads the formulation was applied as per recommended schedule on 0 day, 3rd day and 7th day and in two severely infected cases on 11th day also.

3.15 Statistical analyses:

The data obtained in research work were statistically analyzed by paired t-test and compared as per the standard procedures suggested by Snedecor and Cochran (1994) and Perrie and Watson (1999).

Fig. 5: Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)

Fig. 6: Application of herbal formulation

RESULTS AND DISCUSSION

4. RESULTS & DISCUSSION

The present study entitled "Studies on diagnosis and therapeutics of dermal mycoses in camel calves" was conducted on forty camel-calves irrespective of sex and breed, approximately one year of age at organized herd of Indian Council of Agriculture Research–National Research Centre on Camel, Bikaner. Out of which sixteen camel-calves were found positive for dermal mycoses based on skin scrapings examination in 10% KOH solution.

All the fungal isolates were identified to species level to know the etiological agents responsible for causing dermal mycoses in camel-calves. Mineral status of copper (Cu), cobalt (Co), zinc (Zn) and selenium (Se) and haemato-biochemical parameters were assessed in camel-calves before and after treatment.

Therapeutic efficacy of the herbal formulation was judged by symptomatic, mycological and histopathological examination.

4.1 Clinical manifestations

In the present study clinical manifestations observed in camel-calves were alopecia, itching, thickening of skin and emaciation. Similar clinical manifestations were also reported in camel-calves by Tuteja *et al.* (2010a); (2012) and Wernery *et al.* (2007).

The lesions were dry, hard, rounded, nodular, small disk shape, crusty granulomatus lesions whitish to gray in colour. The lesions were most commonly found on legs, neck, axillaries, chest, abdomen, tail, head and lips (Fig. 7, 9, 11, 13 and 15). Similar findings were observed by Abdurahman and Bornstein (1991); Manefield and Tinson (1996); Tuteja *et al.* (2010b); Abdulaziz *et al.* (2016); Refai *et al.* (2016), Dewal (2017) and Ganguly *et al.* (2017). The causative agent of dermal mycoses in camel calves along with type of lesions, involvement of body parts and associated symptoms are presented in the Table-1.

Table-1: Isolation and identification of causative agents along with main clinical manifestations

S. No.	Fungal Genus	Fungal species	No. of animals	Gross morphology of the lesions	Body parts involved	Associated symptoms recorded	Severity of infection
1	*Microsporum*	*M. nanum*	2	Dry, hard, crusty, fast spreading lesions	Dorsum of the body, scapular region, abdomen and neck	Alopecia, itching and thickening of skin	Moderate, Severe
		M. audouinii	3				Severe
		M. gypseum	1				Moderate
		M. ferrugineum	1				Moderate
2	*Blastomyces*	*B. dematitidis*	2	Abundant patchy ulcerative lesions	Dorsum of the body	Alopecia, itching	Severe
3	*Basidobolous*	*B. ranarum*	1	Round disc-shaped scaly lesions	Dorsal part of body around hump	Alopecia , itching	Moderate
4	*Candida*	*C. albicans*	1	Whitish shallow lesions	Abdominal and thigh regions	Alopecia , itching	Moderate
5	*Histoplasma*	*H. capsulatum*	1	Small nodular lesions, whitish to gray in colour	Dorsum of the body	Alopecia, itching and emaciation	Moderate
6	*Absidia*	*A. corymbifera*	1	Very fast spreading white shallow lesions	Whole body	Alopecia, itching and emaciation	Severe
7	*Aspergillus*	*A. niger*	*1*	Large hard blackish granulomatous lesion	Dorsum of the body , scapular region and legs	Alopecia, itching	Severe
8	*Trichophyton*	*T. verrocosum*	1	Large grayish white granulamatous lesion	Dorsal part of scapular region , abdomen and hump region	Alopecia, itching	Severe
		T. rubrum	1				Severe

Fig.7: Lesions on abdomen and scapular region before treatment

Fig .8: Recovery in camel-calf after treatment

Fig. 9: Lesions on hump and abdomen

Fig. 10: Recovery in camel-calf after treatment

Fig.11: Lesions on abdomen and scapular region before treatment

Fig.12: Recovery in camel-calf after treatment

Fig.13: White shallow lesions on whole body before treatment

Fig.14: Recovery in camel-calf after treatment.

Fig.15: Grayish granulomatous lesions on abdomen and scapular region before treatment

Fig.16: Recovery in camel-calf after treatment

4.2 Isolation and identification of fungal agents by cultural and microscopic examination:

Fungal agents isolated and identified from dermal mycoses affected camel-calves are presented in Table-2 and Fig. 17.

Table-2: Relative frequency of different fungal pathogens of 16 dermal mycoses Infected camel calves

S. No.	Fungal organism	No. of Isolates	Per cent age
1	*Microsporum*	7	43.75
2	*Trichophyton*	2	12.50
3	*Blastomyces*	2	12.50
4	*Candida*	1	6.25
5	*Basidobolous*	1	6.25
6	*Histoplasma*	1	6.25
7	*Absidia*	1	6.25
8	*Aspergillus*	1	6.25
	Total	16	100

In the present study relative frequency of various genera of the fungi identified were *Microsporam spp.* (43.75%), *Trichophyton* and *Blastomyces spp.* (12.5% each), *Candida, Basidobolus, Histoplasma, Absidia* and *Aspergillus spp.* (6.25% each). *Blastomyces, Microsporum* and *Trichophyton spp.* were the most frequent isolates.

Dermatophytes are keratinophylic (keratin digesting) fungi which are common inhabitants of the soil, where they possess the hairs and skin cells shed by animals, as well as all types of keratin products that fall from animals and humans during the natural and continuous cycle of skin and coat shedding. The group of keratinophylic fungi is very large, but only three genera *Microsporum*, *Trichophyton* and *Epidermophyton* are known to cause dermatophytosis and infect the keratinized tissues; hair, skin and nails in all the domestic animals

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worldwide (Aly *et al.* (2000); Aman *et al.* (2001); Elewski, (2000); Nweze, (2001); Roldan *et al.* (2000); Rubio-Calvo *et al.* (2001). *Microsporum and Trichophyton* are most frequently found in animals while the *Epidermatophyton* causes problems mainly in humans (Lewis *et al.* (1991). The particular ability of these fungi to be transmissible to animals, as well as to humans, signifies that they are important veterinary and human pathogens worldwide (Chretien and Garagusi, 1990).

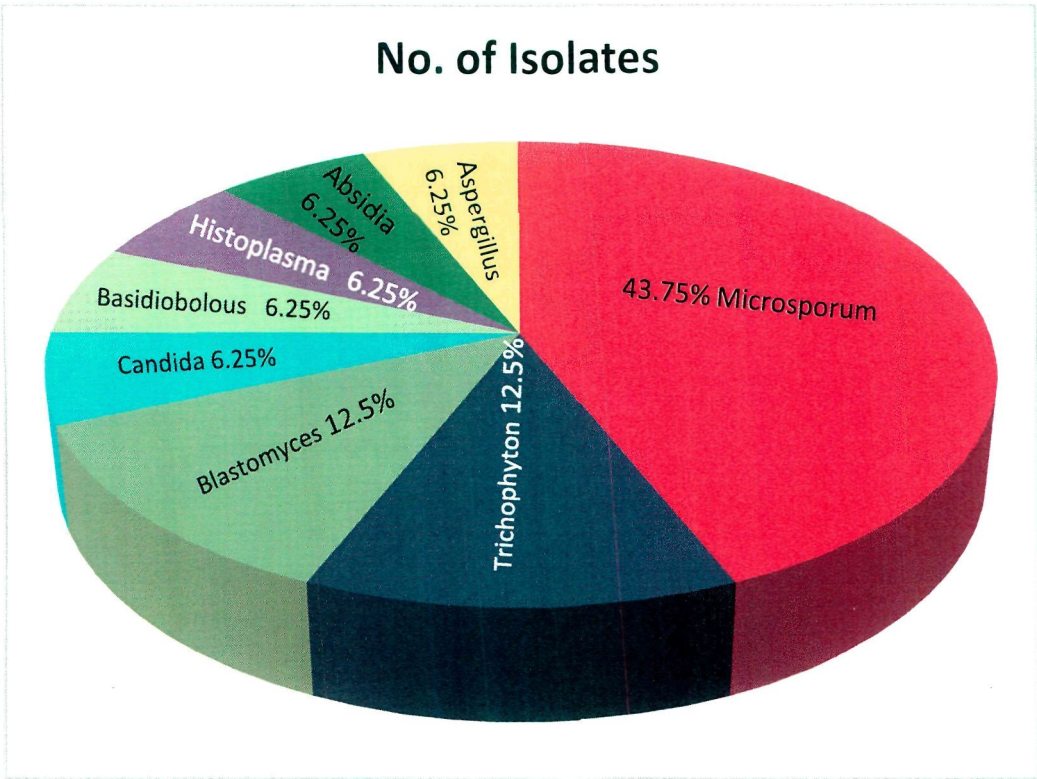

Fig.17: Pie diagram showing relative frequency of different fungal pathogens

1. *Microsporum*:

In the present study, four species of *Microsporum* were observed namely *M. audouinii, M. nanum, M. ferrugineum, M. gypseum.* The lesions were found on dorsum of the body, scapular regions, abdomen and neck of body area. Most of the *Microsporum* spp. is widely distributed in the world while some have restricted geographic distribution. *Microsporum* has the ability to degrade keratin and thus can reside on skin and its appendages and remains non-invasive. The keratinases, proteinases and elastases of the fungus may act as virulence factors. Lesions are small disc-shaped markings and can occur anywhere on the body. Later, the markings develop crusts, which can ooze and may turn bloody on scratching. Hair above the area of the lesion will fall out, due to damage caused by the fungal colonization.

Microsporum colonies are glabrous, downy, woolly or powdery. The growth on SDCA at 28°C may be slow or rapid and the diameter and colour of the colony varies depending upon the species. *Microsporum* produce septate hyphae, micro conidia and macro conidia. Conidiophores are hyphae like. Colony morphology along with colour of the colony and microscopy help in species differentiation.

(i) *M. audouinii*:

In the present investigation *M. audouinii* were isolated in three camel-calf and colonies on SDCA were flat, spreading, grayish-white to light tan-white in colour and have a dense suede-like to downy surface suggestive of mouse fur in texture while in reverse view of plate they were yellow-brown to reddish-brown in colour(Fig.18). Some strains did not show reverse pigment. Most cultures were sterile or produce only occasional thick-walled terminal or intercalary chlamydoconidia(Fig.19). Similar findings were also reported by Tuteja *et al.* (2013a) and Baghza *et al.* (2016).

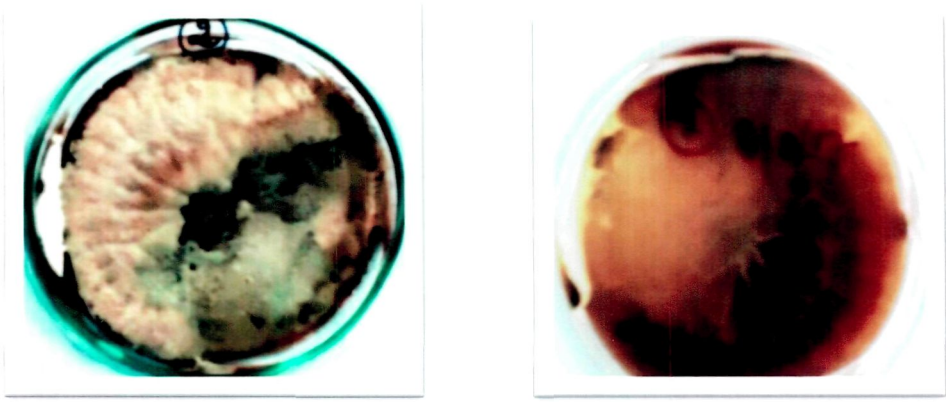

Fig.18: Morphology of colony of *M. audouinii* in front and reverse view of plate

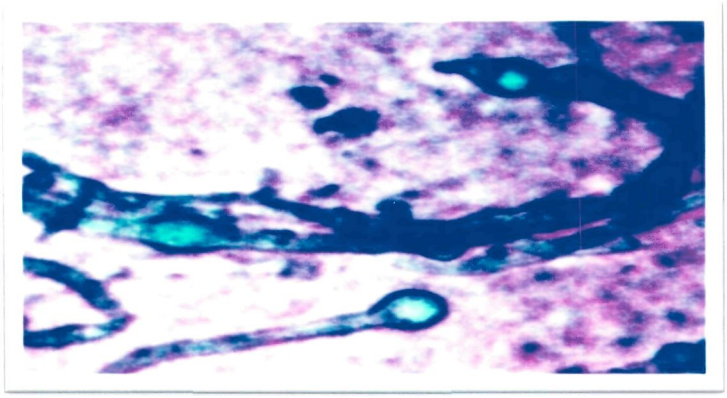

Fig.19: Microphotograph showing intercalary chlamydoconidium

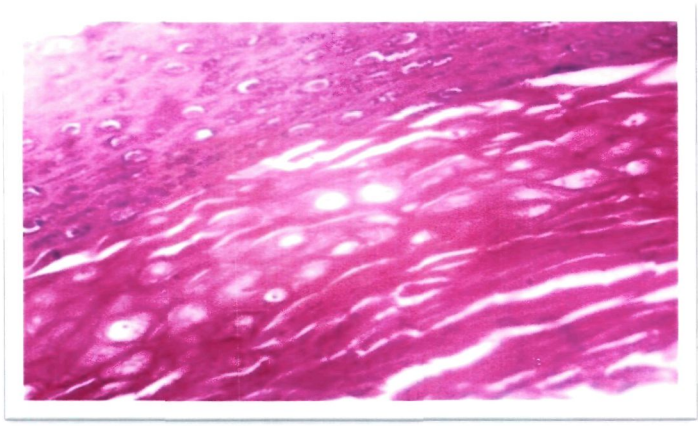

Fig. 20: Hyperkeratosis and acanthosis of epidermis by histopathology (H&E 400X)

ii) *M. nanum*:

In the present investigation *M. nanum* were isolated in two camel-calves and colonies of this species grow moderately on SDCA, colony was powdery, cottony, thin, spreading, velvety or flat and often has some radial, shallow furrows. The colour was white to dark beige in the front and reddish brown from the reverse view of plate (Fig.21). Microscopic examination of fungal culture showed septate hyphae, macroconidia (Fig.22) and microconidia. Similar findings were also reported by Tuteja *et al.* (2013a).

iii) *M. ferrugineum*:

In the present investigation, *M. ferrugineum* was found in one camel calf and colonies on SDCA were slow growing, forming a waxy, glabrous, convoluted thallus with a cream to buff colored surface in front and reverse view of plate (Fig.24). Microscopic irregular branching hyphae with prominent cross walls 'bamboo shaped hyphae' (Fig.25) which is characteristic of this species and chlamydoconidia were also seen. *M. ferrugineum* is a rare isolate in India. Sahai and Mishra (2011) reported M. *ferrugineum* and *M. audouinii* from human patients from central India.

iv) *M. gypseum*:

In the present investigation *M.gypseum* was found in one camel calf and colonies on SDCA were grown moderate rapidly and darken from buff to cinnamon brown, often with granular to sugary texture imparted by heavy sporulation (Fig.27). Microscopic examination showed numerous macroconidia(Fig.28) which were less barrel-shaped with rounded tips and had 6 or fewer cells. Similar findings were also reported in camels by Boever and Rush, (1975); El-Kader, (1985); Mancianti *et al.,* (1988) and Gitao *et al.* (1998). Singal *et al*. (2001) reported *M. audouinii* and *M. gypseum* from *Tinea capitis* cases from North India.

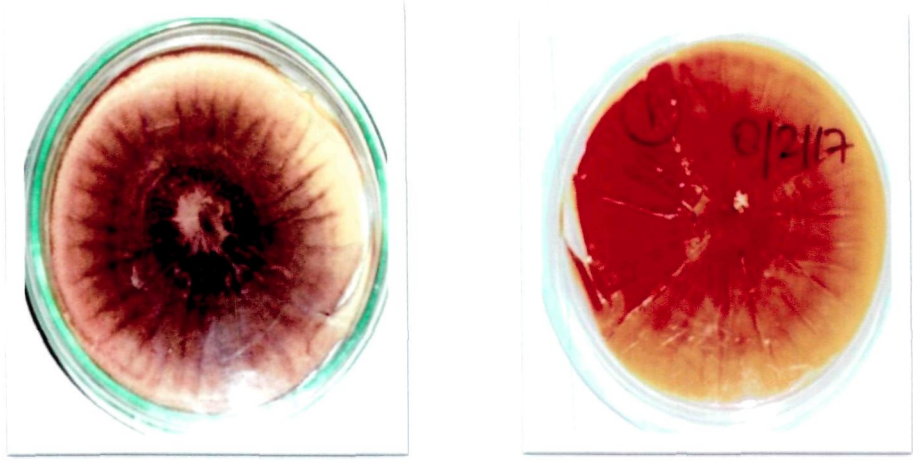

Fig. 21: Morphology of colony of *Microsporum nanum* in front and reverse view of plate

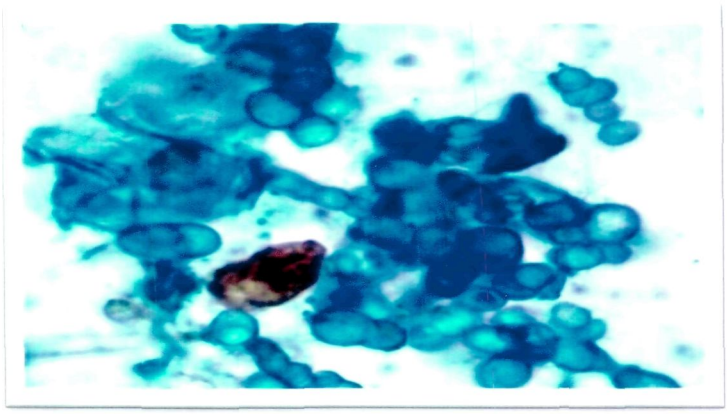

Fig. 22: Microphotograph showing macroconidium

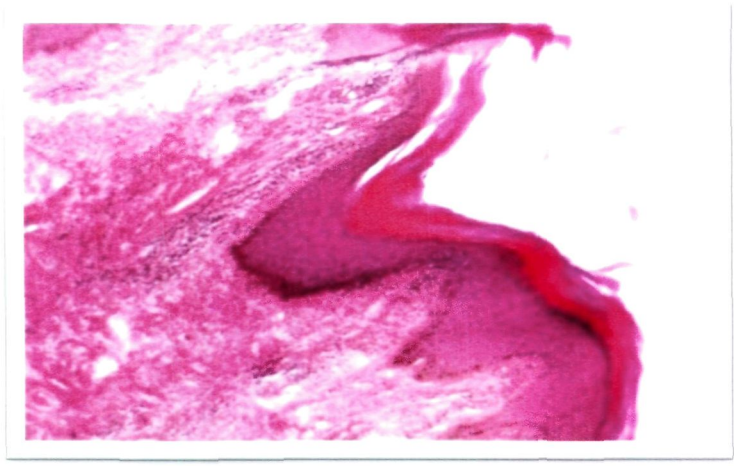

Fig. 23: Hyperkeratosis and acanthosis of epidermis by histopathology (H&E 100X)

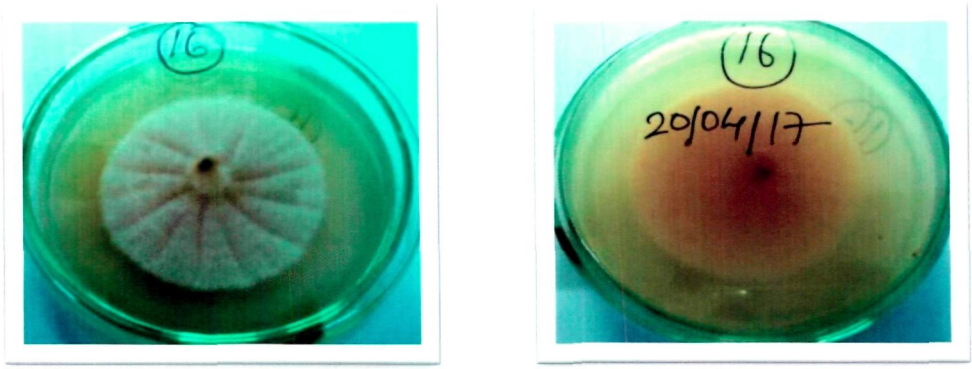

Fig.24: Morphology of colony of *Microsporum ferrugineum* in front and reverse view of plate

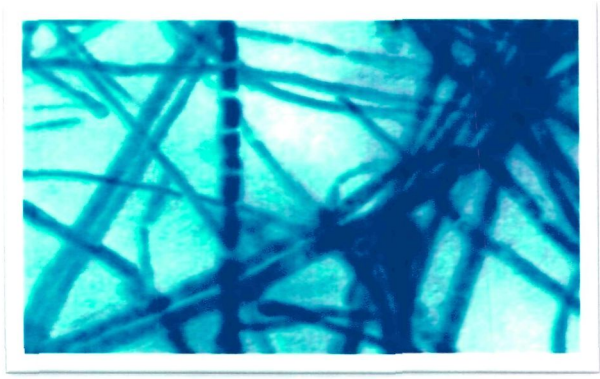

Fig.25: Microphotograph showing bamboo shaped hyphae

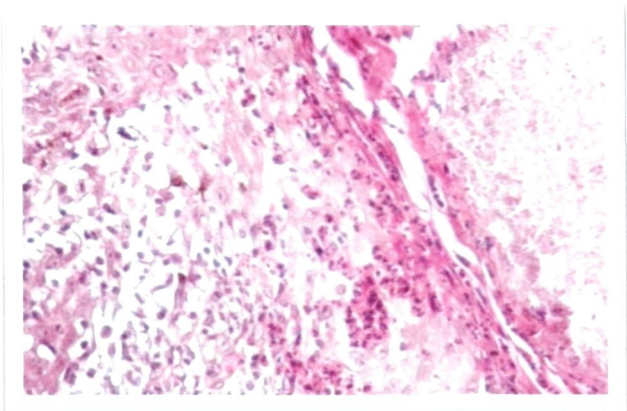

Fig.26: Eosinophilic and mononuclear cellular infiltration in dermal layer by histopathology (H&E 400X)

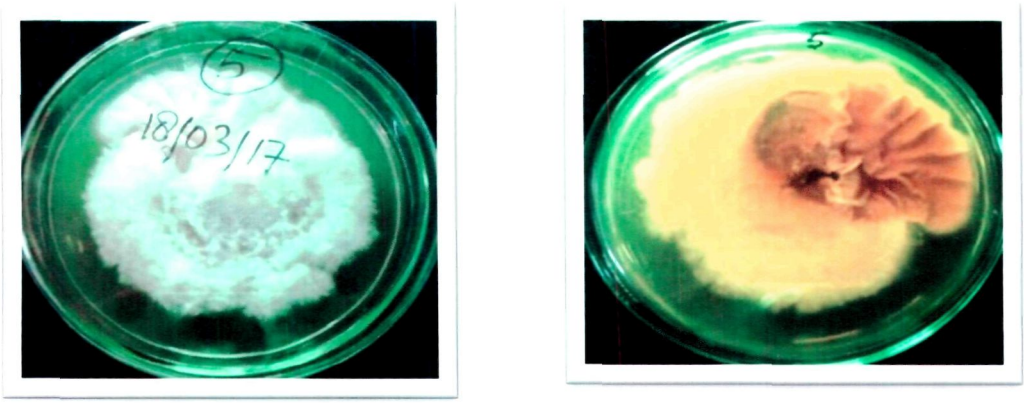

Fig. 27: Morphology of colony of *Microsporum gypseum* in front and reverse view of plate

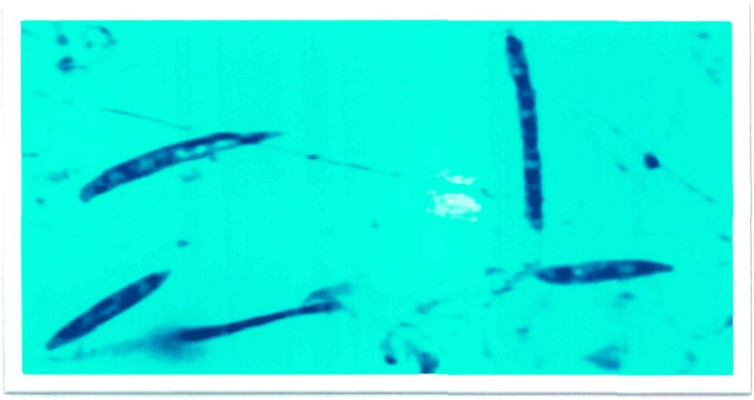

Fig. 28: Microphotograph showing macroconidia

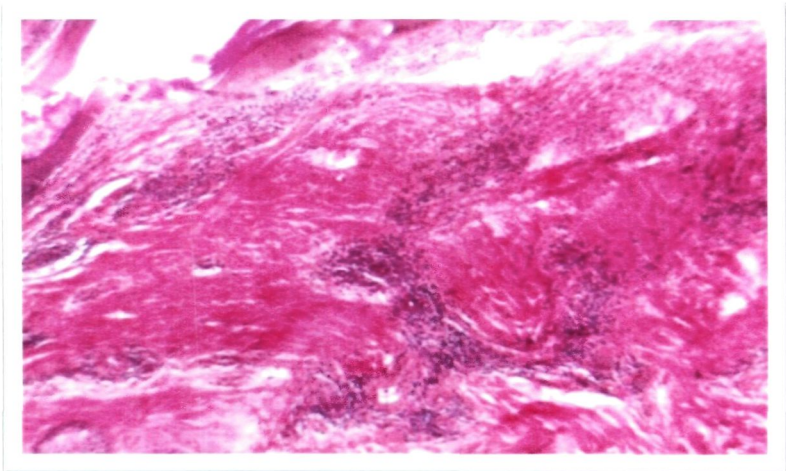

Fig. 29: Mononuclear cellular infiltration along with fibrous tissue proliferation in dermal layer by histopathology (H&E 100X)

2. *Trichophyton:*.

In the present study, two species of *Trichophyton* namely *T. verrucosum* and *T. rubrum* were isolated and identified. The lesions were large grayish white granulomatous at dorsal part of scapular, abdomen and hump regions. Trichophyton has the ability to invade keratinized tissues and possess several enzymes such as acid proteinases, elastase, keratinases and other proteinases which act as virulence factors (Weitzman and Summerbell, 1995).

(i) *T. verrucosum:*

In the present investigation, *T. verrucosun* was isolated from one camel calf. On SDCA, colonies were small, button-or-disk-shaped, white to cream colored, with a suede-like to velvety surface, a raised centre with some submerged growth in front view of plate. In reverse view, pigment varies from non-pigmented to yellow (Fig.30). Microscopic examination of fungal culture showed broad, irregular hyphae with many terminal and intercalary chlamydospores. The tips of some hyphae were broad and club-shaped and occasionally divided (Fig.31). Macroconidia were only rarely produced, but some present, were characteristic tail or string bean shaped. Similar finding were also reported by Colin *et al.*, (2013); El-Kader, (1985); Foutah *et al.* (2012); Tuteja *et al.*, (2013a); Baghza *et al.* (2016) , Dewal (2017) and Ganguly *et al.* (2017).

(ii) *T. rubrum:*

In the present investigation, *T. rubrum* was isolated from one camel-calf. On SDCA, growth rate was slow; cultures showed a violet to red-violet glabrous surface with radial furrows and a deep violet to red-violet on reverse view of plate (Fig.33). Microscopic examination revealed few pyriform lateral microconidia, pencil shaped macro conidia (Fig.34), arthroconidia produced from hyphae. *T. rubrum* were also isolated from camel by Tuteja *et al.* (2013a), from cattle by Singh and Singh, (1970); Chatterjee *et al.* (1978); Sharma *et al.*(1979); Sarkar *et al.* (1985); Mitra, (1988) and from dog by Yamada *et al.* (1991) and Ranganathan *et al.* (1998).

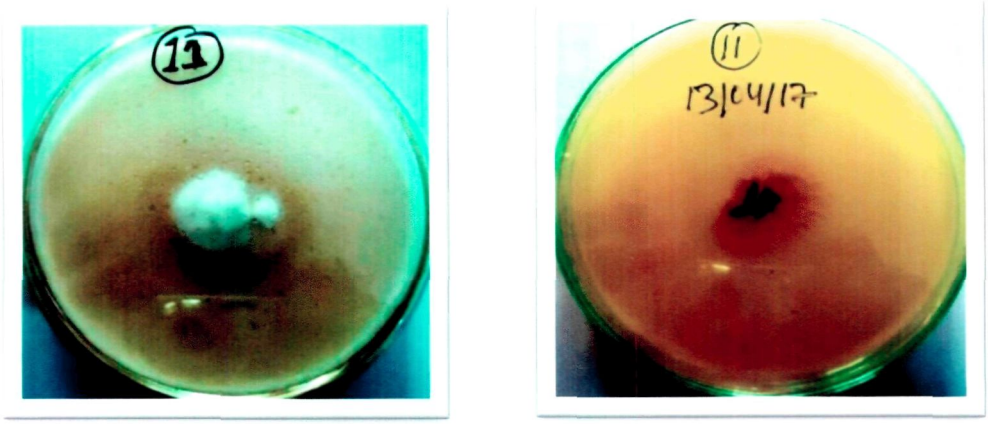

Fig. 30: Morphology of colony of *Trichophyton verrucosum* in front and reverse view of plate

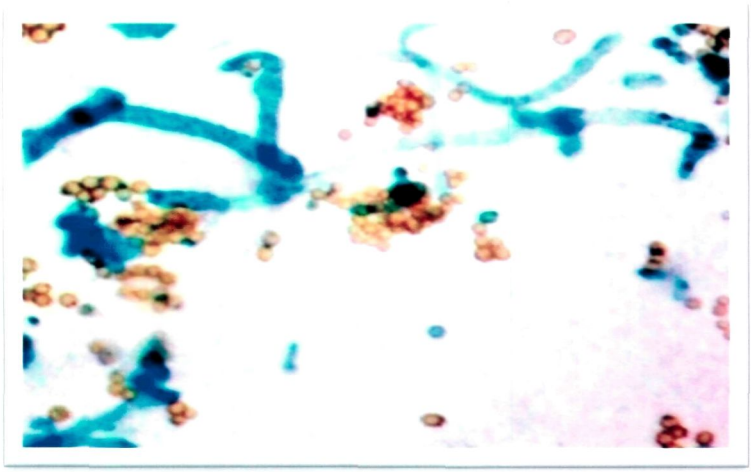

Fig. 31: Microphotograph showing broad and club shaped hyphae

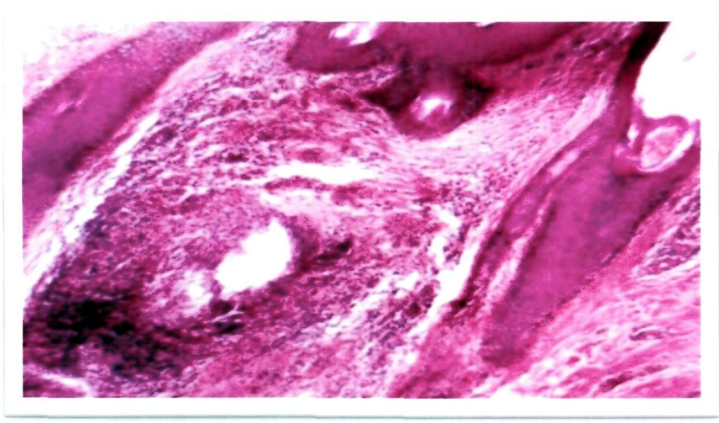

Fig. 32: Hyperplasia of epidermis and mononuclear cellular infiltration in dermal layer by histopathology (H&E 400X)

Fig. 33: Morphology of colony of *Trichophyton rubrum* in front and reverse view of plate

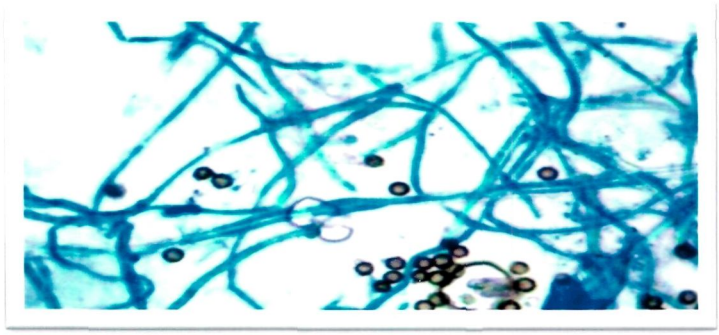

Fig. 34: Microphotograph showing pencil shaped macroconidium

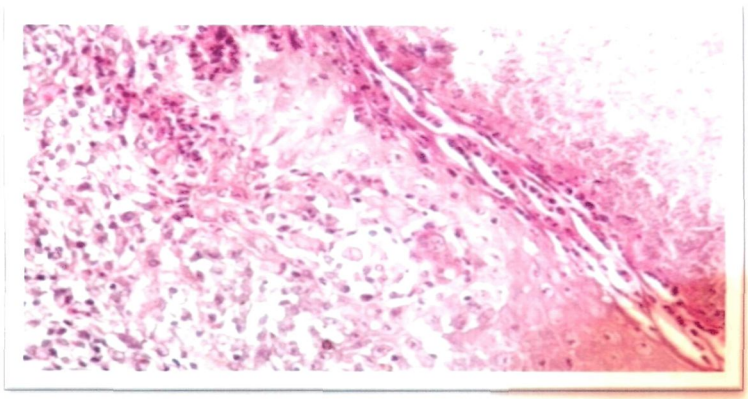

Fig. 35: Mononuclear cellular infiltration and fungal colonies in dermal layer by histopathology (H&E 400X)

Survey of ring worm in camels showed that over 25% of young animals suffered from *T. verrucosum* infection and less than 0.5% had *T. mentagrophytes* (Kuttin *et al,* 1986). *T. verrucosum* was the primary causal agent in young camels and *T. mentagrophytes* was in older camels (Mahmoud, 1993). The peak incidence of disease was autumn and winter, with incidence highest in young growing calves (1-2 years). Lesions were observed primarily on head, neck and shoulder with frequent extension to the flanks and limbs. *T. verrucosum* was isolated as the primary causal organism (Fadlelmula *et al,* 1994). Ebrahimi *et al* (2007) reported *T. verrucosum* and *T. tonsurans* from healthy skin coat of camels from Iran. *T. verrucosum* and *T. mentagrophytes* var. *mentagrophytes* was the common cause of dermatophytosis in alpacas and llamas. Spores of *T. verrucosum* and *T. mentagrophytes* may remain viable for up to 4.5 years in hair and cellular debris scraped off, the animal and left attached to barn walls, fence posts, trees and other fixtures, blankets, leads, grooming apparatus *etc.* (Murray, 1998).

3. *Blastomyces dematitidis:*

In the present investigation, *B. dematitidis* was isolated from two cases. The lesions were abundant patchy and ulcerative found on dorsum of the body. Morphological examination of colony on SDCA revealed membranous and downy to woolly appearance (Fig.36). The surface color was white to beige. It was grown as mold in the environment and yeast in tissues. Microscopic examination revealed ovoid to pyriform, one-celled, smooth-walled conidia borne on short lateral or terminal hyphal branches along with unipolar budding yeast-like cells (Fig.37). Imai *et al.* (2014) reported disseminated *B. dermatitidis* infection in an alpacas (*Vicugna pacos*). Khan *et al* (1982) and Richards (2015) reported *Blastomyces* infection in four camels in mid western USA.

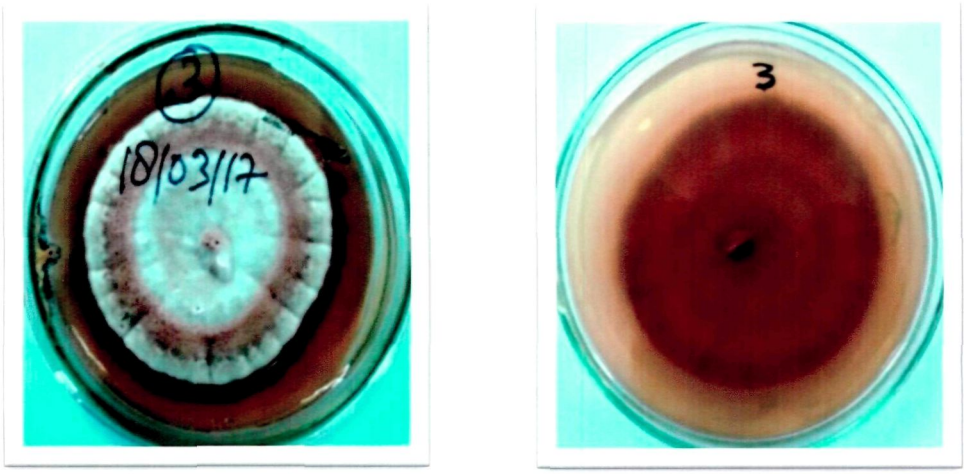

Fig. 36: Morphology of colony of *Blastomyces dermatitidis* in front and reverse view of plate

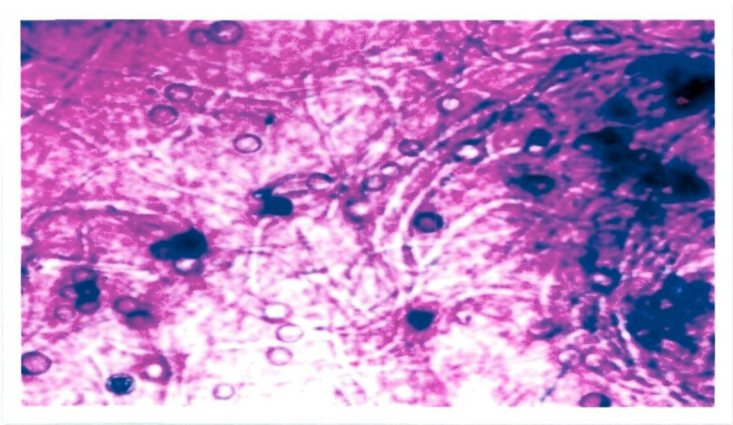

Fig. 37: Microphotograph showing ovoid, terminal hyphae along with unipolar yeast cells

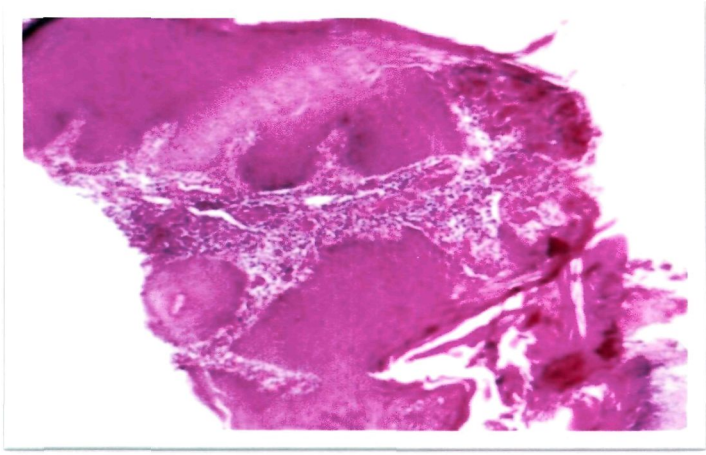

Fig. 38: Hyperplasia of epidermis in dermal layer by histopathology (H&E 400X)

4. *Candida albicans:*

In the present investigation, *C. albicans* was isolated from one case. The lesions were whitish shallow, found on abdomen and thigh regions. Morphological examination of colony on SDCA revealed glistening and smooth with white cream to brown color colonies in front view of plate (Fig39). Microscopically germ tube formation was observed (Fig40). Morphological character of colony and microscopic finding are similar to *C. albicans* as described by Colin *et al.* (2013). Similar findings were also reported by Wernery *et al.* (2007); Tuteja *et al.* (2010a); (2012).

5. *Basidiobolus ranarum:*

In the present investigation, *B. ranarum* was isolated from one case. The lesions were round disc- shaped scaly, found on dorsal part of body and around hump regions. Morphological examination of colony on SDCA revealed moderately fast growing, flat, yellowish-grey to creamy-grey, glabrous colonies becoming radially folded and covered by a fine, powdery(Fig.42), white surface mycelium. Satellite colonies were formed by germinating conidia ejected from the primary colony. Microscopic examination showed the presence of large vegetative hyphae forming numerous rounds, smooth, thick-walled zygospores that have two closely apprised beak-like appendages (Fig.43). Spores were globosely, one-celled, solitary and forcibly discharged from a sporophore. The sporophore had a distinct swollen area just below the spore that actively participates in the discharge of the spore. Similar findings were also reported in horses by Connole, (1973); Miller and Pott, (1980); Miller and Campbell, (1984); Owens *et al,* (1985); Speare and Thomas, (1985) and in dogs by Miller and Turnwald, (1984); Greene *et al,* (2002) and Grooters, (2003).

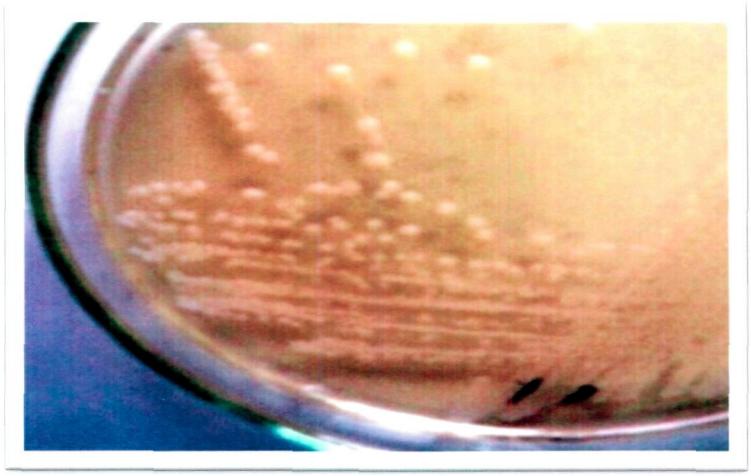

Fig. 39: Morphology of colony of *Candida albicans* in front view of plate

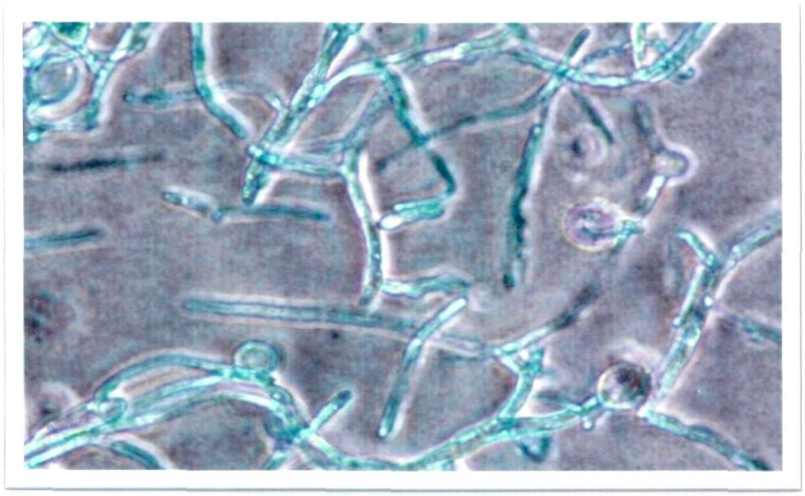

Fig. 40: Microphotograph showing germ tube formation

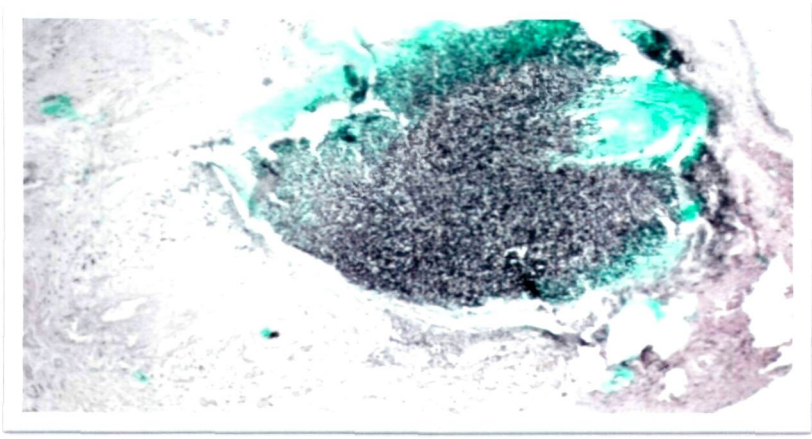

Fig. 41: Black fungal colony in dermal layer by histopathology (H&E 100X)

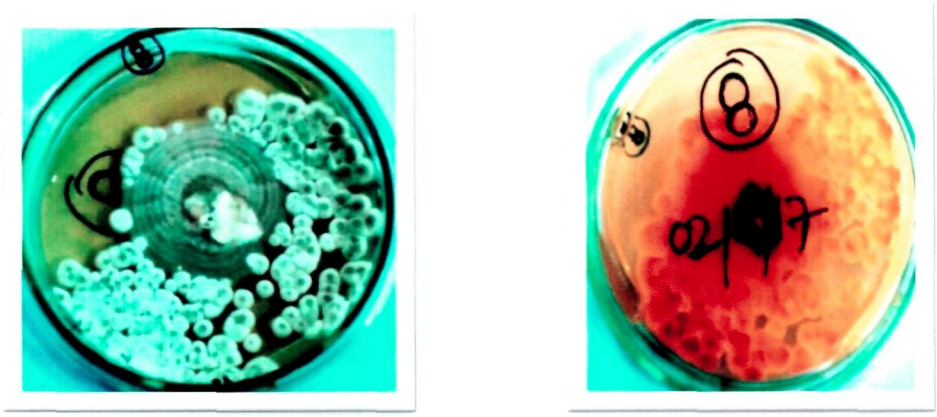

Fig. 42: Morphology of colony of *Basidiobolus ranarum in* front and reverse view of plate

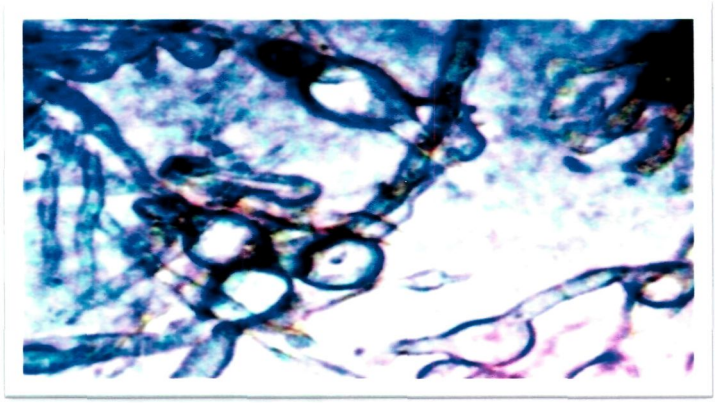

Fig. 43: Microphotograph showing wide septate hyphae with asexual spores

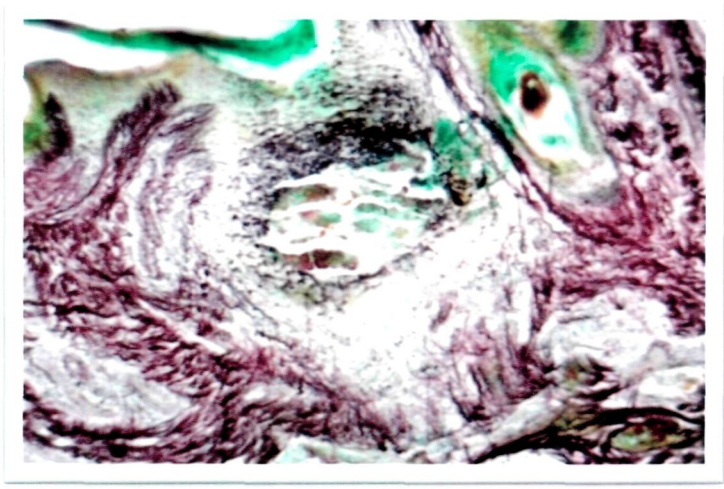

Fig. 44: Black fungal colony with eosinophilic infiltration in dermal layer by histopathology (H&E 100X)

6. *Histoplasma capsulatum*.

In the present investigation, *H. capsulatum* was isolated from one camel-calf. The lesions were whitish to gray in colour and small nodular, found on dorsal part of body. Morphological examination of colony on SDCA revealed flat, spreading, irregular white or buff-brown, suede-like to cottony colonies in front view of plate and pale yellow-brown in reverse view of plate (Fig.45). Microscopic examination showed the presence of characteristic large, rounded, single-celled and tuberculate macroconidia (Fig.46). microconidia were small, round to pyriform, borne on short branches or directly on the sides of the hyphae. Morphological character of colony and microscopic finding were similar to Colin *et al*. (2013). *H. capsulatum* had been reported to cause miliar necroses of the lungs in dromedary camel (Chandel and Kher, 1994).

7. *Absidia corymbifera:*

In the present investigation, *A. corymbifera* was isolated from one camel-calf. The lesions were very fast spreading white shallow found on whole body. Macroscopic examination of colony on SDCA revealed flat, woolly to cottony and olive grey colony in front view of plate, which were mature within 4 days (Fig.48). Microscopic examination of fungal culture showed wide non septate hyphae. The sporangiophores were branched and arise in groups of 2-5 at the internodes. Sporangiophores carried pyriform, relatively small sporangia (Fig.49). Morphological character of colony and microscopic finding are similar to Colin *et al*. (2013) and Tuteja *et al*. (2013c) as described in camels and by Lopez *et al*. (2000) in horses.

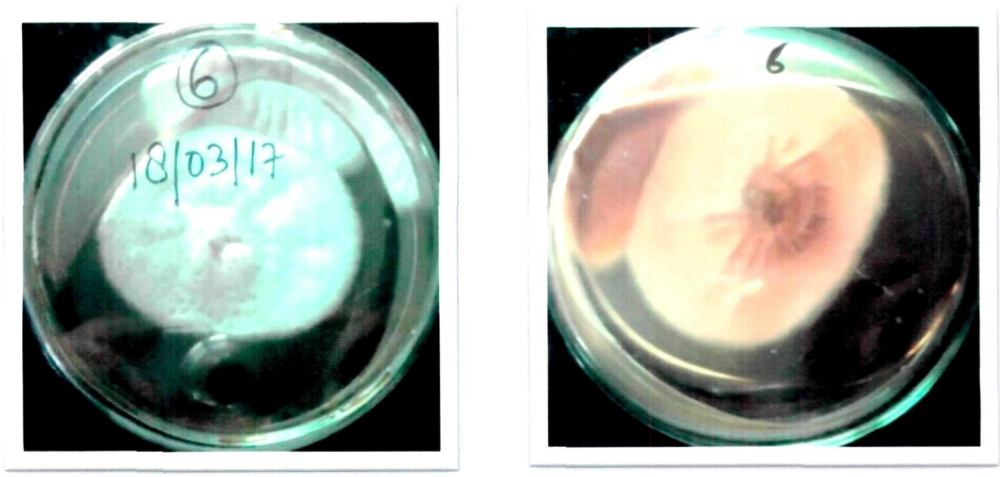

Fig. 45: Morphology of colony of *Histoplasma capsulatum* in front and reverse view of plate

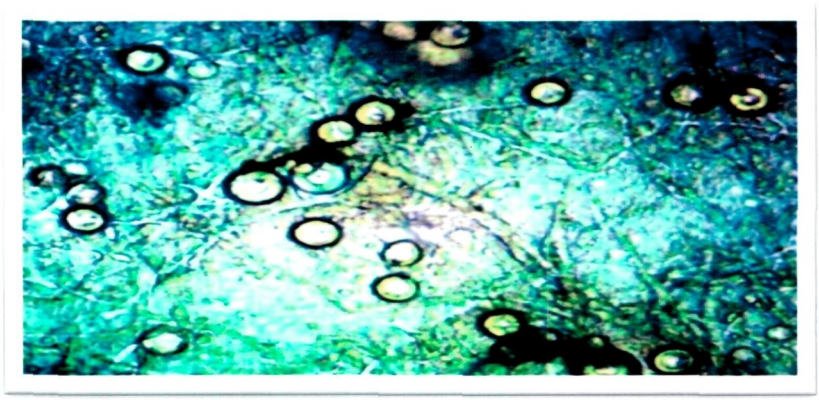

Fig. 46: Microphotograph showing globose shaped macroconidia

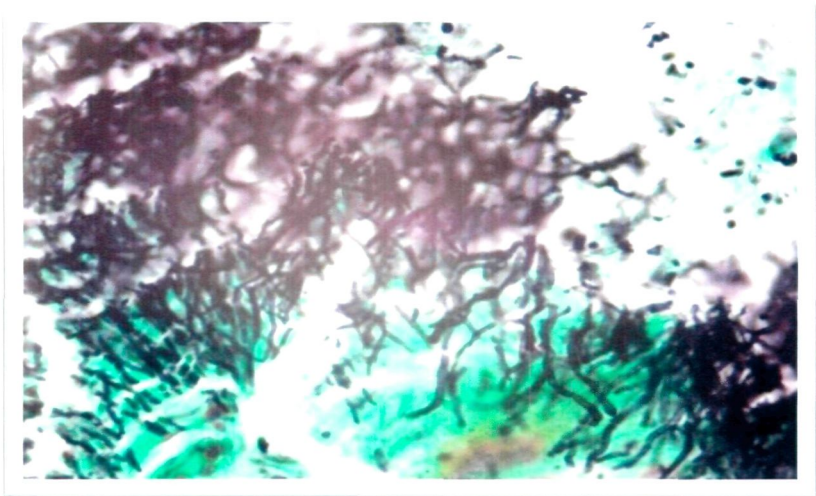

Fig. 47: Black fungal septate hyphae in epidermal layer by histopathology (H&E 100X)

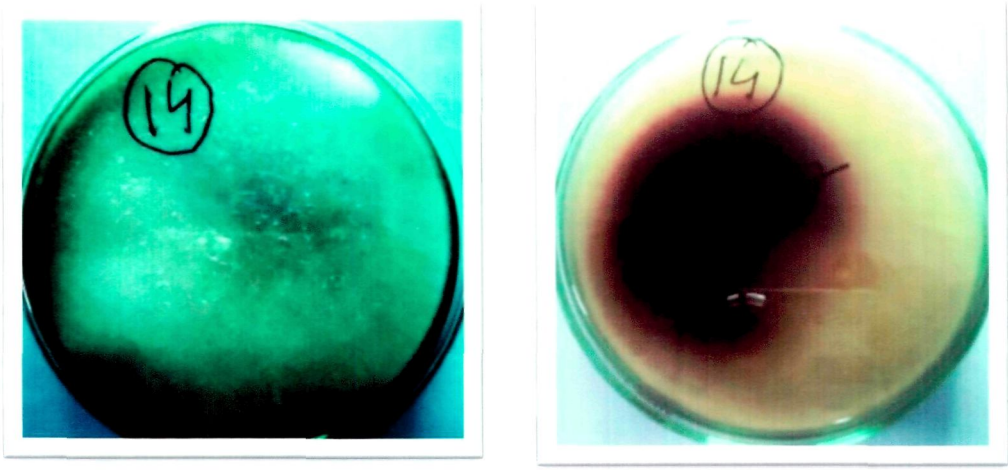

Fig. 48: Morphology of colony of *Absidia corymbifera* in front and reverse view of plate

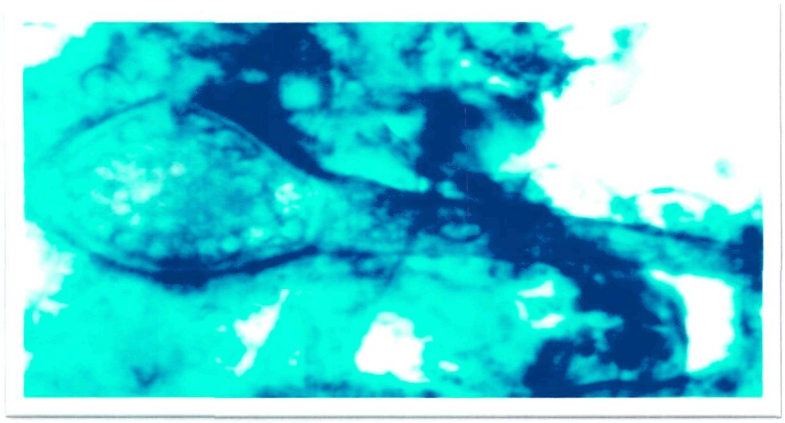

Fig. 49: Microphotograph showing sporangium filled with sporangiospores

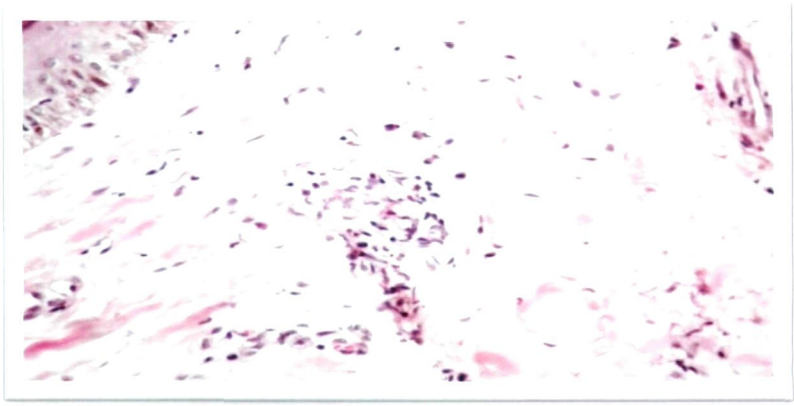

Fig. 50: Mononuclear cellular infiltration in epidermal layer by histopathology (H&E 100X)

8. *Aspergillus niger:*

In the present study, *A. niger* was isolated from one camel-calf. The lesions were large, hard, blackish and granulomatous on dorsal part of body, legs and scapular regions. Blackness of the lesions was also suggestive of this fungus. Macroscopically colour of the colony was black and reverse colour was white to yellow with granular texture . Microscopically conidiophores were thick walled long(Fig.51), smooth, colourless or brown, biseriate phialides and vesicle was round with radiate head (Fig.52). Morphological character of colony and microscopic finding are similar to Colin *et al*.(2013) as described in camels and Pal, (1956) in calf. *A. niger* has also been associated with dermatitis (Pal *et al.* (1987).

4.3 Histopathology of skin biopsy:

Histopathology of skin biopsy revealed skin abnormalities due to fungal infections like hyperkeratosis, acanthosis (Fig.20 and 23) and hyperplasia of epidermis (Fig.32 and 38), Hanaa *et al. (*2011).,mononuclear cells infiltration and fibrous tissue proliferation in dermal layer (Fig.29), Fungal colony(Fig.41) and fungal septate hyphae in epidermal and dermal layers. Singh (1988), Black fungal colonies in epidermis and dermis (Fig.47) surrounded by eosinophil and mononuclear cellular infiltration (Fig.26,35,44,53 and 50). Similar findings were also reported by Foutah *et al*., (2012) and Al–Salihi *et al.* (2014). Histopathology of skin biopsy after treatment revealed no inflammatory changes in epidermis and dermis and absence of fungal colony and hyphae in epidermis and dermis.

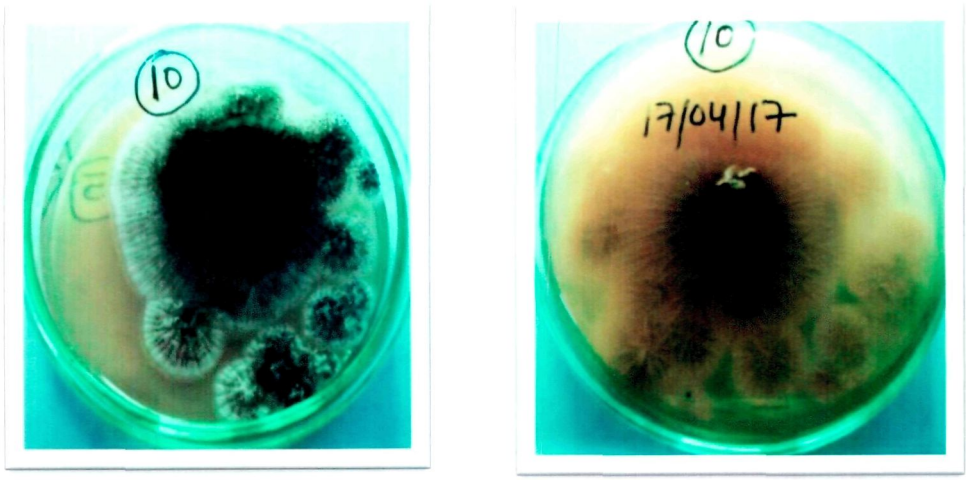

Fig. 51: Morphology of colony of *Aspergillus niger* in front and reverse view of plate

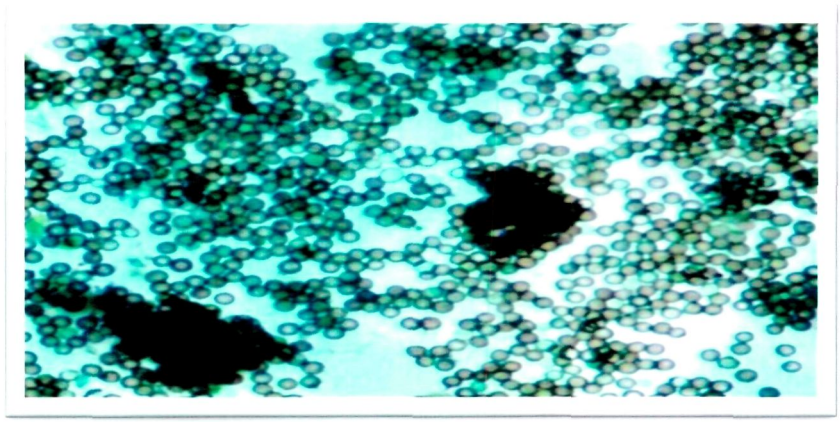

Fig. 52: Microphotograph showing production of carbon black spores From biseriate phialides

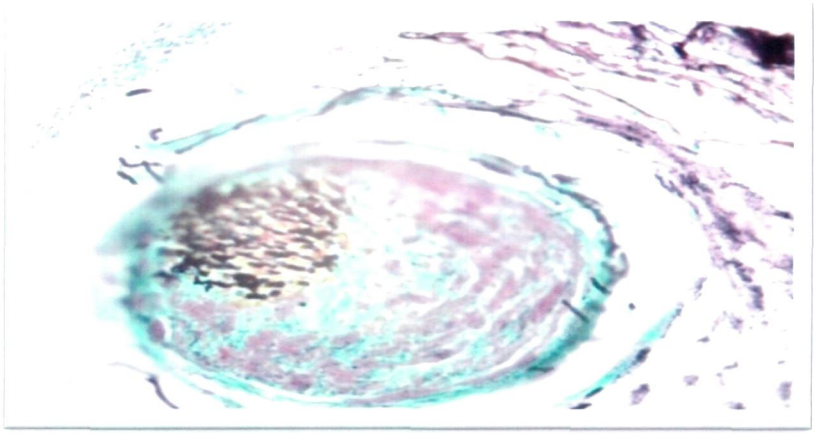

Fig.53: Black fungal colony in epidermal layer by histopathology (H&E 100X)

4.4 Hematological and skin pH estimations:

The mean ± SE values of TEC, hemoglobin, PCV, TLC, Differential leukocyte count and pH of skin of dermal mycoses cases of camel-calves, before and after treatment was presented in Table 3.

Table 3: Blood picture and skin pH of camels-calves before and after treatment. Mean ± SE (n=16)

Parameter	Pre-treatment	1-month post-treatment	2-months post-treatment	P value pre-treatment vs 1 month	P value pre-treatment vs 2 month	Control Range
RBCs (10^6/mm^3)	6.47 ±0.19	7.20 ±0.15	7.69±0.16	0.000	0.000	8.1-8.9
Hb (g %)	10.56±0.17	11.45±0.17	12.06±0.13	0.000	0.000	10.2-10.9
PCV (%)	32.81±0.56	34.31±0.50	34.37±0.41	0.020	0.043	34-35
TLC (10^3/mm^3)	11.93±.22	11.20±.23	10.29±0.21	0.000	0.000	10.8-14.0
Neutrophils (%)	63.94±0.19	62.94±0.32	61.87±0.31	0.002	0.000	64-66
Monocytes (%)	0.81±0.13	0.81±0.139	0.81± 0.10	1.000	1.00	0-1
Lymphocyte (%)	33.00±0.26	33.50±0.40	34.31±0.26	0.240	0.001	32-34
Eosinophils (%)	3.37 ±0.33	3.00 ±0.26	1.94 ±0.25	0.304	0.001	04-06
Basophils (%)	0.13±0.085	0.13±0.085	0.13±0.085	1.000	1.00	00
Skin pH	7.43±0.06	7.34 ±0.59	7.33 ±0.03	1.000	1.00	7.0-7.5

P value less than equal to 0.05 denotes significant difference between treatments P value greater than 0.05 denotes non significant differences between treatments

Analyses of hematological values by paired t-test revealed significant decrease in neutrophils and TLC after one month and two months post treatment values as compared to pre treatment values. This may be due to the fact that secondary bacterial infections invade through discontinuity of the skin integrity caused by the fungal lesions. Significant increase in RBC and Hb were observed after one month and two months of treatment. Similar findings were also reported by Foutah *et al.* (2012) and Dewal, (2017).

Significant increase in PCV was observed after one month and two months of treatment as compared to pretreatment value. Non-significant difference was observed in monocytes, basophils and skin pH after one month and two months of treatment. Mathur *et al.* (2011) observed variations in most of the haematological parameters in dematomycoses in camels occurred within the normal physiological range. High skin pH of camel calves (>7.0) as compared to other animals may be the reason for more occurrence of fungal skin infections in camel. This may be due to their weak immunity and the high pH of the skin as the pH of skin decreases with age (Radostits *et al.,* 1997).

4.5 Serum-biochemical estimation:

Liver enzymes and protein profile of camel calves before and after treatment of dermal mycoses cases is presented in the Table 4.

Analysis of serum biochemical values with paired t-test revealed significant decrease in AST and ALT values in one month and two months post treatment as compared to pre treatment values. Similar findings were also reported by Fatma *et al.*, (2008). There was non-significant decrease in ALKP values after one month of treatment and significant decrease was observed after two month of treatment. Similar findings were also reported by Foutah *et al.*, (2012) and Dewal, (2017).

Table 4: Liver enzymes and protein profile of camel calves before and after treatment. Mean ± SE (n=16)

Parameter	Pre- treatment	1-month post-treatment	2-month post-treatment	p value Pre-treatment vs. 1 month	p value Pre-treatment vs. 2 months	Control Range
AST(U/L)	100.25±13.27	56.68±2.40	46.25±3.17	0.003	0.001	43-60
ALT(U/L)	31.81±4.14	13.44±1.09	14.75±1.27	0.001	0.001	10-12
ALKP(U/L)	188.25±14.06	180.37±15.94	125.69±11.41	0.614	0.003	136-269
T.P. (gm/dl)	5.63± 0.77	6.02± 0.49	5.36±0.19	0.024	0.242	5.1-6.0
Albumin (gm/dl)	2.89±0.11	3.02±0.10	2.59±0.12	0.221	0.040	2.2-3.8
Globulin (gm/dl)	2.74±0.14	2.99±0.13	2.67±0.09	0.068	0.603	2.7-3.2
A/G (ratio)	1.05	1.01	0.97			
P value less than equal to 0.05 denotes significant difference between treatments P value greater than 0.05 denotes non significant differences between treatments						

There was significant increase in total protein, but no significant increase in albumin and globulin values after one month of treatment thereafter change in total protein and globulin was no significant, where as albumin was decreased significantly after two month of treatment. Similar findings were also reported by Foutah *et al.* (2012); Gorakh *et al.* (2006) and Dewal, (2017).

Means values with significant difference turned to normal after recovery. More variation in these values may be due to the fact that concentration of these enzymes is more during bone formation stages. Therefore, its mean concentration is more in growing animals since all the camel calves in this study were approximate one year of age.

4.6 Serum minerals estimation

Mean ± SE value of serum Zn, Co, Cu and Se of camels calves before and after treatment are presented in Table 5.

Table 5: Value of serum minerals of camels before and after treatment (ppm). Mean ± SE (n=16)

Serum mineral	Pre- treatment	1-month post-treatment	2-month post-treatment	p value Pre-treatment vs. 1 month	p value Pre-treatment vs. 2 months	Control Range
Cu	24.51± 0.42	18.88± 2.26	11.45±0.14	0.001	0.000	12.14-13.11
Co	10.11±0.081	22.42±1.51	27.75±0.53	0.000	0.000	23.47-29.43
Se	0.40±0.06	0.41±0.06	0.55±0.07	0.67	0.234	0.324-0.998
Zn	142.54±86.51	82.65±28.27	77.80±25.36	0.54	0.50	16.65-91.54
P value less than equal to 0.05 denotes significant difference between treatments P value greater than 0.05 denotes non significant differences between treatments						

Analysis of serum minerals values by paired t-test revealed significant decrease in copper (Cu) value after one month and two months post treatment as compared to infected camel-calves before treatment. Similar findings were also reported by Pourjafar *et al.*, (2014).

There was significant increase in cobalt (Co) value after one month and two months post treatment as compared to pre treatment values. Similar findings were also reported by Dewal (2017).

There was non-significant increase in selenium (Se) value as reported by Dewal, (2017) while non-significant decrease in zinc (Zn) value in infected camel-calves as compared to one month and two months post treatment as reported by Pourjafar *et al.* (2014).

4.7 Treatment of dermal mycoses in camel calves:

A total of 16 camel calves were subjected to the therapeutic efficacy of herbal formulation developed by ICAR-NRCC, Bikaner. The herbal formulation was applied topically on 0 day, 3rd day and 7th day. After 30 and 60 day of first application of drug, camel-calves were again

examined for symptoms and lesions. Hemato-biochemical examination was done again on 30th and 60th day and histopathological examination on 60th day of first application of herbal formulation. In all the recovered camel calves a total of three applications were required for the total recovery. Since in two animals skin was very hard and lesions were not fully recovered so one more application of the same formulation also applied after 3 days of the third application. Therefore, it is suggested to give four applications of the formulation in those cases where the prognosis is poor. Similar results of herbal formulation were also reported by Dewal (2017).

The recovery in these camel calves were judged on the basis of clinical symptoms like itching, alopecia, emaciation and crusty circular lesions. It was observed that after two months of treatment there was re-growth of hairs and no signs of itching present over the skin lesions, skin became soft and glossy and general condition of animals also improved (Fig. 8, 10, 12, 14 and 16). In the present study, the herbal formulation used for treatment was found to clear all the fungal lesions which were present over the body.

Various ethno veterinary practices are being used for the treatment of dermal mycoses in camel calves. These practices include various hit and trial methods, some of them having side effects like irritation, nausea and diarrhoea.

A variety of common fungicidal and fungistatic agents such as iodine, 5% sulphur in sesame oil, 5% salicylic acid, coal tar phenols (3.25%) with copper acetate (0.58%) and hydroxyquinolines may be applied topically as ringworm ointment onto the affected areas (Wernery and Kaaden, 2002).

Captan is a fungicide for ornamental plants. The use of Captan has been advocated (Ainsworth and Austwick, 1973) but lot of irritation occurs in the affected camels. Treatment of dermatophytoses with

griseofulvin is very effective in cattle (Coetzer *et al.*, 1994), but it causes side effects in camels such as diarrhoea and is therefore not recommended (Schwartz and Dioli, 1992).

Tuteja *et al.* (2012) reported treatment of skin candidiasis in camel calves with three formulations consisting of 2% potassium iodide; 6% sulphur in mustard oil; and 6% sulphur and 3% salicylic acid in mustard oil application topically in naturally occurring cases in camel calves. All the three treatments were found effective with almost similar application schedule but with variable duration of treatment. This is long term treatment schedule with a minimum of eight applications is effective only against skin candidiasis (Tuteja *et al.*, 2012).

SUMMARY AND CONCLUSION

5. SUMMARY AND CONCLUSION

The diagnosis and therapeutics study was conducted during the year 2017 on camel calves were affected with dermal mycosis at Indian council of Agriculture Research, National Research Centre on Camel, Bikaner Rajasthan. A total of 16 dermal mycoses infected camels (of either sex or approximately one year age group) were included in the present study. The major clinical manifestations were itching and alopecia. The mycotic lesions were round, disc shaped and ulcerative most commonly present on dorsum of body, scapular, chest and abdomen regions. Skin scrapings and blood samples were collected from all the affected camel calves aseptically. Microscopic examination, haemato-biochemical examination, skin surface pH examination, histopathology of skin biopsy and culture examination were performed before and after treatment. An effort was made to draw a picture of clinico-diagnostic, haemato-biochemical parameters, and histopathology of skin biopsy of camels suffering from dermal mycoses and evaluate the efficacy of herbal formulation of drug.

Culture examination of skin scraping samples from dermal mycoses suffered camel calves revealed the growth of various fungal species such as *Microsporum nanum, Microsporum audouinii. Microsporum gypeseum, Microsporum ferrugineum, Blastomyces dermatitidis, Basidobolus ranarum, Candida albicans, Histoplasma capsulatum, Absidia corymbifera, Aspergillus niger, Trichophyton verrocosum and Trichophyton rubrum.*

Haematological and skin surface pH results revealed significant decrease in neutrophils, TLC after one month and two month of treatment, significant increased in RBC, PCV and Hb after one month and two month of treatment. While no significant difference was

observed in monocyte, basophils and skin pH after one month and two month of treatment.

Serum biochemical result revealed significant decrease in AST and ALT enzymes values in one and two month of post treatment while non significant decrease ALKP enzymes values after one month of treatment and significant decreases was observed after two month of treatment and significant increase total proteins but non-significant increase albumin and globulin.

Histopathological examination of skin biopsy revealed skin abnormalities due to fungal infections like hyperkeratosis, acanthosis and hyperplasia of epidermis, mononuclear cells infiltration and fibrous tissue proliferation in dermal layer, Fungal colony and fungal septate hyphae in epidermal and dermal layers. Fungal colonies in epidermis and dermis surrounded by eosinophil and mononuclear cellular infiltration. Histopathology of skin biopsy after treatment revealed no inflammatory changes, absence of fungal colony and hyphae in epidermis and dermis.

Analysis of serum minerals revealed significant increase in cobalt (Co) after one month and two month of post treatment as compared to pre treatment, there was non-significant increase in selenium (Se) values and non significant decrease in zinc (Zn) value as infected camel calves as compared to one month and two month of post treatment.

This novel study was intended to test the therapeutic efficacy of herbal drug which has been developed by National Research Centre on Camel, Bikaner. The camel calves were examined for any symptom of dermal mycoses and presence of lesions on body surface. The infected camel calves were subjected for application of the herbal preparation. Application of drug was carried out with a predesigned

protocol on days 0, 3rd and 7th, respectively. Post treatment observations suggested that three applications were sufficient for the complete recovery. The recovery in these camel calves was manifested by subsidence of clinical symptoms like itching, alopecia, crusty circular lesion. It was observed that after treatment the skin became soft and glossy and there was no scar present over the skin thereby improving the appearance of camel calves.

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6. LITERATURE CITED

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ABSTRACT

(ENGLISH & HINDI)

Studies on Diagnosis and Therapeutics of Dermal Mycoses in Camel calves

M.V.Sc. Thesis

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ABSTRACT

The present study was conducted during the year 2017 at the Indian Council of Agriculture Research, National Research Centre on Camel, Bikaner, Rajasthan. A total of 40 camels calves approximate one year of age irrespective of sex and breed were screened for dermal mycoses, out of which 16 camel calves were found positive for dermal mycoses which were included in the present study. The major clinical manifestations were itching and alopecia. The mycotic lesions were round, disc shaped and ulcerative, most commonly present on dorsum of body, scapular, chest and abdomen regions. Microscopic, haemato-biochemical and culture examination were performed before and after treatment to evaluate the efficacy of herbal formulation. Culture examination of skin scrapings revealed presence of *Trichophyton* and *Microsporum spp.* which were the main causative agents of dermal mycoses in camel calves. Hematological and skin surface pH examination revealed significant decrease in neutrophils and TLC while significant increase in PCV, RBC and Hb, non-significant difference in monocytes, basophils and skin pH after one month and two months of treatment. Skin biopsy before treatment revealed skin abnormalities in the dermal and epidermal layer due to fungal infections. After treatment fungal infection was not observed and histopathological changes became normal. There was significant decrease in AST and ALT values after one month and two months of treatment. There was significant decrease in copper while significant increase in cobalt, after one month and two months of treatment. The infected camel calves from dermal mycoses showed complete recovery with three applications of herbal formulation at 0, 3rd and 7th day of clinical examination.

ऊँट के बछड़ों में त्वचीय फंफूद रोग का नैदानिक और चिकित्सीय अध्ययन

स्नातकोत्तर शोध ग्रंथ

ज्नपादकीय रोग विज्ञान एवं निवारण पशु औषध विज्ञान विभाग
पशु चिकित्सा एवं पशु विज्ञान महाविद्यालय
राजस्थान पशु चिकित्सा एवं पशु विज्ञान विश्वविद्यालय, बीकानेर – 334001

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मुख्य उपादेष्टा – डॉ. अन्जु चाहर

अनुक्षेपण

प्रस्तुत अध्ययन वर्ष 2017 के दौरान भारतीय कृषि अनुसंधान परिषद, राष्ट्रीय उष्ट्र अनुसंधान केन्द्र, बीकानेर राजस्थान पर किया गया। प्रस्तुत अध्ययन में त्वचीय फंफूद रोग के लिए कुल 40 ऊँट के बछड़ो (लिंग एवम् नस्ल को छोड़कर) की जाँच की गई, जिनकी उम्र लगभग 1 वर्ष थी। उनमें से 16 ऊँट के बछड़ें त्वचीय फंफूद रोग से ग्रसित पाए गए। मुख्य नैदानिक लक्षण खुजली और केशाभाव थे। फंफूद लक्षण गोल, तश्तरीनुमा और घावयुक्त थे, जो मुख्यतः शरीर के पृष्ठभाग, स्केपुलर, छाती एवम् उदरीय भागों पर थे। जड़ी–बूंटी मिश्रण की प्रभावकारीता का मूल्यांकन करने के लिए उपचार के पहले एवम् बाद में सूक्ष्मदर्शी, रक्तीय–जैवरासायनिक एवं कल्चर परीक्षण किया गया। त्वचीय खरोंच के कल्चर में ट्राइकोफाइटॉन एवं माइक्रोस्पोरम प्रजातियों का पता चला जो ऊँट के बछड़ों में त्वचीय फंफूद रोग के मुख्य कारक थे। रक्तीय और त्वचीय हाइड्रोजन आयन सांद्रता की जाँच से पता चला कि उचार के एक और दो महीने बाद न्यूट्रोफिल्स एवं श्वेत रूधिर कणिकाओं की गणना में महत्वपूर्ण कमी, जबकि पेक्ड सेल वाल्यूम, लाल रूधिर कणिकाओं की गणना और हीमोग्लोबिन में महत्वपूर्ण वृद्धि, मोनोसाइट्स, बेसोफिल और त्वचीय हाइड्रोजन आयन सांद्रता में गैर–महत्वपूर्ण अन्तर पाया गया। उपचार के पहले त्वचीय बायोप्सी जाँच में फंफूद संद्रमण के कारण त्वचीय परत में असमानतायें पायी गयी। उपचार के बाद त्वचीय परत में फंफूद का संक्रमण अनुपस्थित था तथा ऊतक विकृति अध्ययन में परिवर्तन सामान्य हो गया। उपचार के एक और दो महीने बाद ऊँट के बछड़ों में ए.एस.टी. और ए.एल.टी. के स्तर में महत्वपूर्ण कमी पायी गयी। उपचार के एक और दो महीने बाद तांबे के स्तर में महत्वपूर्ण कमी जबकि कोबाल्ट के स्तर में महत्वपूर्ण वृद्धि पायी गयी। त्वचीय फंफूद रोग से ग्रसित ऊँट के बछड़ों में नैदानिक जांच के शून्य, तीसरे और सातवें दिन जड़ी–बूंटी मिश्रण के प्रयोग से पूर्णतया स्वास्थ्य लाभ देखा गया।

APPENDICES

Appendix-1 Hematological values and skin pH of dermal mycoses affected camels calves at 0 day (before treatment)											
Serial no	Animal no	Hb (g/dl)	PCV (%)	RBC	WBC	Skin pH	Neutrophils (%)	Lymphocyte (%)	Monocyte (%)	Eosinophils (%)	Basophils (%)
1	M-165	9.2	28	4.8	12.0	6.5	64	31	02	03	NIL
2	J-305	10.8	32	5.6	11.0	7.5	63	32	01	04	NIL
3	M-94	10.7	33	5.8	10.0	7.5	65	34	01	03	NIL
4	B-796	10.6	32	6.6	11.6	7.5	63	35	01	02	NIL
5	K-271	9.8	28	6.1	12.0	7.5	63	34	01	05	NIL
6	M-159	11.2	33	5.6	12.0	7.5	63	34	01	04	NIL
7	B-729	11.3	32	5.8	14.0	7.5	64	32	00	05	NIL
8	B-798	10.9	33	6.6	12.0	7.5	65	34	00	06	NIL
9	B-725	9.8	32	6.8	11.9	7.5	64	33	01	04	NIL
10	J-313	10.1	33	7.2	12.0	7.5	65	32	01	04	NIL
11	B-802	10.2	34	7.6	11.6	7.5	64	34	01	04	NIL
12	B-806	11.3	35	6.9	13.3	7.5	64	33	00	04	NIL
13	K-277	11.6	35	6.8	11.3	7.5	65	33	01	05	NIL
14	M-167	11.3	36	7.3	12.1	7.5	64	34	01	03	NIL
15	M-100	9.8	35	6.9	11.8	7.5	64	34	00	03	01
16	B-739	10.3	34	7.1	12.3	7.5	63	34	01	02	01
C-A	M-83	10.3	34	8.6	12.3	7.5	65	33	01	06	NIL
C-B	B-641	10.9	34	8.0	12.0	7.5	65	34	01	04	NIL
C-C	B-647	10.6	35	8.0	14.0	7.5	66	32	01	06	NIL

Appendix-2: Hematological values and skin pH of dermal mycoses affected camels calves at 1month of after treatment											
Serial no	Animal no	Hb (g/dl)	PCV (%)	RBC	WBC	Skin pH	Neutrophils (%)	Lymphocyte (%)	Monocyte(%)	Eosinophils (%)	Basophils (%)
1	M-165	10.6	29	6.9	11.5	7.5	64	33	NIL	02	NIL
2	J-305	12.3	34	6.3	10.3	7.5	62	35	01	02	NIL
3	M-94	12.3	37	7.2	9.2	7.5	61	38	NIL	01	NIL
4	B-796	11.9	32	7.1	10.1	7.5	62	34	01	03	NIL
5	K-271	10.9	33	6.4	11.7	7.5	60	34	NIL	O4	NIL
6	M-159	11.8	35	6.3	11.6	7.0	62	33	01	03	NIL
7	B-729	11.3	36	6.9	13.0	7.0	63	31	NIL	O5	NIL
8	B-798	11.9	35	6.9	11.7	7.5	64	32	01	03	NIL
9	B-725	10.3	34	6.9	11.2	7.5	63	32	NIL	04	NIL
10	J-313	10.8	34	7.8	11.3	7.0	64	32	NIL	03	NIL
11	B-802	10.9	33	7.9	10.9	7.0	64	34	01	02	NIL
12	B-806	11.9	35	7.6	12.4	7.5	63	33	01	03	NIL
13	K-277	12.3	34	7.9	10.8	7.5	65	33	01	03	NIL
14	M-167	11.6	35	7.9	11.8	7.0	64	34	01	03	NIL
15	M-100	10.6	34	7.3	10.3	7.5	63	34	01	02	NIL
16	B-739	11.3	33	7.9	11.4	7.5	63	34	01	01	NIL
C-A	M-83	11.2	34	8.8	11.8	7.5	64	33	01	04	NIL
C-B	B-641	11.8	34	8.9	10.6	7.0	64	34	01	03	NIL
C-C	B-647	11.3	35	8.4	13.3	7.5	64	32	01	05	NIL

Appendix-3: Hematological values and skin pH of dermal mycoses affected camels calves at 2month of after treatment											
Serial no	Animal no	Hb (g/dl)	PCV (%)	RBC	WBC	Skin pH	Neutrophils (%)	Lymphocyte (%)	Monocyte (%)	Eosinophils (%)	Basophils (%)
1	M-165	10.9	32	7.2	9.3	7.0	62	35	NIL	02	NIL
2	J-305	12.3	34	6.8	10.0	7.0	61	34	01	02	NIL
3	M-94	11.9	35	6.9	9.0	7.0	60	35	01	03	NIL
4	B-796	12.3	35	7.3	10.0	7.0	60	36	NIL	01	NIL
5	K-271	11.6	36	7.4	10.2	7.5	60	34	01	03	NIL
6	M-159	11.9	35	6.8	10.7	7.0	62	34	01	01	NIL
7	B-729	12.3	35	6.9	12.3	7.0	61	34	01	02	NIL
8	B-798	12.6	34	7.8	11.2	7.0	62	33	01	03	NIL
9	B-725	11.6	33	7.4	10.3	7.0	61	36	01	02	NIL
10	J-313	11.9	33	8.3	10.6	7.0	62	33	01	02	NIL
11	B-802	11.6	33	8.2	9.6	7.0	63	34	01	01	NIL
12	B-806	12.4	35	8.3	10.3	7.0	63	34	01	01	NIL
13	K-277	12.9	34	8.6	9.3	7.0	64	34	01	02	NIL
14	M-167	12.3	35	8.2	10.6	7.0	63	35	01	01	NIL
15	M-100	11.6	33	8.3	10.0	7.0	63	33	01	01	NIL
16	B-739	12.6	34	8.6	10.6	7.0	62	35	01	01	NIL
C-A	M-83	10.3	34	8.9	11.9	7.0	64	34	01	04	NIL
C-B	B-641	10.2	34	8.3	10.8	7.5	64	34	01	04	NIL
C-C	B-647	10.3	34	8.2	12.9	7.5	64	33	01	04	NIL

Appendix-4: Values of serum mineral in dermal mycoses affected camels calves at 0 days(before treatment)					
Serial no	**Animal no**	**Zinc (Zn)**	**Copper(Cu)**	**Cobalt (Co)**	**Selenium (se)**
1	M-165	16.99	27.24	02.93	0.854
2	J-305	10.16	26.75	07.89	01.02
3	M-94	10.84	28.01	09.67	0.193
4	B-796	41.49	28.25	07.54	0.641
5	K-271	15.77	31.87	12.79	0.209
6	M-159	227.82	29.46	05.52	0.306
7	B-729	1406.96	30.05	09.99	0.115
8	B-798	27.64	26.08	10.54	0.406
9	B-725	254.20	25.48	08.95	0.267
10	J-313	27.11	27.13	08.38	0.203
11	B-802	132.44	26.32	13.19	0.201
12	B-806	13.80	25.96	10.73	0.775
13	K-277	09.73	26.70	11.37	0.414
14	M-167	16.21	27.09	14.38	0.452
15	M-100	11.60	26.16	15.5	0.454
16	B-739	57.89	27.70	12.83	0.634
C-A	M-83	91.15	13.11	24.26	0.324
C-B	B-641	16.65	12.10	23.47	0.954
C-C	B-647	74.62	12.14	26.09	0.479

Appendix. 5 :Values of serum mineral in dermal mycoses affected camels calves at 1month after treatment					
Serial no	**Animal no**	**Zinc (Zn)**	**Copper(Cu)**	**Cobalt (Co)**	**Selenium (se)**
1	M-165	17.41	26.28	13.60	0.501
2	J-305	409.37	24.99	13.33	0.835
3	M-94	257.29	26.80	15.73	0.620
4	B-796	148.20	26.00	17.31	0.179
5	K-271	11.01	28.50	18.70	0.370
6	M-159	70.34	30.43	19.67	0.904
7	B-729	14.67	28.62	16.22	0.196
8	B-798	62.03	28.65	21.74	0.257
9	B-725	31.06	11.53	24.29	0.392
10	J-313	30.29	08.77	27.07	0.163
11	B-802	178.39	11.25	30.60	0.150
12	B-806	22.66	09.54	29.74	0.473
13	K-277	17.00	10.19	28.69	0.615
14	M-167	07.89	09.00	27.48	0.298
15	M-100	36.35	10.57	26.77	0.136
16	B-739	08.45	11.01	27.86	0.560
C-A	M-83	91.23	13.15	26.89	0.389
C-B	B-641	16.76	12.10	24.74	0.985
C-C	B-647	74.64	12.16	27.43	0.498

Appendix- 6 :Values of serum mineral in dermal mycoses affected camels calves at 2 month after treatment					
Serial no	**Animal no**	**Zinc (Zn)**	**Copper(Cu)**	**Cobalt (Co)**	**Selenium (se)**
1	M-165	248.09	10.57	28.50	0.639
2	J-305	24.62	11.64	28.91	0.776
3	M-94	26.29	10.74	28.20	0.601
4	B-796	07.20	11.47	32.86	0.380
5	K-271	51.95	11.09	27.64	0.172
6	M-159	278.63	11.19	27.06	0.172
7	B-729	06.10	11.88	30.49	0.769
8	B-798	05.34	11.86	24.52	0.287
9	B-725	01.82	11.86	27.74	0.847
10	J-313	02.80	10.86	26.68	0.529
11	B-802	02.90	11.87	25.86	0.128
12	B-806	221.07	11.22	25.29	0.560
13	K-277	63.80	11.47	27.67	0.741
14	M-167	72.67	11.23	25.55	0.991
15	M-100	08.73	12.71	27.19	0.309
16	B-739	222.73	12.05	29.92	0.866
C-A	M-83	91.54	12.89	28.78	0.398
C-B	B-641	16.87	12.15	26.96	0.998
C-C	B-647	74.64	12.14	29.43	0.480

Appendix 7 :Values of serum biochemistry in dermal mycoses affected camels calves at 0 days(before treatment)							
Serial no	Animal no	ALKP	ALT	AST	Total Protein	Albumin	Globulin
1	M-165	283	22	85	5.8	2.7	3.1
2	J-305	106	10	59	6.3	3.2	3.1
3	M-94	145	29	160	5.5	2.6	2.9
4	B-796	169	10	43	5.1	2.7	2.5
5	K-271	100	22	46	5.4	2.9	2.5
6	M-159	123	22	46	5.4	2.9	2.5
7	B-729	239	13	53	5.9	3.3	2.6
8	B-798	210	73	22	4.9	3.2	1.7
9	B-725	189	41	202	5.3	2.8	2.5
10	J-313	215	46	286	5.9	2.5	3.4
11	B-802	210	37	267	5.5	2.4	3.1
12	B-806	179	38	171	7.2	4.0	3.3
13	K-277	183	43	163	6.3	3.0	3.3
14	M-167	163	37	104	5.9	2.9	3.0
15	M-100	183	43	89	3.9	2.6	3.0
16	B-739	133	30	87	4.9	2.3	2.5
C-A	M-83	269	10	59	6.0	3.0	3.0
C-B	B-641	136	10	51	6.0	2.7	2.3
C-C	B-647	261	12	43	5.1	2.2	2.9

Appendix 8 :Values of serum biochemistry in dermal mycoses affected camels calves at 1month after treatment							
Serial no	**Animal no**	**ALKP**	**ALT**	**AST**	**Total Protein**	**Albumin**	**Globulin**
1	M-165	15	10	45	6.6	3.4	3.2
2	J-305	13	12	63	6.8	3.3	3.5
3	M-94	10	12	60	5.9	3.0	2.9
4	B-796	157	17	35	5.0	2.7	2.3
5	K-271	147	14	52	5.7	3.4	2.2
6	M-159	330	11	62	6.5	3.5	3.1
7	B-729	270	28	51	5.3	2.8	2.5
8	B-798	143	16	46	5.9	3.6	2.3
9	B-725	129	14	55	6.0	2.8	3.2
10	J-313	139	13	49	6.3	2.0	4.3
11	B-802	163	13	63	6.4	3.2	3.2
12	B-806	173	11	67	6.4	3.4	3.0
13	K-277	163	13	70	6.2	2.9	3.3
14	M-167	248	10	66	6.1	3.1	3.0
15	M-100	270	11	64	5.7	2.7	2.9
16	B-739	173	10	59	5.6	2.6	3.0
C-A	M-83	187	11	52	6.0	3.0	3.0
C-B	B-641	113	10	52	5.8	3.8	2.0
C-C	B-647	189	14	56	6.9	3.6	3.3

Appendix 9: Values of serum biochemistry in dermal mycoses affected camels calves at 2month after treatment							
Serial no	**Animal no**	**ALKP**	**ALT**	**AST**	**Total Protein**	**Albumin**	**Globulin**
1	M-165	133	46	126	5.0	2.3	2.6
2	J-305	142	12	113	4.6	2.3	2.3
3	M-94	88	10	40	4.8	2.2	2.6
4	B-796	79	24	46	4.5	2.2	2.3
5	K-271	82	20	33	4.9	2.5	2.4
6	M-159	89	17	29	4.8	2.3	2.5
7	B-729	219	10	67	5.5	2.6	2.8
8	B-798	92	11	50	6.2	3.5	2.7
9	B-725	234	10	50	5.2	2.5	2.6
10	J-313	113	30	50	5.2	2.5	2.7
11	B-802	116	17	60	6.4	3.4	3.0
12	B-806	109	10	44	7.0	3.6	3.4
13	K-277	125	13	45	5.0	2.2	2.8
14	M-167	106	16	71	6.1	2.8	3.2
15	M-100	119	15	48	4.7	2.4	2.3
16	B-739	165	15	54	5.7	2.8	2.9
C-A	M-83	198	11	60	5.9	3.4	3.2
C-B	B-641	196	11	60	6.0	3.7	3.1
C-C	B-647	231	11	60	5.8	2.5	3.2