

EPIDEMIOLOGICAL AND CLINICO - THERAPEUTIC STUDIES ON BACTERIAL SKIN INFECTIONS IN DOGS

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**Thesis submitted in partial fulfilment of the
requirement for the degree of**

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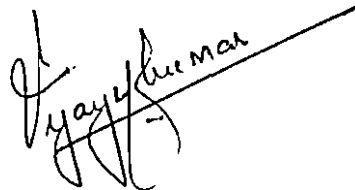
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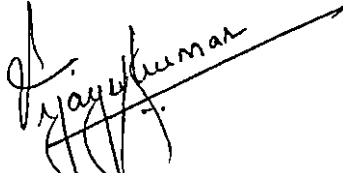
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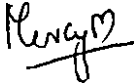
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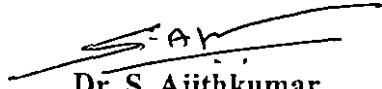
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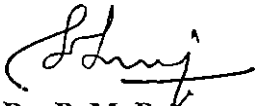
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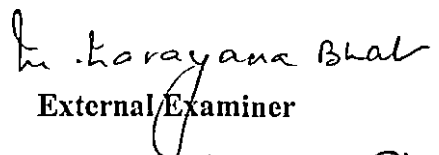
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*Dedicated to
my
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Introduction

1. INTRODUCTION

Dogs are the most loving and loyal companion animals of human beings and the role of dog as a pet is increasing day by day in our society. Skin is the most sensitive part of dog's body and has tremendous aesthetic value for the owners, therefore the skin ailments of dogs become all the more important. Besides, a few canine dermatological disorders hold significance because of their zoonotic nature.

Skin is the largest organ of the body, and act as the anatomic and physiologic barrier between animal and environment. It provides protection from physical, chemical and microbiological injury and its sensory components perceive heat, cold, pain, pruritus, touch and pressure. In addition, the skin is synergistic with internal organ systems and thus reflects pathologic processes that are either primary elsewhere or shared with other tissues.

Reports suggest that in small animal clinics, dermatological disorders constitute the majority of cases and these are estimated to range between 12 to 75 per cent as the chief or concurrent owner complaint. Based on the etiology, the major canine dermatitis have been described as those caused by bacterial, fungi, arthropod parasites, immune-mediation, keratinisation defects, psychogenic reasons, pigmentary abnormalities, neoplasia, endocrine or metabolic disturbances, nutritional deficiency or toxicity.

Cutaneous bacterial infections or bacterial pyoderma are one of the most common canine skin diseases presented to the veterinary dermatologist. The term pyoderma covers many clinical pictures, all of which include some degree of pyogenic skin inflammation associated with bacterial infection. It is manifested as a self-limiting condition or as a treatment resistant, or as a life-threatening infection.

Diagnosis and treatment of cutaneous bacterial infections are often confusing because the condition can be secondary to a number of other integumentary conditions such as atopy, flea bite, mange or hormonal and nutritional imbalances and other systemic diseases. The treatment protocol should be formulated specifically for each individual case. Resolution of secondary pyodermas necessitates identification of the underlying problems and successful treatment of the primary condition. It can be treated topically, systemically or by combination of both for a comparatively longer period of time.

Recurrence of the condition and long duration of treatment often makes the owner frustrated and poses a challenge to the clinician. Henceforth an antibiotic of once a day dose regimen, with oral administration is very necessary for the better management of this disease. Investigation in such an aspect with following objectives will help to suggest ideal therapeutic measures for field application.

1. Study the epidemiology, clinical signs and clinicopathological changes in bacterial skin infections in dogs.
2. Identify the bacterial etiology associated with skin infections and its antibiogram.
3. Evaluate the clinical response of the cases to the treatment regimens adopted.

Review of Literature

2. REVIEW OF LITERATURE

2.1. EPIDEMIOLOGY

2.1.1. Occurrence

Skin conditions are among the most frequent ailments in companion animal practice and may account for more than 20 per cent of patients seen in veterinary clinics (Ihrke, 1987).

It has been estimated that from 20 to 75 per cent cases seen in the average small animal practice have skin problems as a chief or concurrent owner's complaint. (Muller *et al.*, 1989).

Dermatological disorders accounted for 18.80 per cent among all the dogs and most common dermatological disorder seen in dogs were bacterial folliculitis and furunculosis (25.3 per cent) (Scott and Pardis, 1990).

Pal *et al.* (1995) reported that out of 229 cases of canine pyoderma in West Bengal; 57 were superficial pyodermas and 72 were deep pyodermas.

In small animal clinics of Punjab Agricultural University overall incidence of bacterial dermatitis from 825 dogs investigated was found to be 40.24 per cent (Kamboj *et al.*, 1995).

A study conducted in Punjab Agricultural University at Ludhiana revealed that the overall prevalence of bacterial dermatitis was 31.3 per cent (Aujla *et al.*, 1997).

Mathews (1999) reported that the overall occurrence of bacterial dermatitis was 42 per cent in dogs.

Udayasree and Usha (2005) reported that among different dermatological disorders the incidence of canine pyoderma was found to be 12.71 per cent and it was the third most frequently diagnosed skin disorders.

Upadhyay *et al.* (2005) recorded the prevalence of dermatological problems in dogs as 12.75 per cent at Pantnagar.

Bacterial infections of the skin may represent up to 25 per cent of all canine dermatological consultations (Hillier *et al.* , 2006).

2.1.2. Predisposing Factors

2.1.2.1. Age

Canine impetigo (superficial pustular dermatitis, puppy pyoderma) is a pustular disease of dogs from three months to one year old (Codner, 1988)

In German shepherd breed, pyoderma seems to affect primarily middle aged dogs (Krick and Scott, 1989).

There was no age predilection for dermatological disorders as a whole in dogs (Scott and Pardis, 1990).

Aujila *et al.* (1997) reported higher prevalence of bacterial dermatitis in dogs upto 2 years of age.

Age wise prevalence of bacterial dermatitis revealed that dogs below six months of age were more frequently affected with an equal incidence in the age group of one to four years, and above four year group (Mathews, 1999).

It was found that dogs aged between one and four years (46 per cent) were commonly affected with pyoderma, followed by dogs aged between 4 and 8 years (28 per cent) (Patil *et al.* , 1999)

Impetigo affects young dogs before or around the age of puberty (Craig, 2003).

Analysis of percentage of dogs suffering from pyoderma in various age groups revealed that age group between one and four years (36.54 per cent) had the highest incidence followed by age group below six months (26.93 per cent) (Usha and Udayasree, 2005).

2.1.2.2. Sex

Krick and Scott (1989) did not find any significant sex predilection to pyoderma in dogs.

There was no sex predilection for dermatological disorders as a whole in dogs (Scott and Pardis, 1990).

Bitches were susceptible than male dogs to bacterial dermatitis (Aujila *et al.*, 1997).

Incidence of pyoderma was more in male (62 per cent) than in female (38 per cent) (Patil *et al.*, 1999).

Mathews (1999) reported that prevalence of pyoderma in females was 47.6 per cent and in males was 52.40 per cent.

Choi-Won Pil *et al.* (2000) reported that there was no significant sex predilection to canine pyoderma.

Most skin disorders do not show sex predisposition (Hill, 2002).

Senturk *et al.* (2005) reported majority of pyoderma cases from male dogs.

Sex wise prevalence of pyoderma showed that 55.75 per cent were females, and 44.23 per cent were males (Udayasree and Usha, 2005).

The overall highest prevalence was observed in adult male dogs (14.80 per cent) followed by 10.80 per cent in adult female and 7.70 per cent in young dogs (Upadhyay *et al.*, 2005).

There is no significant difference between the occurrence of pyoderma in male and female dogs (Toma *et al.*, 2008).

2.1.2.3. Breed

The higher incidence of pyoderma in German shepherd dogs might be due to an autosomal recessive gene as this is due to cell mediated immunodeficiency and therefore more incidence of recurrent, idiopathic bacterial folliculitis and furunculosis (Wisselink *et al.*, 1985).

The German shepherd dogs accounted for the one third of the case of recurrent idiopathic bacterial folliculitis and furunculosis (Scott and Pardis, 1990).

The breed related prevalence indicated that pure breed dogs were more susceptible to bacterial dermatitis than mixed breeds (Aujila *et al.*, 1997).

Alopecia in dog was more common (53.57 per cent) in long hair breeds than short hair breeds (46.43 per cent) (Chakrabarti *et al.*, 2002).

Udayasree (2004) found that pyoderma has the highest occurrence in German shepherd dogs (32.69 per cent) followed by other breeds which included Boxer, Rottweiler, Cocker spaniel and Dalmatian (26.92 per cent), non descript (19.23 per cent), Labrador (15.38 per cent) and Dachshund (5.78 per cent).

Majority of the cases of pyoderma reported from German shepherd breed (Senturk *et al.*, 2005).

2.1.2.4. Season

Krick and Scott (1989) observed that of the seventeen cases, sixteen dogs were pruritic and it ranged from mild to intense. Of these sixteen, all but one experienced non seasonal pruritus, but some had a summer related pruritus initially.

Kamboj *et al.* (1995) reported that incidence of bacterial dermatitis was maximum during the month of January (48.05 per cent) followed by May (44.87 per cent).

Seasonal prevalence revealed more cases in the month of August (17.01 per cent), followed by July (16.03 per cent) and September (15.03 per cent) and less during November (8.66 per cent) (Upadhyay *et al.*, 2005).

2.2. ETIOLOGY

In canine pyoderma the most frequently isolated pathogenic species was *Staphylococcus intermedius*. It was present on the mucosal sites and may be resident or transient on skin and generally transferred to the skin during grooming. (Berg *et al.*, 1984).

Runte (1985) isolated gram positive, coagulase positive cocci from a case of pruritic bacterial folliculitis in a Dalmatian dog, which were identified as *Staphylococcus intermedius*.

Ferher (1986) identified the association of Protein A with staphylococcal pyoderma in dogs; it is a substance which prematurely triggers the compliment cascade and acts as chemo attractant for neutrophils.

Chalmers and Medleau (1989) isolated *Dermatophilus congolensis* and coagulase positive *Staphylococci* from a case of canine pyoderma.

The cultural examination of touch swabs from lesions at the base of ear, face, neck revealed that the major isolate in fifteen cases of pyodermatitis was *Staphylococcus aureus*. Out of the fifteen cases, six cases were having mixed infections of *Sarcoptes scabie* and *Staphylococcus aureus* (Khosla *et al.* , 1991).

Scott *et al.* (1994) isolated *Staphylococcus intermedius* in pure culture from 20 dogs and *Staphylococcus aureus* in one dog from the cases of canine pyoderma.

Curtis *et al.* (1995) isolated *Staphylococcus intermedius* on bacterial culture of pustular contents obtained from bacterial folliculitis in a two year old German shepherd female dog.

Kamboj *et al.* (1995) identified *Staphylococcus intermedius* (82.96 per cent) as the main organism; other bacterial isolates were *Staphylococcus aureus*, *Proteus spp* *Escherichia coli* and *Bacillus spp* (2.18 per cent each), *Staphylococcus epidermidis*, *Streptococcus spp* and *Pseudomonas spp* (1.31 per cent) in canine pyoderma.

Aujila *et al.* (1997) reported that among various bacteria, *Staphylococcus spp* were most commonly (80 per cent) isolated with *Staphylococcus intermedius*, *Staphylococcus aureus*, *Staphylococcus epidermidis* being recovered in 45 per cent, 22.5 per cent and 12.5 per cent of cases respectively.

Carlotti *et al.* (1999) isolated *Staphylococcus intermedius* (58 per cent), *Staphylooccus aureus* (6 per cent), *Staphylococcus hyicus* and *Staphylococcus hominis* (14 per cent and 8 per cent each) from 50 cases of their study.

Pure cultures of coagulase positive *Staphylococcus intermedius* were grown from most pustules or draining tracts in dogs with pyoderma. Gram negative bacteria such as *Proteus*, *Pseudomonas*, *Escherichia coli*, were culture from samples of canine pyoderma, they grow in conjunction with *Staphylococcus intermedius* (Ihrke *et al.*, 1999).

Predominant bacteria in pyodermic dogs were coagulase positive *Staphylococcus spp*, (82 per cent), coagulase negative *Staphylococcus spp* (12 per cent), *Streptococcus spp* (both α and β hemolytic), *Micrococcus spp*, *Pseudomonas spp*, *Proteus spp* and *Klebsiella spp* were also isolated from pyodermic dogs (Patil *et al.* , 1999).

Chandrasekhar *et al.* (2001) identified 50.98 per cent *Staphylococcus intermedius*, 27.45 per cent *Staphylococcus aureus*, and 21.57 per cent *Staphylococcus epidermidis* in their bacteriological investigation connected with pyoderma in dogs.

Chakrabarti *et al.* (2002) found higher incidence of *Staphylococcus aureus* (50 per cent), followed by *Corynebacterium pyogenes* (20 per cent), *Pseudomonas aeruginosa* and *Escherichia coli* (10 per cent each) in canine pyoderma cases.

Impetigo is a non contagious and usually non pruritic disease caused by coagulase positive *Staphylococcus* bacteria (Craig, 2003).

Bacteriological examination of the swab taken from recently ruptured pustules revealed that the predominant causative agent isolated was *Staphylococcus intermedius* from 90 per cent of the cases under their study. *Escherichia coli*, other *Staphylococci*, *Klebsiella* and *Proteus* were also identified (Horspool *et al.* , 2004).

Superficial infection of the hair follicles is the most common group of bacterial skin diseases in the dog with *Staphylococcus intermedius* being the most common infectious organism isolated in these cases (Ihrke, 2006).

Mycobacterium smegmatis is one of the causative agent for skin lesions in cats. (Rossmerisl Jr. and Manning , 2004).

Isolation and biochemical characterization studies in 18 cases revealed *Staphylococcus intermedius* (61.1 per cent) in 11, *Staphylococcus aureus* (23.3 per cent) in six and atypical coagulase positive *Staphylococci* (5.5 per cent) in one case. *Psuedomonas spp* and *Klebsiella spp* were also isolated in a dog each besides the coagulase positive *Staphylococci* (Seena *et al.*, 2005).

Senturk *et al.* (2005) identified that 45 per cent of bacterial dermatitis was due to *Staphylococcus intermedius*, 15 per cent due to *Staphylococcus aureus*, 30 per cent due to *Staphylococcus epidermidis* and 10 per cent due to *Proteus spp*.

Staphylococcus intermedius was isolated from epidermal collarettes in 18 of 22 dogs with superficial pyoderma but not from healthy dogs (White *et al.* , 2005).

Pseudomonas aeruginosa could be isolated from dogs with lesions of deep pyoderma and it occurred usually along with other bacteria, most notably *Staphylococcus intermedius* (Hillier *et al.*, 2006).

Out of 231 samples of dermatitis, most of the bacterial isolates were *Staphylococcus spp* (115), *Escherichia coli* (23), *Streptococcus spp* (19), *Pseudomonas spp* (20), *Proteus mirabilis* (13), *Pantoea spp* (9), *Enterococcus spp* (6), *Enterobacter spp*.(6), *Acinetobacter* (5) and *Pasteurella spp* (4) were less common and a number of other bacteria detected rarely. Samples from dogs that showed recurrence of pyoderma examined and results revealed that out of the 23

dogs, in nine of them the culture differed from the bacteria found in the first culture (Mueller and Stephen, 2007).

Stegemann *et al.* , (2007) identified the isolates from canine pyoderma as *Staphylococcus intermedius* in 223 cases, *Escherichia coli* in 44 cases, β hemolytic *Streptococcus spp* in 32 cases, *Enterobacter spp* in 30 cases, *Pasteurella multocida* in 19 cases, *Proteus spp* in 16 cases and *Aeromonas* in 14 cases.

Bacteriological examination of ten samples from dermatitis by Sprucek *et al.* (2007) revealed that seven dogs had *Staphylococcus intermedius*, one had *Escherichia coli*, one each with *Streptococcus spp* and *Actinomyces spp* as the causative agents.

Toma *et al.* (2008) isolated *Staphylococcus intermedius* from 29 samples out of forty cases. Other bacteria isolated alone or in association were *Staphylococcus aureus*, *Staphylococcus schleiferi*, coagulase negative staphylococci, *Streptococcus pyogenes*, *Micrococcus*, *Escherichia coli*, *Pseudomonas spp*, *Enterococcus vulneris*, *Enterococcus cloacae*, *Bacillus magaterium*, *Klebsiella spp*, *Burkholderia cepacia* and *Enterobacter agglomerans*.

Microbial culture of the pus from the canine pyoderma revealed that *Staphylococcus spp* was prevalent in 13 dogs, followed by *Psuedomonas spp* in five dogs and both in two dogs (Kumar *et al.*, 2010).

2.3. CLINICAL SIGNS

Distribution of lesions was in dorsal or middle thoracic or lumbar areas or dorsal lumbar areas in surface pyoderma, on dorsal lumbar region, lateral thighs followed by the rump, dorsal thorax and neck region in superficial pyoderma and on different regions in deep pyoderma (Hill and Moriello, 1994).

Duration of clinical signs ranged from two weeks to four years in bacterial dermatitis cases (Scott *et al.*, 1994).

The clinical signs in affected dogs varied from pruritus, hyperemia, edema and alopecia with surrounding matted hairs on body coat from face to back (Rab *et al.*, 1996).

Alopecia in bacterial infections were not symmetrical in nature and had predominant distribution on head, neck, limbs and ear (Chakrabarti *et al.*, 2002).

Clinical presentations of bacterial dermatitis vary with length of hair coat. In short haired dogs small raised tufts of hairs often falls out, leaving patches of focal alopecia. In long haired breeds, owners initially report increased shedding, dull hair coat leading to scaling, hair loss and skin lesions (Craig, 2003).

Pustules, papules, epidermal collarettes, scales and crust were common in superficial pyoderma. Surface ulceration, serous exudation, plaque formation and presence of few satellite pustules were seen in surface pyoderma. In deep pyoderma, symptoms were fistulous tracts, oozing pus, lichenification over joint areas, interdigital pyoderma, deep ulceration of skin and cellulitis (Seena *et al.*, 2005).

Senturk *et al.* (2005) described that skin lesions in bacterial dermatitis generally included patchy, crusted papules, erythema, localized or generalized pustules, alopecia, seborrhea, excoriations, erosions, epidermal collarettes, discrete nodules, alopecic hemorrhagic bullae, cellulitis, irregular ulcers and fistulae.

Epidermal collarettes on ventral abdomen had been described as a clinical sign suggestive of superficial pyoderma in the dog (White *et al.*, 2005).

Hillier *et al.* (2006) reported that skin lesions in pyoderma consisted of erythematous papules, hemorrhagic bullae, ulcers and hemorrhagic crusts confined to the bottom. They also suggested that acute pseudomonal pyoderma syndrome showed clinical signs such as dorsal truncal pain, lethargy, exercise intolerance, anorexia and no skin disease.

2.4. CLINICAL PATHOLOGY

2.4.1. Haematology

Haemogram of dogs affected with bacterial skin infections showed mild leukocytosis and neutrophilia. (Krick and Scott, 1989).

Clinical hematology revealed neutrophilia, lymphopenia in dogs with generalized deep pyoderma (Mason, 1991).

Hematological findings include neutrophilia (27930 cells/ μl) and lymphopenia (588 cells/ μl) in pyodermic patients (Medleau *et al.* , 1991).

There was a peripheral eosinophilia (3.2×10^9 /litre) and unremarkable routine blood biochemistry in a case of bacterial furunculosis (Curtis *et al.*, 1995).

Aujila *et al.* (1997) observed marked leukocytosis (27.88 thousand cells/ mm^3) and absolute neutrophilia (78.2 per cent) in infected dogs.

Bacterial sepsis on wounds in 24 dogs showed neutropenia. The initial mean neutrophils count was 0.63×10^3 / μl (Brown and Rogers, 2001).

Neutrophilia and eosinophilia are suggestive of bacterial and fungal infections. (Kumar *et al.* , 2006).

2.4.2. Biochemical changes

Gowda *et al.* (1982) reported hypocalcemia, hyperphosphataemia, hypercholestremia and hypoglycemia in non specific dermatitis.

In the absence of liver, kidney diseases the abnormal serum protein concentration although not diagnostic, are indicative of chronic inflammation of skin (Wisselink *et al.*, 1985).

Dogs affected with bacterial dermatitis had mildly increased globulin levels of 4.1 to 5.20 g/dl (Krick and Scott, 1989).

Clinical biochemistry often revealed hypergammaglobulinaemia and hypoalbuminaemia in dogs with generalized deep pyoderma (Mason, 1991).

Hyperglycemia (399 mg glucose/dl) and glucosuria (4+ on Clinitest) observed in a cat with superficial pyoderma (Medleau *et al.* , 1991).

The serum phosphorus, cholesterol and blood glucose levels were significantly higher in case of experimentally infected dogs when compared to control group (Pal *et al.*, 1995).

Serum biochemical studies revealed no significant changes in total protein, albumin, total immunoglobulin but circulating immunocomplex analysis showed significant increase as compared to normal controls (Aujila *et al.*, 1997).

Batamuzi *et al.* (1998) reported proteinuria in dogs with recurrent pyoderma, as a consequence of the deposition of immune complexes in the glomerular capillaries. Among the five proteinuric dogs with superficial pyoderma, three had albuminuria, two had globulinuria and none had serum like profile. Of the ten dogs with deep pyoderma, eight had albuminuria, one with globulinuria profile and other had a serum like proteinuria.

Baseline and follow up complete blood count, serum biochemical profiles and urinalysis were within the reference ranges during a study evaluating the efficacy of clindamycin hydrochloride in the treatment of superficial bacterial pyoderma in dogs (Bloom and Rosser, 2001).

Senturk *et al.* (2005) reported that there was no statistical difference in values of serum GGT, ALT, urea and creatinine before and after treatment with rifampicin in pyoderma.

2.4.3. Serum minerals

Fadok (1982) reported a zinc responsive dermatosis in a Great Dane pup. Lesions consisted of crusting, scaling, and erythema around the mouth, chin, eyes, ears, and over pressure points such as the elbows and hocks, and the dog responded well to oral zinc supplementation.

Willemsse (1986) reported that copper was required for melanin production and keratin synthesis, and a deficiency could result in hypopigmentation and a dry, rough coat.

Pal *et al.* (1995) reported that there was significant decrease in level of serum copper (1.83 ± 0.17 mg/100ml) in dogs with experimentally induced pyoderma. The values in control dogs were found to be 2.19 ± 0.16 mg/100ml.

Mathews (1999) reported that the mean serum copper level in 21 dogs with pyoderma was 0.73 ± 0.09 per cent.

A synergistic effect exists between linoleic acid and zinc, with a beneficial effect on the coat of dogs (Kirby *et al.* , 2007).

Krishnamoorthy *et al.* (2008) reported that there is no significant difference in the mean serum copper and iron concentration with respect to age, breed and sex in dogs.

2.5. DIAGNOSIS

2.5.1. Impression smear

Cytology with methylene blue staining revealed degenerated neutrophils with intracellular cocci in all the 17 dogs studied (Krick and Scott, 1989).

Adhesive tape technique with acridine orange staining was used to demonstrate organisms under a fluorescent microscope in the diagnosis of bacterial dermatitis (Mason and Lloyd, 1989).

Cytological examination of pus from lesions revealed suppurative to pyogranulomatous inflammation wherein the majority of neutrophils were degenerated and may contain phagocytosed gram positive cocci (Scott *et al.*,1994).

Impression smears taken from the nasal pyoderma stained with Diff-Quick stain showed numerous eosinophils and neutrophils and no bacteria (Curtis *et al.*, 1995).

Cytopathological examination of intact papule or pustule revealed 3 to 25 neutrophils, 2 to 40 extracellular and 3 to 5 intracellular coccoid bacteria per field. These bacteria had cellular morphology consistent with *Staphylococcus spp* (Bloom and Rosser, 2001).

Craig (2003) diagnosed impetigo from the history, clinical features and was confirmed with stained smear examination and culture of pustular exudates. He also suggested that presence of intracellular cocci were more likely to indicate

active bacterial infection than free extracellular bacteria which may simply be contaminants.

Seena *et al.* (2005) suggested that impression smears can be used for quick and easy confirmation of canine pyoderma.

Bacteria were detected in cytological samples from six of the twelve dogs in which rods were seen in four dogs, cocci in one dog, rods with cocci in one dog (Hillier *et al.*, 2006).

2.5.2. Histopathology

Biopsy results from folliculitis, furunculosis, and cellulitis supported bacterial etiology in 8 cases out of the 17 cases studied (Krick and Scott, 1989).

Skin biopsy with Hematoxylin and Eosin staining revealed several subcorneal pustules, degenerative neutrophils, gram positive cocci, exocytosis of neutrophils and eosinophils in a case of superficial pyoderma (Medleau *et al.*, 1991).

The cellular changes in acute pyoderma included folliculitis and perifolliculitis with infiltration of neutrophils and a few macrophages. (Aujila *et al.*, 1997)

Craig (2003) suggested a 6 mm biopsy punch for providing adequate specimen for histopathology studies. He also suggested that skin should not be aseptically prepared for biopsy as this may remove surface pathology and produce misleading results.

Histopathology of dogs with deep pseudomonal pyoderma was characterized by reverse perforating suppurative folliculitis and furunculosis (Hillier *et al.*, 2006)

Skin biopsy and histology are used to diagnose solar dermatitis, in which secondary bacterial folliculitis is a common phenomenon (Coyner, 2007).

2.5.3. Culture examination

Chandrasekhar *et al.* (2001) described that coagulase positive staphylococci were more prevalent than coagulase negative staphylococci in culture examination of samples from canine pyoderma.

Pardis *et al.* (2001) opined that lesion need not be scrubbed or disinfected prior to obtaining a sample for culture examination.

Craig (2003) suggested that exudates from a freshly ruptured pustule were applied to a sterile swab immersed in transport medium and sent for antibiotic sensitivity testing for diagnosis and treatment of bacterial dermatitis.

Senturk *et al.* (2005) suggested that lesions were not disinfected prior obtaining sample for bacterial culture.

There was no significant differences in the ability to culture *Staphylococcus intermedius*. It does not depend on the the number of *Staphylococcus intermedius* isolates with no-resistance to antimicrobials used or to the number of *Staphylococcus intermedius* isolates resistant to Penicillin G, between dogs with first time pyoderma and dogs with recurrent pyoderma, between dogs that did or did not receive concurrent antimicrobials or between dogs with and without an underlying allergic diseases (White *et al.* , 2005).

The degree of bacterial growth *in vitro* was described as light in 11 dogs, moderate in 6 dogs and heavy in 3 dogs in their 20 cases of pyoderma caused by *Pseudomonas aeruginosa* (Hillier *et al.*, 2006).

Stegemann *et al.* , (2007) confirmed bacterial infection by culture of sample taken before first treatment on Day zero, to determine the efficacy of cefovecin in the treatment of bacterial skin infections in dogs.

2.5.4. Antibiogram

Krick and Scott (1989) opined that antibiotic therapy based on results of culture and antibiotic sensitivity testing had no apparent advantage in terms of the number of relapses following initial therapy and in the number of sustained remissions, as compared to antibiotic therapy selected empirically.

Clavulanate – potentiated amoxicillin @ 26.25mg/lb twice daily for 21 to 28 days was found to be very effective in deep pyoderma (Runte, 1989).

The *in vitro* culture and sensitivity test showed that the bacterial isolates from pyoderma cases were mainly sensitive to penicillin, ampicillin and gentamicin (Khosla *et al.*, 1991).

Scott *et al.* (1994) reported that 90.5 per cent of this isolates were susceptible to tylosin *in vitro*. They also found that *Staphylococcus intermedius* isolates showed *in vitro* susceptibility to erythromycin in dogs that had received prior, usually multiple, systemic antibiotics.

All the staphylococcal isolates were sensitive to cephalixin, chloramphenicol, aminoglycosides (Kamboj *et al.* ,1995).

Antimicrobial drug sensitivity showed that gentamicin and doxycycline were most effective against all strains of *Staphylococci* and *Streptococci* and were resistant to ampicillin, penicillin, bacitracin and polymixin –B (Aujila *et al.*, 1997).

Scott *et al.* (1998) reported that more than one strain of *Staphylococcus intermedius*, which have different patterns of antibiotic susceptibility can exist on a dog's skin.

A strain of *Staphylococcus intermedius* showed resistance to marbofloxacin at day 28, showed no resistance to marbofloxacin at day 0 (Carlotti *et al.*, 1999).

Enterococci, *Staphylococcus aureus* and *Staphylococcus epidermidis* were resistant to first generation cephalosporins (Mason and Kietzman, 1999).

The incidence of antimicrobial resistance was highest for Penicillin G (44 per cent of isolates) in their study (White *et al.*, 2005).

Hillier *et al.* (2006) reported that sulphadiazine/ trimethoprim showed 100 per cent resistance, enrofloxacin 40 per cent resistance, ticarcillin 36 per cent resistance, ciprofloxacin 25 per cent resistance, neomycin six per cent resistance, and gentamicin, amikacin and norfloxacin (each five per cent resistance) and no isolate showed resistance to polymyxin B, ceftazidime and marbofloxacin in antibiogram.

Staphylococcus isolates from a case of infectious dermatitis was mainly sensitive to ciprofloxacin, ampicillin and gentamicin (Kumar *et al.*, 2006).

Cefovecin demonstrated a broad spectrum *in vitro* activity against *Staphylococcus* and *Streptococcus* species. Susceptibility of the most frequently isolated pathogens (*Staphylococcus intermedius*, *Escherichia coli*, hemolytic *Streptococci*, *Pasteurella multocida* and *Proteus spp*) was high, and the data was similar in all the four countries (France, UK, Germany and Spain) in which the studies were conducted (Stegemann *et al.*, 2007).

Out of the ten different antibiotics tested, highest sensitivity was noticed with quinolone group (Vijayakumar and Smitha, 2010).

2.5.5. Molecular techniques

Sauliner *et al.*, (1993) found that Pulse field gel electrophoresis is more discriminating than Random amplified polymorphic DNA for the typing of methicillin resistant *Staphylococcus aureus*.

Strains of *Staphylococcus intermedius* from dogs, pigeons, horses and mink were typed by comparison of rRNA gene restriction fragment length polymorphisms (ribotyping) and the resulting ribotypes examined by cluster-analysis. The data might suggest that pigeons and horses carry *Staphylococcus intermedius* transiently, and might be able to spread these bacteria to other animal species (Hesselbarth and Schwarz, 1995).

A study was conducted to develop rapid multiplex PCR assays for the detection of clinically relevant antibiotic resistance genes in staphylococci and the identification of the staphylococcal species and to compare those PCR assays with standard microbiological methods for susceptibility testing and microbial identification. In this study, a panel of 206 strains of *Staphylococcus aureus* and 188 strains of *Staphylococcus epidermidis* from various sources were tested by conventional susceptibility testing methods as far as by PCR for the antibiotic resistance genes *mecA*, *aac(6')-aph(2'')*, *ermA*, *ermB*, *ermC*, and *msrA*. (Martineau *et al.*, 2000).

As species identification of coagulase negative *Staphylococcus* by fatty acid analyses and biochemical tests were difficult, Enterobacterial repetitive intergenic consensus and BOX PCR seem to be excellent tools for identification of *Staphylococcus epidermidis* (Wieser and Busse, 2000).

A total of 41 *Staphylococcus intermedius* isolates were isolated from skin of healthy members of six phylogenetic groups within the canoidea family from different geographical origin and compared by EcoRI ribotyping and cluster analysis. These ribotype data indicate host-specificity of different types of *Staphylococcus intermedius* and gave possible evidence for host-specificity and co-evolution of bacteria and hosts (Aarestrup, 2001).

Chaieb *et al.*, (2005) studied the antibiotic susceptibility and genotype of strains of *Staphylococcus epidermidis* by ERIC-PCR analysis and PFGE in order to predict their clonality.

Francois *et al.*, (2008) described pulse field gel electrophoresis (PFGE) and multilocus variable number tandem repeat based assay (MLVA) for rapid genotyping of *S. epidermidis* isolates.

Partial *atlE* sequencing (*atlE* nucleotides 2782 to 3114 [*atlE*₂₇₈₂₋₃₁₁₄]) was performed in 41 *Staphylococcus epidermidis* isolates from prosthetic joint infections and 44 isolates from skin as controls. The *atlE*₂₇₈₂₋₃₁₁₄ allele 1 (type strain sequence) was significantly more frequent from prosthetic joint infection strains (Sivadon *et al.*, . 2009)

2.6. TREATMENT

2.6.1. Systemic therapy

Dogs with superficial pyoderma frequently require at least 3 weeks of therapy and should be given antibiotics for 5 days beyond clinical cure (Ihrke, 1984).

Bywater *et al.* (1985) reported that the animals treated with amoxicillin combined with clavulanate showed more rapid resolution of skin lesions than those animals treated with amoxicillin alone.

Ihrke (1987) stated that most superficial pyoderma required about 2 weeks of systemic antibiotics, and the duration of deep pyoderma is highly variable and it require long term therapy. The author reported more success with oxacillin than with other antibiotics.

Scott *et al.* (1989) found that penicillin, oxytetracycline and ampicillin are poor choices for treating canine *Staphylococcal* pyoderma.

Glucocorticoids are contraindicated in the treatment of canine pyoderma (Mason, 1991).

Scott *et al.* (1994) reported complete resolution of infection in 19 of 20 dogs in response to treatment with tylosin for an average of 33 days. Response to treatment was not influenced by age, sex, duration of disease, distribution of lesions, depth of infection, concurrent dermatoses or previous antibiotic therapy.

Kamboj *et al.* (1995) suggested that in case of mixed infections, the use of antibiotics for the elimination of *Staphylococcus intermedius* is sufficient because it is usually the primary pathogen and creates an environment favourable to the growth of other bacteria.

Antibiotics with bacteriostatic effect are suitable for superficial pyoderma. Antibiotics with bacteriocidal effect are the drug of choice for cases of recurrent deep pyoderma and for weakened individuals (Lloyd and Grant, 1996).

Clindamycin hydrochloride @11mg/kg body weight once daily orally showed excellent response to canine pyoderma (Scott *et al.*, 1998).

Excellent response to marbofloxacin @ 2.12mg/kg body weight for an average of 55 days in treatment of canine pyoderma was shown by 84.6 per cent of the dogs. (Carlotti *et al.*, 1999)

Ihrke *et al.* (1999) reported that excellent result of different fluoroquinolones in treatment of canine pyoderma.

Gatifloxacin is a new C-8 methoxy fluoroquinolone, which can produce bacteriostatic action against gyrase mutants in presence and absence of protein synthesis. Its efficacy was proved by Lu *et al.* (1999).

The best known cephalosporin which is used in pyoderma therapy is cephalexin @ 15-30mg/kg body weight twice daily for 3 to 4 weeks (Mason and Kietzman, 1999).

Bloom and Rosser (2001) reported that clindamycin hydrochloride @11mg/kg body weight, once daily for 14-42 days was effective in the treatment of superficial pyoderma.

Scott *et al.* (2001) recommended a minimum of 4-6 weeks of antimicrobial treatment for deep pyoderma. They also recommended the use of immunomodulators like cimetidine and levamisole in the treatment.

Marbofloxacin, a broad spectrum antibiotic developed exclusively for use in animals was found to be very effective in the treatment of canine pyoderma @ 2.75mg/kg body weight once daily for 21-28 days. It get distributed widely throughout the body reaching 1.6 times higher in the skin than in the plasma of dogs (Pardis *et al.* , 2001).

Oral administration of ibafloxacin @ 15mg/kg body weight and marbofloxacin @ 2mg/kg body weight once daily for 3-16 weeks produced similar results in treatment of canine pyoderma under field conditions (Horspool *et al.* , 2004).

Seena *et al.* (2005) suggested that the duration of time required for complete clinical cure in surface pyoderma was 2-4 weeks, superficial pyoderma was 2-3 weeks and in deep pyoderma it was 3-4 weeks.

Senturk *et al.* (2005) reported that use of rifampicin @ 5 mg/kg bodyweight for 10 days was considered successful in 18 out of 20 dogs with bacterial dermatitis.

Udayasree and Usha (2006) used ciprofloxacin @10mg/kg body weight orally once daily for 7 days and enrofloxacin @ 5 mg/kg body weight orally once daily for 21 days successfully in treatment of canine pyoderma.

Hovarth and Neuber (2007) suggested that some antibiotics like penicillin, amoxicillin, streptomycin and ampicillin were unsuitable for treating pyoderma as they did not attain therapeutic concentrations in skin.

Mueller and Stephen (2007) opined that as fluoroquinolones accumulate in inflammatory cells, the severe inflammation associated with deep pyoderma may permit a high local concentration of pradofloxacin in inflamed tissue and a more complete elimination of bacterial organisms.

Administration of cefovecin at 14 day as subcutaneously was highly effective in the treatment of both superficial and deep pyoderma. (Stegemann *et al.*, 2007).

Diesel and Moriello (2008) recommended cyclosporine @ 2.5-5 mg/kg body weight as a new option in the treatment of atopic dermatitis.

Once daily cephalexin @30 mg/kg body weight orally was found to be equally effective as twice daily cephalexin @15 mg/kg body weight in the treatment of canine superficial pyoderma (Toma *et al.*, 2008).

2.6.2. Topical therapy

Benzoyl peroxide, organic iodine compounds, chlorhexidine are able to kill *Staphylococcus intermedius* on the skin (Kwochika and Kowalski, 1991).

Topically administered antibacterials assist with bacterial killing in the superficial layers of the skin where antibiotic concentration was lower than those in well vascularised areas (Hill and Moriello, 1994).

Additional therapy of pyoderma includes bathing the animal with antimicrobial shampoo 3 times per week with a contact time of 10 minutes and localized application of mupirocin at 12 hour interval (Dowling *et al.*, 1996).

Topical treatment of canine skin with fusidic acid, a steroid –like antibiotic, originally isolated from the fungus *Fucidium coccineum*, could offer an additional tool against canine pyoderma , combined with traditional forms of therapy (Kolumies *et al.*, 1998).

Craig (2003) suggested that systemic antibiotics were not commonly needed except in case of intercurrent infections. They also reported that washing the dog once or twice weekly with antibacterial shampoos accelerated healing and ideal contact time for shampooing is around 10 minutes.

Ethyl lactate hydrolysed to ethanol and it lowers skin pH, thus it has similar action to Benzoyl peroxide (de Jaham, 2003).

Superficial pyoderma caused by *Pseudomonas aeruginosa* showed 100 per cent resolution after treatment with 3 per cent silver sulfadiazine cream for 3 weeks twice daily (Hilier *et al.*, 2006).

Hovarth and Neuber (2007) advised a life long shampoo therapy to avoid recurrence of pyoderma. They also suggested that Mupirocin has good activity against gram positive cocci, is bacteriostatic, rather than bactericidal and is not systemically absorbed.

Materials and Methods

3. MATERIALS AND METHODS

The present study was carried out in the Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary and Animal Sciences, Mannuthy for a period of one year during February 2009 to January 2010.

3.1. MATERIALS FOR STUDY

Dogs presented to veterinary teaching hospitals of Mannuthy and Kokkalai were included in the study. Forty dogs with clinical signs suggestive of bacterial dermatitis were subjected to detailed clinical examination and bacteriological examination. Thirty two confirmed cases were included in the study. The prescribed proforma was filled up after collecting detailed information such as the appearance of lesion, its spread to contact animals and human beings, feeding practices, vaccination and deworming schedule, bathing and grooming practices, method of disinfection of kennel, presence of ectoparasites etc. as suggested by Muller *et al.* (1989).

3.2. CLINICAL EXAMINATION

Thorough clinical examination of the dogs showing dermatological problems was carried out and examined for the presence of lesions suggestive of bacterial dermatitis, pruritus, hair loss and presence of ectoparasites.

3.3. COLLECTION OF CLINICAL MATERIAL

Sterile skin swab, blood samples from dogs suggestive of having bacterial dermatitis were collected for laboratory examination.

3.3.1. Method for Collection of Material for Bacterial Culture

Before collecting samples, hair around the lesions was clipped and swabbed with 70 per cent alcohol and air dried. A touch swab was collected using sterile swab kept in sterile tubes. In case of pustules, after swabbing with

70 per cent alcohol they were opened using sterile scalp blade or needle and touch swab from exudate was collected. In cases of crusty lesions, crust or scab was lifted and touch swab collected from beneath the crust or scab.

3.4 ISOLATION AND IDENTIFICATION OF BACTERIA

3.4.1. Glassware and Reagents

Borosil, Qualigens brand of glass ware, analytical grade of chemicals, reagents, culture media (Hi –Media) were used for the study.

3.4.2. Preparation of Glassware and Culture Media

The petri dishes and test tubes were kept in 0.1 per cent hydrochloric acid overnight. They were washed in running tap water and immersed in detergent solution for one day. The petri dishes and test tubes were thoroughly washed with running tap water followed by distilled water. The glassware were dried and sterilised in hot air oven at 160 °C for one hour.

The culture media was reconstituted in double distilled water according to the manufacturer's instructions. It was then sterilized by autoclaving at 121 °C, 15 lbs pressure for 15 minutes. It was cooled to 45 °C, poured to sterile petri dishes and test tubes and incubated at 37 °C for 24 hours to test the sterility.

3.4.3. Isolation of Bacteria

The swabs were put in sterile phosphate buffered saline (pH 7.2), mixed well and a drop of the inoculum was streaked aseptically on sterile nutrient agar plates. These plates were incubated at 37 °C for 24 to 48 hours.

Single colonies were selected for preparing pure culture and used for further identification. Pure cultures were preserved on nutrient agar slants by at 4 °C and the organisms were subcultured at 2 week interval.

3.4.4. Identification of Bacteria

The isolates were stained by Gram's Method and the preliminary tests were done based on it. The morphological, cultural, biochemical and sugar fermentation tests of the isolates belonging to different species were determined as per the methods described by Cowan (1974).

The culture media used in present study are

1. Nutrient agar
2. Mueller Hinton agar
3. Peptone water
4. Brain heart infusion broth

Selective media used in the present study are

1. Mannitol salt agar
2. Blood agar
3. Eosin methylene blue agar
4. McConkey agar
5. Starch agar
6. Baird Parker agar
7. Edwards medium

The biochemical tests employed for the identification of the isolates were

1. Catalase test
2. Oxidase test
3. Oxidation- fermentation test
4. Carbohydrate utilization test
5. Motility test
6. Nitrate reduction test
7. Methyl red test
8. Voges-Proskeur test

9. Indole production test
10. Citrate utilization test
11. Urease production test
12. Triple sugar iron agar test
13. Coagulase test (Plate. 7)

3.5. ANTIBIOGRAM

3.5.1. Materials

Mueller –Hinton agar (MHA) was used to study the antibiotic sensitivity pattern of the isolates. The following antibiotic discs with known concentration as noted in micrograms (mcg) were used (Hi-Media).

| | | |
|------------------|------------------|-------------|
| 1. Amoxyclav | Ac ³⁰ | 30 mcg/disc |
| 2. Ciprofloxacin | Cf ³⁰ | 30 mcg/disc |
| 3. Clindamycin | Cd ² | 2 mcg/disc |
| 4. Cephalexine | Cp ³⁰ | 30 mcg/disc |
| 5. Erythromycin | E ¹⁵ | 15 mcg/disc |
| 6. Gatifloxacin | Gf ³⁰ | 30 mcg/disc |

3.5.2. Method

In vitro antibiotic sensitivity of the organisms were studied using disc diffusion technique (Barry, 1976). Five colonies of each pure culture were picked up with sterile nichrome loop and were used as the inoculum in four milliliter of sterile peptone water and kept at room temperature for 4 hours to develop turbidity. Inoculum was applied uniformly on the surface of MHA, using a sterile cotton swab and the plate kept covered for 15 minutes at room temperature for drying the inoculum. Antibiotic discs were then placed on the surface of the agar 20 mm apart and they were gently pressed on the surface of the agar to ensure contact. The plates were incubated at 37⁰C for 18 to 24 hours.

3.5.3. Interpretation

The zone of inhibition of bacterial growth around each disc was measured and interpreted as sensitive, moderately sensitive or resistant by comparing with the ranges given by manufacturer.

3.6. COLLECTION OF BLOOD FOR HAEMATOLOGICAL EXAMINATION

About three milliliter of blood was collected from cephalic vein of affected dogs in sterile syringes containing EDTA as anticoagulant at the rate of 1 mg/ml of blood and specimen examined on the same day.

A drop of blood was collected on clean grease free slide to prepare a blood smear (Benjamin, 1985).

3.6.1. Haematological Parameters

The following haematological parameters were estimated.

3.6.1.1. Haemogram

3.6.1.1.1. Haemoglobin (Hb)

Haemoglobin was estimated by acid-haematin method using Sahli's haemoglobinometer and expressed as gram percentage (Jain, 1986).

3.6.1.1.2. Packed Cell Volume (PCV)

Packed cell volume was estimated by Wintrobe's method as per Coles (1986) and expressed as per cent.

3.6.1.1.3. Total Erythrocyte Count

Erythrocytes were counted using Haemocytometer and Hayem's fluid as detailed by Wintrobe *et al.* (1981)

3.6.1.2. Leucogram

3.6.1.2.1. Total Leucocyte Count (TLC)

Total leucocyte count was estimated using Thoma's fluid as per Coles (1986) and value expressed as 10^3 cells/mm³ of blood.

3.6.1.2.2. Differential Leucocyte Count (DLC)

Blood smear was stained by Leishman's stain and 100 leukocytes were counted under oil immersion objective and differential counts were expressed as percentage (Benjamin, 1985).

3.7. METHOD OF COLLECTION OF BLOOD FOR BIOCHEMICAL EXAMINATION

Five milliliter of blood was collected in a test tube for separating serum. The separated serum, after slow centrifugation at 3000 rpm for ten minutes without disruption of clot, was transferred to a serum vial. Sera thus separated were stored at -20⁰C till further analysis.

Disposable clean micropipette tips were used to draw serum from the vials for various biochemical estimations.

Capillary blood from the ear tip was used for blood glucose estimation of affected dogs with glucometer (Ez -Smart -168 Blood glucose monitoring system) as per the manufacturer's instruction.

3.7.1. Biochemical Examination

Biochemical estimations of serum total protein, albumin, globulin, albumin globulin ratio, cholesterol, serum calcium, iron, copper, zinc, were carried out on stored serum samples.

3.7.1.1. Total Protein and Albumin

Serum total protein was estimated by modified Biuret method described by Weichselbaum (1946), while albumin was estimated by bromcresol green dye binding method as described by Doumas *et al.* (1971). Total protein and albumin were estimated by spectrophotometry in Merck 200 spectrophotometer using commercially available kits (Agappe Diagnostics). Serum globulin and A/G ratio were calculated from the above obtained values.

3.7.1.2. Cholesterol

Serum cholesterol was estimated by cholesterol oxidase peroxidase method. It was estimated colorimetrically by spectrophotometry in Merck 200 spectrophotometer using commercially available kits (Agappe Diagnostics) (Bruss, 1997).

3.7.1.3. Serum Calcium

Serum calcium was estimated using Atomic Absorption Spectrometer (Perkin-Elmer 3110) at wavelength of 422.7 nm.

3.7.1.5. Serum Iron

Serum Iron was estimated using Atomic Absorption Spectrometer (Perkin-Elmer 3110) at wavelength of 248.3 nm.

3.7.1.6 Serum Zinc

Serum Zinc was estimated using Atomic Absorption Spectrometer (Perkin-Elmer 3110) at wavelength of 213.9 nm.

3.7.1.4. Serum Copper

Serum Copper was estimated using Atomic Absorption Spectrometer (Perkin-Elmer 3110) at wavelength of 324.8 nm.

3.8. CLINICAL CASES

The cases were divided into four treatment groups. Gatifloxacin, amoxicillin-clavulanic acid, cephalixin and clindamycin hydrochloride were used in each treatment group. Animals were allotted to each group randomly. On the day of presentation, the clinical samples were collected aseptically with sterile swab and subjected to culture and sensitivity tests. Cases reviewed after 72 hours and changes in the therapy made in required cases.

Treatment response was evaluated at weekly intervals for three weeks in the course of treatment and score was assigned according to Bloom and Rosser (2001).

3.8.1. Group I

Treated with Gatifloxacin @ 5mg/kg body weight once daily for 14 days (Gatiquin 200mg, 400mg).

3.8.2. Group II

Treated with Amoxicillin-clavulanic acid combination @ 12.5 mg/kg body weight twice daily orally for 14 days (Clavet 250mg, 500 mg).

3.8.3. Group III

Treated with Cephalixin @ 25 mg/kg body weight once daily for 14 days (Lixen pet dry syrup 250mg/5ml).

3.8.4. Group IV

Treated with Clindamycin-hydrochloride @ 11 mg/kg body weight for 14 days (Clinvet oral suspension 25mg/ml).

-
1. Gatiquin 200mg, 400mg - Cipla Ltd., Vijayawada
 2. Clavet 250mg, 500 mg - Cipla Ltd., Vijayawada
 3. Lixen pet 38.4 mg/60 ml -Virbac Animal Health India Pvt. Ltd. Mumbai
 4. Clinvet oral suspension 20 ml - Cipla Ltd., Vijayawada

3.9. STATISTICAL ANALYSIS

Epidemiological data were collected as per proforma and analyzed (Snedecor and Cochran, 1994). In all the clinical trials, efficacy was assessed based on observation of the clinical improvement and culture results.

3.10. RIBOTYPING PCR ANALYSIS

3.10.1. DNA Isolation

3.10.1.1. Reagents

1. Tris phosphate EDTA glacial acetic acid buffer (TAE buffer) (x 50, pH 8.0)
 - Tris base - 242 g
 - Glacial acetic acid - 57.1 ml
 - 0.5 M EDTA solution – 100 ml
 - Milli Q water -1000 ml
 - Working solution:
 - Stock solution (50x) - 10 ml
 - Triple distilled water - 490 ml
2. Lysozyme (Sigma-Aldrich)
 - Bacterial lysosome -10 mg/ml
3. Proteinase K (Sigma- Aldrich)
 - Proteinase K - 20 mg/ml

3.10.1.2. Procedure (Kaliya *et al.* , 1999)

1.The staphylococcal isolates were grown overnight on Trypticase soy agar and a pure colony was inoculated into Trypticase soy agar slant and kept at 37⁰ C for 24 hours. It was then inoculated into Nutrient broth for incubated at 37⁰ C for 18 hours in shaking incubator.

2. Centrifuged 1.5 ml of culture in nutrient broth at 10000 rpm for 1 minute.
3. Resuspended in 150µl TAE buffer (x1)

4. Added 10 μ l of lysosyme for lysis and incubated at 37⁰ C for 30 minutes.
5. Suspension was boiled for 10 minutes.
6. Centrifuged at 5000 rpm for 5 minutes.
7. Supernatant was transferred.
8. 5 μ l of proteinase K was mixed with supernatant by vortex.
9. Incubated at 37⁰ C for 30 minutes.
10. Suspension was boiled for 1:5 minutes to stop enzyme activity.
11. Centrifuged at 10000 rpm for 1 minute and transferred the supernatant.
12. Extracted DNA stored at -80⁰C until PCR was performed.

3.10.2. Ribotyping

3.10.2.1. Reagents (Genei Bangalore)

1. Forward primer (0.2 μ M)

5' TTGTACACACCGCCCGTCA 3'

2. Reverse primer (0.2 μ M)

5' GGTACCTTAGATGTTTCAGT 3' (Primers were selected as

described by Jensen *et al.* (1993)

3. Buffer x10

4. Taq DNA polymerase

Taq DNA polymerase enzyme with a concentration of 5U/ μ l was used.

5. Magnesium chloride (25mM)

6. Deoxyribonucleotide triphosphate (dNTP)

dNTP of 200 μ M was used.

7. Distilled water

8. DNA Molecular Size Markers

A Gene Ruler TM 1 kilobase pair DNA ladder 0.5 μ g/ μ l was used (Fermentas)

3.10.2.2. Reconstitution of reagents

The Ribotyping PCR was performed in a total volume of 25 μ l

| | |
|-------------------|--------------|
| Forward primer | 0.5 μ l |
| Reverse primer | 0.5 μ l |
| dNTP | 0.5 μ l |
| MgCl ₂ | 1.5 μ l |
| Buffer | 2.5 μ l |
| Taq polymerase | 0.2 μ l |
| Distilled water | 17.3 μ l |

3.10.2.3. Preparation of Mastermix for seven samples and control

| | |
|-------------------|---------------|
| Forward primer | 4 μ l |
| Reverse primer | 4 μ l |
| dNTP | 4 μ l |
| MgCl ₂ | 12 μ l |
| Buffer | 20 μ l |
| Taq polymerase | 1.6 μ l |
| Distilled water | 138.4 μ l |

3.10.2.4. Ribotyping PCR

To each PCR tube 23 μ l of mastermix and 2 μ l of template DNA were added. The PCR amplification was carried out in an automated thermal cycler (Applied Biosystems, Germany) for 2 hrs.

3.10.3. Detection of PCR Products by Electrophoresis

The amplified ribo PCR products were detected by electrophoresis in 1.5 per cent agarose gel in TAE buffer.

3.10.3.1. Reagents

| | |
|------------------------------|-----------|
| 1. TAE buffer (x 50, pH 8.0) | |
| Tris base | - 242 g |
| Glacial acetic acid | - 57.1 ml |
| 0.5 M EDTA solution | - 100 ml |

Milli Q water -1000 ml

Working solution:

Stock solution (50X) - 10 ml

Triple distilled water - 490 ml

2. Ethidium bromide solution

A stock solution of 5mg/ml ethidium bromide was prepared using TAE buffer working solution. Ethidium bromide working solution was prepared in the concentration range of 0.5 mg/ml using TAE buffer working solution stored at 4⁰C in amber coloured bottles.

3. Agarose gel 0.375 g

TAE buffer 25 ml

4. Loading dye

6x DNA loading dye was used (Fermentas)

3.9.3.2. Procedure

Agarose gel electrophoresis was carried out in horizontal submarine electrophoresis unit (Genei Bangalore). Agarose (1.5 per cent) was dissolved in TAE buffer by heating and cooled to 50⁰C. To this ethidium bromide was added at a final concentration of 0.5 µg/ml. The gel was placed in the gel tray in buffer tank after a period of 30 minutes for solidification of the gel. TAE buffer was added until it covered the top of the gel completely. Fifteen microlitre of the PCR product was mixed with three microlitre of loading dye and samples were loaded into respective wells carefully and 1 kilobase pair molecular marker was loaded into the last well.

Electrophoresis was carried out at room temperature at 60 V for 40-45 minutes depending on the length of the gel or until the dye had migrated more than half the length of the gel. The gel was visualized under UV transilluminator (Fotodyne, USA) and the results were documented in a gel documentation system (Alpha Innotech, USA).

Results

4. RESULTS

A total of 7771 dogs were brought to University Veterinary teaching Hospitals Mannuthy (3034) and Kokkalai (4737) from February 2009 to January 2010 with different clinical illness. Of these 933 animals presented with dermatological problems in the clinics, and 127 animals were treated for bacterial dermatitis. Bacterial isolation was attempted from skin lesions in 45 cases and growth could be observed in 42 animals. Among the infected group, 32 animals were selected at random and divided into four groups each consisting of eight animals and the efficacy of four antibiotics were evaluated.

4.1. OCCURRENCE

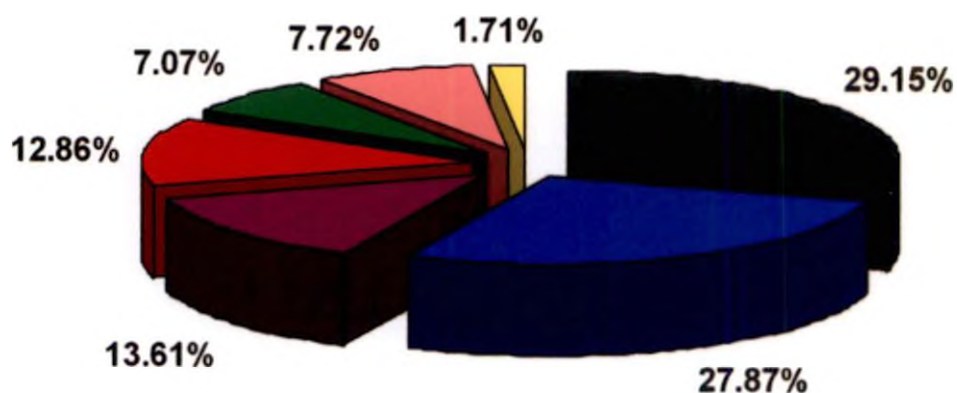
Dermatological problems constituted 12.06 per cent in the dogs presented to University Veterinary Hospitals, Mannuthy and Kokkalai with various clinical illness. It includes ectoparasitic infestation, fungal dermatitis, bacterial dermatitis, nutritional dermatoses, malassezia dermatitis, demodecosis and dermatitis due to other reasons (Table 1 and Fig. 1).

The overall occurrence of bacterial skin infections in the present study was 13.61 per cent of the total cases presented with dermatological problems. This accounted for 1.63 per cent of the total canine cases presented in the two University Veterinary hospitals at Mannuthy and Kokkalai.

4.1.1. Age wise Occurrence

The highest occurrence was noticed in dogs of one to three years of age (42.85 per cent) followed by one month to six months of age (23.83 per cent), six months to one year of age (14.28 per cent), one year to six years of age and above six year (9.52 per cent each) among the 42 infected animals (Table 2 and Fig. 2)

Fig. 1. Occurrence of dermatological problems in dogs presented to University Veterinary Hospitals during February 2009 to January 2010



- Ectoparasitic infestation
- Fungal dermatitis
- Bacterial dermatitis
- Nutritional dermatitis
- Demodecosis
- Malasseziosis
- Other reasons

Table 1. Occurrence of dermatological problems in dogs presented to University Veterinary hospitals during February 2009 to January 2010

| Dermatological problems | Number of cases | Per cent |
|--------------------------|-----------------|------------|
| Ectoparasite infestation | 272 | 29.15 |
| Fungal dermatitis | 260 | 27.86 |
| Bacteria dermatitis | 127 | 13.61 |
| Nutritional dermatitis | 120 | 12.86 |
| Demodicosis | 66 | 7.07 |
| Malassezia dermatitis | 72 | 7.71 |
| Other reasons | 16 | 1.74 |
| Total | 933 | 100 |

Table 2. Age wise occurrence of bacterial skin infections in dogs (n=42)

| Age groups | Number of infected animals taken for study | Per cent |
|---------------------|--|------------|
| 1 month to 6 months | 10 | 23.83 |
| 6 months to 1 year | 6 | 14.28 |
| 1 to 3 years | 18 | 42.85 |
| 3 to 6 years | 4 | 9.52 |
| Above 6 years | 4 | 9.52 |
| Total | 42 | 100 |

4.1. 2. Breed wise Occurrence

Highest occurrence was observed in German Shepherd (26.19 per cent), followed by Labrador Retriever (19.04 per cent), Rottweiler (9.51 per cent), non descript, Dachshund, Pug, Bull Mastiff (7.14 per cent each), Boxer, Spitz and Doberman (4.76 per cent each) and Dalmatian (2.42 per cent). (Table 3 and Fig. 4)

4.1.3. Month wise Occurrence

Bacterial skin infection was reported throughout the year. However more number of cases were brought to the hospitals during the month of April (16.67 per cent), followed by September (14.28 per cent), March (11.93 per cent), July, November (9.52 per cent each), February, August, May (7.14 per cent), October, December, January (4.76 per cent each) and June (2.38 per cent) (Table 4 and Fig. 3)

4.1.4. Sex wise Occurrence

Among the infected group of 42 animals, 24 animals were females (57.14 per cent) and 18 animals were males (42.86 per cent). (Table 5 and Fig. 5)

4.2. CLINICAL SIGNS

Majority of the animals brought with the complaint of pruritus (47.61 per cent) and alopecia (71.42 per cent). It varied from localized to generalised. Results of observed primary and secondary lesions are presented in the Table 6. Generalized lesions observed in 17 animals (40.48 per cent) and localized lesions in 25 animals (59.52 per cent).

4.3. MANAGEMENT

In infected group of 42 animals, 26 (61.90 per cent) were given bath regularly and 16 animals (38.10 per cent) were not given regular bath. Majority of the animals (85.72 per cent) of animals were kept in kennel and only 14.28 per cent were kept strictly indoors.

Table 3. Breed wise occurrence of bacterial skin infections in dogs (n=42)

| Breed | Number of infected animals | Per cent |
|--------------------|----------------------------|------------|
| German Shepherd | 11 | 26.19 |
| Labrador Retriever | 8 | 19.04 |
| Rottweiler | 4 | 9.51 |
| Dachshund | 3 | 7.14 |
| Pug | 3 | 7.14 |
| Bull Mastiff | 3 | 7.14 |
| Non descript | 3 | 7.14 |
| Boxer | 2 | 4.76 |
| Spitz | 2 | 4.76 |
| Doberman | 2 | 4.76 |
| Dalmatian | 1 | 2.42 |
| Total | 42 | 100 |

Table 4. Month wise occurrence of bacterial skin infections in dogs (n=42)

| Month | Number of infected animals | Per cent |
|---------------|----------------------------|------------|
| February 2009 | 3 | 7.14 |
| March | 5 | 11.93 |
| April | 7 | 16.67 |
| May | 3 | 7.14 |
| June | 1 | 2.38 |
| July | 4 | 9.52 |
| August | 3 | 7.14 |
| September | 6 | 14.28 |
| October | 2 | 4.76 |
| November | 4 | 9.52 |
| December | 2 | 4.76 |
| January 2010 | 2 | 4.76 |
| Total | 42 | 100 |

Table 5. Sex wise occurrence of bacterial skin infections in dogs (n=42)

| Sex | Number of infected animals | Per cent |
|--------------|----------------------------|------------|
| Female | 24 | 57.14 |
| Male | 18 | 42.86 |
| Total | 42 | 100 |

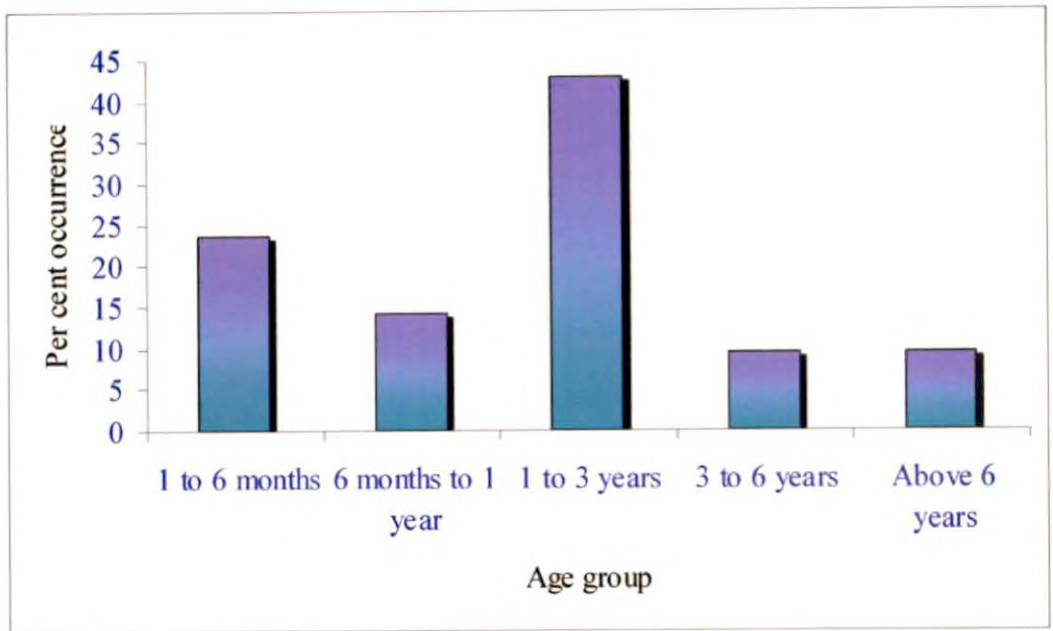


Fig. 2. Age wise occurrence of bacterial skin infection in dogs

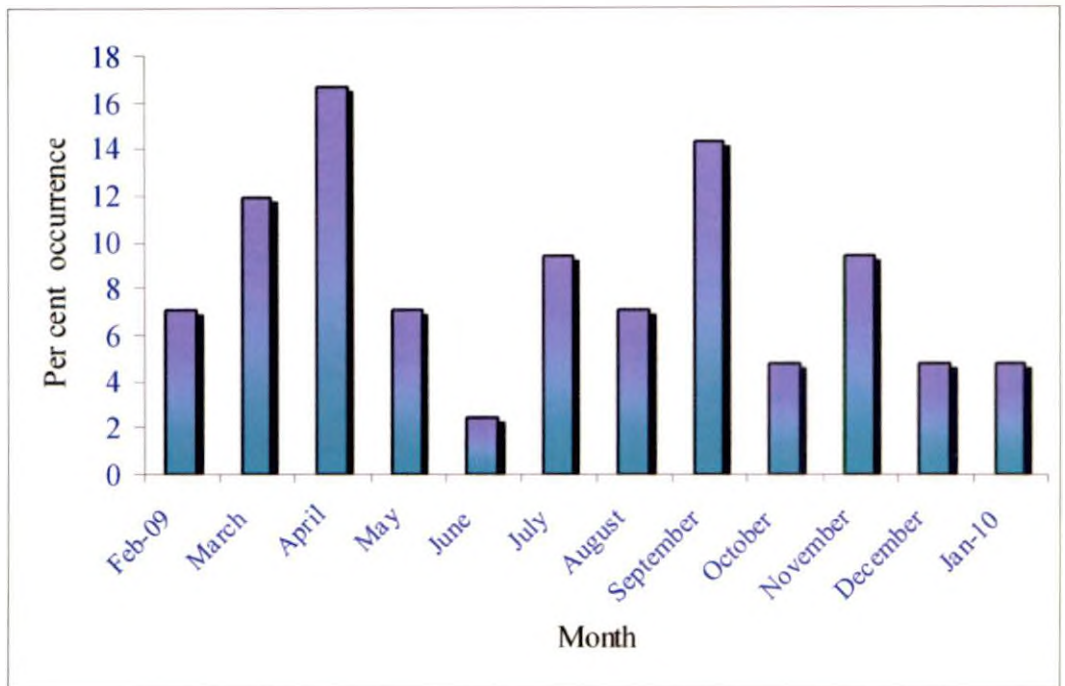


Fig. 3. Month wise occurrence of bacterial skin infection in dogs

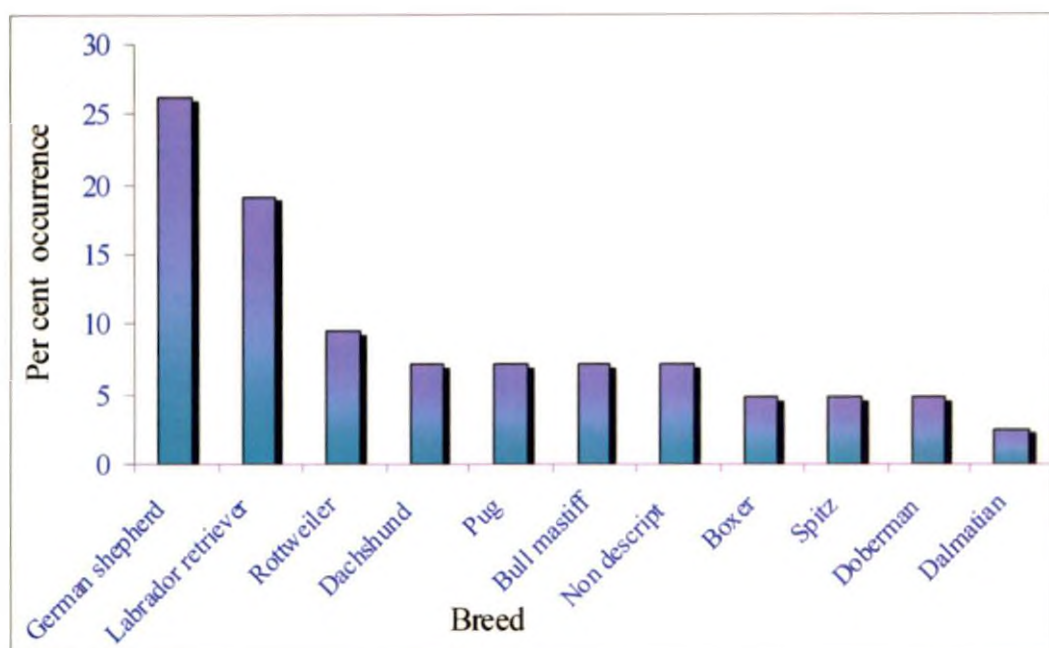


Fig. 4. Breed wise occurrence of bacterial skin infection in dogs

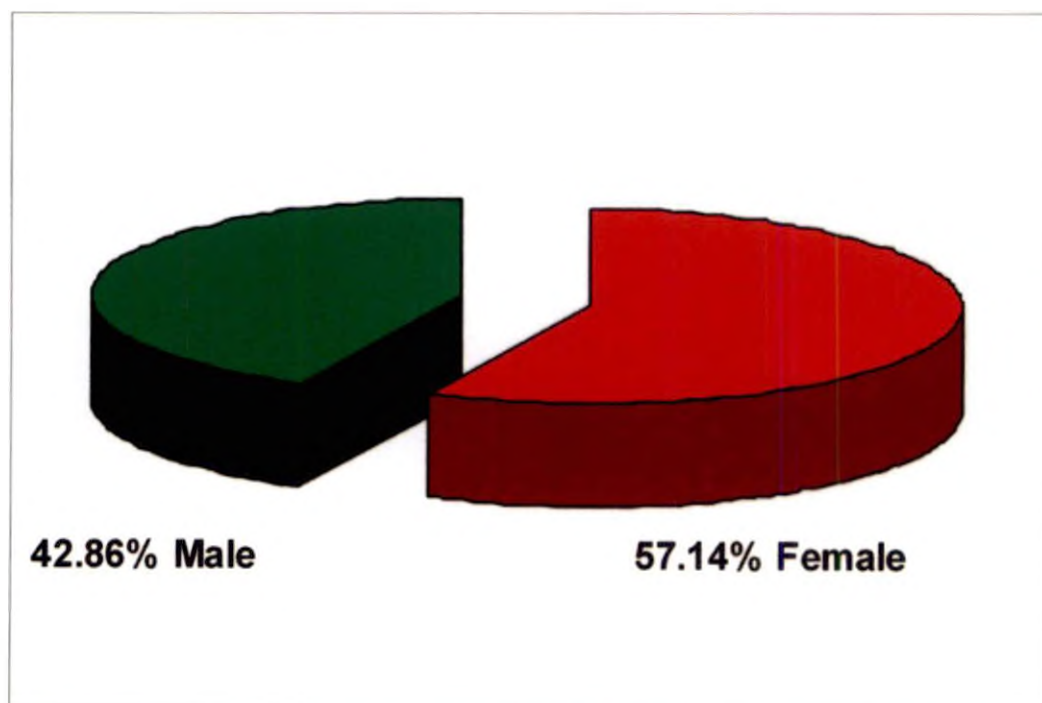


Fig. 5. Sex wise occurrence of bacterial skin infection in dogs

Table 6. Primary and secondary lesions in infected animals (n=42)

| Lesions | Number of animals | Per cent |
|--------------------------|-------------------|----------|
| 1.Primary lesions | | |
| Papule | 4 | 9.52 |
| Pustule | 1 | 2.38 |
| Nodule | 2 | 4.76 |
| Fistulous tract | 1 | 2.38 |
| Erythema | 10 | 23.80 |
| Alopecia | 30 | 71.42 |
| 2.Secondary lesions | | |
| Scale | - | - |
| Crust | 1 | 2.38 |
| Ulcer | 7 | 16.66 |
| Epidermal collarette | 3 | 7.14 |
| Erosion | - | - |
| Plaque | 1 | 2.38 |
| Hyper pigmentation | 1 | 2.38 |
| Lichenification | 1 | 2.38 |
| Pruritus | 20 | 47.61 |
| Pain | 4 | 9.52 |
| Keratinisation | 1 | 2.38 |
| Edema | 1 | 2.38 |
| Abscess | 1 | 2.38 |
| Contact animals affected | 2 | 4.76 |

Regular cleaning of kennel was practiced by 69.45 per cent of owners. Disinfectants/ chemicals/washing powder was used for cleaning kennel by 61.11 per cent of owners. Regular grooming was practiced only in 28.58 per cent of animals in the infected group. Ectoparasites were present in 11 animals (26.19 per cent). Majority of the animals in the infected group were dewormed and vaccinated regularly (66.7 per cent and 52.38 per cent respectively). (Table 7)

4.4. CULTURE AND SENSITIVITY TEST

4.4.1. Bacteriological Findings

Exudates, touch swab from beneath the scab and crust lesions of all suspected cases (45 cases) of canine bacterial dermatitis were collected on sterile swabs. They were cultured in nutrient agar plates and incubated for 24 hours at 37°C and stained by Gram's staining (Plate 1 and 2). Out of the 45 cases, bacterial growth was obtained in 42 cases. Among the 32 cases under detailed study, mixed cultures were obtained from two cases and the 34 isolates from 32 cases were subjected to identification.

Out of the 34 isolates obtained *Staphylococcus spp* (Plate 3 and 4) were most commonly isolated viz. *Staphylococcus epidermidis* (32.35 per cent), *Staphylococcus hyicus* (20.58 per cent), *Staphylococcus intermedius* (14.73 per cent), *Staphylococcus aureus* (8.82 per cent) (Plate 5, 6), *Micrococcus spp* (2.94 per cent), *Pseudomonas fluorescense* (2.94 per cent) (Plate 8A and 8B), *Pseudomonas aeruginosa* (11.76 per cent) and *Klebsiella pneumoniae* (5.88 per cent) were also obtained. Mixed cultures included *Staphylococcus epidermidis* and *Klebsiella pneumoniae* in one case and *Micrococcus spp* and *Klebsiella pneumoniae* in another case (Table 8 and Fig. 6).

4.4.2. Antibigram

In vitro antibiotic sensitivity studies of different isolates from 32 clinical cases of bacterial skin infections showed that *Staphylococcus epidermidis* had 100

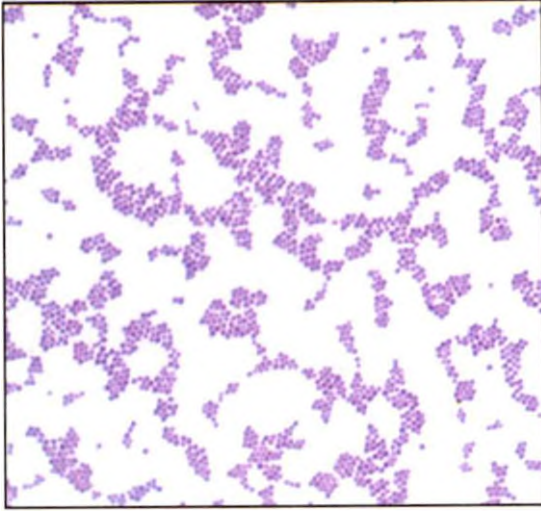


Plate 1. Microphotograph of *Staphylococcus spp*
(Gram staining x1000)

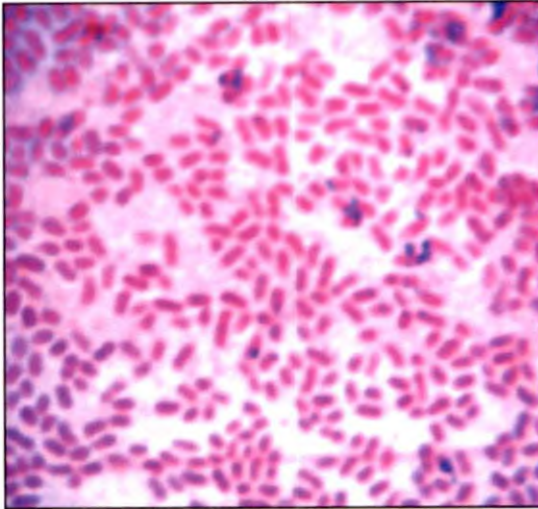


Plate 2. Microphotograph of *Klebsiella spp*
(Gram staining x1000)

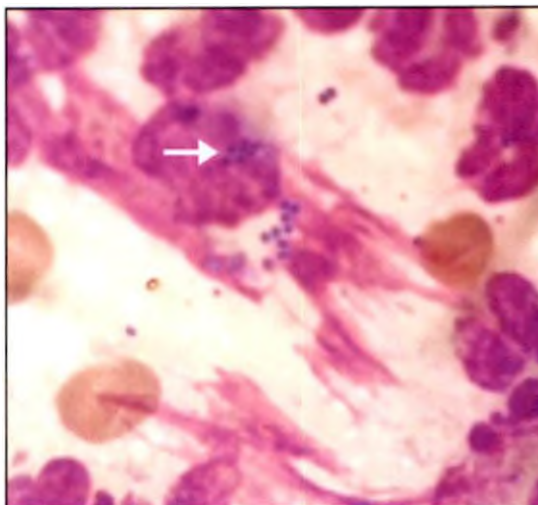


Plate 3. Microphotograph of Skin
impression with cocci inside
the neutrophil
(Leishman's staining x1000)



Plate 4. Hemolytic colonies of *Staphylococcus spp* on Blood agar

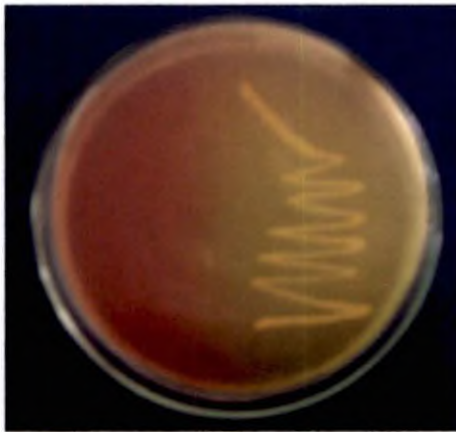


Plate 5. *Staphylococcus aureus* on Mannitol salt agar

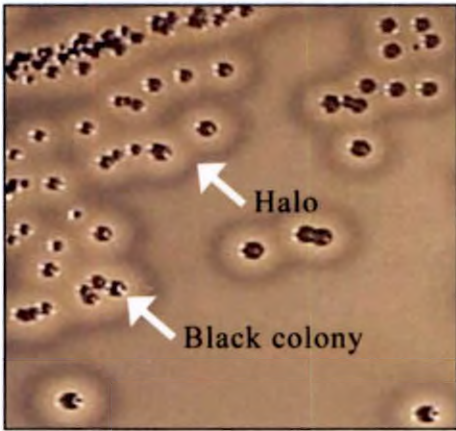


Plate 6. *Staphylococcus aureus* on Baird - Parker agar

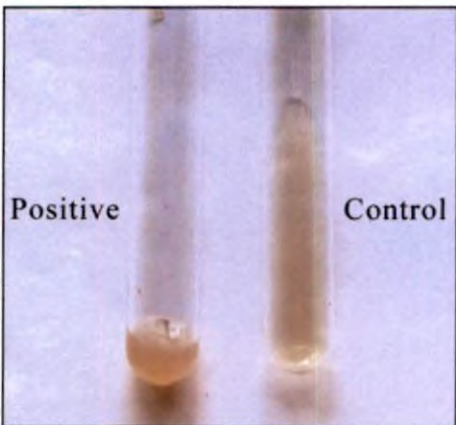


Plate 7. Coagulase test

Plate 8: *Pseudomonas fluorescence*

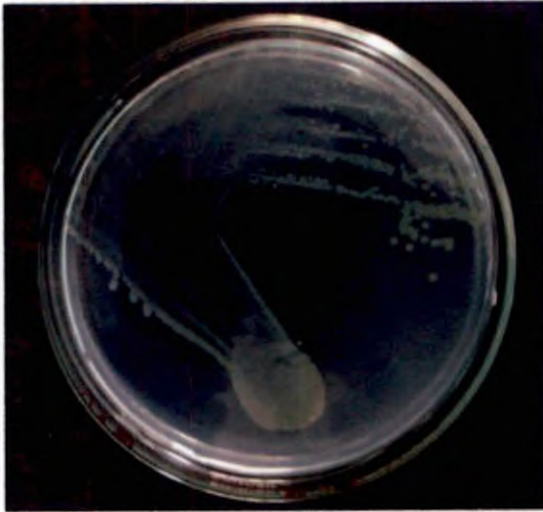


Plate 8A. *Pseudomonas fluorescence* on nutrient agar

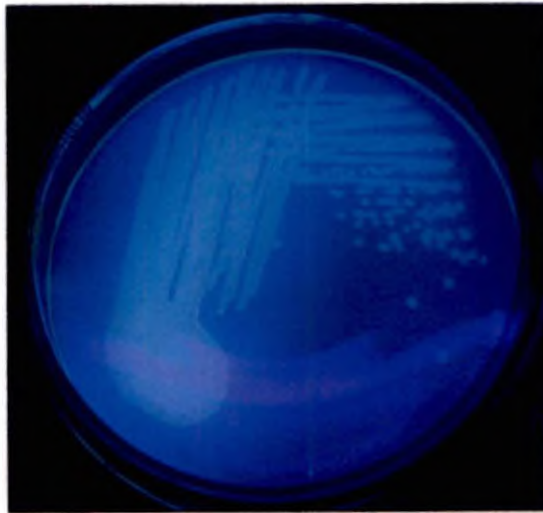


Plate 8B. Diffusible fluorescent pigment by *Pseudomonas fluorescence* on Pseudomonas agar

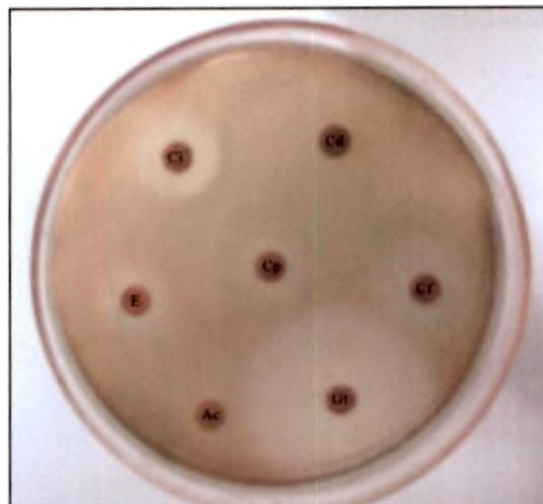


Plate 8C. Antibiogram of *Pseudomonas fluorescence*

- Gt - Gatifloxacin
- Cp - Cephalixin
- Cd - Clindamycin Hydrochloride
- Ac - Amoxicillin-clavulanic acid
- Ci - Ciprofloxacin
- E - Erythromycin
- Cf - Ceftriaxone

Table 7. Management details of infected animals (n=42)

| Management practice | | Number of infected animals taken for study | Per cent |
|---------------------|-------------------------|--|----------|
| Bathing | Regular | 26 | 61.90 |
| | Not regular | 16 | 38.10 |
| Keeping animals | Indoor | 6 | 14.28 |
| | Kennel | 36 | 85.72 |
| Cleaning of kennel | Regular | 25 | 69.45 |
| | Not regular | 11 | 30.55 |
| Cleaning of kennel | Chemicals/disinfectants | 22 | 61.11 |
| | Washing only | 14 | 38.89 |
| Grooming | Regular | 12 | 28.58 |
| | Not regular | 30 | 71.42 |
| Ectoparasites | Present | 11 | 26.19 |
| | Absent | 31 | 73.81 |
| Deworming | Regular | 28 | 66.70 |
| | Not regular | 14 | 33.30 |
| Vaccination | Regular | 22 | 52.38 |
| | Not regular | 20 | 47.62 |

Table 8: Bacterial isolates from bacterial skin infections (n=34)

| Serial number | Bacteria | Number | Per cent |
|---------------|-----------------------------------|-----------|------------|
| 1 | <i>Staphylococcus epidermidis</i> | 11 | 32.35 |
| 2 | <i>Staphylococcus hyicus</i> | 7 | 20.58 |
| 3 | <i>Staphylococcus intermedius</i> | 5 | 14.73 |
| 4 | <i>Staphylococcus aureus</i> | 3 | 8.82 |
| 5 | <i>Micrococcus spp.</i> | 1 | 2.94 |
| 5 | <i>Pseudomonas aeruginosa</i> | 4 | 11.76 |
| 6 | <i>Pseudomonas fluorescense</i> | 2 | 2.94 |
| 7 | <i>Klebsiella pneumoniae</i> | 1 | 5.88 |
| | Total | 34 | 100 |

per cent sensitivity to gatifloxacin, and amoxicillin- clavulanic acid combination. Sensitivity to erythromycin was shown by 90.09 per cent of the isolates and 81.81 per cent of the isolates showed sensitivity to ciprofloxacin and cephalexin equally. In the present study 72.72 per cent of them showed sensitivity to clindamycin hydrochloride. Resistance to clindamycin hydrochloride, erythromycin and ciprofloxacin was shown by 27.27 per cent, 9.09 percent and 9.09 percent of the isolates respectively. Another 18.18 percent and 9.09 per cent of isolates showed intermediate sensitivity to cephalexin and ciprofloxacin respectively.

All isolates of *Staphylococcus hyicus* showed 100 per cent sensitivity to gatifloxacin and ciprofloxacin while 71.42 per cent were sensitive to amoxicillin-clavulanic acid combination, clindamycin hydrochloride and erythromycin each and 85.71 per cent of isolates were sensitive to cephalexin. Intermediate sensitivity to clindamycin hydrochloride and cephalexin were shown by 14.28 per cent of the isolates each and 28.56 per cent and 14.28 per cent of the isolates showed resistance to erythromycin and clindamycin hydrochloride respectively. Another 28.56 per cent showed intermediate sensitivity to amoxicillin- clavulanic acid combination.

All isolates of *Staphylococcus intermedius* showed sensitivity to gatifloxacin. Sensitivity to clindamycin hydrochloride, erythromycin, amoxicillin-clavulanic acid combination, cephalexin were shown by 60 per cent of the isolates each and 40 per cent were sensitive to ciprofloxacin. Intermediate sensitivity to cephalexin and ciprofloxacin was shown by 20 per cent and 40 per cent isolates respectively. Resistance to clindamycin hydrochloride, erythromycin and amoxicillin-clavulanic acid combination was shown by 40 per cent of the isolates each and 20 per cent of the isolates were resistant to cephalexin and ciprofloxacin each.

Isolates of *Staphylococcus aureus* showed 100 per cent sensitivity to gatifloxacin, ciprofloxacin and amoxicillin- clavulanic acid combination each. Sensitivity to clindamycin hydrochloride and erythromycin was shown by 66.67 per cent of the isolates and 33.33 per cent of the isolates were sensitive to cephalexin. Resistance to cephalexin was shown by 66.67 per cent of the isolates and 33.33 per cent of the isolates were resistant to clindamycin hydrochloride and erythromycin equally.

Micrococcus spp showed 100 per cent sensitivity to ciprofloxacin, cephalexin, erythromycin and gatifloxacin each. It showed 100 per cent resistance to amoxicillin- clavulanic acid combination and clindamycin hydrochloride.

Pseudomonas aeruginosa isolates showed 100 per cent sensitivity towards gatifloxacin and ciprofloxacin. Sensitivity to amoxicillin- clavulanic acid combination was shown by 75 per cent of the isolates and 50 per cent of bacterial isolates showed sensitivity to erythromycin and clindamycin hydrochloride each. Another 25 per cent showed sensitivity to cephalexin. Resistance to clindamycin hydrochloride and erythromycin was shown by 50 per cent of the isolates each and 25 per cent showed resistance to cephalexin and amoxicillin-clavulanic acid combination each. Intermediate sensitivity towards cephalexin was shown by 50 per cent of the isolates.

Pseudomonas fluorescense was sensitive to gatifloxacin alone and resistant to all other antibiotics in the study group. (Plate 8C)

Klebsiella pneumoniae isolates showed 100 per cent sensitivity to gatifloxacin and resistant to a clindamycin hydrochloride, amoxicillin- clavulanic acid combination, and cephalexin. But 50 per cent of the isolates were sensitive to erythromycin and ciprofloxacin each (Table 9).

Table 9: *In vitro* antimicrobial sensitivity patterns of bacterial isolates from bacterial skin infection cases

| Bacterial isolate | No: of Isolates | Sensitive (Per cent) | | | | | | Resistant (Per cent) | | | | | | Intermediate sensitive (Per cent) | | | | | |
|-----------------------------------|-----------------|----------------------|-------------|-------------|-------------|--------------|-------------|----------------------|-------------|-------------|-------------|-------------|----|-----------------------------------|-------------|----|-------------|---|----|
| | | Ac | Cf | Cd | Cp | E | Gf | Ac | Cf | Cd | Cp | E | Gf | Ac | Cf | Cd | Cp | E | Gf |
| <i>Staphylococcus epidermidis</i> | 11 | 11 (100) | 9 (81.8) | 8 (72.7) | 9 (81.8) | 10 (90.9) | 11 (100) | - | 1 (9.09) | 3 (27.3) | - | 1 (9.1) | - | - | 1 (9.09) | - | 2 (18.2) | - | - |
| <i>Staphylococcus hyicus</i> | 7 | 5 (71.4) | 7 (100) | 5 (71.4) | 6 (85.7) | 5 (71.4) | 7 (100) | - | - | 2 (28.6) | 1 (14.3) | 2 (28.6) | - | 2 (28.6) | - | - | 1 (14.3) | - | - |
| <i>Staphylococcus intermedius</i> | 5 | 3 (60) | 2 (40) | 3 (60) | 3 (60) | 3 (60) | 5 (100) | 2 (40) | 1 (20) | 2 (40) | 1 (20) | 2 (40) | - | - | 2 (40) | - | 1 (20) | - | - |
| <i>Staphylococcus aureus</i> | 3 | 3 (100) | 3 (100) | 2 (66.7) | 1 (33.3) | 2 (66.7) | 3 (100) | - | - | 1 (33.3) | 2 (66.7) | 1 (33.3) | - | - | - | - | - | - | - |
| <i>Micrococcus spp.</i> | 1 | - | 1 (100) | - | 1 (100) | 1 (100) | 1 (100) | 1 (100) | - | 1 (100) | - | - | - | - | - | - | - | - | - |
| <i>Pseudomonas aeruginosa</i> | 4 | 3 (75) | 4 (100) | 2 (50) | 1 (25) | 2 (50) | 4 (100) | 1 (25) | - | 4 (100) | 2 (50) | 2 (50) | - | - | - | - | 1 (25) | - | - |
| <i>Pseudomonas fluorescence</i> | 1 | - | - | - | - | - | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | - | - | - | - | - | - | - |
| <i>Klebsiella pneumoniae</i> | 2 | - | 1 (50) | - | - | 1 (50) | 2 (100) | 2 (100) | 1 (50) | 2 (100) | 2 (100) | 1 (50) | - | - | - | - | - | - | - |

Figures in parenthesis indicate sensitivity or resistance to antibiotics in parenthesis

Ac- Amoxicillin- clavulanic acid combination Cf- Ciprofloxacin Cd-Clindamycin hydrochloride Cp-Cephalexin E- Erythromycin Gt- Gatifloxacin

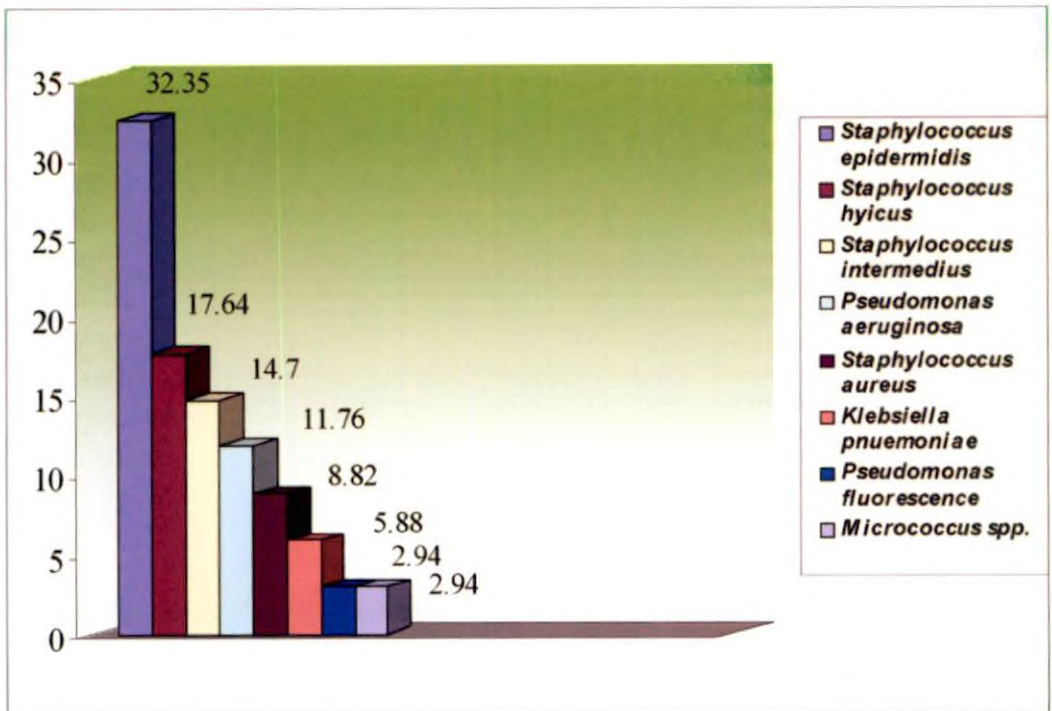


Fig.6. Percentage distribution of bacterial isolates from cases of bacterial dermatitis

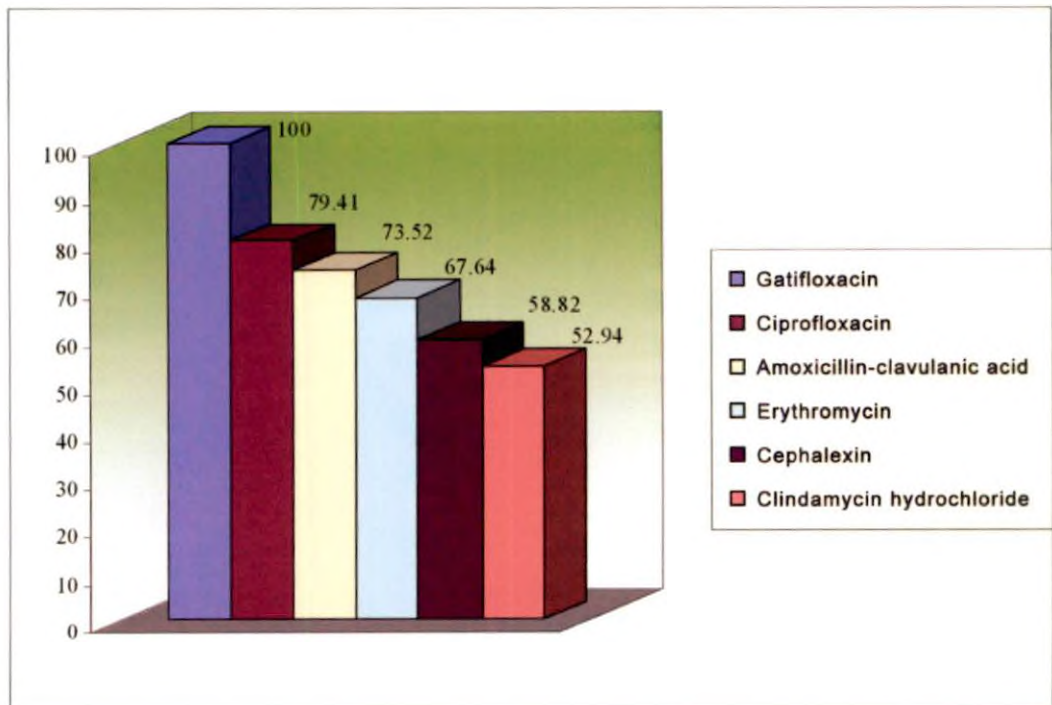


Fig. 7. Percentage distribution of antimicrobial sensitivity pattern of 34 bacterial isolates

4.5. HAEMATOLOGICAL PARAMETERS

Haematological parameters of infected and control group are presented in the Table 10.

4.5.1. Haemoglobin

Mean haemoglobin content of infected group was 11.74 ± 0.32 g/dl and that of control group was 13.36 ± 0.64 g/dl. Statistical analysis using 't' revealed no significant difference existed between haemoglobin content of infected and control group.

4.5.2. Packed cell volume

Infected group had lower mean packed cell volume (33.75 ± 1.77 per cent) when compared to the mean value of control animals (36.89 ± 1.27 per cent). ($P < 0.05$)

4.5.3. Total erythrocyte count

Mean total erythrocyte count of infected group ($4.63 \pm 0.32 \times 10^6 / \text{mm}^3$) was lower from the total erythrocyte count of the control group ($6.24 \pm 0.46 \times 10^6 / \text{mm}^3$). ($P < 0.05$)

4.5.4. Total leucocyte count

Mean total leucocyte count of infected group ($11.31 \pm 1.68 \times 10^3 / \text{mm}^3$) showed numerically lower mean value as compared control group ($14.37 \pm 0.46 \times 10^3 / \text{mm}^3$). Statistical analysis using 't' test revealed no significant difference existed between total leucocyte count of infected and control group.

4.5.5. Differential count

Differences in the count of different cells observed were as follows, neutrophils (69.33 ± 1.84 in the infected group and 62.33 ± 1.87 in the control group), lymphocytes (27.83 ± 2.29 in the infected group and 28.33 ± 3.74 in the control group), eosinophils (1.75 ± 0.74 in the infected and 5.78 ± 1.56 in the

control group), monocytes (0.42 ± 0.34 in the infected and 3.33 ± 2.12 in the control group). Statistical analysis using 't' test revealed that significant variation existed between values of neutrophils and eosinophils between infected and control group. ($P < 0.05$)

4.6. BIOCHEMICAL PARAMETERS

Biochemical parameters of infected and control group are presented in the Table 11.

4.6.1. Blood Glucose

Infected group showed numerically lower blood glucose level (89.8 ± 2.92 mg/dl) compared to the mean value of control group (105.22 ± 11.03 g/dl). But both the values were within the normal range. Statistical analysis using 't' test revealed no significant difference existed between blood glucose level of infected and control group.

4.6.2. Cholesterol

Mean value of cholesterol in the infected group and control group was 90 ± 8.89 mg/dl, 78 ± 8.22 mg/ dl respectively. However, no significant difference existed between the values of cholesterol of the two groups.

4.6.3. Total protein

Mean value of total protein in the infected group (7.72 ± 0.52 g/dl) is numerically higher than that in the control group (6.63 ± 0.57 g/dl). Statistical analysis using 't' test revealed no significant difference existed between total protein level of infected and control group.

4.6.4. Albumin

Mean albumin value of infected group (2.26 ± 0.15 g/dl) is lower than that in the control group (3.17 ± 0.25 g/dl). ($P < 0.05$)

4.6.5. Globulin

Mean globulin value of infected group is 5.36 ± 0.54 g/dl and that in the control group is 3.47 ± 0.44 g/dl. Statistical analysis using 't' test revealed no significant difference existed between globulin level of infected and control group.

4.6.7. A/G Ratio

Mean A/G ratio of infected group (0.66 ± 0.17) is numerically lower than that in the control group (0.93 ± 0.13). Statistical analysis using 't' test revealed no significant difference existed between globulin level of infected and control group.

4.7. SERUM MINERALS

The mean values of calcium, iron, copper, zinc is as follows, 11.95 ± 1.99 mg/dl, 354.96 ± 45.24 μ g/dl, 49.5 ± 3.49 μ g/dl, 240.6 ± 27.63 μ g/dl respectively. On the other hand mean values in the control group were 10.15 ± 1.78 mg/dl, 105 ± 15.93 μ g/dl, 115 ± 20.08 μ g/dl, 51 ± 1.52 μ g/dl respectively. Statistical analysis using 't' test revealed significantly higher iron and zinc values in infected animals and numerically lower copper values in infected group (Table 12).

4.8 .TREATMENT TRIAL

4.8.1. Group 1(Plate.9)

Eight animals of the infected group were treated with gatifloxacin @ 5mg/kg body weight once daily for 14 days. Complete cure was observed in five animals after treatment for 7 days and continued the treatment for seven more days for complete resolution of lesions. One animal showed improvement in condition after 14 days of treatment only and one showed clinical improvement but not cured completely. Recurrence of lesion observed in one case after one month.

Plate 9 : Group 1 Treated with Gatifloxacin



Plate 9A. 0 day



Plate 9B. 3rd day

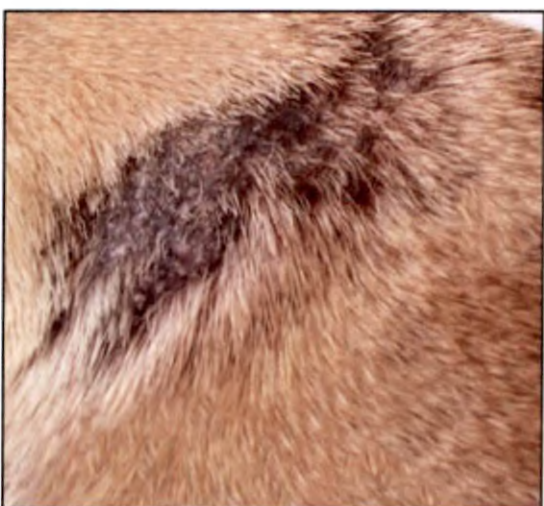


Plate 9C. 8th day

Table 10: Haematological parameters of infected group with that of control group

| Haematological parameters | Mean values \pm SD | | t values |
|--|----------------------|-------------------|---------------------|
| | Control (n=6) | Infected (n=42) | |
| Haemoglobin (g/dl) | 13.36 \pm 0.64 | 11.74 \pm 0.32 | 1.408 ^{NS} |
| Packed cell volume (per cent) | 36.89 \pm 1.27 | 33.75 \pm 1.77 | 1.115 ^S |
| Total erythrocyte count ($10^6/\text{mm}^3$) | 6.24 \pm 0.46 | 4.63 \pm 0.32 | 2.166 ^S |
| Total leukocyte count ($10^3/\text{mm}^3$) | 14.37 \pm 0.46 | 11.31 \pm 1.69 | 2.123 ^{NS} |
| Neutrophils (per cent) | 62.33 \pm 1.87 | 69.33 \pm 1.84 | 2.182 ^S |
| Lymphocytes (per cent) | 28.33 \pm 3.74 | 27.833 \pm 2.29 | 0.087 ^{NS} |
| Eosinophils (per cent) | 5.78 \pm 1.56 | 1.75 \pm 0.74 | 2.178 ^S |
| Monocytes (per cent) | 3.33 \pm 2.12 | 0.42 \pm 0.34 | 2.505 ^{NS} |

P<0.05

^S- Significant variation^{NS}-Nonsignificant variation

Table 11: Biochemical parameters of infected group with that of control group

| Biochemical parameters | Mean values \pm SD | | t values |
|------------------------|----------------------|-----------------|----------------------|
| | Control (n=6) | Infected (n=42) | |
| Blood glucose (mg/dl) | 105.22 \pm 11.03 | 89.8 \pm 2.91 | 2.5605 ^{NS} |
| Cholesterol (mg/dl) | 78.00 \pm 8.22 | 90 \pm 8.90 | 0.6455 ^{NS} |
| Total protein (g/dl) | 6.63 \pm 0.57 | 7.72 \pm 0.51 | 0.922 ^{NS} |
| Albumin (g/dl) | 3.17 \pm 0.25 | 2.26 \pm 0.15 | 2.4442 ^S |
| Globulin (g/dl) | 3.47 \pm 0.44 | 5.36 \pm 0.54 | 1.7441 ^{NS} |
| A/G ratio | 0.93 \pm 0.13 | 0.93 \pm 0.13 | 0.767 ^{NS} |

P<0.05

^S- Significant variation^{NS}-Nonsignificant variation

Table 12: Serum mineral status of infected group with that of control group

| Minerals | Mean values \pm SD | | t values |
|------------------------------------|----------------------|--------------------|----------------------|
| | Control (n=6) | Infected (n=42) | |
| Calcium (mg/dl) | 10.15 \pm 1.78 | 11.95 \pm 1.99 | 0.438 ^{NS} |
| Iron ($\mu\text{g}/\text{dl}$) | 105 \pm 15.93 | 354.96 \pm 45.33 | 2.24 ^S |
| Copper ($\mu\text{g}/\text{dl}$) | 115 \pm 20.08 | 49.5 \pm 3.49 | 1.9237 ^{NS} |
| Zinc ($\mu\text{g}/\text{dl}$) | 51 \pm 1.52 | 240.6 \pm 27.63 | 3.0326 ^S |

P<0.05

^{NS}-Nonsignificant variation^S- Significant variation

4.8.2. Group II (Plate.10)

Eight animals were treated with cephalexin @ 25 mg/kg body weight once daily for 14 days. Four cases showed complete resolution of lesions after seven days of treatment but continued the treatment for 14 days. Excellent response to treatment observed in seven cases and one animal developed anaemia and died during the course of treatment due to heavy hookworm infestation. But resolution of the lesions was noticed in this case also.

4.8.3. Group III (Plate. 11)

Eight cases of bacterial dermatitis treated with amoxicillin-clavulanic acid combination @ 12.5 mg/kg body weight twice daily for 14 days. All the animals showed good response to treatment after 2 weeks except one case and the drug was changed to gatifloxacin but no clinical cure was observed inspite of the change in drug.

4.8.4 Group IV (Plate.12)

Eight animals were treated with clindamycin hydrochloride @ 11 mg/kg body weight once daily for 14 days. The *in vitro* culture and sensitivity test revealed resistance to the drug and hence the drug was changed to gatifloxacin in one case, amoxicillin clavulanic acid combination in two cases and ciprofloxacin in one case. In case of the above mentioned cases, one dog showed slight improvement in condition after treatment with clindamycin hydrochloride, eventhough bacterial isolate showed resistance to the drug. Three animals treated with clindamycin hydrochloride for 14 days showed complete resolution of lesions. One animal showed sensitivity to clindamycin hydrochloride but did not respond to the treatment with the antibiotic.

Clinical score of animals after treatment was recorded as per Bloom and Rosser (2001) (Table.13).

Plate 10: Group 2 Treated with Cephalexin



Plate 10 A. 0 day



Plate 10 B. 7th day



Plate 10 C. 15th day

Plate 11: Group 3 treated with amoxicillin-clavulanic acid combination



Plate 11 A. 0 day



Plate 11 B. 3rd day



Plate 11 C. 8th day

Plate 12: Group 4 Treated with Clindamycin hydrochloride



Plate 12A. 0 day



Plate 12B. 3rd day



Plate 12C. 8th day

Table 13: Therapeutic response in different groups

| Group I (Gatifloxacin) (n=8) Case no: | Clinical score | Group II (Cephalexin) (n=8) Case no : | Clinical score | Group III (Amoxicillin- clavulanic acid) (n=8) Case no : | Clinical score | Group IV (Clindamycin Hydrochloride) (n=8) Case no : | Clinical score |
|--|-------------------|--|-------------------|--|-------------------|--|-------------------|
| 1373 | Recurrence | 6710 | Good | 2483 | Fair | 5013 | Poor |
| 1387 | Good | 6010 | Good | 5013 | Excellent | 5019 | Poor |
| 6021 | Poor | 6127 | Fair | 5019 | Good | 2286 | Fair |
| 5640 | Excellent | 10671 | Good | 6137 | Good | 6856 | Poor |
| 8118 | Poor | 6333 | Good | 9128 | Good | 6696 | Poor |
| 2021 | Excellent | 9236 | Excellent | 1571 | Excellent | 6875 | Good |
| 8081 | Fair | 6856 | Fair | 6840 | Poor | 8118 | Poor |
| 9091 | Good | 1126 | Good | 7071 | Excellent | 10671 | Good |

Recurrence -Relapse of lesions

Excellent -Complete remission of lesions

Good - Primary lesions resolved but secondary lesions evident

Fair - Partial improvement but secondary lesions still evident

Poor - No improvement in condition (Bloom and Rosser, 2001)

4.9. RIBOTYPING for *Staphylococcus epidermidis*

Out of the eleven isolates of *Staphylococcus epidermidis* seven were typed by Ribotyping fingerprinting of 16srRNA as it was the most commonly isolated pathogen in this study. The different isolates were grouped into three different genotypes arbitrarily designated as a, b, c based on their band patterns. (Plate 13)

Genotype b occurred at a frequency of 57.14 per cent with a maximum of four isolates followed by genotype a with two isolates (28.57 per cent) and genotype c with one isolate (14.29 per cent).

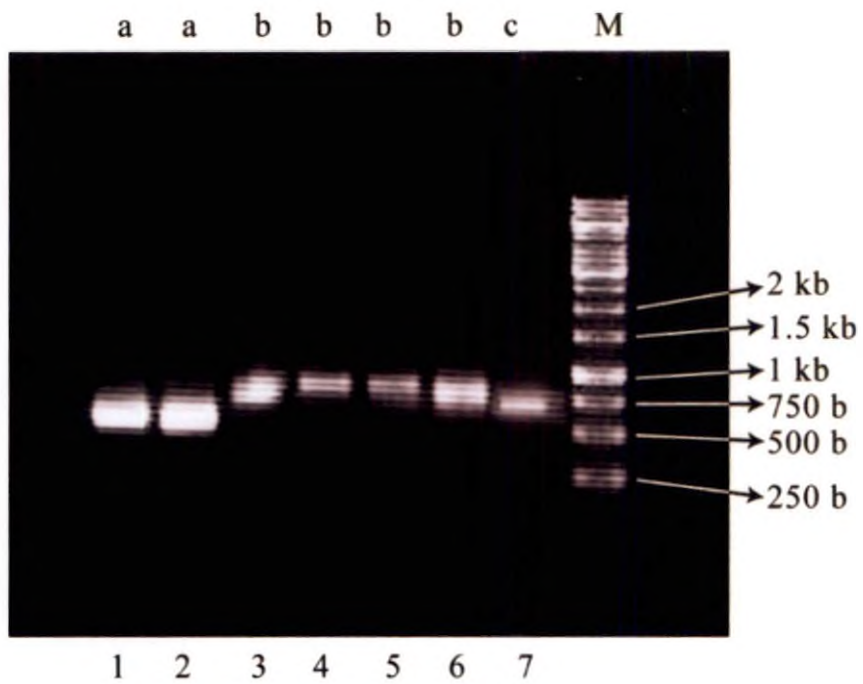


Plate 13. Ribotyping finger prints of seven strains of *Staphylococcus epidermidis*

Discussion

5. DISCUSSION

Incidence of bacterial skin infections and recurrent pyoderma is high in small animal practice and is often difficult to treat. The treatment protocol should be formulated specifically for each individual case. Resolution of secondary pyoderma necessitates identification of underlying problems and successful treatment of primary condition.

5.1. OCCURRENCE

The overall occurrence of dermatological problems in dogs as per the hospital records during the study period was 12.06 per cent. The result is not comparable with the finding of Kamboj *et al.* (1995), Scott and Pardis (1990) and Udayasree and Usha (2005) who reported that the overall prevalence of dermatological disorders was 18.93 per cent, 20 per cent and 24.45 per cent respectively. But Aujila *et al.* (1997) reported a lower incidence of 9.3 per cent in Punjab. Lower incidence in the present study might be due to the better skin care given by the pet owners.

Among different dermatological disorders, the overall occurrence of bacterial skin infections in the present study was 13.61 per cent and it is the third most frequently diagnosed skin disease. It is in close accordance with the finding of Udayasree and Usha (2005) who reported 12.71 per cent incidence of canine pyoderma. The incidence of bacterial dermatitis in the present study revealed was less than that reported by Kamboj *et al.* (1995), Aujila *et al.* (1997) and Mathews (1999). This difference might be due to difference in the type and size of canine population studied.

5.1.2. Age wise Occurrence

Analysis of percentage of dogs suffering from bacterial skin infections in various age group revealed that age group between one to three years (42.85 per cent) had the highest incidence followed by age group between one month to six

month old (23.83 per cent). This is in agreement with Krick and Scott (1989), Aujila *et al.*, (1997), Bloom and Rosser (2001) and Udayasree and Usha (2005).

However Mathews (1999) reported that dogs below six months of age were more frequently affected by bacterial skin infections followed by equal incidence in age group of 1 to 4 years and above four years.

5.1.3. Breed wise Occurrence

Highest occurrence was observed in German Shepherd (26.19%), followed by Labrador Retriever (19.04%), Rottweiler (9.51%), non descript, Dachshund, Pug, Bull Mastiff (7.14% each), Boxer, Spitz, Doberman (4.76% each) and Dalmatian (2.3%). The highest incidence of pyoderma in German shepherd breed was reported by Wisselink *et al.* (1985), Scott and Pardis (1990), Udayasree (2004), and Senturk *et al.* (2005). However Mathews (1999) reported that non descript dogs were more affected (33.33 per cent) followed by German shepherd (23.8 per cent).

Scott and Pardis (1990) observed that Doberman and German shepherd dogs had predilection for folliculitis and furunculosis. But in the present study less incidence of bacterial dermatitis was noticed in Doberman and highest incidence in German shepherd and a comparative high incidence noticed in Labrador retriever breed. Similar finding was reported by Udayasree (2004).

5.1.4. Month wise Occurrence

Maximum number of cases with bacterial skin infections brought to the hospitals was during the month of April (16.6%), followed by September (14.28%), March (11.9%), July, November (9.52% each), February, August, May (7.14% each), October, December, January (4.76% each) and June (2.38%). The observation was not in agreement with previous workers, Kamboj *et al.* (1995) and Upadhyay *et al.* (2005) who reported highest incidence in January and

August respectively. The higher incidence of bacterial skin infection was reported in April and March might be due to summer season.

5.1.5. Sex wise occurrence

Sex wise occurrence of bacterial skin infection showed that 57.14 per cent were females and 42.85 per cent were males in the infected group. This finding was in close accordance with Aujila *et al.* (1997) and Udayasree and Usha (2005). But Mathews (1999) and Upadhyay *et al.* (2005) reported higher occurrence of bacterial dermatitis in male dogs. Krick and Scott (1989), Scott and Pardis (1990), Chi –Won Pil *et al.* (2000) reported that there was no significant sex predilection to canine pyoderma.

5.2. CLINICAL SIGNS

Dogs with bacterial skin infections may present with a range of lesions, in any combination. These include pustules, papules, crust, epidermal collarette, alopecia, scale, erythema, pruritus and hyperpigmentation. Here we observed pustules (2.38 per cent), papules (9.52 per cent), crust (2.38 per cent), ulcer (16.66 per cent), epidermal collarette (7.14 per cent), alopecia (71.42 per cent), scale (0 per cent), erythema (23.8 per cent), pruritus (47.61 per cent), hyperpigmentation (2.38 per cent), pain (9.52 per cent), keratinisation (2.38 per cent) and abscess (2.38 per cent). Contact animals are affected in 4.76 per cent of the cases. Here in one case, infection transmitted from dam to puppies; and in another case, infection transmitted between two adult dogs in the same kennel.

The findings on different lesions in the present study was in accordance with Udayasree (2004) who classified the cases in to surface, deep and superficial pyoderma based on clinical signs. But the percentage of occurrence of different lesions was not similar. Her observations were as follows: papule(15.38 per cent), pustule (65.38 per cent), nodule (0 per cent), fistulous tract (11.5 per cent), erythema (73.07 per cent), alopecia (65.38 per cent), scale (11.5 per cent), crust (19.23 per cent), ulcer (11.5 per cent), epidermal collarette (30.76 per cent),

plaque (0 per cent), erosion (23.07 per cent), hyperpigmentation (15.38 per cent), pruritus (61.53 per cent), pain (3.84 per cent) and edema (11.55 per cent). Seena *et al.* (2005) observed pustules (77.72 per cent), papules (45.5 per cent), epidermal collarettes (27.3 per cent), scales (18.18 per cent) and crusts (9.11 per cent).

Variability in clinical signs was present in each individual case as it depends on many factors including the underlying condition as well as the stage of infection when the animal was examined. Previous workers, Hill and Moriello (1994), Craig (2003), Senturk *et al.* (2005) also reported the above described clinical signs in bacterial dermatitis.

Generalized lesions observed in 17 animals (40.47 per cent) and localized lesions in 25 animals (59.52 per cent). Udayasree (2004) observed only localized lesions in all cases.

5.3. MANAGEMENT

Bathing of dogs with shampoos mechanically remove tissue debris, exudates and reduce the bacterial population on the skin. Majority of animals in the infected group were given regular bath (61.90 per cent). This may be explained by the fact that frequent bathing increases the water absorption into the skin, which softens keratin (Muller *et al.* 1989). Soulsby (1982) also stated that frequent use of alkaline soap or shampoo during bath acted as a predisposing factor to the skin diseases. But this finding is not in accordance with Dowling (1996) as he advised to bathe dogs with a shampoo to avoid pyoderma.

Grooming was not practiced in majority of animals (71.42 per cent) in the infected group. Similar observation was made by Hill and Moriello (1994) that poor grooming may alter the cutaneous climate sufficiently to predispose animal to pyoderma. Therefore it can be inferred that grooming improves circulation in

skin and removes the matted hairs, scales and infected debris, which helps to make the coat less susceptible to bacterial infections.

Ectoparasites were present in 26.19 per cent of infected animals and absent in majority of the cases, 73.80 per cent. But and Craig *et al.* (2003) and Seena *et al.* (2005) reported parasitism as predisposing factor for canine pyoderma. The observation of the present study might be due to the avoidance of dogs with severe ectoparasites from the study group. The previous vaccination and deworming history of the animal was recorded as baseline information. Majority of animals in the infected group were dewormed and vaccinated regularly. In dogs skin conditions associated with viruses and internal parasites are quite rare. Examples would be rare conditions of footpad keratosis associated with canine distemper virus, pododermatitis due to hook worm larvae, pruritus associated with intestinal helminth hypersensitivity and cutaneous nodules caused by *Dirofilaria immitis* larvae (Hill, 2002).

Majority of the owners (61.11 percent) used some disinfectants to clean the kennel. Primary irritants (disinfectants, oil, and fertilizers) would be more common problems in animals and produce reactions on skin in areas of flank, feet, rear of the leg (Muller *et al.*, 1989).

In the present study we tried to collect information regarding type of food, frequency of feeding, duration of animal in out door, beginning of lesions, seasonal recurrence etc. but did not get sufficient response from clients. Skin conditions associated with diet include food intolerance, zinc responsive dermatoses and seborrhea. In addition dogs with chronic sun irradiation can develop photosensitization reactions and squamous cell carcinoma (Muller *et al.* , 1989), solar dermatitis (Coyner, 2007).

5.4. CULTURE AND SENSITIVITY TEST

5.4.1. Bacteriological Findings

The cases of bacterial skin infections were diagnosed by culture of pus or exudates obtained from lesions. Identification of bacteria was made on the basis of cultural, morphological and biochemical characteristics. Out of the 42 cases recorded during the study period of one year, 32 cases were taken for detailed study.

The bacterial isolates obtained from pyoderma cases included both gram positive and gram negative organisms. Among the gram positive organisms, *Staphylococcus epidermidis* (32.35 per cent) was the most commonly isolated organism followed by *Staphylococcus hyicus* (20.58 per cent), *Staphylococcus intermedius* (14.7 per cent), *Staphylococcus aureus* (8.82 per cent) and *Micrococcus spp* (2.94 per cent). Gram negative organisms such as *Pseudomonas aeruginosa* (11.76 per cent), *Klebsiella pneumoniae* (5.88 per cent) and *Pseudomonas fluorescens* (2.94 per cent) were also isolated. Mixed culture was obtained from two cases that contained *Klebsiella pneumoniae* and *Staphylococcus epidermidis* in one case and *Klebsiella pneumoniae* with *Micrococcus spp* in the other.

This finding confirmed *Staphylococcus epidermidis* as the major skin pathogen in dogs. But Berg *et al.* (1984), Aujila *et al.* (1997), Horspool *et al.* (2004), Udayasree (2004) reported *Staphylococcus intermedius* as the common infectious organism obtained from the cases under their study. Aujila *et al.* (1997), Chandrasekhar *et al.* (2001), Senturk *et al.* (2005) reported *Staphylococcus epidermidis* as one of the causative agents in canine pyoderma. Senturk *et al.* (2005) observed 30 per cent of the organisms obtained from the cases under their study were *Staphylococcus epidermidis*. Aujila *et al.* (1997) recovered *Staphylococcus epidermidis* from 22.5 per cent of the cases.

Carlotti *et al.* (1999) isolated *Staphylococcus hyicus* (14 per cent) as another important skin pathogen from bacterial skin infections in dogs. The present study revealed *Staphylococcus intermedius* (16 per cent) as the third most important pathogen causing bacterial dermatitis in dogs. This finding is not in accordance with Berg *et al.* (1984), Aujila *et al.* (1997), Horspool *et al.* (2004), Udayasree (2004) reported *Staphylococcus intermedius* as the common infectious organism obtained from the cases under their study.

Aujila *et al.* (1997), Carlotti *et al.* (1999), Udayasree (2004) isolated *Staphylococcus aureus* from 22.5 per cent, 10 per cent, and 30.76 per cent of cases respectively. But in the present study only 8 per cent of the isolates were *Staphylococcus aureus*.

Patil *et al.* (1999) and Toma *et al.* (2008) isolated *Micrococcus spp* as one of the causative agent in canine bacterial skin infections.

Two species of *Pseudomonas* could be identified in the present study *ie*, *Pseudomonas fluorescens* (2.94 per cent) and *Pseudomonas aeruginosa* (11.76 per cent). Udayasree (2004), Seena *et al.* (2005), Mueller and Stephan (2007), Toma *et al.* (2008) and Kumar (2010) recovered *Pseudomonas spp* from the cases of canine pyoderma. Hillier *et al.* (2006) isolated *Pseudomonas aeruginosa* from twenty cases of deep pyoderma.

Klebsiella pneumoniae was isolated from one case along with *Staphylococcus epidermidis* and with *Micrococcus spp* in another case. Presence of gram negative bacteria along with pyogenic *Staphylococci* was considered to be due to secondary invasion (Ihrke, 1987). Udayasree (2004), Horspool *et al.* (2004) and Seena *et al.* (2005) reported the isolation of *Klebsiella* from few cases.

5.4.2. Antibiogram

It is of paramount importance to conduct *in vitro* antibiotic sensitivity test to help the clinician for the appropriate selection of antibiotic because many bacterial species have become highly resistant to antimicrobials through their indiscriminate and inappropriate use. Multiple drug resistance was shown by many organisms. The results of *in vitro* antibiotic sensitivity tests were suggestive of an internal genetic structure of each organism.

Variations occurred in the antibiotic sensitivity of different species of organisms from place to place. Following antimicrobial therapy, bacteria might develop resistance by the change in the bacterial genome by conjugation or by transfer of R- plasmids between bacteria. Muller *et al.* (1989) suggested that culture and sensitivity test must be performed in chronic, refractory cases of pyoderma to guide the selection of the most appropriate drug. But Krick and Scott (1989) opined that antibiotic therapy based on results of culture and antibiotic sensitivity testing had no apparent advantage in terms of number of relapses following initial therapy and number of sustained remissions when compared to antibiotic therapy selected empirically.

Antimicrobial drug sensitivity showed that gatifloxacin (100 per cent), ciprofloxacin (79.41 per cent), amoxicillin-clavulanic acid (73.52 per cent), clindamycin hydrochloride (52.94 per cent), cephalexin (58.82 per cent) and erythromycin (67.64 per cent) sensitivity. Resistance to gatifloxacin, ciprofloxacin, amoxicillin-clavulanic acid, clindamycin hydrochloride, cephalexin and erythromycin were zero per cent, 20.59 per cent, 26.48 per cent, 47.06 per cent, 41.18 per cent and 32.36 per cent respectively.

Gatifloxacin and ciprofloxacin are fluoroquinolones. Gatifloxacin is a new C-8 methoxy fluoroquinolone which showed 100 per cent sensitivity to all the isolates. Fluoroquinolones are a group of antibiotics with considerable application in veterinary dermatology. They are rapidly bactericidal against a wide variety of

clinically important organisms including *Staphylococcus intermedius* and gram negative enteric bacilli by virtue of the interference with the supercoiling of bacterial chromosomal material. The only major contradiction is that it can not be used in young, rapidly growing dogs as they induce noninflammatory erosive arthropathy. Most veterinary dermatologists reserve fluoroquinolones for use after culture and sensitivity testing or for *staphylococci* which prove resistance to clindamycin, sulphonamides and clavulanic acid-potentiated amoxicillin. (Ganiere *et al.* , 2004). Vijayakumar and Smitha (2010) reported that out of ten different antibiotics tested in their study highest sensitivity was noticed with quinolone group. Other available fluoroquinolones in veterinary market are orbifloxacin, marbofloxacin and difloxacin.

An increase in β lactamase, a plasmid mediated enzyme production among *staphylococci*, the principal pathogen in pyoderma has been seen in recent years. Thus only those penicillins with resistance to β lactamase (Amoxicillin-clavulanic acid combination) are likely to be of value in treating canine pyoderma (Harvey and Hunter, 1999). Amoxicillin-clavulanic acid combination proved 73.52 per cent sensitivity to the bacterial isolates in the present study. It is almost in accordance with finding of Stegemann *et al.* (2007) who reported efficacy of amoxclav as 92.5 per cent.

Clindamycin hydrochloride (a lincosamide antibiotic) is a semisynthetic derivative of lincomycin. It inhibits protein synthesis in susceptible organisms by binding to 50S ribosomal subunits. Clindamycin is active against most aerobic gram positive cocci including *Staphylococci* and many anaerobic and microaerophilic gram negative and gram positive organisms. Clindamycin accumulates in leucocytes, which allows it to act on intracellular bacteria and be transported to and liberated at the sites of infection. Tissue concentration of clindamycin is higher than those in plasma. These considerations make clindamycin a very attractive choice for the treatment of deep staphylococcal pyoderma in dogs (Harai and Lincoln, 1989). However in the present study

52.94 per cent of the isolates were sensitive to clindamycin hydrochloride. Bloom and Rosser (2001) reported that out of the 17 cultures in their study, 72 per cent were susceptible to clindamycin. But Toma *et al.* (2008) observed only 29 per cent isolates which were susceptible to clindamycin in their study.

Cephalosporins are β lactam antibiotics and act by inhibiting the cell wall synthesis. In veterinary dermatology, the principle use of cephalosporins is in the clinical management of canine pyoderma associated with *Staphylococcus intermedius* and appear to be slow in acquiring resistance to cephalosporins (Mason and Keitzmann, 1999). The best known cephalosporin which is used in pyoderma therapy is cephalexin. Here 68 per cent of the isolates showed sensitivity to cephalexin.

Erythromycin is a macrolide antibiotic inhibiting bacterial ribosomal protein synthesis. They are narrow spectrum antibiotics showing excellent result in treatment of bacterial skin infections in dogs. It often shows cross resistance with lincosamides. In the present study, 67.64 per cent of the isolates showed sensitivity to erythromycin. Ihrke (1984) and Udayasree (2004) reported 46 per cent and 85 per cent susceptibility to erythromycin respectively.

Variations occurred in the antibiotic sensitivity in the present study from previous workers may be due to difference in place and period.

5.5. HAEMATOLOGICAL PARAMETERS

Infected group of dogs had significantly lower mean values of hemoglobin content, packed cell volume, total erythrocyte count, total leucocyte count (11.74 ± 0.32 , 33.75 ± 1.76 , 4.63 ± 0.32 , 11.31 ± 1.68) respectively when compared to respective mean values of control animals (13.36 ± 0.64 , 36.89 ± 1.27 , 6.24 ± 0.46 , 14.37 ± 0.46). This indicated anaemia in diseased dogs and could be explained with significantly lower copper values in infected animals obtained in serum mineral estimation since lowering of the copper content in blood was a

constant finding in anaemia (Maynard *et al.*, 1979). The value of total leucocyte count is within the normal range (8.2 to 13.5 thousand cells/mm³). Udayasree (2004) also obtained total leucocyte count as $11.08 \pm 0.61 \times 10^3$ cells/mm³. But this leucopenia in infected animals is not in agreement with previous workers Krik and Scott (1989) and Aujila *et al.* (1999) and Kumar *et al.* (2006) as they reported leucocytosis in infected animals.

Differential leucocyte count in infected animals indicated neutrophilia (69.333 ± 1.844), reduced levels of eosinophils and monocytes (1.75 ± 0.74 and 0.42 ± 0.34 respectively). But Curtis *et al.* (1995) reported a peripheral eosinophilia in a case of bacterial furunculosis. Neutrophilia in infected animals was reported by Mason (1991) and Medleau (1991) Kumar *et al.* (2006). But neutropenia in bacterial dermatitis was reported by Brown and Roggers (2001) and they opined that it might be due to increased demand of neutrophils in marked inflammation and bacterial sepsis. Number lymphocytes (27.83 ± 2.29) were similar to that of control group in the present study. But lymphopenia was reported by Mason (1991) and Medleau *et al.* (1991) in diseased dogs.

5.6. BIOCHEMICAL PARAMETERS

5.6.1. Total protein, Albumin, Globulin, Albumin Globulin Ratio (A/G)

In the present study the mean values of total protein, albumin, globulin, A/G were as follows *ie*, 7.72 ± 0.52 g/dl, 2.26 ± 0.15 g/dl, 3.47 ± 0.44 g/dl, 0.92 ± 0.13 . The value of albumin was significantly lower than the mean value of control animals and value of total protein, globulin and A/G ratio are higher than that of control animals. This finding is in agreement with Mason (1991) who reported hypergammaglobulinemia and hypoalbuminemia in dogs affected with bacterial dermatitis. Krick and Scott (1989) reported mildly increased levels of globulin level (4.1 to 5.2 g/dl) in affected dogs. A mild increase in total protein value in present study may be due to increased inflammatory response due to infection.

5.6. 2. Blood glucose and Cholesterol

Infected dogs in the present study presented blood glucose level (89.80 ± 2.91 mg/dl) and cholesterol level (90 ± 8.90 mg/dl) and that of normal control animals was (105.22 ± 11.03 mg/dl, and 78.00 ± 8.22 mg/dl respectively). This finding was in agreement with Gowda *et al.* (1982) who reported hypercholesterolemia and hypoglycemia in experimentally infected dogs. But this finding was not in accordance with Medleau *et al.* (1991) and Pal *et al.* (1995) as they reported hyperglycemia in infected cases.

The serum biochemistry estimation was only used for identifying an underlying problem, which might be a contributing factor in the development of disease and not for the diagnosis of disease.

5.7. SERUM MINERAL STATUS

5.7.1. Calcium

Mean value of serum calcium obtained in the present study was 11.95 ± 1.99 mg/dl as against the normal value of 10.15 ± 1.78 mg/dl. There is no statistical difference between the values of infected and control group. Calcium is present in the body in larger amounts than any other cation. Ninety nine percent of calcium occurs in skeleton and teeth, the remaining one percent is widely distributed throughout the body as essential constituent of most living cells and tissue fluids. Estimation of calcium in the present study is based on the facts that zinc absorption in the intestine may be inhibited by excessive levels of dietary calcium and thus leads to zinc deficient dermatitis.

5.7.2. Iron

Mean values of iron in infected animals was 354.96 ± 45.33 μ g/dl and it is significantly higher ($P < 0.05$) than the normal value. Iron requirement for domestic animals was influenced by age, growth rate and availability of dietary iron source (Smith, 1997).

5.7.3. Copper

Mean value of copper in infected and control group were $49.5 \pm 3.49 \mu\text{g/dl}$ and $115 \pm 20.08 \mu\text{g/dl}$ respectively. It is considerably lower ($P < 0.05$) than the control mean value. This finding is in accordance with Pal *et al.* (1995), Mathews (1999) and Udayasree (2004) who reported a decrease in level of copper in cases of bacterial dermatitis. Copper is involved in the process of osteogenesis, helps in pigmentation and keratinisation of hair, essential for erythropoiesis, involved in ceruloplasmin, copper containing enzyme regulating iron absorption. As it is required for pigmentation and keratinisation of tissue, a decreased copper level in infected group may aggravate the lesions caused by bacterial infections.

5.7.4. Zinc

Mean value of zinc in the infected group ($240.6 \pm 27.63 \mu\text{g/dl}$) was significantly higher than the normal control value ($51 \pm 1.52 \mu\text{g/dl}$). It is not in agreement with the previous workers Pal *et al.* (1995) and Udayasree (2004) as they reported significantly lower values of zinc in pyodermic dogs. In addition skin lesions were caused in animals when a zinc deficiency existed as it was a component of over 70 metalloenzymes that affected carbohydrate, lipid, protein and nucleic acid synthesis or degradation. Lesions in zinc associated pyoderma are mainly seen over the elbow, hock and other pressure points.

Serum mineral examination of infected animals in the present study suggest that increase in zinc and iron indicated that these minerals would have limited the absorption of copper (Underwood, 1981). As it is required for pigmentation and keratinisation of tissue, a decreased copper level in infected group may aggravate the lesions caused by bacterial infections.

5.8. CLINICAL RESPONSE TO TREATMENT TRIAL

Treatment of canine bacterial skin infections depends on the extent and depth of lesion, owner and patient compliance and the underlying disease. Topical or systemic treatment can be adopted. For systemic therapy prescribe an

appropriate antibiotic (based on sensitivity testing) that penetrates skin for an adequate period of time and avoid concurrent use of corticosteroids.

5.8.1. Group 1

Eight animals of the infected group were treated with gatifloxacin @ 5mg/kg body weight once daily for 14 days. Complete cure observed in five animals after treatment for 7 days and continued the treatment for seven more days for complete resolution of lesions. One animal showed improvement in condition after 14 days of treatment only and one animal showed clinical improvement but not cured completely. Recurrence of lesion observed in one case after one month. It is observed that 75 per cent of animals in the group I showed excellent clinical response to gatifloxacin.

Gatifloxacin is a new generation C-8 methoxy flouroquinolone and its bacteriostatic effect was proved by Lu *et al.* (1999). Excellent clinical response obtained in dogs treated with gatifloxacin in the present study is supported by similar findings by many previous workers. Most veterinary dermatologists reserve fluoroquinolones for use after culture and sensitivity testing for *Staphylococci* which prove resistance to clindamycin, sulphonamides and clavulanic acid-potentiated amoxicillin (Ganiere *et al.* 2004). Carlotti *et al.* (1999) and Padis *et al.* (2001) reported excellent response to marbofloxacin @ 2.12 mg/kg body weight and 2.75 mg/ kg body weight once daily orally respectively in canine pyoderma. Udayasree and Usha (2006) used ciprofloxacin @10 mg/kg body weight for seven days and enrofloxacin @ 5 mg/kg body weight orally once daily successfully in the treatment of canine pyoderma.

In the present study gram negative organisms *Klebsiella pneumoniae*, *Pseudomonas fluorescence* and *Pseudomonas aeruginosa* obtained from four different cases. Here *Pseudomonas fluorescence* showed sensitivity towards gatifloxacin alone and responded to the treatment. Ihrke (1999) suggested

fluoroquinolones as the drug of choice in skin infections caused by *Pseudomonas aeruginosa*.

Recurrence in one case was treated with cephalexin @ 25 mg/kg body weight for 14 days and resolution of lesions observed. Relapse was observed in the animal again and it may be due to some other underlying diseases.

5.8.2. Group II

Eight animals were treated with cephalexin @ 25 mg/kg body weight once daily for 14 days. Four cases showed complete resolution of lesions after seven days of treatment but continued the treatment for 14 days. Clinical response to treatment was observed in seven cases and one animal died after resolution of lesions during the course of treatment. It is observed that 100 per cent of animals in the group II showed clinical cure on treatment with cephalexin. This finding was in accordance with Seena *et al.* (2005) whose therapeutic evaluation revealed that 100 per cent of dogs treated with cephalexin showed recovery from canine pyoderma. Toma *et al.* (2008) also reported that 97.5 per cent of the dogs showed excellent response to cephalexin @ 30 mg/kg body weight once daily for 14 to 42 days. But Scott *et al.* (1994) reported clinical cure in 47 to 49 per cent of dogs treated with cephalexin.

5.8.3. Group III

Eight cases of bacterial dermatitis treated with amoxicillin-clavulanic acid combination @ 12.5 mg/kg body weight twice daily for 14 days. All the animals showed good response to treatment after two weeks except one case and changed the drug of choice to gatifloxacin and no clinical cure observed. It is observed that 87.5 per cent of animals in the group III showed good clinical response to amoxicillin-clavulanic acid combination. This observation is in agreement with Stegemann *et al.* (2007) who reported recovery of dogs with superficial pyoderma was 90.3 per cent when treated with amoxicillin-clavulanic acid combination.

But Scott *et al.* (1994) observed only 43 per cent response to amoxicillin-clavulanic acid combination.

5.8.4. Group IV

Seven animals were treated with clindamycin hydrochloride @ 11 mg/kg body weight once daily for 14 days. Four animals showed resistance to the drug on antibiogram and changed the antibiotic according to the result. Two animals treated with clindamycin hydrochloride for 14 days showed complete resolution of lesions. One animal showed sensitivity to clindamycin hydrochloride but not responded to treatment and changed the antibiotic of choice to amoxicillin-clavulanic acid combination. It is observed that only 37.5 per cent of animals showed clinical cure on treatment with clindamycin hydrochloride. This finding is not agreement with previous workers. Because Scott *et al.* (1998) reported that response to treatment with clindamycin hydrochloride @ 11 mg/kg body weight once daily orally for 21 days was excellent in 100 per cent of the dogs and relapses occurred in 25 per cent of dogs after three months. Bloom and Rosser (2001) reported clinical response rate to clindamycin hydrochloride as 71.4 per cent. In addition to it they observed that, of the six dogs that had poor response to therapy, three of the dog's cultures revealed an initial sensitivity to clindamycin on *in vitro* testing, and follow up cultures indicated resistance to clindamycin.

5.9. RIBOTYPING

Ribotyping is a method that can identify and classify bacteria based upon differences in rRNA. Ribotyping involves the fingerprinting of genomic DNA restriction fragments that contain all or part of the genes coding for the 16S or 23S rRNA.

Ribotyping is a highly sensitive and precise method for the identification, classification, observation, and tracking of various bacteria from the sample of interest. This helps to distinguish organisms within the same species, which

might help in the identification of the disease-causing organism and finding an appropriate treatment for it.

In the present study we identified three strains of *Staphylococcus epidermidis*. The predominant genotype b seen in four cases (1kb to 750bp) showed sensitivity to amoxicillin-clavulanic acid, gatifloxacin, cephalexin, erythromycin, ciprofloxacin and clindamycin hydrochloride and complete clinical cure also observed in these cases. The two dogs which had infection with genotype a (250 bp to 500 bp) showed a very unique pattern of occurrence of lesions with diffused ulcers throughout the body. Eventhough the bacterial isolates showed sensitivity to all the antibiotics under study satisfactory clinical cure was not obtained in these cases. It may be the most pathogenic strain of *Staphylococcus epidermidis* in this area. Genotype c (500 bp to 750 bp) seen in one case showed resistance to amoxicillin-clavulanic acid, cephalexin and clindamycin hydrochloride, whereas sensitivity to ciprofloxacin and gatifloxacin. However the animal showed clinical cure on treatment with amoxicillin-clavulanic acid combination.

It can be concluded that ribotyping can be used to determine if an infection is caused by a single strain or by multiple strains. In an outbreak of an infection in a large population, ribotyping can detect the source of infection, identify its mode of transmission, and monitor the distribution and occurrence of a particular strain of bacteria. In clinical studies, ribotyping can identify the role of an organism in the occurrence of the disease and monitor the treatment regimens.

Summary

6. SUMMARY

The present study “Epidemiological and clinico-therapeutic studies on bacterial skin infection in dogs” was carried out to know the epidemiology, bacterial etiology, antibiogram and comparative efficacy of four different antibiotics in the treatment. A total of 7771 dogs were brought to University Veterinary teaching Hospitals at Mannuthy and Kokkalai from February 2009 to January 2010 with different clinical illness. Among the 933 animals presented with skin lesions in the clinics, cultural examination of skin swab from lesions was carried out in 45 cases and bacterial growth could be observed in 42 animals. Among the infected group, 32 animals were selected at random and divided in to four groups each consisted of eight animals. Epidemiological data of infected animals were collected as per the proforma.

Highest per cent of infection was noticed in dogs of 1-3 year age group and in the German Shepherd breed of dogs. Females were more affected when compared to males. Increased numbers of infected cases were reported in the month of April.

Detailed clinical examination of infected animals revealed pruritus, alopecia, ulcer, erythema and papule as the major skin lesions. Confirmatory diagnosis was carried out by the culture examination of exudates and touch swab from the lesions. As per the epidemiological data collected using the proforma, majority of the owners were unaware of grooming their pets regularly and avoiding disinfectants in kennel. But the status of deworming and vaccination of pets was good.

Haematological parameters revealed significantly lower values for packed cell volume, total erythrocyte count and neutrophilia ($P < 0.05$) in infected dogs when compared to normal control animals. Statistical analysis of biochemical parameters such as blood glucose level, cholesterol, total protein, globulin and

A/G ratio revealed no statistical difference between infected and control group. But the infected animals exhibited significant hyperalbuminemia. Serum mineral estimation of infected animals showed lower mean value for copper ($P<0.05$) and higher mean value for iron ($P<0.05$) and zinc.

The bacterial isolates obtained from pyoderma cases included both gram positive and gram negative organisms. Among the gram positive organisms *Staphylococcus epidermidis* (32.35 per cent) was most commonly isolated organism, followed by *Staphylococcus hyicus* (20.58 per cent), *Staphylococcus intermedius* (14.7 per cent), *Staphylococcus aureus* (8.82 per cent), *Micrococcus spp* and (2.94 per cent). Gram negative organisms such as *Pseudomonas aeruginosa* (11.76 per cent), *Klebsiella pneumoniae* (5.88 per cent) and *Pseudomonas fluorescens* (2.94 per cent) were also identified. Mixed culture was obtained from two case that contained *Klebsiella pneumoniae* and *Staphylococcus epidermidis* in one case and *Klebsiella pneumoniae* with *Micrococcus spp* in the other.

Overall antibiotic sensitivity pattern of 34 isolates showed maximum sensitivity to gatifloxacin (100 per cent) followed by ciprofloxacin (79.41 per cent), amoxicillin-clavulanic acid combination (73.52 per cent), erythromycin (67.64 per cent), cephalixin (58.82 per cent) and clindamycin hydrochloride (52.94 per cent).

Suspected cases were divided into four groups each consisting of 8 animals at random and treated with any one of the selected antibiotic under study (gatifloxacin, amoxicillin-clavulanic acid combination, cephalixin, clindamycin hydrochloride) on day zero and reviewed after 72 hours. Required changes in the treatment were advised according to the clinical response and result of antibiogram.

Clinical response to therapy was monitored at day seven, 14 and 21. Excellent clinical response was reported in two animals treated with gatifloxacin, three animals treated with amoxicillin – clavulanic acid combination and in one animal treated with cephalixin. Good clinical response observed in five animals treated with cephalixin, three animals treated with amoxicillin-clavulanic acid combination, two animals with gatifloxacin and clindamycin hydrochloride each. Fair response was observed in two animals treated with cephalixin, in one animal with gatifloxacin, two animals with amoxicillin-clavulanic acid combination and in one animal with clindamycin hydrochloride. Poor response was shown by two animals treated with gatifloxacin, one animal with amoxicillin-clavulanic acid combination and in five animals with clindamycin hydrochloride. Recurrence was reported in one dog treated with gatifloxacin.

In vitro antibiotic sensitivity analysis revealed that gatifloxacin is the most effective antibiotic in the treatment of bacterial skin infections in dogs and amoxicillin-clavulanic acid combination and cephalixin could be used as a second choice based on the antibiogram. But highest rate of clinical cure (100 per cent) was showed in group treated with cephalixin, followed by groups treated with amoxicillin clavulanic acid combination and gatifloxacin. Treatment of pyoderma requires a long term antibiotic therapy even after the resolution of lesions. So antibiotics that had once daily dose regimen such as gatifloxacin, cephalixin will satisfy the owner's compliance.

16srRNA ribotyping was used to type seven isolates of *Staphylococcus epidermidis*, the most commonly isolated pathogen in this study. Three different genotypes were identified among which type b was predominated. Based on the severity of lesions and less response to treatment, it can be concluded that type a is the most pathogenic strain.

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EPIDEMIOLOGICAL AND CLINICO - THERAPEUTIC STUDIES ON BACTERIAL SKIN INFECTIONS IN DOGS

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ABSTRACT

The present study was mainly focused on epidemiological, diagnostic and clinico-therapeutic aspects of bacterial skin infections in dogs. A total of 42 animals were observed and diagnosed bacterial skin infection by culture of exudates or touch swab from skin lesions, out of the 933 animals brought to the University Veterinary Hospitals at Kokkalai and Mannuthy from February 2009 to January 2010 with different dermatological problems. More incidence of bacterial skin infections was observed in female dogs and in German shepherd breed. Detailed clinical examination of infected animals revealed pruritus, alopecia, ulcer, erythema and papule as the major clinical signs.

Infected animals showed statistically significant reduction in mean values of packed cell volume and total RBC count in infected animals. Serum biochemical examination revealed significantly higher mean value for serum albumin and hypoglycemia in infected dogs. Serum mineral estimation of infected animals showed lower mean value for copper and higher mean value for zinc.

Out of the 34 bacterial isolates, *Staphylococcus epidermidis* (32.35 per cent) was the most commonly isolated organism followed by *Staphylococcus hyicus* (20.58 per cent), *Staphylococcus intermedius* (14.7 per cent), *Staphylococcus aureus* (8.82 per cent) and *Micrococcus spp* (2.94 per cent). Gram negative organisms such as *Pseudomonas aeruginosa* (11.76 per cent), *Klebsiella pneumoniae* (5.88 per cent) and *Pseudomonas fluorescence* (2.94 per cent) were also obtained.

In vitro antibiotic sensitivity analysis revealed that gatifloxacin is the most effective antibiotic in the treatment of bacterial skin infections in dogs and amoxicillin-clavulanic acid combination and cephalexin could be used as a second choice based on the antibiogram. But highest rate of clinical cure (100 per

cent) was showed in group treated with cephalixin, followed by groups treated with amoxicillin clavulanic acid combination and gatifloxacin.

16srRNA ribotyping was used to type seven isolates of *Staphylococcus epidermidis*, the most commonly isolated pathogen in this study. Three different genotypes were identified among which type b was predominated. Based on the severity of lesions and less response to treatment, it can be concluded that type a is the most pathogenic strain.

Appendix

PROFORMA

Sl. No:

Case No:

Date:

Name and address of the owner:

Phone No.

Details of the animal:

Breed:

Colour:

Age:

Sex:

Weight:

Parity:

Identification mark:

Complaint :

History :

General appearance :

Good Fair Poor Thin Stunted

Behaviour :

Frenzy Mania Active Restlessness Dullness Depressed

Expression :

Anxious Woebegone Lethargic

Bodily condition :

Normal Obese Thin Emaciated Hide bound

Skin changes

Elasticity

Present Absent :

Present Absent

Epilation:

Hair coat

Good Poor Rough Others (broken, easily pulled off) :

Annular Linear Central healing roped Configuration

Distribution :

Generalized localised Asymmetrical Bilateral symmetrical
 Patchy Scattered

Depth of lesion:

Elevated Surface Deep

Consistency :

Soft Fluctuant Atrophied

Quality :

Dry Moist Greasy
 Bleeding Purulent

Colour of Alopecia

lesion:

Present Absent :

If present

Localized Generalized Symmetrical
 Asymmetrical

Pruritus

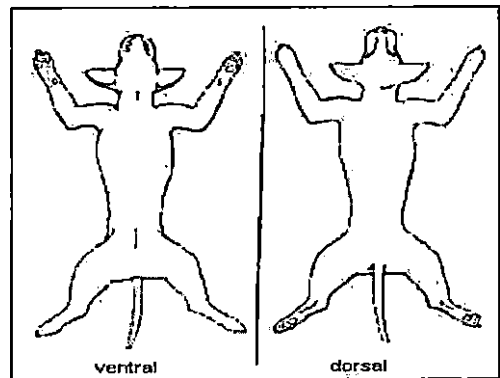
Present Absent Constantly Occasionally Only at night :

Food consumption:

Normal Less Anorectic

Whether it was purchased:

Yes No



If purchased : Kennel Pet shop Private

TYPE OF LESIONS

Primary

Macule tumour
Plaque wheal
Nodule papule
Pustule patch

Secondary

Scales Crusts
Scars Ulcers
Excoriation Lichenification
Hyperpigmentation Hyperkeratosis
Hypopigmentation Abscess
Erosions Callus
Erythema Alopecia
Exfoliation

Clinical signs:

When the problem began?

Has it spread? : Yes No If Yes, where.....

Date of last whelping :

Mating history :

Are the symptoms seasonal : Yes No If Yes, Year round Seasonal Non seasonal

In which season? : Summer Rainy Winter

Whether the animal is kept indoor?

Duration in indoor : Duration in outdoor:

.....

Whether any other contact animals affected? Yes No

Whether any contact human affected? Yes No

Management details:

Whether it is given bath : Yes No

Frequency of bath : Daily Once in 2 days Once in week Once in forth night
 Once in a month Once in 2 months

Soaps /shampoos used : Used Not If Yes, Name:

Do you groom the dog? : Yes No Occasionally Just before bath After bath

Diet :

Frequency of feeding : Time of feeding:

Type of food House hold Pet foods :

Dewormed ?Not : Date of last deworming:

Details about vaccination :

Ectoparasites present Biting flies Lice Ticks Fleas :

Culture results: