EVALUTION OF HAEMATO – BIOCHEMICAL CHANGES ASSOCIATED WITH CIPROFLOXACIN THERAPY IN CANINE PYODERMA

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DECLARATION

I hereby declare that the thesis entitled **"EVALUATION OF HAEMATO-BIOCHEMICAL CHANGES ASSOCIATED WITH CIPROFLOXACIN THERAPY IN CANINE PYODERMA"** is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled **"EVALUATION OF HAEMATO-BIOCHEMICAL CHANGES ASSOCIATED WITH CIPROFLOXACIN THERAPY IN CANINE PYODERMA"** is a record of research work done independently by **Dr. Jessy. V.,** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, associateship or fellowship to her.

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Jessy. V

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LIST OF ABBREVIATIONS

@	At the rate of
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
A:G	Albumin globulin ratio
AST	Asparatate amiontrasferase
BUN	Blood urea nitrogen
DLC	Differential leucocyte count
DTNB	5, 5'-dithiobis-2-nitrobenzoic acid
EDTA	Ethylene diamine tetraacetic acid
ESR	Erythrocyte sedimentation rate
TEC	Total erythrocyte count
GLDH	Glutamate dehydrogenase
GPX	Glutathione peroxidase
GSD	German shepherd dog
GSH	Reduced glutathione
Hb	Haemoglobin
i.m.	Intramuscularly
i.p.	Intraperitoneally
i.v.	Intravenously
IFCC	International Federation of Clinical Chemistry
IU	International Unit
LDH	Lactate dehydrogenase
MDA	Malondialdehyde
MDH	Malate dehydrogenase
NAD	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide dehydrogenase
NPN	Non protein nitrogen
PCV	Packed cell volume
s.c.	Subcutaneously
SOD	Superoxide dismutase
TCA	Trichloroacetic acid
TEC	Total erythrocyte count
TLC	Total leucocyte count
ß-NAG	Beta N-acetyl glucosamine
γGT	Gamma glutamyl transferase

Introduction

1. INTRODUCTION

Antibiotics are chemical substances produced by various species of microbes that suppress the growth of other microbes and may eventually destroy them. An antibiotic may be qualified as safe if it is effective selectively on microorganisms without causing any harm to the individual.

Even though antibiotics have revolutionised the management of many illnesses successfully, they have several adverse effects. Toxic or adverse drug reaction is due to excess pharmacological action of the drug which results from overdosage, frequent usage, prolonged administration and non rational use. The major adverse effects of the antibiotics include hypersensitive reactions, target organ toxicities, carcinogenicity, mutagenicity, impaired immune response and antibiotic drug interactions. Of these, target organ toxicity is the most important which include nephrotoxicity, hepatotoxicity, gastrointestinal disturbances, neurotoxicity, cardiotoxicity, tendonitis, tendon rupture and haemotoxic reactions.

Long-term antibiotic administration is necessary in some cases to control bacterial infections, even though present guidelines recommend short courses of antibiotics. In such cases drug induced nephrotoxicity and hepatotoxicity are the deleterious consequences. Prolonged use of aminoglycoside antibiotics possesses a high tubulotoxic potential when a several-dose daily regimen is used. Beta lactam antibiotics and quinolones cause interstitial nephritis based on hypersensitive reactions. Sulfonamides can form insoluble crystals which lead to hematuria, albuminuria, crystalluria and acute renal insufficiency (Wawruch *et al.,* 2002). Some fluoroquinolones like grepafloxacin, temafloxacin, trovafloxacin have been abandoned because of severe and fatal reactions. Chronic hepatitis has been induced by isoniazid, beta lactam antibiotics, erythromycin and nitrofurantoin. Tetracyclines cause liver damage on overdosage. Many drugs alter biochemical and haematological parameters but may not be clinically relevant.

Prolonged antibiotic treatment is often advised for diseases like ehrlichiosis, cystitis, pyelonephritis, pyoderma. In ehrlichiosis, continuous or long term administration of antibiotics, such as tetracycline has apparently contributed to delayed haematological recovery in dogs. Pyelonephritis and cystitis should be treated aggressively with antibiotics, based on urine culture and sensitivity test for 4-6 weeks. Current guidelines for treatment of deep pyoderma emphasise that prolonged therapies are required and treatment period of 21 days are often advocated.

Pyoderma, a bacterial skin infection is one of the most common condition seen in small animal practice and yet also one of the most frustrating disease to treat. Among the dermatologic diseases in dogs, canine pyoderma is the second most frequently reported skin disease after flea dermatitis (Ihrke *et al.,* 1999; Scott *et al.,* 1994). Around 90 % of pyoderma cases in dogs are associated with *Staphylococcus* sp. These bacteria are not virulent and infection tends to develop secondarily to an underlying cutaneous, metabolic, immunologic diseases or ectoparasitic infestation. Occasionally other microorganisms like *Escherichia coli, Pseudomonas* sp., *Proteus* sp. and *Klebsiella* sp. may colonise the skin and cause pyoderma.

The basic principles of systemic antibiotic therapy in pyoderma include the selection of an appropriate antibiotic, establishment of an optimal dosage, and maintenance of that dosage for enough time to ensure cure. Successful treatment in pyoderma requires the administration of an appropriate systemic antibiotic for a minimum of 14 days. The choice of antibiotics for administration against pyoderma has expanded considerably over recent years. This includes erythromycin, clindamycin, fluoroquinolones, rifampicin, potentiated sulphonamides, tylosin, amoxicillin-clavulanate, cephalexin, lincomycin. The adverse effects of trimethoprim-sulphonamide combinations on prolonged administration are reversible non regenerative anaemia and keratoconjunctivitis sicca (Campbell, 1999). Even though clindamycin showed excellent efficacy in

the treatment of pyoderma, vomiting is one of the adverse effects noticed (Scott *et al.,* 1998). The efficacy of cephalosporins is also promising, but it produces low level of toxicities like vomiting, diarrhoea and reduced appetite (Bruner, 2006).

Fluoroquinolones are commonly chosen to treat pyoderma as this drug is both efficacious and comparatively cheaper compared to cephlosporins, clindamycin and lincomycin. In a study conducted in University Veterinary Hospitals, Mannuthy and Kokkalai, during the time period from March 2003 to April 2004, the overall occurrence of dermatological problems was 18.93 % of which the incidence of canine pyoderma was 12.71 %. The findings of the study also concluded that ciprofloxacin showed an excellent response in the treatment of canine pyoderma and ciprofloxacin continues to be most efficient drug in the treatment of pyoderma with promising results. But the literature regarding the adverse effects of ciprofloxacin on prolonged administration in dogs are sparse.

Hence the present study was undertaken with the objective to evaluate the haematological and biochemical changes following prolonged administration of ciprofloxacin in the treatment of canine pyoderma.

Review of Literature

2. REVIEW OF LITERATURE

The goal of antibiotic therapy is to facilitate the body to eliminate infectious organism without causing any toxic effect to the host cells. Although antibiotics have revolutionised the management of many illnesses, they often have several side effects. These adverse effects of antibiotics may cause a failure in antibiotic treatment.

2.1 IMPORTANT ANTIBIOTICS AND THEIR SIDE EFFECTS

Antimicrobial drugs are the greatest contributions of the 20th century to therapeutics. Their advent changed the outlook of the physician about the power the drugs can have on diseases. They are one of the most frequently used as well as misused drugs.

Vassileva *et al.* (1998) reviewed the antimicrobial photosensitive reactions like bullous phototoxic eruptions, porphyria and pseudoporphyria caused by sulphonamides, tetracyclines, quinolones and griseofulvin. For quinolones, the reported incidence of photosensitivity varies from 1 to 4 per cent, for ciprofloxacin 10 per cent and 19 per cent for fleroxacin.

Wawruch *et al*. (2002) surveyed the hepatotoxic reactions of several antibiotics. Hepatitis with multilobular necrosis and liver damage may be caused by a number of antibiotics such as isoniazid, nitrofurantoin, oxacillin and erythromycin. Tetracyclines on overdosage resulted in liver damage. Nephrotoxic risk of aminoglycoside, vancomycin, frusemide, cephalosporin, and amphotericin B increase with age of the patient, prolonged treatment and pre-existing renal diseases. Aminoglycosides possess a high tubulotoxic potential when a several-dose daily regime is used. Interstitial nephritis may be related to beta lactam antibiotics and quinolones; sulfonamides form insoluble crystals which lead to hematuria, albuminuria, crystalluria and acute renal insufficiency.

Navarro and John (2006) reported the drug induced hepatotoxicity by the use of trovafloxacin, tetracyclines, sulphonamides, amoxycillin-clavulanic acid and erythromycin with a predominant increase in ALP, ALT and total bilirubin level.

Webb *et al*. (2006) determined the effects of enteral administration of doxycycline, amoxycillin, cephalexin and enrofloxacin @ 10, 30, 30 and 20 mg/kg body weight respectively for a typical clinical duration of seven days on haematological parameters like platelet count, haematocrit and TLC in healthy dogs and found that antimicrobial administration did not cause any significant difference in these parameters.

2.1.1 Penicillins

Penicillins have unique advantages such that members of this group of antibiotics are currently the drugs of choice for a large number of infectious diseases.

A study in 240 piglets from 24 litters was conducted to assess the toxicity of procaine penicillin. Each piglet was given 0.5 ml iron dextran and 1 ml (300000 IU) procaine penicillin i.m. and after 30 minutes, all the piglets started shivering, vomiting, several of them collapsed and 13 died. No specific gross necropsy findings were present but clear amber fluid was present in the thorax, pericardium and abdomen (Sanford, 1991).

Larrey *et al.* (1992) reported amoxycillin-clavulanic acid induced hepatotoxicity in 15 males. The study confirmed that amoxycillin-clavulanic acid @ 0.5 to 0.6 g/day for an average period of 18 days caused acute cholestatic hepatitis with an increased ALT, AST and ALP levels.

See and Lee (2006) examined a case of 49 year old male treated with ampicillin/sulbactam @ 3 g i.v. every eight hours for nine days for diabetic foot infection. On the ninth day of treatment yellowish discolouration of skin and tea colored urine was noted. Also there was an increase in serum direct and total bilirubin, ALP, γGT but ALT, AST and prothrombin time were normal. Jaundice subsided one month after withdrawal of the drug.

Abbas (2007) reported a case of 57 year old male having mediastinal abscess treated with piperacilin/tazobactam @ 4.5 g i.v. every eight hours. On the 25th day of treatment, the patient developed leucopenia, the TLC was 1900/µl with zero per cent neutrophils, 87 per cent lymphocytes, five per cent eosinophils and eight per cent monocytes, while Hb and platelets remained unchanged.

2.1.2 Sulphonamides

The sulphonamides were the first effective chemotherapeutic agent employed systemically for the prevention and cure of bacterial infections. They have a wide range of antimicrobial activity against both gram positive and gram negative bacteria.

Jenkins *et al.* (1970) evaluated the haematological data of patients treated with trimethoprim-sulphamethoxazole @ 250 and 500 mg/kg body weight daily for a period of three months for chronic bronchitis. There was a consistent, but significant decrease in Hb, TLC and neutrophil counts in eight out of the ten cases.

Toth and Derwelis (1980) reported a case of sulphadiazine-trimethoprim induced hepatitis in a six year old St. Bernard with cystitis. Results of blood test confirmed hepatitis as there was an increase in ALT, AST and ALP levels.

Anderson *et al.* (1984) reported a case of hepatocellular damage in a 12 year old Basenji dog treated with 240 mg sulphadiazine-trimethoprim bid for two weeks and 125 mg cyclophosphamide for recurrent *Escherichia coli* cystitis and multiple myelomma. One week later, the animal developed icterus, bilirubinemia and elevated ALP and ALT levels suggesting a diagnosis of cholestatic liver disease. The dog returned to normal with no further evidence of hepatocellular damage, after the withdrawal of the drug.

A clinical case of generalised demodectic mange with deep pyoderma treated with trimethoprim-sulphamethazole revealed hematuria after six weeks of therapy. Whole blood clotting time and blood chemistry were normal. The main haematological findings were thrombocytopenia with low PCV, Hb concentration and TEC. Platelet counts gradually returned to normal over the next three weeks (McEwan, 1992).

Hepatotoxicosis was reported in a seven month old Schnauzer dog treated for pyoderma with trimethoprim-sulphadiazine administered @ 30 mg/kg body weight for two weeks. After the treatment period the animal showed signs of vomiting and anorexia. Serum biochemical analysis revealed high ALT, AST, ALP, γGT and total bilirubin level (Rowland *et al.,* 1992).

Frank and Egenwall (1994) reported comatose state with spastic convulsions in a two year old female German short haired pointer on ingestion of sulphanilamide containing ointment. Sulphanilamide toxicity was diagnosed on the basis of the neurological disturbances and the concomitant presence of sulphanilamide crystals in the urine.

Brown and Rogers (2001) studied the drug-associated neutropenia in 29 dogs of which three dogs developed neutropenia upon treatment with trimethoprim-sulphadiazine.

2.1.3 Aminoglycosides

Aminoglycosides are a therapeutically essential class of antibiotics whose usefulness is often restricted by their nephrotoxic and ototoxic potential.

Patel *et al.* (1975) in their study in rats found that gentamicin given @ 30 mg/kg body weight for 15 days s.c. showed a significant increase in BUN by day 15 whereas at 60 mg/kg body weight, BUN increased by day 5. They also found the appearance of significant quantities of lysosomal enzymes in the urine early in the course of gentamicin treatment.

Dellinger *et al.* (1976) examined the nephrotoxicity of cephalothin and gentamicin independently and in combination in rats and found that gentamicin @ 6, 12, 25 and 50 mg/kg body weight per day produced tubular necrosis which increased linearly with the increasing dosage. Blood urea nitrogen and serum creatinine values were significantly higher in the group of animals receiving gentamicin at the dose rate of 50 mg/kg body weight per day.

Garg *et al.* (1989) reported some of the haemato-biochemical changes during the administration of gentamicin @ 5 mg/kg body weight at eight hour interval for seven days in buffalo calves. The daily i.m. administration of gentamicin did not produce any significant adverse effect on TEC, TLC, DLC, Hb and PCV. The levels of ALT, AST and electrolytes (sodium, potassium and chloride) showed no change, but serum ALP, creatinine and BUN were found increased; a significant increase in creatinine and BUN suggested the renal damage.

Fadel and Larkin (1996) monitored the nephrotoxic effects of gentamicin in lambs @ 80 mg/kg body weight s.c., every eight hour interval with three doses. Three days after treatment, there were significant increase in TLC and neutrophil count, a decrease in lymphocyte count, but no significant changes in other

haematological parameters. An increase in the plasma creatinine concentration was observed in the gentamicin treated lambs from 14 days after treatment which became significant on 23rd day after treatment. No significant difference was observed in plasma concentration of urea, γGT, triacylglycerides, inorganic phosphates, potassium, total protein, albumin and globulin.

Gupta and Verma (1998) stated that the i.m. injection of gentamicin @ 4 mg/kg body weight at six hour interval for seven days in male guinea pigs did not produce any difference in serum total protein concentration between the treatment and control group, but found a significant increase in serum creatinine and BUN concentrations in gentamicin administered group. There was a significant increase in copper and a decrease in manganese concentration of serum. Blood samples collected on ninth day of experiment showed no significant difference in PCV, TEC and Hb concentration, but a significant decrease in TLC was observed in gentamicin administered animals when compared to controls. Also there was no significant difference on per cent values of neutrophils and lymphocytes between both the experimental group and control group, but reduction in absolute count of neutrophils was observed in gentamicin administered animals as compared to controls.

Erdem *et al.* (2000) assessed the nephrotoxicity of gentamicin in rats and found that the gentamicin @ 100 mg/kg/day for eight days produced a significant increase in serum creatinine and BUN. Lipid peroxidation was augmented; GPX and SOD activities were suppressed by gentamicin administration. These changes were brought back to the normal level by taurine treatment.

Jayanthi *et al.* (2000) evaluated the toxicity of gentamicin in chicks and found that kidney was the primary target organ during gentamicin toxicity. In birds that received higher doses of gentamicin @ 20 mg/kg body weight once daily for three days by s.c. route showed acute tubular necrosis and desquamative

changes. The hepatic changes were congestion, haemorrhage, and multi focal areas of necrosis.

Vijaykumar *et al.* (2000) confirmed the nephrotoxicity of gentamicin in rats after receiving a dose of 80 mg/kg body weight i.p. for eight days. Results showed a significant nephrotoxicity as evidenced by increase in BUN, serum creatinine, decreased creatinine clearance, renal tubular necrosis and glucosuria. Also a significant increase in lipid peroxidation products was noticed suggesting the involvement of oxidative stress.

Ali *et al.* (2001) studied the influence of sex on the renal toxicity of gentamicin and the results indicated that there was a small but statistically significant sex difference in the vulnerability of Sprague-Dawley rats to gentamicin nephrotoxicity. The creatinine and urea levels in plasma were higher in males than in females.

Yazar *et al.* (2003) evaluated the effects of aminoglycoside antibiotics on renal SOD, GPX, GSH and MDA. The aminoglycosides like gentamicin, kanamycin and streptomycin @ 100 mg/kg body weight for four days s.c. decreased renal GSH levels in all experimental groups. There was no change in SOD, GPX, MDA, total bilirubin, total protein, glucose, creatinine, uric acid, sodium and potassium levels.

Stokes and Forrester (2004) reviewed that the systemic use of aminoglycoside antibiotics has been known to cause nephrotoxicity in animals. A case of fatal nephrotoxicosis in cat was associated with topical administration of gentamicin @ 500 mg twice to a soft tissue wound. Serum gentamicin concentration was more than five times the therapeutic concentrations following eight hours after receiving the treatment and the cat was diagnosed as oliguric with renal failure. In four cats another aminoglycoside, paramomycin administered @ 70 - 208 mg/kg body weight orally every 12 h for less than five

days developed renal failure with vomiting, lethargy, dehydration and azotemia, which reverted within six to 11 months.

Derakhshanfar *et al.* (2007) conducted a study in rats with gentamicin @ 80 mg/kg body weight i.m. once daily for 10 days and found that gentamicin produced remarkable nephrotoxicity which was characterised by an increase in BUN and a statistically significant decrease in creatinine clearance as compared with the control rats. A comparable increase in fractional sodium excretion and a significant decrease in serum potassium reabsorption were noticed in gentamicin alone treated rats.

2.1.4 Fluoroquinolones

The introduction of fluorinated 4-quinolones, such as ciprofloxacin, moxifloxacin, gatifloxacin represents a particularly important advance because these agents have broad antimicrobial activity and are effective on oral administration for the treatment of a wide range of infectious diseases.

Fluoroquinolones group are highly effective at extremely low concentrations when compared to other antibiotics. They are becoming the drug of choice for serious gram negative infections in animals because of their similar antimicrobial spectrum to aminoglycosides. Due to the wide spectrum of bactericidal activity, oral efficacy and good tolerability it is being extensively employed for the blind therapy against infections.

Hooper and Wolfson (1985) reviewed the toxicities of fluoroquinolones in humans and reported toxic effects on gastrointestinal tract, cartilage and central nervous system. They also observed the development of crystalluria with high dose of fluoroquinolones especially norfloxacin. A low frequency or no abnormalities on blood counts and liver function were found in fluoroquinolone administration.

Jones and Smith (1997) reported a case of quinolone induced hepatitis in a 21 year old male treated with ofloxacin and ciprofloxacin for productive cough with increased serum biochemical parameters such as AST, ALT and bilirubin.

Lucena *et al.* (2000) reported three cases of acute hepatitis after trovafloxacin therapy @ 200 mg/day for one week in three males for respiratory infection. Serum biochemistry indicated acute liver injury with high ALT, AST, ALP and bilirubin levels.

Sarkozy (2001) reviewed the adverse effects of quinolones as gastrointestinal disturbances, dizziness, depression, non-inflammatory erosive arthropathies, and photosensitisation and occasionally increase in hepatocellular enzymes like ALT and AST, BUN, crystalluria and decrease in haematocrit.

Yazar and Tras (2001) found that enrofloxacin @ 10 mg/kg body weight for three days in Balb/C mice caused a decrease in GPX activity at 24, 48 and 72 h and danofloxacin at the same dose rate caused an increase in SOD activity at 24 and 48 h after the injection.

Kilic *et al.* (2003) showed that ciprofloxacin and pefloxacin @ 50 mg/kg body weight for 10 days in male albino rats showed a significant anti-inflammatory activity to the formalin induced oedema. There was a significant reduction in the TLC but no significant difference was observed in other haematological parameters like TEC, Hb concentration and haematocrit values.

Zimpfer *et al.* (2004) reported a case of 22 year old man who developed hepatic failure after intake of ciprofloxacin @ 2×250 mg for seven days for a non specific bacterial infection of the skin. Seven days after ciprofloxacin intake, the laboratory findings showed the typical pattern of acute liver failure with elevated ALT, AST, ALP, γGT, bilirubin and reduced prothrombin time. Liver biopsy revealed extensive hepatocellular necrosis. Liver function tests and levels of ALT

and AST improved rapidly, reaching normal levels within one week after corticosteroidal therapy.

Baykal *et al.* (2005) reported that the rats treated with ciprofloxacin @ 400 mg/kg body weight for four, seven and 14 days showed no significant differences in sodium, potassium levels as well as pH values with the control animals. Furthermore, no significant difference was observed for the creatinine clearance in all groups of animals and the weight of the rats remained unchanged. Urine ß-NAG values were significantly higher in four day treatment group when compared with control rats but there was no significant difference between four and seven days treated rats. These results indicate that ciprofloxacin in this dose comparable to those commonly prescribed, does not induce nephrotoxicity.

2.1.5 Tetracyclines

Tetracyclines are bacteriostatic antibiotics with activity against a wide range of aerobic and anaerobic gram positive and gram negative bacteria and as an additive to animal feeds to facilitate growth.

Aronson (1980) reviewed the untoward effects of tetracyclines such as phototoxicosis, hepatic failure characterised by fatty metamorphosis and gastro-intestinal upsets. Other changes associated with the daily i.v. injection of minocycline for one month included decrease in TEC, Hb and heamatocrit in all dogs given 10 mg/kg body weight, increase in ALT and AST in one dog given 20 mg/kg body weight and increased urinary calcium, sulfobromophtalein retention, decreased food consumption and marked loss in body weight @ 40 mg/kg body weight /day.

Machado *et al.* (2003) reported the possible effects of tetracycline administration to rats on the tenth day of pregnancy, on kidney and liver development of the offspring. The pregnant rats were given tetracycline

hydrochloride i.p. @ 25, 50, 75 and 100 mg/kg body weight. The liver of the newborns in the treated groups presented lesions such as hepatocyte vacuolisation, sinusoidal dilatation, foci of necrosis, inflammation and haematopoietic cells which were intense as the dose increased. The kidneys of the offspring also had moderate amount of vacuolisation in the convoluted tubules and Henle's loop, foci of necrosis in tubular epithelium which showed a positive correlation with increased dose rate.

Vijaykumar *et al.* (2004) reported that the drug induced hepatotoxicity was an established cause of acute hepatic failure in dogs. Oxytetracycline administered @ 22 mg/kg body weight orally bid for 14 days in dogs with *Ehrlichia canis* infection showed an elevated level in the serum biochemical values such as ALT, ALP, bile acids, bilirubin and the liver biopsy section showed foamy cytoplasm with mild to moderate hydropic changes at the end of the treatment. There was no change in mean Hb, PCV and TEC. Liver weight also increased in group of animals treated with oxytetracycline. Leucocytosis and monocytosis observed on day 0 of trial returned to normal following treatment with oxytetracycline.

Gnanasoundari (2006) observed that prolonged administration of oxytetracycline @ 200 mg/kg body weight i.p. in rats resulted in significant elevation in serum hepatospecific markers such as AST, ALT, LDH and bilirubin. The levels of lipid peroxidation markers and lipid peroxides in liver were also found elevated. Oxytetracycline also caused a significant reduction in activities of SOD, GPX, GSH, catalase, vitamin C and vitamin E in liver.

Vijaykumar *et al.* (2008) evaluated the haemato-biochemical changes in dogs with primary hepatic disorders caused by liver diseases, oxytetracycline and pyometra induced hepatitis, steroid hepatopathy and hepatitis due to leptospirosis. In oxytetracycline induced hepatitis marked increase in serum ALT, ALP, bilirubin and bile acids; decrease in erythron values and neutrophilic leucocytosis were noticed.

2.1.6 Chloramphenicol

Saba *et al.* (2000) reported the toxic effect of the prolonged use of chloramphenicol in rats, which produced a significant increase in ALT, AST, BUN and creatinine.

2.2 CANINE PYODERMA

Bacterial pyoderma is among the most common causes of skin diseases in dogs and it is very pleomorphic in nature. The most important organism causing canine pyoderma involve *Staphylococcus intermedius*, which is a member of normal flora in most dogs and colonises the upper respiratory tract, oral cavity and sometimes the perianal region and the external ear canal.

Pyodermas may be classified according to their primary etiology. But a more useful classification is based on the depth of involvement of the skin, as surface, superficial and deep pyodermas.

Surface pyoderma are characterised by superficial erosions associated with increased colonisation by pathogenic *Staphylococcus* sp. Superficial pyoderma involves the epidermis or the superficial portion of the hair follicle. Deep pyodermas are less common than superficial pyoderma and involve tissues deeper than the hair follicles, including the dermis and even the subcutaneous layer (Codner, 1988a; Ihrke, 1987; Craig, 2003).

2.2.1 Signalment

2.2.1.1 Age Wise Occurrence

Slade *et al.* (1984) studied the prevalence of pyoderma in 26 dogs with an age of six months to 13 years and observed more incidences in three and half years.

In the study of Wisselink *et al.* (1985) pyoderma infection was found more common with seven years of age.

Retrospective analysis of 17 cases of bacterial folliculitis, furunculosis and cellulitis in GSD revealed that the age of the affected animals was within a normal range of three months to 13 years and the disease seemed to affect primarily the middle aged dogs with mean of five years (Krick and Scott, 1989).

Aujla *et al*. (1997) reported greater prevalence of bacterial dermatitis in dogs up to two years of age (26.48 %).

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

Udayasree (2004) reported that the dogs between the age group of one to four years were more frequently affected with pyoderma.

2.2.1.2 Breed Wise Occurrence

According to Krick and Scott (1989), GSD was more prone to recurrent bacterial folliculitis, furunculosis and cellulitis.

Pal *et al*. (1995) also reported that the clinical cases of canine pyoderma were more in GSD compared to other breeds.

Carlotti *et al*. (1999) observed that 23.1 per cent GSD, 10.3 per cent Labrador, 7.7 per cent Boxer, 7.7 per cent Cocker Spaniel, 7.7 per cent crossbreeds, 7.7 per cent English Setter and 39 per cent others were susceptible to pyoderma.

Mathews (1999) reported that the non-descript dogs were more affected (33.3 per cent) with pyoderma followed by GSD (23.8 per cent).

Udayasree (2004) reported that pyoderma had the highest occurrence in GSD (32.69 per cent), followed by other breeds which included Rottweiler, Spitz, Boxer, 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).

2.2.1.3 Sex wise Occurrence

Wisselink *et al.* (1985) reported that out of 23 GSD with deep pyoderma, 15 were intact males and two of the eight females were ovariohysterectomised.

Krick and Scott (1989) reported that out of 17 GSD with bacterial folliculitis, furunculosis and cellulitis, six were intact males, three intact females, two castrated males and six spayed females.

In a study of pyoderma in 45 dogs by Frank and Kunkle (1993), 22 were males and 23 were females.

Mathews (1999) reported the prevalence of bacterial dermatitis in females as 47.6 and males as 52.4 per cent.

The sex wise prevalence of pyoderma was highest in females with 55.77 per cent (Udayasree, 2004).

2.2.2 Etiology

Staphylococcus intermedius is the primary bacterial pathogen of the skin in dogs and is the most common isolates in canine pyoderma (Berg *et al.*, 1984).

Ihrke (1984) reported that the most common canine pyoderma is caused by coagulase positive *Staphylococcus* sp.

According to Ihrke (1987), the factors leading to the initiation of pyoderma may be pre-existing diseases like seborrhea and allergy, poor grooming, ectoparasites, immune incompetence, endocrinopathies such as hypothyroidism and Cushing's disease and the injudicious use of corticosteroids.

Codner (1988a) explained the predisposing factors in canine pyoderma as poor grooming, macerated skin, ectoparasites, seborrhea, allergic skin diseases, endocrine abnormalities, immunosuppression by glucocorticoids and inherent immune disorder. The author also reviewed the most important bacterial cause of canine pyoderma as *Staphylococcus intermedius*, the secondary pathogens being *Escherchia coli*, *Pseudomonas* sp. and *Proteus* sp.

Mason and Lloyd (1989) investigated the role of allergy in the pathogenesis of pyoderma. Intradermal injections of *Staphylococcal* antigens in normal dogs elicited epidermal damage and there was an increased percutaneous absorption of radiolabelled *Staphylococcal* antigens by mast cell degranulation. These findings suggested that epidermal permeability caused by hypersensitive reaction of *Staphylococcal* antigen from the stratum corneum caused the lesions of pyoderma.

Hill and Moriello (1994) reviewed canine atopy as one of the underlying causes that predisposes to canine pyoderma.

Batamuzhi *et al*. (1998) estimated the protein fraction in urine from proteinuric dogs with and without pyoderma by agarose gel electrophoresis. Among the five dogs with superficial pyoderma, three had albuminuria, two had globinuria and none had serum-like eletrophoretic profile. Of the dogs with deep

pyoderma, eight had an albuminuria profile; one each had a serum-like proteinuria and a globinuria profile.

2.2.3 Clinical Signs

Wisselink *et al.* (1985) mentioned the lesions in pyoderma as fistulisation, cluster of pustules, ulceration, alopecia, hyperpigmentation and thickening of the skin. The lesions also had haemopurulent exudates.

Superficial pyoderma is characterised by crusted papules and pruritus and in deep pyodermas, clinical signs included seropurulent debris drains from ruptured pustules, erythema, hyperpigmentation, maceration, indurations and swelling (Ihrke, 1987)

The clinical signs in pyoderma also include papules, pustules, crusting, epidermal collarettes, alopecia, erythema, hyperpigmentation, ulcers, draining tracts and scaling (Craig, 2003; Horvath and Neuber, 2007)

The clinical symptomology reported by Seena *et al.* (2005) was fistulous tracts with oozing pus, lichenification over joint areas, erythema, pustules, pain, deep ulceration of skin, plaque formation and matting of hairs.

2.2.4 Clinical Pathology

2.2.4.1 Hematology

Fadok (1982) reported a case of zinc responsive dermatosis in a six month old Great Dane dog with slight leucocytosis, neutrophilia, monocytosis and eosinophilia.

Wisselink *et al.* (1985) reported the clinical pathology in canine pyoderma as slightly decreased PCV (36-39 per cent) and leucocytosis (15.6- 20.2 × $10^3/mm^3$).

Krick and Scott (1989) investigated the haematological parameters in seventeen GSD with canine pyoderma and results revealed a mild leucocytosis in eight dogs and neutrophilia in four dogs.

Aujla *et al*. (1997) reported that the stress due to dermatitis and bacterial toxins have resulted in marked leucocytosis (27 – 88 × $1000/mm^3$) and absolute neutrophilia (78.20 per cent).

Mathews (1999) reported that the PCV value of 21 dogs with pyoderma as 43.3 per cent and TLC as 14926 cells/mm^3.

Udayasree (2004) reported that the Hb and PCV values obtained in dogs with bacterial dermatitis were within the normal range. The slight elevation in the leucocyte count could be due to bacterial infection.

2.2.4.2 Biochemical Parameters

Fadok (1982) reported that the chemistry panel of zinc responsive dermatosis in a six month old Great Dane dog was within the normal limits.

Wisselink *et al.* (1985) reported elevated total protein concentrations (7 g/dl), concomitant hypoalbuminemia (1.6-3 g/dl) in canine pyoderma. Plasma concentration of urea, ALP and ALT were within reference limits.

Krick and Scott (1989) estimated the serum biochemical profile in canine pyoderma and observed a mild increase in globulin levels (4.1 - 5.2 g/dl) in three cases.

Pal *et al*. (1995) studied the different biochemical attributes of blood and serum and found that the serum cholesterol and blood glucose levels were significantly higher in canine pyoderma.

Aujla *et al.* (1997) analysed the biochemical characteristics in bacterial dermatitis and found no significant change in total proteins, albumin and total immunoglobins.

Udayasree (2004) reported that the total protein, albumin, A:G were within the normal range.

2.2.4.3 Mineral Studies

Pal *et al*. (1995) noticed a significant decrease in the levels of serum calcium, copper, zinc and iron in experimentally infected dogs with pyoderma.

In a study of clinical implication of sodium:potassium in dogs, Pak (2000) reported a normal level of sodium, but an elevated potassium level in pyoderma infection.

2.2.5 Culture and Sensitivity Test

According to Frank and Kunkle (1993), the antimicrobial resistance and susceptibility patterns for the *Staphylococcus intermedius* isolated from 44 dogs with pyoderma revealed that 16 dogs with first time pyoderma and 28 dogs with recurrent pyoderma were susceptible to enrofloxacin and ciprofloxacin.

Kamboj *et al.* (1995) reported that 89 per cent of *Staphylococcal* isolates obtained from canine bacterial dermatitis were more sensitive to cephalexin and amikacin (100 per cent), followed by cloxacillin (93.59 per cent), amoxycillin

(91.13 per cent), gentamicin, kanamycin, lincomycin and chloramphenicol (89.65 per cent).

Staphylococcus intermedius isolated from canine pyoderma were highly resistant to ampicillin, amoxycillin/clavulanic acid and enrofloxacin (Dowling, 1996).

Kruse *et al*. (1996) reported in a retrospective study on the antimicrobial susceptibility pattern of *Staphylococcus* sp. isolated from dermatitis in dogs that all the isolates were sensitive to cloxacillin, cephalexin and quinolones such as enrofloxacin and ciprofloxacin.

Antimicrobial resistance and susceptibility patterns for the *Staphylococcus* sp. isolated from 18 dogs with superficial bacterial dermatitis revealed that enrofloxacin was most sensitive in all dogs followed by erythromycin and ampicillin (Bloom and Rosser, 2001).

As per Petersen *et al*. (2002), *Pseudomonas aerugenosa* and *Staphylococcus intermedius* isolated from canine skin were more sensitive to ciprofloxacin.

2.2.6 Treatment

Oral, systemic antibiotics are the first choice for the treatment of canine bacterial pyoderma. The choice of an appropriate antibiotic can be made empirically or based on the results of a culture and susceptibility test.

Ihrke (1984) recommended penicillins, potentiated sulphonamides, chloramphenicol, macrolides, lincosamides, aminoglycosides and cephalosporins for the treatment of pyoderma.

Antibiotics that are effective against *Staphylococcus intermedius* are appropriate for treating canine pyodermas and those with proven efficacy for this include erythromycin, lincomycin, beta-lactamase resistant penicillins, trimethoprim potentiated sulphonamides, chloramphenicol, cephalosporins and amoxycillin-clavulanic acid combinations (Codner, 1988b).

Mason (1991) suggested the use of erythromycin, lincomycin, clavulanic acid-potentiated sulphonamides for the empirical treatment of canine pyoderma.

Campbell (1999) indicated the use of sulphadimethoxine/ormethoprim for the treatments of skin, soft tissue and urinary tract infections of dogs caused by *Staphylococcus* sp., *Escherchia coli*, and *Proteus* sp.; the treatment should be continued for a minimum of two days past clinical resolution.

Craig (2003) reported the management of pyoderma with fluoroquinolones, cephalosporins, amoxycillin, potentiated sulphonamides and lincosamides based on antibiotic sensitivity test.

Horvath and Neuber (2007) reviewed the use of fluoroquinolones, cephalosporins, amoxycillin, doxycycline, clindamycin, lincomycin and potentiated sulphonamides for the treatment of canine pyoderma.

2.2.6.1 Efficacy of Fluoroquinolones in the Treatment of Canine Pyoderma

Hill and Moriello (1994) reported the use of quinolone group of antibiotics for the treatment of deep pyoderma in dogs. Enrofloxacin and ciprofloxacin were the drugs of choice for severe deep pyoderma with mixed infections.

Curtis *et al.* (1995) reported two cases of eosinophilic folliculitis and furunculosis in dogs. In the first case treatment with enrofloxacin @ 5 mg/kg body weight once daily for one week cured peripheral eosinophilia (3.2×10^9/L)

with hair regrowth at the lesions. The second case treated with clindamycin @ 11 mg/kg body weight and oral prednisolone 1.1 mg/kg body weight once daily also healed the lesions within ten days.

Dowling (1996) reported that enrofloxacin was the first choice of antibiotic for deep pyoderma and short term therapy for recurrent pyoderma. It had an excellent antibacterial activity against *Staphylococcus intermedius* and *Pseudomonas* sp.

Carlotti *et al*. (1999) investigated the effectiveness and safety of marbofloxacin in the treatment of difficult cases of canine pyoderma. Marbofloxacin was administered orally @ 2.12 mg/kg body weight, once daily in 54 dogs for time periods varying from ten to 213 days. Thirty three dogs showed an excellent response, one animal showed a clear improvement, one a slight improvement and remaining four dogs showed no response after 11 to 60 days of treatment. Fifteen dogs relapsed over the follow-up period of three to 191 days.

Paradis *et al.* (2001) conducted an open clinical trial in 72 dogs to evaluate the clinical efficacy of marbofloxacin in the treatment of canine pyoderma and assessed the treatment response as 86.1 per cent successful, 8.3 per cent improvement and 5.6 per cent failure after 28 days of treatment.

Horspool *et al*. (2004) assessed the relative efficacy of ibafloxacin and marbofloxacin in the treatment of canine pyoderma. The therapeutic regime consisted of ibafloxacin and marbofloxacin @ 15 and 2 mg/kg body weight respectively for two weeks. Results showed that one week after the cessation of the therapy, 74 and 81 per cent of dogs in ibafloxacin and marbofloxacin respectively responded to therapy and one month after the therapy, 70 per cent in both the groups were classified as cured or improved.

Udayasree (2004) reported that ciprofloxacin and enrofloxacin were found to be the most effective antibiotics against bacterial isolates of canine pyoderma.

Bruner (2006) stated that marbofloxacin @ 2.5 - 5 mg/kg body weight orally once daily was highly efficient in the treatment of cutaneous infections, superficial and deep pyoderma.

Hillier *et al.* (2006) conducted study with systemic antibiotics such as enrofloxacin @ 6 - 13 mg/kg body weight once daily (six dogs) and 5 - 12 mg/kg body weight bid (two dogs), norfloxacin @ 18 - 23 mg/kg body weight once daily (two dogs) and 11 - 22 mg/kg body weight (three dogs), marbofloxacin @ 3 - 5 mg/kg body weight once daily (three dogs) and cephalexin @ 20 - 25 mg/kg body weight twice daily (two dogs) for the treatment of canine pyoderma caused by *Pseudomonas aerugenosa* in 18 dogs. The duration of treatment was three to 12 weeks and found that there was complete resolution of the clinical signs in 16 dogs, except each one in enrofloxacin and norfloxacin treated group.

Mueller and Stephen (2007) compared the efficacy of pradofloxacin @ 3 mg/kg body weight once daily and amoxycillin/clavulanic acid @ 12.5 mg/kg body weight twice daily for the treatment of deep bacterial pyoderma for a period of three weeks. This comparison showed that out of 56 dogs treated with pradofloxacin, 48 dogs achieved clinical remission, four showed improvment, four did not respond. There was no recurrence of clinical signs even after 11 weeks. Out of 51 dogs treated with amoxycillin/clavulanic acid, 37 dogs achieved clinical remission, three showed improvement, five showed no response and in six dogs, clinical signs recurred within two weeks after therapy emphasising that pradofloxacin was most effective in the treatment for canine pyoderma.

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2.2.6.2 Adverse Effects of Fluoroquinolones

Carlotti *et al.* (1999) evaluated the safety of marbofloxacin @ 2 mg/kg body weight once daily in the treatment of canine pyoderma and found that none of the 39 dogs in the study showed any adverse effects, even when the duration of antibiotic therapy was as long as seven months which is often required in cases of deep pyoderma.

Ihrke *et al*. (1999) reviewed the adverse effects of fluoroquinolones which occurred with the use of higher doses and were not significant. Mild gastrointestinal disturbances like nausea, vomiting and diarrhoea were observed which were neglectable.

Mohanasundaram and Mohanasundaram (2001) evaluated the adverse effects of ciprofloxacin with reference to arthopathy in young rats by administering ciprofloxacin @ 80 mg/kg body weight/day in two divided doses for 15, 30 and 45 days to find out the minimum duration for which ciprofloxacin can be administered safely and to know the extend to which arthopathic changes were reversible. In this study, one month old rats treated with ciprofloxacin did not show any radiological evidence of arthopathy in plain x-ray in the weight bearing joints when treated up to 45 days. There was an increase in serum ALP which was proportionate to the duration of therapy and the values returned to normal within 15 days after withdrawal of the therapy indicating the changes were transient and reversible.

Paradis *et al.* (2001) evaluated the adverse effects associated with marbofloxacin treatment in canine pyoderma for 28 days and found that six out of 81 dogs showed listlessness, anorexia, vomiting, polydipsia and flatulence which resolved despite continuation of the treatment.

Horspool *et al.* (2004) evaluated the efficacy and safety of ibafloxacin and marbofloxacin @ 15 and 2 mg/kg body weight respectively orally, once daily for three week period. The study revealed that out of the 245 dogs, only one dog treated with ibafloxacin developed gastrointestinal disturbances after two to three weeks of treatment and one dog treated with marbofloxacin showed signs of an allergic-type reaction after eight days of treatment confirming the safety of fluoroquinolones in the long term treatment of canine pyoderma.

Marbofloxacin @ 5.5 mg/kg body weight per day is generally well tolerated with a low occurrence of adverse effects such as inappetance, decreased activity and vomiting (Bruner, 2006).

Mueller and Stephen (2007) compared the safety and efficacy of pradofloxacin, a new fluoroquinolone with amoxycillin/clavulanic acid in the treatment of canine deep pyoderma. The suspected adverse effects noticed when treated with pradofloxacin @ 3 mg/kg body weight once daily for three weeks were polydipsia and polyuria and diarrhoea. All drug related adverse effects disappeared after treatment termination, providing evidence that pradofloxacin is apparently safe drug for long term therapy of canine deep pyoderma.

2.2.6.3. Other Antibiotics used against Pyoderma and their Adverse Effects

Treatment of pyoderma caused by *Staphylococcus* sp. with clavulanate-potentiated amoxycillin and amoxycillin orally @ 12.5 mg/kg body weight and 10 mg/kg body weight twice daily for 14 days independently revealed the success rate as 90 and 86 per cent respectively (Bywater *et al.,* 1985).

Codner *et al.* (1988b) reported that the side effects of trimethoprim-sulphonamide combinations in the treatment of canine pyoderma as reversible non regenerative anaemia and keratoconjunctivitis sicca. The side effects of chloramphenicol were decreased antibody production and a reversible depression

of the hepatic microsomal enzymes which prolonged the action of some drugs. They also described the limitations in the use of aminoglycosides for the treatment of pyoderma due to its nephrotoxicity on prolonged use.

Moriello (1989) recommended the use of trimethoprim-sulphadiazine @ 30 mg/kg body weight twice daily for the first occurrence of pyoderma. In recurring cases of pyoderma, oxacillin was recommended @ 20 mg/kg body weight thrice daily and the therapy was intended for at least three weeks.

Khosla *et al*. (1991) conducted a study in fifteen dogs suffering from pyoderma caused by *Staphylococcus aureus* treated with benzathine penicillin parentrally twice a week and observed that nine cases recovered completely within 15 days. Rest six cases were diagnosed as mixed infection of *Sarcoptes scabiei* and *Staphylococcus aureus* and were treated with ivermectin along with antibacterial therapy.

Scott *et al.* (1994) evaluated the efficacy of tylosin tablets for the treatment of pyoderma in dogs @ 20 mg/kg body weight twice daily for an average period of 33 days. Out of the 21 dogs treated with tylosin, 19 dogs showed an excellent response (90.51 per cent) with no adverse drug effects.

Lloyd *et al*. (1997) conducted a study to evaluate the efficacy of co-amoxyclav (amoxycillin and clavulanic acid) @ 12.5 mg/kg body weight twice daily and 25 mg/kg body weight twice daily orally for a period of 12 weeks for eliminating the lesions of canine pyoderma. There was no significant difference between the responses obtained with the two dose rates. The response rate was 91.5 per cent for folliculitis, 87.5 per cent for furunculosis and 60 per cent for cellulitis.

Scott *et al.* (1998) investigated the efficacy of clindamycin in the treatment of canine pyoderma @ 11 mg/kg body weight once daily for an average period of

45 days. Clindamycin produced an excellent result in 100 per cent of the dogs treated and the only side effect noticed was vomiting.

Campbell (1999) indicated the use of sulphadimethoxine/ormethoprim for the treatment of skin, soft tissue and urinary tract infections of dogs caused by strains of *Escherchia coli*, *Staphylococcus* sp. and *Proteus* sp. The treatment was followed by the risk of adverse effects such as keratoconjunctivitis sicca and hypothyroidism when treatment is required beyond 21 days.

Potentiated amoxycillin or cephalosporins were reported as highly effective in the treatment of most clinical cases of canine pyoderma (Harvey and Hunter, 1999).

Mason and Kietzmann (1999) reviewed the use of cephalosporins @ 15 - 30 mg/kg body weight twice daily for a period of seven to 14 days for superficial pyoderma and three to four weeks for deep pyoderma. The most important side effects reported were hypersensitivity reactions, gastrointestinal disturbances but none exhibited any signs of nephrotoxicity.

Bloom and Rosser (2001) evaluated the clinical efficacy of clindamycin @ 11 mg/kg body weight once daily over a period of 14 to 42 days. Excellent response was observed in 71.4 per cent of dogs with 14 to 28 days. Baseline and follow up of complete blood counts, serum biochemical profiles and urinalysis were within reference ranges during the study. No side effects or adverse reactions to clindamycin were noticed.

Mellor *et al.* (2005) reviewed a case report of neutrophilic dermatitis and vasculitis with immune-mediated hemolytic anaemia and thrombocytopenia occurring as a suspected adverse drug reaction to carprofen along with cefalexin and co-amoxyclav prescription in a dog. Haematological examination revealed a marked normocytic, normochromic, non regenerative anaemia with TEC, Hb,

PCV values below the reference range. No significant abnormalities were detected in parameters like urea, glucose, creatinine, total protein, albumin, globulin, sodium, potassium, calcium, ALP, ALT, AST, total bilirubin and cholesterol.

Seena *et al.* (2005) investigated the therapeutic efficacy of trimethoprim-sulphamethazole (30 mg/kg body weight twice daily), lincomycin (22 mg/kg body weight twice daily) and cephalexin (25 mg/kg body weight twice daily) for a period of one week and continued for a week in surface and superficial pyoderma and two weeks in deep pyoderma even after clinical recovery. Therapeutic evaluation revealed that the per cent recovery of dogs treated with trimethoprim-sulphamethazole, lincomycin and cephalexin were 66.6, 83.3 and 100 respectively.

Bruner (2006) reported the use of cefpodoxime proxetil, a group of cephalosporin @ 5 - 10 mg/kg body weight orally once daily for a maximum period of 28 days in the treatment of bacterial skin diseases caused by *Escherchia coli*, *Staphylococcal* sp. and *Proteus* sp. Even though cefpodoxime proxetil is well tolerated in dogs, it has low level of toxicity with clinical signs of vomiting, diarrhoea and decreased appetite.

Cefovicin demonstrated statistical non-inferiority as compared with amoxycillin/clavulanic acid in the treatment of bacterial skin infections in dogs. The treatment response was 96.9 and 92.5 per cent for cefovecin and amoxycillin/clavulanic acid respectively (Stagemann *et al.,* 2007).

Materials and methods

3. MATERIALS AND METHODS

The study was conducted in dogs presented to the Veterinary College Hospital, Mannuthy, for the treatment of pyoderma during the period of August 2007 to March 2008. Twenty one dogs with clinical signs of pyoderma treated with ciprofloxacin were selected for the study. The signalment and previous history of the dogs were recorded and subjected to detailed anamnesis, clinical and bacteriological examination. The clinical signs of pyoderma like papules, pustules, hyperpigmentation, purulent discharge, nodules, matting of hairs were evaluated and also examined for the presence of ectoparasites.

3.1 OUTLINE OF THE STUDY

3.1.1 Pre-treatment evaluation

General information and dermatologic history of the patient were collected from the owner on the pre-treatment day (0^{th} day). A detailed physical examination was performed and the dogs with specific clinical signs or lesions indicative of pyoderma were documented.

3.1.1.1 Collection of Clinical Materials

Skin swabs and blood samples were taken from the dogs having clinical signs suggestive of pyoderma.

3.1.1.1.1 Collection of Skin Swabs

Clipped the hairs around the lesions. Gently swabbed the area with 70 % alcohol and air-dried. A touch swab of the exudates was collected aseptically and placed in a disposable sterile tube.

3.1.1.1.2 Collection of Blood Samples

About 10 ml of blood was collected from the cephalic or saphenous vein of the animal in a clean dry sterile syringe using scalp vein set.

A small drop of fresh blood was placed on smooth clean grease free glass slide towards an edge and drew the spreader slide gently into the drop of blood to make a thin smear for DLC.

Three ml of the blood was transferred to a clean dry sterile vial with sodium salt of EDTA as anticoagulant (@ 1 mg/ml of blood) for the estimation of Hb, TEC, PCV, ESR and TLC.

The remaining blood was then transferred to a clean dry sterile vial without anticoagulant for getting serum for biochemical analysis. The sera were then separated and stored in a sterile vial at -25°C for further use.

3.1.1.2 Treatment Schedule

The pyoderma confirmed dogs were treated with ciprofloxacin (Ciplox 250; Cipla Ltd.) @ 10 mg/kg body weight once daily orally for a period of 14 days.

3.1.2 Interim Evaluation

An interim evaluation was performed on seventh and 14th day of treatment to assess the clinical response. Blood samples were collected on seventh and 14th day of therapy for assessment of the haemato-biochemical changes.

3.1.3 Post Treatment Evaluation

A post treatment evaluation was conducted on fifth day after the completion of the therapy for assessment of clinical response. Blood samples were also collected on this day to evaluate the haemato-biochemical picture.

3.2 ISOLATION AND IDENTIFICATION OF THE BACTERIAL ORGANISM

3.2.1 Isolation of Bacteria

The skin swabs were put in the sterile peptone water and incubated at 37°C for 24 h and a drop of the inoculum was streaked aseptically on sterile nutrient agar plates. These plates were incubated for 24 - 48 h at 37°C.

Plates were examined after 24 - 48 h. Single colonies were selected and a representative sample was streaked on nutrient agar slants for further identification. Slants were preserved by storing in refrigerator at 4^{o}C.

3.2.2 Identification of the Bacterial Organism

3.2.2.1 Gram's Staining Method

A 24 h old culture was smeared on a clean slide, air dried and passed over the flame for fixation. The smear was then stained with crystal violet for one minute followed by Gram's iodine for another one minute. The stained smear was decolourised with ethyl alcohol for 15 seconds and then counterstained with dilute carbol fuchsin for 30 seconds. After each step the slide was washed with water. The stained slide was air dried and subjected to microscopic examination under oil

immersion objective. The bacteria were studied and each isolate was recorded as gram positive or gram negative cocci, bacilli or coccobacilli.

3.3 ANTIBIOGRAM

3.3.1 Materials

Mueller-Hinton agar was used to study the antibiotic sensitivity pattern of the isolates. The following antibiotic discs with known concentration as noted in µg/disc were used (Hi-Media Laboratories Private Limited, Mumbai, India).

Antibiotic discs	Concentration of the antibiotic in the disc	Disc code
Ciprofloxacin	5 µg/disc	RC
Gentamicin	10 µg/disc	GM
Cefotaxime	10 µg/disc	Cc
Amoxycillin	10µg/disc	Ax
Cephalexin	10µg/disc	CF

3.3.2 Method

Antibiotic sensitivity tests were done as per the standard disc diffusion method of Bauer *et al.* (1966).

Sterile Mueller-Hinton agar plates were prepared with a medium thickness of about four mm for rapidly growing aerobic organisms. Pure culture was used as inoculum. Three to four similar colonies from the cultures were selected and transferred them into about 5 ml of tryptone soya broth. Incubated at 35°C for 2 - 8 h

till light to moderate turbidity was obtained to yield a uniform suspension containing 10^5 - 10^6 cells/ml.

A sterile non-toxic cotton swab was dipped into the standardised inoculum and rotated the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streaked the entire agar surface of the plate with the swab three times, turning the plate at 60°C angle between each streaking. The inoculum was allowed to dry for 5 - 15 minutes with lid in place.

The antibiotic discs were applied aseptically in the centre at least 24 mm apart. The plates were incubated immediately at 37°C and examined after 14 - 19 h or later if necessary. The zones showing complete inhibition were measured and recorded the diameters of the zones to the nearest millimeter.

3.4 EXAMINATION OF BLOOD FOR HAEMATOLOGICAL PARAMETERS

3.4.1 Haemoglobin Estimation

Haemoglobin was estimated photometrically by cyanhaemoglobin method using Hemoglobin kit, Beacon Diagnostics Pvt. Ltd.

Principle

In alkaline medium haemoglobin and its derivatives are oxidised in presence of potassium ferricyanide and converted to methaemoglobin which reacts then with potassium cyanide to form purple red coloured cyanhaemoglobin complex, the intensity of which is measured in a spectrophotometer (Spectronic 1001) at 546 nm.

Reagents

Drabkin's solution

Sodium bicarbonate	1 g
Potassium cyanide	50 mg
Potassium ferricyanide	200 mg
Distilled water	1000 ml

Procedure

	Blank	Test
Working Drabkin's solution	5 ml	5 ml
Blood	-	20 µl

Mixed well and allowed to stand at room temperature for 5 minutes. Measured the absorbance of test against blank at 546 nm. Took the absorbance of cyanhaemoglobin standard directly (without adding working reagent), against blank at 546 nm.

Calculation

$$\text{Blood haemoglobin in g/dl} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 15.06 \text{ (standard concentration)}$$

3.4.2Total erythrocyte count

Total erythrocytes were counted using standard dilution technique using Hayem's fluid as described in Benjamin (1985) and value expressed as $\times 10^6$ cells/mm^3 of blood.

3.4.3 Packed Cell Volume

Packed cell volume was estimated by Wintrobe's method as described in Benjamin (1985) and expressed as percentage.

3.4.4 Erythrocyte Sedimentation Rate

Erythrocyte sedimentation rate was estimated by Wintrobe's method for one hour as described in Benjamin (1985) and expressed as mm/hour.

3.4.5 Total Leucocyte Count

Total leucocytes were counted using standard dilution technique using Thoma's fluid as described in Benjamin (1985) and value expressed as $\times 10^3$ cells/ mm^3 of blood.

3.4.6 Differential Leucocyte Count

Blood smears were stained with Leishman's stain and 100 leucocytes were counted under oil immersion objective. The differential count was expressed as percentage as described in Benjamin (1985).

3.5 ESTIMATION OF SERUM BIOCHEMICAL PARAMETERS

3.5.1 Alanine aminotransferase

Alanine aminotransferase was determined photometrically based on reference method of International Federation of Clinical Chemistry (IFCC) in semiautomatic

blood analyzer (Microlab 200) using Ecoline ALAT kit, (Merck Specialities Pvt. Ltd.).

Principle

$$\text{L-alanine} + \text{2-Oxoglutarate} \xleftrightarrow{\text{ALT}} \text{L-Glutamate} + \text{Pyruvate}$$

$$\text{Pyruvate} + \text{NADH} + H^{+} \xleftrightarrow{\text{LDH}} \text{L-Lactate} + NAD^{+}$$

The rate of NADH consumption is measured photometrically at 340 nm and is directly proportional to the ALT concentration in the sample.

Reagents

Reagent 1:	TRIS buffer	pH 7.5	100 mM/L
	L-Alanine		500 mM/L
	LDH		≥ 1.2 kU/L
Reagent 2 :	2-Oxoglutarate		15 mM/L
	NADH		0.18 mM/L

Assay Procedure

Wavelength	340 nm
Light path	1 cm
Temperature	25°C

Procedure

Preparation of Reaction Solution

Mixed reagent 1 and 2 in the ratio 4:1 and proceeded as follows.

Sample	100µl
Reaction solution	1000 µl

Mixed well and after 1 minute read the decrease in absorbance every minute for 3 minutes.

Calculation

ALT activity [IU/L] = ΔA/minute × factor

ΔA / minute = mean absorbance change/minute

Factor = 1746

3.5.2 Asparatate aminotransferase

Asparatate aminotransferase was determined photometrically based on reference method of IFCC in semiautomatic blood analyzer (Micro lab 200) using Ecoline ASAT kit, (Merck Specialities Pvt Ltd).

Principle

$$\text{L-Asparatate + 2-Oxoglutarate} \xleftrightarrow{\text{AST}} \text{L-Glutamate + Oxaloacetate}$$

$$\text{Oxaloacetate + NADH + } H^{+} \xleftrightarrow{\text{MDH}} \text{L-Malate + } NAD^{+}$$

The rate of NADH consumption is measured photometrically at 340 nm and is directly proportional to the AST concentration in the sample.

Reagents

Reagent 1: TRIS buffer	pH	7.8	80 mM/L
L-Asparatate			240 mM/L
MDH			≥ 420 mM/L
LDH			≥ 600 mM/L
Reagent 2 : 2-Oxoglutarate			12 mM/L
NADH			0.18 M/L

Assay Procedure

Wavelength	340 nm
Light path	1 cm
Temperature	37°C

Procedure

Preparation of Reaction Solution

Mixed reagent 1 and 2 in the ratio 4:1 and proceeded as follows.

Sample	100µl
Reaction solution	1000 µl

Mixed well and after 1 minute read the decrease in absorbance every minute for 3 minutes.

Calculation

AST activity [IU/L] = ΔA/minute × factor

ΔA / minute = mean absorbance change/minute

Factor = 1746

3.5.3 Total Protein

Total protein was determined photometrically by biuret method in semiautomatic blood analyzer (Microlab 200) using Ecoline Total Protein kit, (Merck Specialities Pvt Ltd).

Principle

Proteins which contain peptide linkage form a violet coloured complex with copper in alkaline medium. The intensity of the colour is directly proportional to the number of peptide linkages present which is a measure of the concentration of protein.

Reagents

Reagent 1: Sodium hydroxide	100 mM/L
Potassium sodium tartarate	16 mM/L
Reagent 2 : Sodium hydroxide	100 mM/L
Potassium sodium tartarate	16 mM/L
Potassium iodide	15 mM/L
Copper sulphate	6 mM/L
Reagent 3: Standard protein solution	5 g/dl

Assay Procedure

Wavelength	540 nm
Light path	1 cm
Temperature	37°C
Measurement	Against reagent blank

Procedure

Preparation of Reaction Solution

Mixed reagent 1 and 2 in the ratio 4:1 and proceeded as follows

	Blank	Sample	Standard
Sample/ Standard	-	20 µl	20 µl
Distilled water	20 µl	-	-
Reaction solution	1000 µl	1000 µl	1000 µl

Mixed, incubated for 5 minutes at 37°C and read the absorbance against the reagent blank within 60 minutes.

Calculation

$$\text{Total protein [g/dl]} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{\text{Concentration of standard}}{\text{Volume of sample}} \times 100$$

3.5.4 Albumin

Albumin was determined photometrically based on bromocresol green method in semiautomatic blood analyzer (Microlab 200) using Ecoline Albumin kit, (Merck Specialities Pvt. Ltd.).

Principle

Serum albumin forms a yellow-green to green-blue complex at a slightly acidic pH, which is measured photometrically.

Reagents

Reaction solution:	Citrate buffer	pH 4.2	30 mM/L
	Bromocresol green		0.26 mM/L
Albumin standard			5 g/dl

Assay Procedure

Wavelength	546 nm
Light path	1 cm
Temperature	37°C
Measurement	Against reagent blank

Procedure

	Blank	Sample	Standard
Sample/ Standard	-	10µl	10 µl
Distilled water	10µl	-	-
Reaction solution	1000µl	1000 µl	1000 µl

Mixed, incubated for 10 minutes at 37°C and read the absorbance against the reagent blank within 60 minutes.

Calculation

$$\text{Albumin [g/dl]} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{\text{Concentration of standard}}{\text{Volume of sample}} \times 100$$

3.5.5 Albumin Globulin Ratio

A:G was calculated from the total protein and albumin concentration.

3.5.6 Cholesterol

Cholesterol was determined photometrically by CHOD-PAP method in semiautomatic blood analyzer (Microlab 200) using Ecoline Cholesterol kit, (Merck Specialities Pvt Ltd).

Principle

Cholesterol and its esters are released from lipoproteins by detergents. Cholesterol esterase hydrolyses the esters. In the subsequent oxidation by cholesterol oxidase, H_2O_2 is liberated. The colorimetric indicator, quinoneimine is generated from 4-aminoantipyrene and phenol by H_2O_2 under the catalytic action of peroxidase (Trinder's reaction).

Reagents

Reaction solution:	PIPE's buffer	pH 7.5	99 mM/L
	Salicylic alcohol		3.96 mM/L
	4-aminoantipyrine		0.5 mM/L
	Cholesterol esterase		≥ 100 IU/L
	Cholesterol oxidase		≥ 100 IU/L
	Peroxidase		≥ 1000 IU/L
Cholesterol standard			200 mg/dl

Assay Procedure

Wavelength	500 nm
Light path	1 cm
Temperature	37°C
Measurement	Against reagent blank

Procedure

	Blank	Sample	Standard
Sample/ Standard	-	10 µl	10µl
Distilled water	10µl	-	-
Reaction solution	1000µl	1000 µl	1000 µl

Mixed, incubated for 5 minutes at 37°C. Read the absorbance against the reagent blank within 60 minutes.

Calculation

$$\text{Cholesterol [mg/dl]} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{\text{Concentration of standard}}{\text{Volume of sample}} \times 100$$

3.5.7. Blood Urea Nitrogen

Blood urea nitrogen was determined photometrically according to urease GLDH method in semiautomatic blood analyzer (Microlab 200) using Ecoline Urea kit, (Merck Specialities Pvt. Ltd.).

Principle

$$\text{Urea} + 2\ H_2O \xrightarrow{\text{Urease}} 2\ NH_4^+ + 2\ HCO_3^-$$

$$\text{2-Oxaloacetate} + NH_4^+ + \text{NADH} \xrightarrow{*\text{GLDH}} \text{L-glutamate} + NAD^+ + H_2O$$

*GLDH: Glutamate dehydrogenase

The decrease in NADH consumption is measured photometrically at 340 nm.

Reagents

Reagent 1: TRIS buffer	pH 7.8	99 mM/L
2-Oxoglutarate		7 mM/L
ADP		0.6 mM/L
Urease		≥ 6 kU/L
GLDH		≥ 1 kU/L
Peroxidase		≥ 1000 IU/L
Reagent 2: NADH		0.25 mM/L
Reagent 3: Urea standard solution		50 mg/dl

Assay Procedure

Wavelength	340 nm
Light path	1 cm
Temperature	37°C

Procedure

Preparation of Monoreagent Solution

Mixed reagent 1 and 2 in the ratio 4:1. Left the monoreagent for at least 30 min. at room temperature before using.

	Sample	Standard
Sample/Standard	10µl	10µl
Monoreagent	1000 µl	1000 µl

Mixed, incubated for 30-40 seconds at 37°C and read absorbance A1 and after exactly 60 seconds read absorbance A2.

Calculation

$$\text{Urea [mg/dl]} = \frac{\Delta A \text{ of sample}}{\Delta A \text{ of standard}} \times \frac{\text{Concentration of standard}}{\text{Volume of sample}} \times 100$$

ΔA = Mean absorbance change of A_1 and A_2

3.5.8 Creatinine

Creatinine was determined photometrically based on Jaffe kinetic method without deproteinisation in semiautomatic blood analyzer (Microlab 200) using Merckotest Creatinine kit, (Merck Specialities Pvt Ltd).

Principle

Creatinine in alkaline medium reacts with picric acid and forms an orange red colour of creatinine picrate. The intensity of the colour is a measure of the amount of creatinine present. At a low concentration of picric acid used in this method, precipitation of proteins does not take place.

Reagents

Reagent 1: NaOH 313 mM/L

Phosphate	12.5 mM/L
Reagent 2: Picric acid	8.73 mM/L
Reagent 3: Creatinine standard	1.0 mg/dl

Assay Procedure

Wavelength	492 nm
Light path	1 cm
Temperature	37°C

Procedure

Preparation of Reaction Solution

Mixed reagent 1 and 2 in the ratio 1:1. Left the monoreagent for at least 10 min. at room temperature before using.

	Sample	Standard
Sample/Standard	100 µl	100 µl
Reaction solution	1000 µl	1000 µl

Mixed and read absorbance A1 and after 60 seconds read absorbance A2 after further 120 seconds.

Calculation

$$\text{Creatinine[mg/dl]} = \frac{\Delta A \text{ of sample}}{\Delta A \text{ of standard}} \times \frac{\text{Concentration of standard}}{\text{Volume of sample}} \times 100$$

ΔA = Mean absorbance change of A_1 and A_2

3.5.9 Estimation of Sodium and Potassium

The sodium and potassium level in serum was measured in a flame photometer (Systronics 128).

Principle

The solution containing the substance to be measured is sprayed as a fine mist into a flame. In the flame the solution evaporates and the substance is converted to atomic state. The thermal energy of the flame excites the electrons so that they are able to absorb the thermal energy and move into the higher energy orbit. When the electrons return to lower energy orbits, the energy absorbed is released as quanta of light. The light intensity is measured at a wavelength of 589 nm for sodium and 768 nm for potassium which is directly proportional to the concentration of the substance in the flame. Before the analysis of unknown fluids, the system is standardised with solutions of known concentration.

Preparation of Standard Stock Solutions of Sodium and Potassium

Sodium

Dissolved 1.169 g of pure dry sodium chloride in 100 ml of deionised glass distilled water. This gave 200 mEq/L of solution (1 mEq/L = 23 ppm).

Potassium

Dissolved 74.6 mg of pure dry potassium chloride in 100 ml of deionised glass distilled water. This gave 10 mEq/L of solution (1 mEq/L = 39ppm).

Preparation of Working Standards

Sodium Standard I

Took 0.4 ml of the standard stock solution of sodium and diluted to 100 ml with deionised glass distilled water (0.8 mEq/ L).

Sodium Standard II

Took 1.5 ml of the standard stock solution of sodium and diluted to 100 ml with deionised glass distilled water (3 mEq/L).

Potassium Standard I

Took 0.2 ml of the standard stock solution of potassium and diluted to 100 ml with deionised glass distilled water (0.02 mEq/L).

Potassium Standard II

Took 1.5 ml of the standard stock solution of potassium and diluted to 100 ml with deionised glass distilled water (0.15 mEq/L).

Preparation of Samples

Diluted 0.2 ml of sera samples with 19.8 ml of deionised glass distilled water.

Procedure

Calibrated the instrument with lower and higher concentrations of sodium and potassium working standards. Diluted serum sample was then sprayed into the flame and the response obtained was compared with those obtained from the standards. The value was expressed as mM/L.

3.5.10 Estimation of Serum Glutathione Activity

Glutathione is measured by its reaction with 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB) to give a yellow coloured complex with absorption maximum at 412 nm (Moron *et al*., 1979).

Reagents

Phosphate buffer	pH 8.0	0.2 *M*
TCA		25 %
TCA		5 %
DTNB		0.6 mM
GSH standard		10 mg/dl

Procedure

Preparation of Protein Free Filtrate from Serum

125 µl of 25 % TCA was added to 500 µl of serum samples taken in ependoff tubes, mixed well and cooled on ice bath for 5 minutes. The mixture was further diluted with 575 µl of 5 % TCA and centrifuged for 5 minutes at 5000 rpm. The supernatant was taken for further procedure.

Supernatant		300 µl
Phosphate buffer	0.2 *M*	700 µl
Freshly prepared DTNB		2 ml
Mixed well and read the yellow colour formed at 412 nm.		

Preparation of Standard Curve of GSH

Dissolved 10 mg standard reduced glutathione in 100 ml 5 % TCA and took the following different GSH concentration for the calibration curve.

Tube No	GSH standard (µl)	Phosphate buffer 0.2 *M* (µl)	Corresponding GSH concentration (µg)
S_1	20	980	2
S_2	40	960	4
S_3	60	940	6
S_4	80	920	8
S_5	100	900	10

Added 2 ml of freshly prepared DTNB in all the above tubes. Mixed well and read the absorbance at 412 nm.

A graph was plotted between optical density and concentration of the standards. Knowing the optical density of the unknown samples, the corresponding concentration of GSH was read directly from the calibration curve and expressed as mg/dl.

3.6 STATISTICAL ANALYSIS OF THE DATA

The results obtained from the experiment were analysed and tested for significance using student's t test as described by Snedecor and Cochran (1994) using computerised software program SPSS (SPSS/PC + statistic 10.0 SPSS Inc. Chicago, IL., 2000).

Results

4. RESULTS

Clinical signs of pyoderma were observed in 21 dogs irrespective of age and breed reported to the Veterinary College Hospital, Mannuthy. Among these, 12 dogs successfully completed 14 days treatment schedule and post treatment evaluation. The results of these cases are presented here.

4.1 SIGNALMENT

4.1.1 Age wise occurrence

Age wise occurrence of pyoderma is given in Table 1. Among the total of 21 animals, the highest occurrence of pyoderma was noticed in dogs of one to three years of age group (38.10 %) followed by less than one year (23.81 %), three to five years (19.05 %) and above five years (19.05 %). Comparison of age wise occurrence of pyoderma is given in Fig. 1.

4.1.2 Breed wise occurrence

Various breeds of dogs with confirmed clinical signs of pyoderma were GSD, Labrador, Rottweiler, Spitz, Basset Hound, Cocker Spaniel, Dalmatian and non-descript. The highest occurrence was observed in GSD (38.10 %), followed by Labrador (14.29 %), non-descript (14.29 %), Rottweiler (9.52 %), Spitz (9.52 %) and other breeds (14.29 %) which included Basset Hound, Cocker Spaniel and Dalmatian. The breed wise occurrence of pyoderma is given in Table 2 and comparison of the age wise occurrence of pyoderma is given in Fig. 2.

Table 1. Age wise occurrence of canine pyoderma

Age group	Number of dogs with pyoderma (n = 21)	Percentage
Less than 1 year	5	23.81
1 - 3 year	8	38.10
3 - 5 year	4	19.05
Greater than 5 year	4	19.05

Table 2. Breed wise occurrence of canine pyoderma

Breeds of dog	Number of pyoderma infected dogs (n=21)	Percentage
GSD	8	38.10
Labradror	3	14.29
Spitz	2	9.52
Rottweiler	2	9.52
Non-descript	3	14.29
Others	3	14.29

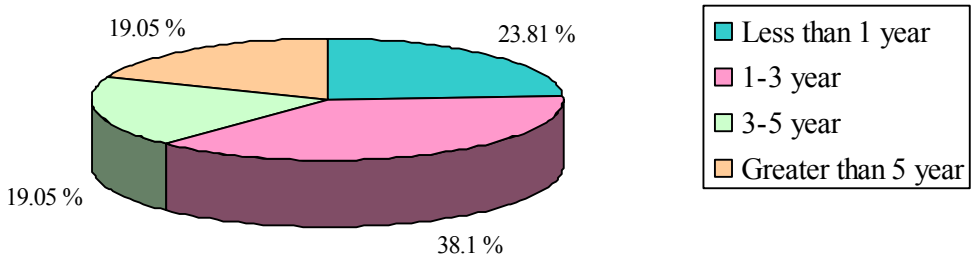

Fig. 1. Age wise occurrence of canine pyoderma

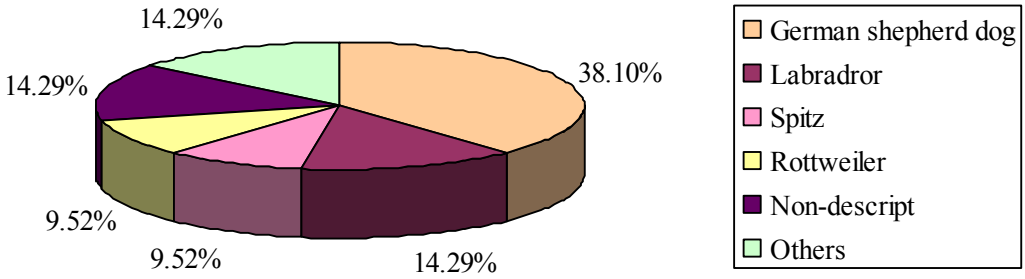

Fig. 2. Breed wise occurrence of canine pyoderma

4.1.3 Sex wise Occurrence

Out of the 21 dogs reported with pyoderma, eleven (52.38 %) were male and remaining ten were female (47.62 %). The prevalence was higher in males than in females.

4.2 CLINICAL SIGNS IN PYODERMA

The clinical signs and lesions noted in the 12 dogs were combinations of papules and pustules, matting of hairs, erosions, cellulitis, alopecia, scales, hyperpigmentation and pruritus (Plate 1.A).

4.3 CULTURAL EXAMINATION OF THE SKIN SWAB

Aseptically collected skin swabs, streaked on nutrient agar plates were examined for the presence of bacterial growth after 24 h of incubation. Bacterial growth obtained on nutrient agar was then identified as gram positive cocci by Gram's staining method.

4.4 ANTIBIOTIC SENSITIVITY TEST

The isolated gram positive cocci obtained by cultural examination were subjected to antibiotic sensitivity test and were found sensitive to ciprofloxacin and moderately sensitive to gentamicin, amoxycillin, cefotaxime and cephalexin. Comparison of the sensitivity of gram positive cocci to different antibiotics is shown in Plate 2.

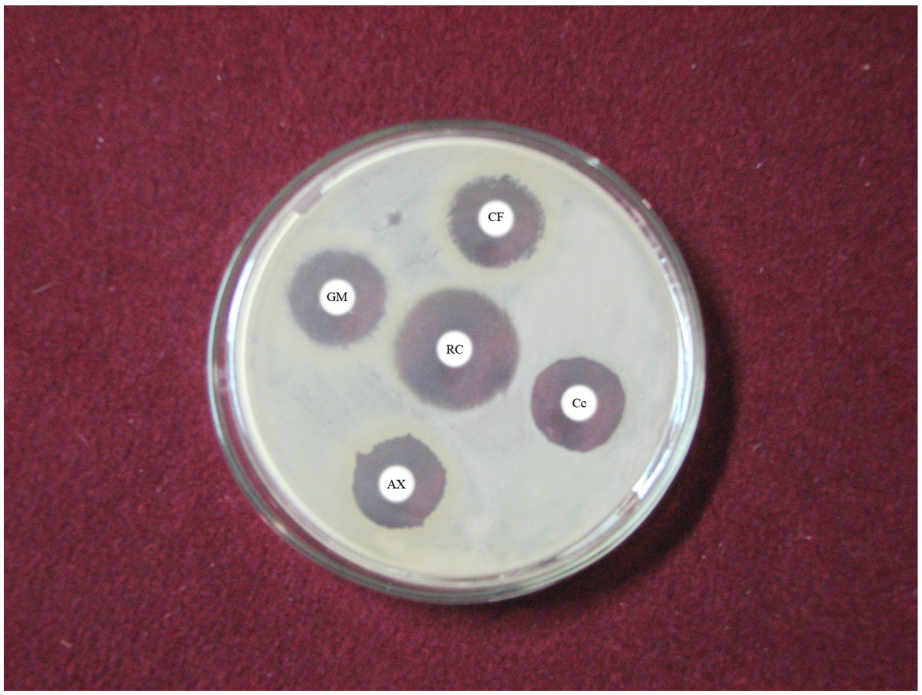

Plate 2. Antibiogram of the gram positive cocci isolated from skin swabs of pyoderma affected dog

RC	Ciprofloxacin
GM	Gentamicin
Cc	Cefotaxime
Ax	Amoxycillin
CF	Cephalexin

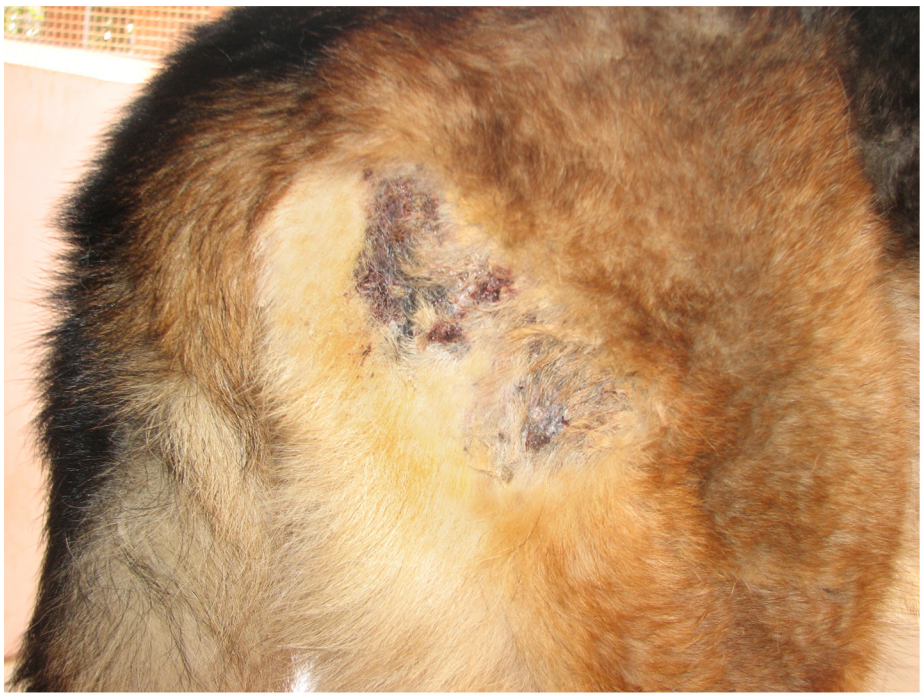

Plate 1 A. Clinical signs of pyoderma with alopecia, erythema & moist area of exudation over lateral thigh in GSD

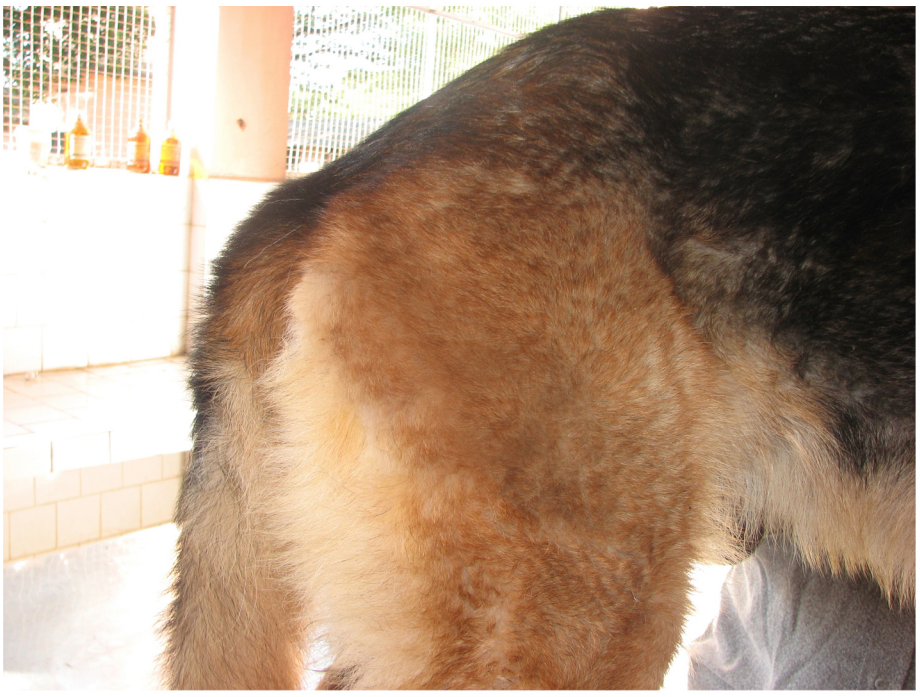

Plate 1 B. Clinical response after 14 days of ciprofloxacin therapy

4.5 HAEMATO-BIOCHEMICAL CHANGES IN DOGS WITH PYODERMA BEFORE TREATMENT

4.5.1 Haematological Changes

The haematological changes in dogs with pyoderma are presented in Table 3. The mean TEC on the initial day of treatment was 5.37 ± 0.68 × 10^6/mm^3 which lies within the normal range. A mild leucocytosis was observed in pyoderma infected dogs and the value was 19788.46 ± 1665.53/mm^3. No change in the Hb, PCV and ESR values were observed in pyoderma infected dogs before treatment when compared to normal reference values. Differential leucocyte count showed a mild neutrophilia with a value of 67.77 ± 5.74 %. The lymphocytes, monocytes and eosinophils were within the normal range.

4.5.2 Biochemical Changes

The biochemical parameters were within the normal range and are presented in Table 4. Hepatocellular enzymes like ALT and AST activities were found to be 27.31 ± 0.26 and 33.62 ± 4.39 IU/L respectively showing no variation from reference level. Serum concentration of total protein, albumin, A:G lies within the normal range. Serum cholesterol value was within the normal range (174.23 ± 24.44 mg/dl). Blood urea nitrogen and creatinine values were also observed in the normal range with a mean value of 26.23 ± 3.72 and 1.30 ± 0.23 mg/dl respectively. The concentration of electrolytes such as sodium and potassium were within the normal range. The mean reduced glutathione value in pyoderma infection was 5.20 ± 0.70 mg/dl and within the normal range.

Table 3. Haematological values in dogs with pyoderma

Parameters	Values
TEC ($\times 10^6$cells/mm^3)	5.37 ± 0.68
TLC (cells/mm^3)	19788.46 ± 1665.53
Hb (g/dl)	14.89 ± 1.50
PCV (%)	39.23 ± 3.58
ESR (mm/h)	1.15 ± 0.02
DLC (%) Neutrophil Lymphocyte Monocyte Eosinophil	 67.77 ± 5.74 20.46 ± 2.01 2.46 ± 0.37 1.38 ± 0.24

Table 4. Level of serum biochemical parameters in dogs affected with pyoderma

Parameters	Concentration
ALT (IU/L)	27.31 ± 0.26
AST (IU/L)	33.62 ± 4.39
Total protein (g/dl)	6.08 ± 0.64
Albumin (g/dl)	2.40 ± 0.27
A:G	0.62 ± 0.07
Cholesterol (mg/dl)	174.23 ± 24.44
BUN (mg/dl)	26.23 ± 3.72
Creatinine (mg/dl)	1.30 ± 0.23
Sodium (mM/L)	165.86 ± 15.62
Potassium (mM/L)	6.43 ± 0.75
GSH (mg/dl)	5.20 ± 0.70

4.6 EFFICACY OF CIPROFLOXACIN THERAPY

Almost all the lesions have healed in all the dogs and recovered completely after 14 days of ciprofloxacin treatment (Plate 1.B)

4.7 HAEMATO-BIOCHEMICAL CHANGES IN DOGS WITH PYODERMA AFTER TREATMENT

4.7.1 Haematological Changes

4.7.1.1 Total Erythrocyte Count

The mean TEC of pyoderma infected dogs treated with ciprofloxacin is given in the Table 5. A mild increase in these values were observed on day 14 ($6.72 \pm 0.76 \times 10^6$ cells/mm^3) and 5^{th} day after the course of the treatment ($6.74 \pm 1.07 \times 10^6$ cells/mm^3). But these changes in TEC were not significant in comparison with the observations on the initial day.

4.7.1.2 Total Leucocyte Count

An increase in TLC was observed before ciprofloxacin treatment with a mean value of 19788.46 ± 1665.53 cells/mm^3. This revealed leucocytosis in pyoderma infected dogs which decreased gradually on 7^{th}, 14^{th} day of treatment and 5^{th} day after the therapy. The values are presented in Table 5. A marked decrease was observed between the TLC values on 7^{th} day (16008.23 ± 1391.40 cells/mm^3), 14^{th} day of treatment (13908.77 ± 1273.25 cells/mm^3) and 5^{th} day after the therapy (10963 ± 1086.78 cells/mm^3), which was significantly different from that of 0^{th} day. Statistical

Table 5. Haematological parameters on 0^{th}, 7^{th}, 14^{th} day of treatment and 5^{th} day after therapy with ciprofloxacin in canine pyoderma

Parameters	0^{th} day	7^{th} day	14^{th} day	5^{th} day after therapy
TEC ($\times 10^6$cells/ mm^3)	5.37 ± 0.68	5.77 ± 0.47	6.72 ± 0.76	6.74 ± 1.07
TLC (cells/mm^3)	19788.46* ± 1665.53	16008.23* ± 1391.40	13908.77* ± 1273.25	10963* ± 1086.78
Hb (g/dl)	14.89 ± 1.50	15.75 ± 1.09	14.14 ± 0.65	14.66 ± 0. 89
PCV (%)	39.23 ± 3.58	40.46 ± 3.25	39.92 ± 2.71	40.54 ± 2.28
ESR (mm/h)	1.15 ± 0.02	1.68 ± 0.53	1.17 ± 0.69	1.55 ± 0.45
DLC (%)				
Neutrophil	67.77* ± 5.74	68.39* ± 5.21	63.92* ± 4.28	61.69* ± 3.62
Lymphocyte	20.46* ± 2.01	21.31* ± 1.55	26.62* ± 1.44	29.31* ± 1.17
Monocyte	2.46 ± 0.37	2.85 ± 0.48	3.85 ± 0.97	4.69 ± 1.27
Eosinophil	1.38 ± 0.24	1.92 ± 0.45	2.23 ± 0.99	2.54 ± 1.38

* Significant at 5 % level

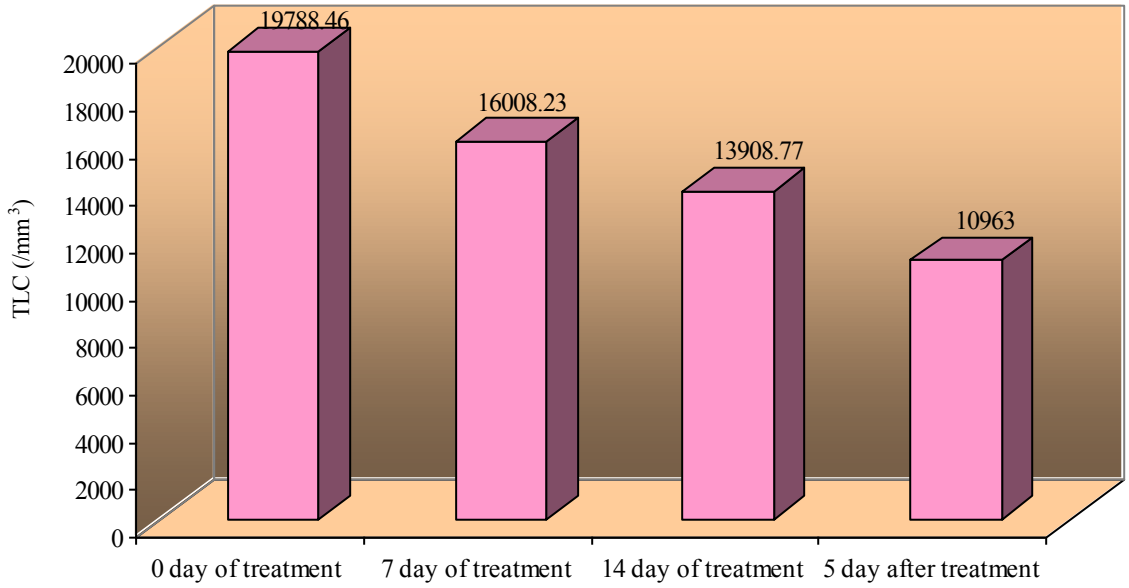

Fig. 3. Blood leucocyte count of dogs on 0th, 7th, 14th day of treatment and 5th day after treatment with ciprofloxacin against pyoderma

analysis using student's t-test showed a difference between the 7th and 14th day of treatment, 7th day of treatment and 5th day after the therapy. There was also a progressive decrease in the values and the difference was significant ($P \leq 0.05$) from 14th day of treatment until 5th day after the treatment. Comparison of the mean values of TLC is shown in Fig. 3.

4.7.1.3 Haemoglobin

The haemoglobin values of ciprofloxacin treated dogs are presented in Table 5. No change in the Hb values were observed on 7th, 14th day of treatment and 5th day after the treatment as compared to that on 0th day.

4.7.1.4 Packed Cell Volume

The mean PCV of pyoderma infected dogs treated with ciprofloxacin are given in the Table 5. The PCV values obtained on 0th, 7th, 14th day of treatment and 5th day after the treatment were also similar and within the normal range.

4.7.1.5 Erythrocyte Sedimentation Rate

Erythrocyte sedimentation rate of ciprofloxacin treated dogs on 0th, 7th, 14th day of treatment and 5th day after treatment are presented in Table 5. Before treatment with ciprofloxacin the mean ESR value was 1.15 ± 0.02 mm/h. On 7th day of therapy, there was an increase in the ESR value but was not significant. On 14th day of therapy also showed a progressive increase to 1.17 ± 0.69 mm/h. On the 5th day after therapy, a further increase (1.55 ± 0.45 mm/h) was noted which too did not differ significantly from 0th, 7th and 14th day of treatment.

4.7.1.6 Differential Leucocyte Count

4.7.1.6.1 Neutrophils

The neutrophil counts of pyoderma infected dogs treated with ciprofloxacin are given in the Table 5. The mean neutrophil count decreased from a value of 67.77 ± 5.74 % on 0^{th} day of treatment, which was slightly higher than the normal neutrophil count, to a mean of 63.92 ± 4.28 % on 14^{th} day of treatment. A further decrease was observed on day 5 after the completion of therapy with a mean value of 61.69 ± 3.62 %. Thus, a significant decrease ($P \leq 0.05$) in neutrophil count was observed before and after the ciprofloxacin treatment. The mean neutrophil count of 7^{th} day of treatment was 68.39 ± 5.21 %, which again showed a significant decrease ($P \leq 0.05$) between 14th day and 5^{th} day after the completion of therapy. Comparison of the mean values of neutrophils is given in Fig: 4.

4.7.1.6.2 Lymphocytes

Haematological examination showed a significant decrease ($P \leq 0.05$) in lymphocyte count with a mean value of 20.46 ± 2.01 % before the treatment when compared to normal reference range. The mean values are presented in Table 5. Following ciprofloxacin treatment, the lymphocyte value increased progressively to 29.31 ± 1.17 % on 5^{th} day after the therapy through 21.31 ± 1.55 and 26.62 ± 1.44 % on 7^{th} and 14^{th} day of treatment respectively. The difference was significant from 14^{th} day until 5^{th} day after therapy. Treatment with ciprofloxacin was also associated with significant increase ($P \leq 0.05$) in mean lymphocyte values during the progression of treatment between 7^{th} and 14^{th} day of treatment, 7^{th} day of treatment and 5^{th} day after the therapy and 14^{th} and 5^{th} day after completion of therapy. Comparison of the mean values of lymphocytes is given in Fig. 4.

4.7.1.6.3 Monocyte

The monocyte counts of pyoderma infected dogs treated with ciprofloxacin are given in Table 5. Before treatment, the monocyte count was 2.46 ± 0.37 % which became 3.85 ± 0.97 and 4.69 ± 1.38 % on 14th day of treatment and 5th day after the treatment respectively. Comparison of the mean values of monocyte is given in Fig. 4.

4.7.1.6.4 Eosinophils

The mean eosinophil count on 0th, 7th, 14th day of treatment and 5th day after the treatment were 1.38 ± 0.24, 1.92 ± 0.45, 2.23 ± 0.99 and 2.54 ± 1.38 % respectively. Statistical analysis showed no significant difference between the eosinophil counts on 0th, 7th, 14th day of treatment and 5th day after the treatment. The values on the respective days are given in Table 5. and comparison of the mean values of eosinophil is given in Fig. 4.

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4.7.2 Biochemical Parameters

4.7.2.1 Alanine Aminotransferase

The mean values of ALT activity following ciprofloxacin therapy are given in the Table 6. The ALT values on 0th, 7th, 14th day of treatment and 5th day after the treatment were 27.31 ± 0.26, 27.54 ± 2.89, 30.77 ± 2.80 and 30.77 ± 2.17 IU/L respectively.

4.7.2.2 Asparatate Aminotransferase

The mean AST values of ciprofloxacin treated dogs are presented in the Table 6. The values were 33.62 ± 4.39, 36.54 ± 4.28, 36.46 ± 4.07 and 37.08 ± 4.10 IU/L

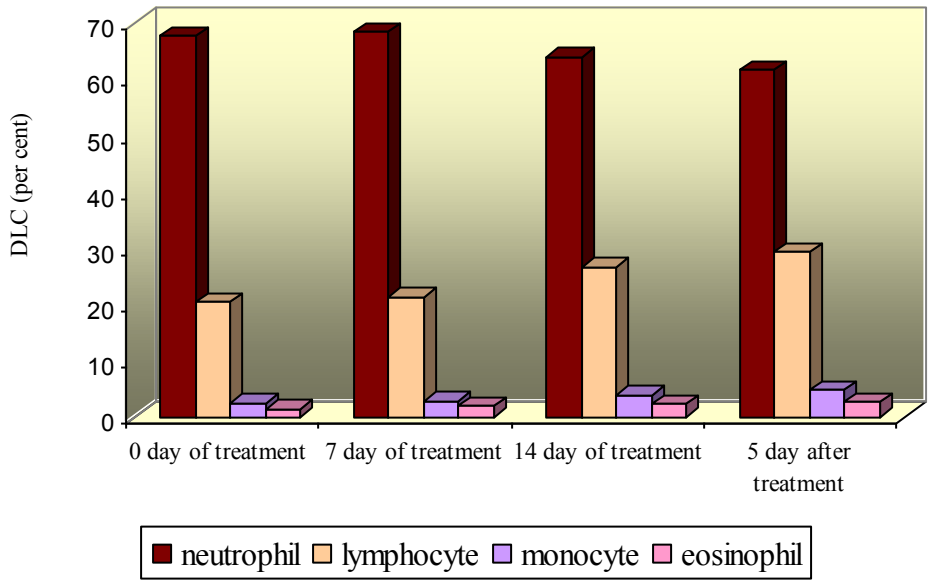

Fig. 4. Differential leucocyte count of dogs affected with pyoderma on before, during and after treatment with ciprofloxacin

on 0^{th}, 7^{th}, 14^{th} day of treatment and 5^{th} day after the treatment respectively and the values were within the normal range.

4.7.2.3 Total Protein

The total protein values on 0^{th}, 7^{th}, 14^{th} day of treatment and 5^{th} day after the treatment in pyoderma infected dogs treated with ciprofloxacin are given in Table 7. The initial mean total protein value was 6.08 ± 0.64 g/dl, which was at the base line of the reference range. After one week of therapy, mean total protein increased but insignificantly to 7.23 ± 0.46 g/dl. One week later the value was 7.85 ± 0.59 g/dl which also showed no significant difference. After completion of the therapy, on 5^{th} day, the mean total protein value increased to 8.08 ± 0.96 g/dl, which was within the normal range.

4.7.2.4 Albumin

The albumin concentrations of ciprofloxacin treated dogs were within the normal range and are presented in Table 7. The values were 2.40 ± 0.27, 3.12 ± 0.40, 3.75 ± 0.87 and 3.81 ± 1.28 g/dl on 0^{th}, 7^{th}, 14^{th} day of treatment and 5^{th} day after the treatment respectively.

4.7.2.5 Albumin Globulin Ratio

The mean A:G are presented in Table 7. Before treatment, the mean A:G was 0.62 ± 0.07. Treatment with ciprofloxacin had no significant effect on the A:G on 7^{th}, 14^{th} day of treatment and 5^{th} day after treatment. One week following the therapy, the dogs showed an elevated A:G of 1.14 ± 0.49 which increased progressively to 1.68 ± 1.03 on 14^{th} day of treatment and to 2.02 ± 1.42 on 5^{th} day after completion of the therapy.

4.7.2.6 Cholesterol

The cholesterol values are given in Table 7. The initial cholesterol value before treatment was 174.23 ± 24.44 mg/dl indicating no change from normal values. The mean cholesterol values on 7^{th}, 14^{th} day of treatment and 5^{th} day after the therapy were 165.08 ± 20.75, 150.69 ± 17.54 and 160.85 ± 18.16 mg/dl respectively.

4.7.2.7 Blood Urea Nitrogen

The mean BUN values are given in Table 8. Clinical biochemistry tests showed no significant abnormalities in BUN values on 0^{th}, 7^{th}, 14^{th} day of treatment and 5^{th} day after the treatment. The initial mean BUN value was 26.23 ± 3.72 mg/dl and that on 7^{th} day of treatment was 28.00 ± 3.10 mg/dl. The values on 14^{th} day of treatment and 5^{th} day after the treatment was 27.15 ± 2.70 and 27.31 ± 2.20 mg/dl respectively which was similar and within the normal range.

4.7.2.8 Creatinine

The mean creatinine values are given in Table 8. The creatinine values obtained were found to be within the normal range. The mean values on 0^{th} and 7^{th} day of treatment were 1.30 ± 0.23, 1.69 ± 0.46 mg/dl which did not differ significantly. The mean creatinine values increased slightly to 2.24 ± 0.99 g/dl on14^{th} day of treatment and to 2.43 ± 1.38 mg/dl on 5^{th} day after the therapy. Even though a slight increase in creatinine value was noted, no significant difference was observed between before and after treatment.

Table 6. Hepatocellular enzyme activity before and after treatment with ciprofloxacin in dogs with pyoderma

Parameters	0^{th} day	7^{th} day	14^{th} day	5^{th} day after therapy
ALT (IU/L)	27.31 ± 0.26	27.54 ± 2.89	30.77 ± 2.80	30.77 ± 2.17
AST (IU/L)	33.62 ± 4.39	36.54 ± 4.28	36.46 ± 4.07	37.08 ± 4.10

Table 7. Serum concentrations of total protein, albumin, A:G, cholesterol on 0^{th}, 7^{th}, 14^{th} day of treatment and 5^{th} day after therapy in pyoderma infected dogs treated with ciprofloxacin

Parameters	0^{th} day	7^{th} day	14^{th} day	5^{th} day after therapy
Total Protein (g/dl)	6.08 ± 0.64	7.23 ± 0.46	7.85 ± 0.59	8.08 ± 0.96
Albumin (g/dl)	2.40 ± 0.27	3.12 ± 0.40	3.75 ± 0.87	3.81 ± 1.28
A:G	0.62± 0.07	1.14 ± 0.49	1.68 ± 1.03	2.02 ± 1.42
Cholesterol (mg/dl)	174.23 ± 24.44	165.08 ± 20.75	150.69 ± 17.54	160.85 ± 18.16

4.7.2.9 Sodium

The mean sodium values on 0^{th}, 7^{th}, 14^{th} day of treatment and 5^{th} day after the therapy are given in Table 8. The values obtained were 165.86 ± 15.62, 165.69 ± 13.51, 168.71 ± 13.31 and 172.74 ± 14.07 mM/L on 0^{th}, 7^{th}, 14^{th} day of treatment and 5^{th} day after the treatment respectively and were within the normal range.

4.7.2.10 Potassium

The mean values of potassium in pyoderma infected dogs treated with ciprofloxacin on 0^{th}, 7^{th}, 14^{th} day of treatment and 5^{th} day after the therapy were 6.43 ± 0.75, 6.53 ± 0.52, 7.19 ± 0.72 and 7.54 ± 1.16 mM/L respectively. The values were within the normal range and did not differ significantly between 0^{th}, 7^{th}, 14^{th} day of treatment and 5^{th} day after the treatment (Table 8)

4.7.2.11 Reduced Glutathione

The mean values of reduced glutathione on 0^{th}, 7^{th}, 14^{th} day of treatment and 5^{th} day after the therapy in pyoderma infected dogs treated with ciprofloxacin were 5.20 ± 0.70, 5.00 ± 0.51, 5.22 ± 0.87 and 5.74 ± 1.18 mg/dl respectively. No significant difference was observed between the reduced glutathione before, during and after treatment. The corresponding values on the respective days are given in Table 8.

Table 8. Serum concentrations of BUN, creatinine, sodium, potassium and GSH in dogs with pyoderma before and after treatment with ciprofloxacin

Parameters	0^{th} day	7^{th} day	14^{th} day	5^{th} day after therapy
BUN (mg/dl)	26.23± 3.72	28.00 ± 3.10	27.15 ± 2.70	27.31 ± 2.20
Creatinine (mg/dl)	1.30 ± 0.23	1.69 ± 0.46	2.24 ± 0.99	2.44 ± 1.38
Sodium (mM/L)	165.86 ± 15.62	165.69 ± 13.51	168.71 ± 13.31	172.74 ± 14.07
Potassium (mM/L)	6.43 ± 0.75	6.53 ± 0.52	7.19 ± 0.72	7.54 ± 1.16
GSH (mg/dl)	5.20 ± 0.70	5.00 ± 0.51	5.22 ± 0.87	5.74 ± 1.18

Discussion

5. DISCUSSION

Antibiotics are vital medicine used for the treatment of bacterial infections in animals and human beings. An accurate understanding of their effects on homeostasis is important when selecting the class of antimicrobial to prescribe. It is difficult to make these decisions on the basis of information contained in the literature because only few studies have been performed to evaluate the *in vivo* effects of antimicrobials at relevant dosages and durations in dogs. Adverse effect of the antibiotics often result from prolonged administration which is essential for many infectious diseases. Pyoderma is a pyogenic bacterial infection of the skin and is one of the most common causes of skin diseases in dogs. Prolonged administration of many antibiotics is being used to cure pyoderma but have several adverse effects. Nowadays, ciprofloxacin has become an increasingly important antibiotic in the treatment of canine pyoderma and the therapy is often advocated for two to three weeks. Reports are not available on whether the prolonged administration of this antibiotic cause any adverse effect in dogs.

In the present study, the haemato-biochemical parameters were analysed in dogs affected with pyoderma, which had undergone treatment with ciprofloxacin for two weeks. Twenty one dogs referred to Veterinary College Hospital, Mannuthy from August 2007 to March 2008 with clinical signs of pyoderma were selected for the study. Following history and physical examination, blood was collected for the determination of haemato-biochemical parameters according to standard procedure.

5.1 SIGNALMENT

5.1.1 Age wise Occurrence

In this study, the percentage of dogs suffering from pyoderma revealed that the highest incidence of canine pyoderma was in one to three years of age

group, followed by age groups of less than one year, three to five years and above five years. This is in accordance with the findings of Udayasree (2004), who reported the highest incidence in one to four years age group, but the next highest incidence was in less than six months group which is contradictory to the results obtained in the present study. According to Aujla *et al*. (1997) the greatest prevalence of bacterial dermatitis in dogs was up to two years of age. The findings of the present study disagree with the findings of Mathews (1999) who reported that dogs below six months are frequently affected by bacterial skin infections. In another report, pyoderma mainly affected dogs of five years of age (Krick and Scott, 1989). Wisselink *et al.* (1985) reported that dogs of six months to six years of age were more commonly affected with pyoderma.

5.1.2 Breed wise Occurrence

The most commonly affected breed with pyoderma in the present study was GSD. This is in accordance with the findings of Udayasree (2004). Krick and Scott (1989) also reported the highest incidence of pyoderma in GSD. According to Pal *et al*. (1995) and Mathews (1999), the highest incidence of pyoderma was in non-descript dogs. The higher incidence of pyoderma in GSD might be due to an autosomal recessive gene, as this leads to a cell mediated immunodeficiency and therefore more incidence of idiopathic bacterial folliculitis and furunculosis (Wissselink *et al.,* 1985).

5.1.3 Sex wise Occurrence

Sex wise prevalence of pyoderma was highest in male than in female dogs. This is in accordance with the findings of Mathews (1999). On the contrary, Krick and Scott (1989) and Aujla *et al*. (1997) reported that the disease had high incidence in females than in males, the reason being frequent exposure of females to various stress factors including oestrus, whelping, nursing and close confinement with pups. Since the number of dogs in the present study was

limited, a detailed demographic study involving a wide range of age, breed and sex would be required for a proper conclusion of signalment to be made.

5.2 CLINICAL SIGNS IN PYODERMA

The dogs selected for the study had clinical signs such as papules and pustules, matting of hairs, erosions, cellulitis, alopecia, scales, hyperpigmentation and pruritus. Similar clinical signs were observed in many studies in dogs affected with pyoderma (Wisselink *et al.*, 1985; Seena *et al.,* 2005; Horvath and Neuber, 2007). In addition to these clinical signs, Ihrke (1987) reported the presence of maceration, induration and seropurulent debris drains from ruptured pustules. Craig (2003) reported epidermal collarettes and draining tracts along with the above lesions.

5.3 CULTURAL EXAMINATION OF THE SKIN SWABS

In an attempt to identify the microorganism responsible for pyoderma, skin swabs were collected and allowed to grow in nutrient agar. The bacterial growth obtained on nutrient agar was identified as gram positive cocci by Gram's staining method. Wisselink *et al.* (1985) isolated coagulase positive *Staphylococcus* sp. from skin scrapings of 23 dogs affected with pyoderma. *Staphylococcus* sp. was isolated from eight of the eleven cultures in a study conducted by Krick and Scott (1989) in canine pyoderma. Among the various bacterial isolates, *Staphylococcus* sp. was the most common isolate according to Aujla *et al*. (1997) and Stagemann *et al.* (2007). Bloom and Rosser (2001) also reported that 20 out of 21 dogs had positive cultures of *Staphylococcus* sp. Carlotti *et al.* (1999) and Mellor *et al.* (2005) reported that *Staphylococcus* sp. was the most frequent isolate followed by *Proteus* sp., *Pseudomonas* sp. and *Escherchia coli.* All the above studies reveal that *Staphylococcus* sp., the gram positive cocci is the most common bacteria responsible for the development of pyoderma in dogs. Since the isolated bacteria were gram positive cocci, it might

be the *Staphylococcus* sp. responsible for the cause of pyoderma in the present study.

5.4 ANTIBIOTIC SENSITIVITY TEST

Ciprofloxacin was found to be the most sensitive drug in the present antibiogram study as compared to other antibiotics. So it was selected for the treatment of pyoderma in dogs. The isolates of gram positive cocci were sensitive to ciprofloxacin followed by gentamicin, amoxycillin, cefotaxime and cephalexin. Petersen *et al.* (2002) reported that 99 % of *Staphylococcus intermedius* isolated from skin were sensitive to ciprofloxacin. Kruse *et al.* (1996) reported that all isolates of *Staphylococcus* sp. from dermatitis in dogs were sensitive to quinolones such as ciprofloxacin and enrofloxacin. The findings of the present study also concurs with the findings of Frank and Kunkle (1993) and Bloom and Rosser (2001) who studied the antimicrobial resistance and susceptibility patterns of *Staphylococcus intermedius* isolated from dogs with pyoderma and showed that all bacteria were sensitive to enrofloxacin. The finding of the present study is not in agreement with the observations of Kamboj *et al.* (1995) and Mueller *et al*. (1998) who reported the sensitivity of cloxacilin to *Staphylococcus* sp. isolates from canine bacterial dermatitis as 93.59 and 96 % respectively. Aujla *et al.* (1997) studied the antimicrobial drug sensitivity and showed that gentamicin and doxycycline as most effective against both *Staphylococcus* sp. and *Streptococcus* sp.

5.5 HAEMATO-BIOCHEMICAL CHANGES IN DOGS WITH PYODERMA BEFORE TREATMENT

5.5.1 Haematological Changes

The TEC of pyoderma infected dogs lies within the normal range of 5.5 - 8.5 × 10^6 cells/mm^3 reported by Meinkoth and Clinkenbeard (2000). The findings

in the present study is also in accordance with the findings of McEwan (1992), who reported a TEC of 5.4 × 10^6 cells/mm^3 in a two year old Scotttish terrier with pyoderma. Similar findings were also observed in a study conducted by Mathews (1999).

The leucocyte count showed a mild leucocytosis. The reference range of TLC in dogs was 6000 – 7000 cells/mm^3 (Meinkoth and Clinkenbeard, 2000). Similar findings were also observed by Fadok (1982) in a zinc responsive dermatosis. The findings of Wisselink *et al.* (1985) in deep pyoderma also confirmed mild leucocytosis with a TLC of 17.9 × 10^3 cells/mm^3. The findings of the present study are also comparable with the TLC of 21.9 × 10^3 cells/mm^3 (Krick and Scott, 1989) and 27.8 × 10^3 cells/mm^3 (Aujla *et al*., 1997) in bacterial dermatitis. However, TLC reported by Mathews (1999) and Udayasree (2004) were within the reference range. The leucocytosis observed in pyoderma infected dogs might be due to the stress of dermatitis and bacterial toxins.

The haemoglobin value was observed in the normal range (12 - 18 g/dl) which is in accordance with Meinkoth and Clinkenbeard (2000). The findings of Mathews (1999) and Udayasree (2004) also suggested that Hb value did not show any significant variation from normal range in pyoderma infected dogs. Dogs with pyoderma showed a PCV, which falls within the normal range of 37 - 55 % reported by Meinkoth and Clinkenbeard (2000). The PCV value in GSD with deep pyoderma ranged from 36 to 39 % (Wisselink *et al.,* 1985). The findings of Udayasree (2004) also concluded that the PCV value in canine pyoderma was 36.37 ± 17.37 %. A PCV value of 43.3 ± 7.3 % was reported by Mathews (1999) in bacterial dermatitis. The mean ESR obtained in the present study before treatment was within the normal range reported by Meinkoth and Clinkenbeard (2000). Mathews (1999) reported the ESR value in dogs with bacterial dermatitis as 4 ± 1.3 mm/h, which was comparatively higher than the value obtained in the present study.

The neutrophil count in dog with pyoderma before treatment showed a mild neutrophilia. This is in accordance with the findings of Fadok (1982) who reported mild neutrophilia in zinc responsive dermatitis in a Great Dane. Mathews (1999) and Udayasree (2004) have reported a neutrophilia with a mean value of 71.9 ± 5.8 and 71 ± 0.86 % respectively in canine pyoderma. Krick and Scott (1989) and Aujla *et al*. (1997) have reported a neutrophil count of 78.20 and 76.36 % in dogs with bacterial dermatitis. The slight increase in neutrophil count in the pyoderma affected dogs in the present study may be due to the mild nature of the infection. The lymphocyte count obtained in this study is within the normal range reported by Meinkoth and Clinkenbeard (2000), which is also in accordance with the reports of Mathews (1999) and Udayasree (2004). The monocyte count is also in accordance with the findings of Udayasree (2004) who reported a normal monocyte count in pyoderma infection. However, Mathews (1999) reported a monocyte count of 0.6 ± 1.1 % in dogs with bacterial dermatitis. Dogs with zinc responsive dermatitis revealed monocytosis as reported by Fadok (1982). The mean eosinophil count lies within the normal range similar to the findings of Mathews (1999) and Udayasree (2004) who reported a mean eosinophil count of 3.2 ± 2 and 2.75 ± 2.02 % respectively. However, an increase in eosinophil count was observed in zinc responsive dermatitis in a Great Dane (Fadok, 1982).

The haematological analysis of the blood samples suggested that no variation in TEC, Hb, PCV, ESR and DLC (lymphocyte, monocyte and eosinophil) from normal range occurred with pyoderma infection.

5.5.2 Biochemical Changes

The ALT and AST activities of dogs with pyoderma in the present study were within the normal ranges reported by Kaneko *et al.* (1997). Wisselink *et al.* (1985) also opined that the ALT value lies within the normal range in GSD.

Based on ALT and AST activities, it could be suggested that the pyoderma infection might have not caused any damage to liver tissue.

To study the functional status of liver, total protein, albumin and A:G were evaluated. Total protein value was 6.08 ± 0.64 g/dl. Kaneko *et al.* (1997) reported a normal value of 5.4 - 7.1 g/dl. Attri *et al.* (2005) reported that the total protein value obtained in bacterial dermatitis was 6.23 ± 0.81 g/dl, which lies in the base line of reference range. Fadok (1982) and Aujla *et al*. (1997) reported no change in total protein value for pyoderma infection. However, Wisselink *et al.* (1985) reported a total protein concentration of 7 g/dl in dogs with deep pyoderma and suggested as high due to elevated β and γ globulins. The total protein concentration in the study was comparatively higher than the total protein concentration (5.33 ± 0.82) reported by Udayasree (2004). Dogs with pyoderma had a mean albumin value (2.40 ± 0.27 g/dl), which lies within the normal range reported by Kaneko *et al.* (1997). The findings of the present study are in agreement with the findings of Fadok (1982) suggesting that albumin concentration is not altered in canine pyoderma. This value is slightly higher than the albumin concentration reported by Aujla *et al*. (1997) and Udayasree (2004). The A:G of pyoderma infected dogs was 0.62 ± 0.07. The normal reference A:G in dogs is 0.59 - 1.11 (Kaneko *et al.,* 1997). Udayasree (2004) also could not find any significant difference in A:G in dogs with pyoderma. Serum cholesterol concentration was within the normal range. The result correlated well with the findings of Mathews (1999) who reported a normal cholesterol value in dogs with bacterial dermatitis. The normal levels of total protein, albumin and A:G obtained in the present study suggests that pyoderma infection did not cause any change in the functional status of liver.

Pyoderma infected dogs had a mean BUN value of 26.23 ± 3.72 mg/dl on the initial day of the treatment. Kaneko *et al.* (1997) reported a normal value of 10 - 30 mg/dl for BUN in dogs. Fadok (1982) and Wisselink *et al.* (1985) also reported similar observations. Mathews (1999) obtained a BUN value, which was

also within the normal range but comparatively lower than the values obtained in the present study. The dogs under study showed a mean creatinine value of 1.30 ± 0.23 mg/dl. A normal creatinine value of 1 - 2 mg/dl was reported by Kaneko *et al.* (1997). This is in accordance with the findings of Fadok (1982).

The mean sodium and potassium values of pyoderma infected dogs were within the normal range. Pak (2000) reported a normal level of sodium but elevated potassium in pyoderma infection. Results of BUN, creatinine and mineral status showed that pyoderma infection did not exert any nephrotoxicity. Also there was no significant difference in the GSH concentration in dogs with pyoderma infection indicating that no oxidative damage might have occurred in pyoderma infection.

5.6 HAEMATO-BIOCHEMICAL CHANGES IN DOGS WITH PYODERMA DURING AND AFTER TREATMENT

Among the twenty one dogs studied, twelve completed the full course of antibiotic therapy and post treatment evaluation. The dogs confirmed to be affected with pyoderma were given ciprofloxacin @ 10 mg/kg body weight orally, once daily for 14 days. The dosage and duration of administration of the antibiotic used in the study were determined on the basis of dose regimens typically used by general practitioners. Blood samples were taken at weekly interval during the treatment and 5th day after the therapy for analysing haematological and biochemical parameters.

5.6.1 Haematological Changes

5.6.1.1 Total Erythrocyte Count

The TEC obtained did not show any significant difference between before, during and after treatment. These values were within the normal range reported

by Meinkoth and Clinkenbeard (2000). The result correlate well with the findings of Kilic *et al.* (2003) who reported an insignificant increase in TEC in rats during 10 days treatment with ciprofloxacin in an induced inflammatory condition. Similar observations were also made by Hooper and Wolfson (1985), Garg *et al.* (1989) and Fadel and Larkin (1996) in humans, buffalo calves and lambs respectively. However, McEwan (1992) reported a case of anaemia in pyoderma infected dog treated with trimethoprim-sulphamethazole for six weeks and TEC returned to normal within three weeks on withdrawal of the treatment. Vijaykumar *et al.* (2008) also showed that oxytetracycline decreased the TEC on prolonged administration.

5.6.1.2 Total Leucocyte Count

The present data shows a mild leucocytosis in dogs selected for the treatment. A progressive significant decrease in TLC was observed during treatment and then returned to normal level. This is in accordance with the findings of Kilic *et al.* (2003) who observed a gradual decrease in TLC in rats which had undergone ciprofloxacin treatment for ten days against induced inflammation. The significant decrease noticed in the TLC in guinea pigs treated with gentamicin for seven days might have been due to direct cytotoxic effect of the antibiotic as an antigen in combination with leucocyte protein thereby resulting in lysis or agglutination of the leucocytes (Gupta and Verma, 1998). Fadel and Larkin (1996) mentioned that gentamicin induced leucopenia in lambs after three days treatment could be due to necrosis and inflammation at the injection sites. A decrease in TLC was also reported in a 57 old year male who had undergone treatment with piperacillin/tazobactam up to 25 days, confirming leucopenia as a serious adverse effect of β-lactam antibiotic (Abbas, 2007).

5.6.1.3 Haemoglobin

The haemoglobin values obtained were within the normal range. Similar findings were also reported in rats with induced inflammation treated with ciprofloxacin (Kilic *et al.,* 2003). Hooper and Wolfson (1985) reported that no change in the Hb concentration occurred in humans on fluoroquinolone administration. Treatment with gentamicin could not produce any adverse effect on Hb value in buffalo calves, lambs and guinea pigs according to Garg *et al.* (1989), Fadel and Larkin (1996) and Gupta and Verma (1998) respectively. The findings of Vijayakumar *et al.* (2004) suggested that oxytetracycline treatment did not cause any change in the already decreased Hb value in dogs with ehrlichiosis while Aronson (1980) showed a decrease in Hb value in dogs given minocyline @ 10 mg/kg body weight for one month. The Hb value remained unchanged on prolonged administration of β-lactam antibiotic in a 57 year old male (Abbas, 2007). The main haematological finding as reported by McEwan (1992) was a low Hb concentration of 9.7 g/dl in a dog treated with trimethoprim-sulphamethazole against pyoderma which returned to normal of 12.1 g/dl three weeks after the completion of the therapy.

5.6.1.4 Packed Cell Volume

The PCV value in the present study did not differ significantly between prior to, during and after treatment. The values fall within the normal range and are in agreement with earlier reports. Hooper and Wolfson (1985) did not find any change in PCV values on fluoroquinolone administration in human beings. The findings in the present study are comparatively lower than the PCV values in ciprofloxacin and pefloxacin treated rats against induced inflammatory process reported by Kilic *et al.* (2003). Garg *et al.* (1989), Fadel and Larkin (1996) and Gupta and Verma (1998) had reported that gentamicin administration did not affect the PCV value in guinea pigs, lambs and buffalo calves respectively. Similar findings were also observed in dogs treated with oxytetracycline

(Vijayakumar *et al.,* 2004). However, Aronson (1980) noticed a decreased PCV value in dogs given minocycline for one month. The studies of McEwan (1992) showed a low PCV of 31.2 % in a dog treated with trimethoprim-sulphamethazole for 42 days. Mellor *et al.* (2005) found a decreased PCV value, which returned to normal in a dog treated with carprofen along with cefalexin and co-amoxyclav.

5.6.1.5 Erythrocyte Sedimentation Rate

The values of ESR also showed no significant differences between the treatment days and after the therapy. These values fall within the normal range reported by Meinkoth and Clinkenbeard (2000).

5.6.1.6 Differential Leucocyte Count

5.6.1.6.1 Neutrophils

In this study, a mild increase in neutrophil count was observed, which decreased significantly after the treatment. Brown and Rogers (2001) reported a drug induced neutropenia in three dogs treated with trimethoprim-sulphadiazine, which might be due to the cytotoxic effect on stem cells and circulating cells. Jenkins *et al.* (1970) reported a significant decrease in neutrophil count in human beings with long term use of trimethoprim-sulphonamide which might be attributed to sulphonamide. Bloom and Rosser (2001) found that the neutrophil percent was within the reference ranges during the treatment with clindamycin in pyoderma condition. The findings of Fadel and Larkin (1996) showed a significant increase in neutrophil:lymphocyte, which was explained as nephrotoxic effects of gentamicin or by necrosis at the injection sites or a combination of these two. However, the findings of Garg *et al.* (1989) disagree with these reports. The author reported that no adverse effect occurred on DLC following daily intramuscular administration of gentamicin for seven days.

Vijaykumar *et al.* (2008) reported a neutrophilic leucocytosis in oxytetracycline induced hepatitis. Abbas (2007) noticed an initial neutrophil count of 61 %in a 57 year old man, which reached zero per cent on 25th day of treatment with piperacilin/tazobactam. This neutropenia might be attributed to the bone marrow suppression caused by cumulative piperacilin dose.

5.6.1.6.2 Lymphocytes

Lymphocyte showed a significant increase after the treatment with ciprofloxacin as compared to day 0 observation. Abbas (2007) reported a lymphocyte count of 24 %on the initial day of treatment which changed to 87 %by 25th day of treatment in human beings while Garg *et al.* (1989) could not find any variation in lymphocyte count in gentamicin administered rats. Fadel and Larkin (1996) found a decrease in the number of lymphocytes following three days treatment with gentamicin in lambs, which might be attributed to the nephrotoxic effects of gentamicin or necrosis at the injection site. According to Bloom and Rosser (2001) clindamycin administration did not produce any variation in lymphocyte count following treatment in canine pyoderma.

5.6.1.6.3 Monocyte

A slight increase in monocyte was seen on completion of the treatment and 5th day after treatment. But the change was insignificant. This is in accordance with the findings of previous workers, Bloom and Rosser (2001), who reported that the antibiotic clindamycin did not produce any significant change in the monocyte count in superficial bacterial dermatitis in dogs. Garg *et al.* (1989) reported that daily i.m. administration of gentamicin for seven days in buffalo calves did not produce any significant variations in DLC. Abbas (2007) reported that piperacilin/tazobactam administration for 25 days in a 57 year old man did not cause any change in the monocyte count, even though variation were observed

in other DLC parameter. Vijaykumar *et al.* (2004) noticed monocytosis in *Ehrlichia canis* infection, which returned to normal following treatment with oxytetracycline.

5.6.1.6.4 Eosinophils

There was no variation in the eosinophil count before and after treatment. Similar results were obtained with clindamycin, when used in superficial pyoderma in dogs (Bloom and Rosser, 2001). Garg *et al.* (1989) could not find any significant variation in the eosinophil count in buffalo calves administered with gentamicin i.m. for seven days. The findings of the present study are also in agreement with the findings of Abbas (2007).

No significant difference was observed in haematological parameters such as TEC, Hb, PCV, ESR and DLC (lymphocyte, monocyte and eosinophil) with ciprofloxacin treatment, except for TLC and neutrophil count. The neutrophilic leucocytosis observed on day 0 of treatment returned to normal with the progression of the treatment suggesting that ciprofloxacin could have subsided the infection.

5. 6.2 Biochemical Parameters

5.6.2.1 Alanine Aminotransferase

Liver injury is generally indicated by elevation in the levels of serum aminotransferases. Theses enzymes are cytoplasmic or mitochondrial in origin and released into blood as a result of hepatic damage.

During and after treatment with ciprofloxacin, ALT values did not show any variation from the normal range. Treatments with gentamicin (Garg *et al.,* 1989), carprofen along with cefalexin and co-amoxyclav (Mellor *et al.,* 2005) and

ampicillin/sulbactam (See and Lee, 2006) did not produce any increase in ALT activity in buffalo calves, dog and man respectively. However, Zimpfer *et al.* (2004) reported an elevated ALT activity in a patient treated with ciprofloxacin for seven days during a non-specific bacterial infection of the skin, which might be due to the cytotoxic effect of the drug in hepatocytes. Aronson (1980) reviewed that there was an increase in the level of serum ALT in a dog treated with tetracycline @ 20 mg/kg body weight/day. Anderson *et al.* (1984) noticed hepatitis in Basenji dog with increased ALT activity following sulphadiazine-trimethoprim therapy against *Escherichia coli* cystitis and the level of ALT reduced to normal, when the drug was discontinued. The studies of Vijaykumar *et al.* (2004; 2008) showed a marked elevation in ALT value in dogs treated with oxytetracycline.

5.6.2.2 Asparatate Aminotransferase

The serum AST values of dogs before and after treatment with ciprofloxacin did not differ significantly. The findings of Hooper and Wolfson (1985) are also in agreement with the findings of the present study, that fluoroquinolone administration does not change the AST value on prolonged administration in human beings. This is in agreement with the findings of Garg *et al.* (1989) who reported that gentamicin administration for a week in buffalo calves did not alter AST level. Furthermore, the reports of See and Lee (2006) also support the present observation, where they noticed normal level of AST in a 49 year old male treated with ampicillin/sulbactam. Mellor *et al.* (2005) observed the AST values within the normal range on administration of carprofen along with cefalexin and co-amoxyclav in a dog. However, Zimpfer *et al.* (2004) reported an elevated AST value, indicating acute liver damage with ciprofloxacin treatment for seven days in a 22 year old male during a non specific bacterial infection of the skin. An increase in AST level was observed when minocycline was administered for one month @ 20mg/kg body weight in a dog (Aronson, 1980).

5.6.2.3 Total Protein and Albumin

Since most serum proteins are synthesised by the liver it is an important analyte in monitoring liver function. In the present study, the serum total protein level obtained was near the base line of normal range (6.08 ± 0.64 g/dl) which increased gradually (8.08 ± 0.96 g/dl) but within the normal level. Similarly, albumin also showed a mild increase for the initial value but the increase was not significant. Present observations are in accordance with the findings of Fadel and Larkin (1996) who reported that gentamicin administration in lambs too produced a change in the levels of total protein and albumin but it was insignificant statistically. Hooper and Wolfson (1985) reported that fluoroquinolone administration in human beings did not change the levels of total protein and albumin. Mellor *et al.* (2005) also could not find any variation in the levels of total protein and albumin concentration from the normal range on administration of carprofen along with cefalexin and co-amoxyclav in a dog.

5.6.2.4 Albumin Globulin Ratio

The A:G before and after treatment with ciprofloxacin were not statistically significant and within the normal range of A:G reported by Kaneko *et al.* (1997).

5.6.2.5 Cholesterol

The serum cholesterol concentrations obtained during and after treatment were within the normal range reported by Kaneko *et al.* (1997).

The value of biochemical parameters such as ALT, AST, total protein, albumin, A:G and cholesterol were within the normal range before, during and after treatment suggesting that no liver damage might have occurred with 14 days of ciprofloxacin treatment in canine pyoderma.

5.6.2.6 Blood Urea Nitrogen and Creatinine

To determine whether the prolonged treatment of ciprofloxacin produced any renal deterioration, the NPN compounds such as BUN and creatinine levels were analysed. Elevated levels of these NPN compounds are considered as an indication of renal failure.

Blood urea nitrogen and creatinine values did not show any significant difference between 0^{th}, 7^{th}, 14^{th} day of treatment and 5^{th} day after the completion of the treatment. This is in accordance with the findings of Baykal *et al.* (2005). Sarkozy (2001) reviewed the possibility of increased BUN in patients treated with ciprofloxacin. The antibiotic, gentamicin was known to produce marked renal damage with an elevated BUN and creatinine levels (Patel *et al.,* 1975; Dellinger *et al.,* 1976; Garg *et al.*, 1989; Gupta and Verma, 1998; Erdem *et al.,* 2000; Ali *et al.,* 2001; Derakhshanfar *et al.,* 2007).

5.6.2.7 Sodium and Potassium

The serum sodium and potassium levels showed no significant difference before and after treatment. Similar observations were reported in rats orally administered with ciprofloxacin (Baykal *et al.,* 2005). Administration of carprofen along with cefalexin and co-amoxyclav in a dog also showed no change in sodium and potassium concentration (Mellor *et al.*, 2005). Yazar *et al.* (2003) reported that the different aminoglycoside antibiotics such as gentamicin, kanamycin, streptomycin and amikacin treatments, for four days in mice did not alter the serum sodium and potassium levels. However, a comparable increase in sodium excretion was noticed following gentamicin administration in rats without any effect on potassium level (Derakhshanfar *et al.,* 2007).

The BUN, creatinine, and electrolytes such as sodium and potassium concentration obtained in the present study prior to, during and after treatment were not altered from the normal level. These findings could suggest that ciprofloxacin therapy of 14 days in pyoderma cannot cause any nephrotoxicity.

5.6.2.8 Reduced Glutathione

Reduced glutathione defends the cells against the toxic effects of free radicals. There was no significant difference in the GSH concentration between before, during and after treatment. Yazar and Tras (2001) reported that enrofloxacin and danofloxacin enhanced the production of reactive oxygen species in liver and phagocytic cells which inactivate the GPX activity and induce SOD activity. Yazar *et al.* (2003) reported that the aminoglycosides such as gentamicin, kanamycin and streptomycin decreased the renal tissue GSH level in Balb/C mice suggesting a tissue injury at high dosage or on prolonged administration.

Reduced glutathione concentration during and after treatment was insignificant suggesting no oxidative stress could have occurred in prolonged ciprofloxacin treatment.

The present study suggests that prolonged ciprofloxacin treatment does not alter the haematological parameters such as TEC, Hb, PCV, ESR and DLC (lymphocyte, monocyte and eosinophil) except for the TLC and neutrophil count. As the condition of the animal improved with ciprofloxacin treatment, the neutrophilic leucocytosis returned to normal level. The overall biochemical data suggest that ciprofloxacin treatment does not exert any toxicity in hepatic and renal systems. Ciprofloxacin also produced an excellent response in dogs treated @ 10mg/kg body weight orally once daily for 14 days against pyoderma irrespective of the breeds.

Summary

6. SUMMARY

The present study was undertaken with the objective to evaluate any variation in haemato-biochemical parameters in dogs, following prolonged ciprofloxacin therapy against canine pyoderma.

The study was conducted in different breeds of dog presented to the Veterinary College Hospital, Mannuthy, for the treatment of pyoderma during the period from August 2007 to March 2008. Among the twenty one dogs studied, twelve completed the full course of antibiotic therapy and post treatment evaluation. The dogs with clinical signs confirming pyoderma were treated with ciprofloxacin @ 10 mg/kg body weight orally once daily for a period of 14 days. Blood samples were collected on day 0, 7 and 14 of treatment and day 5 on completion of the therapy to evaluate the haemato-biochemical picture during ciprofloxacin therapy. The blood samples were analysed for the haematological parameters such as Hb, TEC, TLC, PCV, ESR and DLC. The sera samples were subjected to biochemical analysis. The analysed parameters were ALT, AST, total protein, albumin, A:G, cholesterol, urea, creatinine, GSH and the electrolytes, sodium and potassium.

The occurrence of pyoderma was highest in one to three years of age group (38.10 %), followed by less than one year (23.81 %), three to five years (19.05 %) and above five years (19.05 %) and among the total of 21 dogs. Among the breeds, highest occurrence was observed in GSD (38.10 %), followed by Labrador (14.29 %), non-descript (14.29 %), Rottweiler (9.52 %), Spitz (9.52 %) and other breeds (14.29 %) which included Basset Hound, Cocker Spaniel and Dalmatian. The higher incidence of pyoderma in GSD might be due to an autosomal recessive gene, which leads to a cell mediated immunodeficiency resulting in bacterial folliculitis and furunculosis. Out of the 21 dogs reported with pyoderma, the prevalence was higher in males (52.38 %) than in females (47.62 %).

The clinical signs and lesions were combinations of papules and pustules, matting of hairs, erosions, cellulitis, alopecia, scales, hyperpigmentation and pruritus. Overall antibiotic sensitivity pattern of the gram positive cocci isolates from skin swabs showed maximum sensitivity to ciprofloxacin followed by cefotaxime, cephalexin, gentamicin and amoxicillin.

The data of TEC showed no significant difference between prior, during and after treatment, even though a mild increase was observed on 7^{th} and 14^{th} day of treatment. The mean TLC in dogs before treatment showed leucocytosis (19788.46 ± 1665.53/mm^3) which might be due to bacterial infection. A marked decrease was observed in the TLC values on 7^{th} day (16008.23 ± 1391.40/mm^3), 14^{th} day of treatment (13908.77 ± 1273.25/mm^3) and 5^{th} day after the therapy (10963 ± 1086.78/mm^3) which produced a significant difference from the 0^{th} day of treatment. No change in the Hb values were observed on 7^{th}, 14^{th} day of treatment and 5^{th} day after the treatment as compared to that on 0^{th} day of therapy. The PCV values did not show any variation between weekly intervals of treatment and also after completion of therapy. No variation was also observed in ESR. Differential leucocyte count showed mean neutrophil counts on 0^{th}, 7^{th} day of treatment as 67.77 ± 5.74 and 68.39 ± 5.21 % respectively. A significant decrease was noticed in these values on 14^{th} day of treatment and 5^{th} day after treatment which were 63.92 ± 4.28 and 61.69 ± 3.62 % respectively. Following ciprofloxacin therapy, the initial lymphocyte value of 20.46 ± 2.01 increased progressively to 29.31 ± 1.17 % on 5^{th} day after the therapy through 21.31 ± 1.55 and 26.62 ± 1.44 % on 7^{th} and 14^{th} day of treatment respectively. Pyoderma infected dogs showed no variation in the monocyte and eosinophil count before, during and after treatment. Altogether, the haematological data suggest that except TLC and DLC (neutrophil and lymphocyte counts), all other parameters such as TEC, Hb, PCV, ESR, monocyte and eosinophil count did not exhibit any significant difference prior to, during and after treatment. As the treatment progressed and condition of animals improved,

the level of these parameters returned to normal. Neutrophilic leucocytosis may be due to bacterial infection, which subsided with ciprofloxacin treatment.

The biochemical data of the present study suggested that the pyoderma infection did not cause any change in the functional status of liver and kidney. Treatment with ciprofloxacin did not result in any significant change in the ALT and AST values. Total protein, albumin and A:G were also within the normal range prior to, during and after treatment. The concentration of cholesterol was within the normal range in the course of ciprofloxacin therapy. No variations in BUN and creatinine values were observed before, during and after therapy. The electrolytes, sodium and potassium were in the normal range during ciprofloxacin treatment. Reduced glutathione levels also did not show any variation during the course of study, indicating no oxidative stress following ciprofloxacin therapy. The overall observations on haematological and biochemical parameters suggest that the drug ciprofloxacin @ 10mg/kg body weight orally once daily is not causing any damage to liver tissues on prolonged administration for the treatment of canine pyoderma up to two weeks.

Thus the present study revealed that ciprofloxacin could be used effectively in treating canine pyoderma up to two weeks without causing any adverse effects in the functional status of liver and kidney. The study also showed that the drug, ciprofloxacin was having maximum antibacterial activity against gram positive cocci and it could be used as the better drug to treat canine pyoderma.

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EVALUTION OF HAEMATO – BIOCHEMICAL CHANGES ASSOCIATED WITH CIPROFLOXACIN THERAPY IN CANINE PYODERMA

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ABSTRACT

The present study was undertaken with the objective to assess the haemato-biochemical changes following prolonged ciprofloxacin therapy in dogs affected with pyoderma. The study was conducted in dogs presented to the Veterinary College Hospital, Mannuthy, during the period from August 2007 to March 2008. Among the twenty one dogs studied, twelve completed the full course of antibiotic therapy and post treatment evaluation. The pyoderma confirmed dogs were treated with ciprofloxacin @ 10 mg/kg body weight once daily orally for a period of 14 days. Blood samples collected on day 0, 7 and 14 of treatment and fifth day after the completion of therapy was subjected to haemato-biochemical analysis.

Signalment indicated an age wise occurrence of pyoderma and was highest in one to three years of age group (38.10 %). Breed wise occurrence was highest in GSD (38.10 %) and sex wise prevalence was higher in males (52.38 %).

Clinical signs and lesions noted in the 21 dogs were combinations of papules and pustules, matting of hairs, erosions, cellulitis, alopecia, scales, hyperpigmentation and pruritus. The antibiotic sensitivity pattern of the gram positive cocci isolates from skin swabs showed maximum sensitivity to ciprofloxacin followed by cefotaxime, cephalexin, gentamicin and amoxycillin.

Haematological examination of the blood samples showed no change in TEC, Hb, PCV, ESR and DLC (moncyte and eosinophil) between before, during and after treatment. But a significant variation in TLC, neutrophil and lymphocyte were noticed in dogs with pyoderma. As the treatment progressed, the condition of the animal improved which resulted in decreasing TLC and neutrophil count to normal level.

The data obtained on hepatocellular enzymes ALT, AST and other biochemical parameters such as total protein, albumin, A:G and cholesterol suggest that treatment with ciprofloxacin might not have produced any adverse effect on liver tissue. Serum BUN and creatinine levels were found within the normal range. The electrolytes, sodium and potassium were also not altered during the course of treatment. These observations suggest that prolonged administration of ciprofloxacin, at the dose rate mentioned, is not capable of causing any nephrotoxicity. Reduced glutathione also support the above conclusion by eliminating the chance of having any oxidative stress.

The present study conclude with the findings that prolonged ciprofloxacin treatment does not produce any deterioration in hepatic and renal system of dogs affected with pyoderma, suggesting ciprofloxacin treatment as a safe and efficacious drug for the complete cure of canine pyoderma.