

Haematological, biochemical, histopathological studies and isolation of bacteriological agents from canine pyoderma cases in Karnataka

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Abstract

The present study was carried out to investigate the haematology, serum biochemistry, histopathology and isolation of causative bacterial agents from canine pyoderma cases. The highest occurrence of canine pyoderma was recorded in the age group between one to six years and particularly in male dogs. Affected dogs showed significant ($P \leq 0.05$) decrease in the haematological values like haemoglobin, total erythrocyte count and significant increase in the total leucocytes, absolute neutrophil and eosinophil counts. Reduced albumin, increased cholesterol levels in serum biochemistry and infiltration of neutrophils with nuclear debris in the superficial keratin layer of the epidermis in histopathological analysis were recorded in pyoderma-affected dogs. Forty bacterial isolates were identified as *S. intermedius* (n=17), *S. schleiferi* (n=10), *S. aureus* (n=5), *P. aeruginosa* (n=5) and *E. coli* (n=3) based on cultural and biochemical characters. The study was concluded with significant changes in haematological, serum biochemical parameters and isolation of *S. intermedius* from canine pyoderma cases as a major bacteriological agent.

Keywords: Canine, Cultural and biochemical characters, Pyoderma, *Staphylococcus intermedius*, *Staphylococcus schleiferi*

Highlights

- The highest occurrence of pyoderma observed with respect to age, sex and breed was one to six years of age, male dogs and Labrador Retriever, respectively.
- Differences in the levels of blood and serum parameters were recorded in infected dogs.
- *Staphylococcus intermedius* was isolated as a major bacterial isolate along with other members of the genus *Staphylococcus* spp. and Gram-negative organisms.

INTRODUCTION

Pyoderma is the most commonly encountered dermatological disorder in pet animals, especially in dogs, due to certain characteristics and features of its skin, like thin stratum corneum with less lipid material and unprotected hair follicles that have increased risk for bacterial invasion and subsequent colonization and overgrowth. The most common underlying causes observed for canine pyoderma are atopy, parasitic infestations, endocrine disorders and food allergies Khinchi (2019). Pyoderma is a cutaneous infection of the skin with pyogenic (pus-forming) bacteria, characterized by primary skin lesions, including papules and pustules, followed by secondary skin lesions crusting, epidermal collarettes, alopecia, scaling, erythema, pruritus, lichenification and hyperpigmentation (Chaudhary *et al.*, 2019). Normal resident and transient

bacteria of canine skin includes *Staphylococcus* spp., *Streptococcus* spp., *Micrococcus* spp., *Acinetobacter* spp., and *Bacillus* spp., *Corynebacterium* spp., *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas* spp., respectively. It not only has a zoonotic significance but also has the possibility of frequent recurrence (Guardabassi *et al.*, 2008). In view of the above common causes, this work was conducted to learn the haematology, serum biochemistry, histopathology, and causative bacterial agents for canine pyoderma.

MATERIALS AND METHODS

Source and selection of study animals

Dogs presented to a small animal outpatient section of Veterinary College Hospital, Hebbal, Bangalore, Karnataka Veterinary Animal and Fisheries Sciences University (KVAFSU), and different Veterinary Hospitals

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of Bangalore city, with various dermatological disorders, especially those suggestive of pyoderma were considered for the study. A total of ten apparently healthy dogs in the age group of six months to eight years, which were presented for general check-ups and vaccination, were randomly selected and considered as a control group. Forty dogs with different breeds aged between less than one year to above twelve years were selected for the study after a detailed history of recurrent pyoderma and a thorough physical examination with clinical signs. The occurrence of canine pyoderma was categorized based on age, gender and breed. Dogs with external parasites like ticks, mites, fleas and fungal infections, sarcoptic and demodectic mange were eliminated from the study based on thorough physical examination of the hair coat and skin scraping examination, respectively.

Sample collection and processing

Skin swabs: Skin swab samples were collected aseptically from the selected dogs by using sterile swabs (M/s. Himedia Pvt Ltd, Mumbai) and inoculated into brain heart infusion broth (BHIB) and incubated overnight at 37°C. After incubation, a loopful culture from BHIB was streaked onto brain heart infusion agar (BHIA) and incubated overnight at 37°C to isolate the organisms. Isolated bacteria were inoculated into differential and selective media based on their appearance on Gram's staining and colony morphology. The isolated bacterial agents were subjected to various biochemical tests viz. catalase test, coagulase test, oxidase test, IMViC tests (Indole, Methyl red (MR), Voges-Proskauer (VP) and Citrate utilization test) (Cruickshank *et al.*, 1975) and by using Hi Staph kit (M/s.

Himedia Pvt Ltd, Mumbai) for the identification of *Staphylococcus* bacteria at the species level.

Blood and serum samples: Blood was collected in EDTA vials from all selected dogs (healthy and suspected animals) for estimation of different haematological parameters like Packed Cell Volume (PCV), Haemoglobin (Hb), Total Leucocyte Count (TLC), Total Erythrocyte Count (TEC), neutrophils, lymphocytes, eosinophils and monocytes by using Automatic Haematology Analyser (Mindray BC-2800 Vet, Chain). Serum samples were collected in clot activator vials and subjected for estimation of total protein, serum albumin, globulin, albumin: globulin ratio and total cholesterol by using a semiautomatic biochemistry analyser (Micro labs RX 50, India) and kits from Span Diagnostics Ltd.

Skin biopsy: The skin biopsies were collected from the dogs showing recurrent dermatological lesions. Hair was clipped over and around the lesion and swabbed with 70 percent alcohol, taking utmost care not to disturb the surface lesions. Primary skin lesions and transition lesions were selected for biopsy. Representative tissue samples were obtained by elliptical excision or by using 6 mm punch biopsy needle under local anaesthesia using 2% lignocaine. The samples were fixed in 10 percent neutral buffered formalin immediately and processed by routine paraffin embedding technique. Cut sections of 4-5 µm were stained with Haematoxylin and Eosin (Luna, 1968).

Statistical analysis

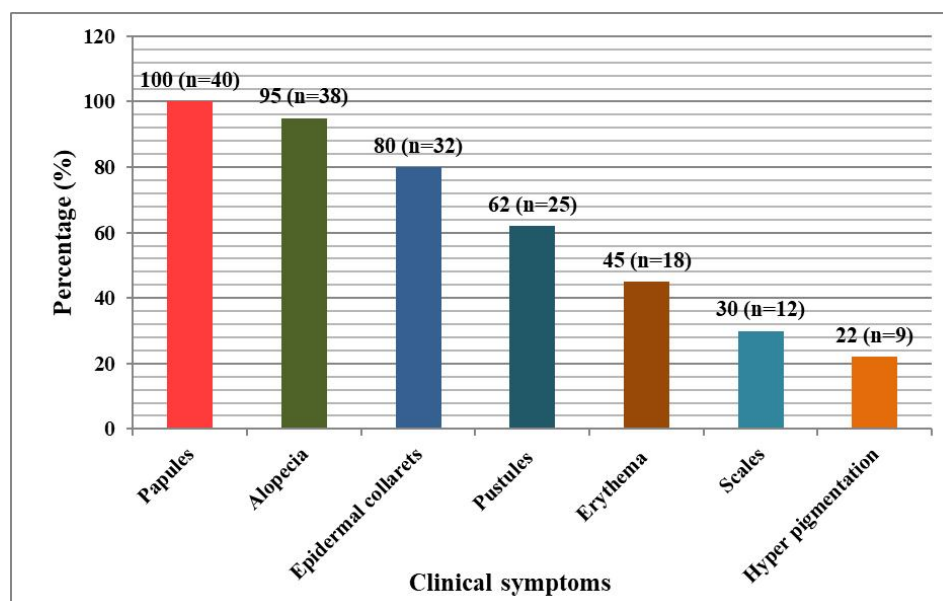
The data was subjected to statistical analysis using T-test and ANOVA as per the procedure described by Gupta, 1998 and Graph Pad Prism Software.

RESULTS

All the affected dogs exhibited pruritus and other different clinical symptoms such as papules, alopecia, pustules, etc. (Fig. 1 and Graph 1).

Canine pyoderma was more frequent in male dogs (n=26, 65%) than female dogs (n=14, 35%). The highest occurrence was observed in Labrador Retriever and age group between one to six years (Table 1 and 2).

The haematological mean values of Hb



Graph 1. Clinical symptoms exhibited by pyoderma affected dogs

Table 1. Age-wise occurrence of canine pyoderma (n=40)

< 1 year		1 – 6 years		6 – 12 years		> 12 years	
No.	%	No.	%	No.	%	No.	%
04	10%	18	45%	12	30%	06	15%

Table 2. Breed-wise occurrence of canine pyoderma (n=40)

Breed	No. of cases	Percentage (%)
Labrador Retriever	09	22.5
Non-descript	07	17.5
Golden Retriever	05	12.5
Rottweiler	04	10
Siberian Husky	04	10
Pug	03	7.5
Shih Tzu	03	7.5
Boxer	02	05
Dachshund	02	05
Cocker Spaniel	01	2.5

(13.03±0.42 g/dL), PCV (39.29±2.14%) and TEC (6.51±0.19 x 10⁶/μL) were recorded in apparently healthy animals. Whereas dogs with pyoderma recorded a mean Hb of (10.49±0.30 g/dL), PCV (38.84±1.19%) and TEC (4.25±0.14 x 10⁶/μL). The results showed a significant (P≤0.05) decrease in the values of haemoglobin and TEC. No significant difference was observed in PCV of dogs with pyoderma when compared to apparently healthy dogs. The mean TLC (9.33±0.81 x 10³/μL), absolute neutrophil (6.69±2.44 x 10³/μL), lymphocyte (2.41±1.19 x 10³/μL), eosinophil (0.135±0.22 x 10³/μL) and monocytic counts (0.068±0.017 x 10³/μL) were observed in apparently healthy dogs. In dogs with pyoderma, the mean values of TLC (12.30±0.50 x 10³/μL), absolute neutrophil (9.64±5.25 x 10³/μL), lymphocyte (2.08±1.45 x 10³/μL), eosinophil (0.49±0.050 x 10³/μL) and monocyte counts (0.062±0.020 x 10³/μL) were observed (Table 3). There was an increase in the values

Table 3. Mean haematological values of healthy and canine pyoderma affected dogs (Mean ± S.E)

Parameters	Healthy group (n=10)	Pyoderma cases (n=40)
Hb (g/dL)	13.03±0.42	10.49±0.30
PCV (%)	39.29±2.14	38.84±1.19
TEC (x10 ⁶ /μL)	6.51±0.19	4.25±0.14
TLC (x10 ³ /μL)	9.33± 0.81	12.30±0.50
Neutrophils (x10 ³ /μL)	6.69±2.44	9.64±5.25
Lymphocytes (x10 ³ /μL)	2.41±1.19	2.08±1.45
Eosinophils (x10 ³ /μL)	0.135±0.22	0.496±0.050
Monocytes (x10 ³ /μL)	0.068±0.017	0.062±0.020

of TLC, absolute neutrophil and eosinophil in dogs with pyoderma compared to the healthy group. No significant differences were observed in absolute monocyte and lymphocyte counts compared to healthy dogs.

The mean total serum protein (6.81±0.22 g/dL), albumin (3.25±0.04 g/dL), globulin (3.56±0.20 g/dL), albumin and globulin ratio (0.85±0.11) and total cholesterol (84.57±6.81 mg/dL) were recorded in apparently healthy animals. Dogs with canine pyoderma had the mean total protein (6.29±0.10 g/dL), albumin (2.49±0.11 g/dL), globulin (3.79±0.07 g/dL), albumin and globulin ratio (0.72±0.04) and total cholesterol values (124.32±22.14 mg/dL) (Table 4).

Table 4. Mean serum protein profile and cholesterol values of healthy and pyoderma affected dogs (Mean ± S.E)

Parameters	Healthy group (n=10)	Canine pyoderma cases (n=40)
Total protein (g/dL)	6.81±0.22	6.29±0.10
Albumin (g/dL)	3.25±0.04	2.49±0.11
Globulin (g/dL)	3.56±0.20	3.79±0.07
A:G ratio	0.85±0.11	0.72±0.04
Total cholesterol (mg/dL)	84.57±6.81	124.32±22.14

Dogs with recurrent pyoderma had a reduction in their albumin levels when compared to that of the apparently healthy group. There was no difference observed in total protein, globulin and albumin and globulin ratio values when compared with apparently healthy dogs. Dogs with recurrent pyoderma had increase in cholesterol levels when compared to that of healthy group.

Histologically, the lesion from the skin biopsy showed focal abscess with accumulation of neutrophils and nuclear debris in epidermal regions. Epidermis showed spongiosis, presence of apoptotic cells with

condensed nucleus. Infiltration of mononuclear cells with predominant population of neutrophils and eosinophils were observed in epidermis. Subcorneal pustules showed neutrophils, eosinophils and debris with the presence of haemorrhages. Dermal blood vessels were occluded with neutrophils and showed perivascular dermatitis. Lympho-histocytic and neutrophilic infiltrates were seen around the apocrine sweat gland with intact hair

follicles (Fig. 2).

All forty samples showed positive results for the growth of microorganisms in BHIB and BHIA. Thirty two bacterial isolates (80%) revealed Gram positive cocci in clusters or bunches on Grams staining and positive for catalase and coagulase test, suggestive of coagulase positive *Staphylococcus* genus. The colonies on mannitol salt agar produced creamy greyish white to golden yellow color colonies and were further identified at species level based on the biochemical profile using Hi Staph kit as *Staphylococcus intermedius* (17 no., 53%), *Staphylococcus schleiferi* (10 no., 32%) and *Staphylococcus aureus* (5 no., 15%). Five bacterial isolates (12.5%) on BHIA showed bluish green pigmentation and appeared as Gram negative rods on Grams staining and were assumed as *Pseudomonas aeruginosa* (*P. aeruginosa*). The colonies on subculturing in MacConkey agar exhibited no change in colour of the colonies, suggestive of lactose non fermentors and biochemical tests profile were catalase and oxidase positive, IMViC --- +.

Three bacterial isolates (7.5%) were found to be Gram negative coccobacilli on Grams staining; after subculturing of these isolates on MacConkey agar, they produced pink colour colonies, which was suggestive of members of lactose fermenting Enterobacteriaceae family. Isolates were confirmed as *Escherichia coli* (*E. coli*) based on metallic sheen growth on eosin methylene blue agar and biochemical IMViC test results ++--.

DISCUSSION

Pyoderma (pus in the skin) is a common skin disease in dogs mainly caused by bacteria, also known as bacterial skin infection. The age-wise observations of this study were in agreement with the findings of Khinchi (2019), Anikar *et al.* (2021) and Naveena *et al.* (2023). However, in contrast Reddy *et al.* (2014) and Kuhrana *et al.* (2016) reported that dogs less than one year of age were mostly affected with pyoderma. The occurrence of pyoderma was high in male dogs, which was similar to the studies of Kumar and Haque (2015), Asmita *et al.* (2015), Khurana *et al.* (2016), Khinchi (2019) and Anikar *et al.* (2021) and also this may be attributed to pet owners passion towards keeping a male than female dogs, preference and purpose of dog keeping, the difference in market values etc. These findings did not coincide with studies of Reddy *et al.* (2014), who reported a higher prevalence of pyoderma in female than male dogs. This increased incidence of pyoderma in females may be attributed to the stress associated with oestrus, pregnancy, whelping

and nursing. In breed-wise occurrence, Khinchi (2019) and Anikar *et al.* (2021) showed the highest occurrence in Labrador Retriever, which was in concordance with our results. The least incidence of pyoderma was reported in Shih Tzu, Dachshund and Cocker Spaniel; this could be due to their rarity as a pet population and the sample number in the study area and managerial care given by owners.

The haematological parameter of apparently healthy dogs was within the normal reference range, as quoted by Reddy *et al.* (2014). The blood parameters (Hb, PCV and TEC) of dogs with pyoderma were in line with the findings of Nair and Nauriyal (2007). The decrease in the values of haemoglobin and TEC might be due to the loss of skin protein. An increase in TLC, absolute neutrophil count and absolute eosinophil count of dogs with pyoderma was recorded in the present study. This increased leucocytosis could be due to a higher cutaneous inflammatory reaction. The results coincided with the findings of Gera *et al.* (2009).

The mean total protein, globulin, albumin and globulin ratio values of canine pyoderma were within the normal range, which was similar to the findings of Benjamin (2007). In the present study, it was observed that mean albumin and total protein values were lower than the control group. This might be due to loss of albumin through injured skin. The higher level of cholesterol recorded in the study might be due to a high-fat diet, lack of exercise, steroid medication and stress associated with canine pyoderma, as reported by Gera *et al.* (2009).

The predominance of gram-positive *Staphylococcus* spp. in this study was in accordance with the findings of Shah *et al.* (2017), Ankita and Gandge (2018), Chaudhary *et al.* (2019), Naveena *et al.* (2023). The main causative agent of canine pyoderma is known to be *Staphylococcus pseudintermedius*, which belongs to the *Staphylococcus intermedius* group (SIG); this group contains *Staphylococcus intermedius* (*S. intermedius*) and *Staphylococcus delphini* (*S. delphini*) as other organisms (Thomas, 2010). However, in the present study, *S. intermedius* was isolated as a major pathogen of pyoderma, which was in line with the reports of Reddy *et al.* (2014). Based on biochemical characteristics, 10 out of 32 dogs, *S. schleiferi* were isolated, which was in accordance with the findings of Reddy *et al.* (2014). Five *Staphylococcus aureus* (*S. aureus*) and five *P. aeruginosa* along with *Staphylococcus* spp. were isolated in this study, similar to the findings of Siugzdaite *et al.* (2008). Three *E. coli*, along with *Staphylococcus* spp. isolates, were



Fig. 1. Alopecia, erythema, moist eczema, hyperpigmentation, pustules, crust and epidermal collate on face, forehead and ventral abdominal region of dogs with pyoderma

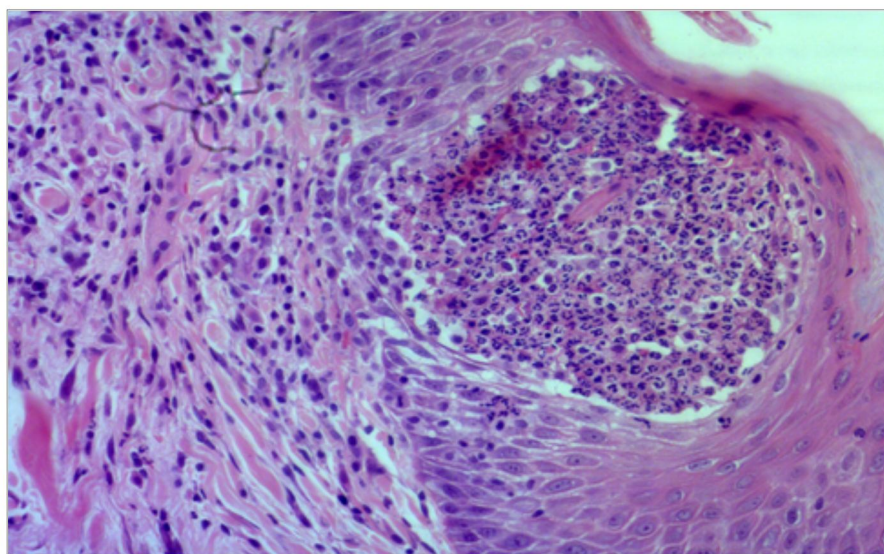


Fig. 2. Histopathology of skin biopsy showing focal abscess with accumulation of neutrophils and nuclear debris in epidermis

obtained in the study and were in agreement with the work of Udayasree and Pillai (2006). The presence of Gram-negative bacteria is considered to be due to secondary invasion.

The present study concluded with significant changes in blood and serum parameters and isolation of *S. intermedius* from skin swabs as a major causative agent of canine pyoderma.

Conflict of interest: The authors declares that there is no conflict of interest in the present research work.

Author's contributions: MC: Presented research work from Master's degree programme; PTR: Major advisor, guided the 1st author for above degree programme;

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- KMC: Guided the 1st author for carrying out research work related to microbiology and prepared manuscript; VL, KRA, GJR: Advisory committee members and guided the 1st author for carrying out research work related to medicine and pathology and have contributed equally to this research work.
- Data availability statement:** All the data pertaining to this original research work is available with the corresponding author.
- Ethical statement:** All the skin swab samples from dogs presented to out patient section, Dept. of Medicine, Veterinary College, Bengaluru were collected with the consent of the owners after their request consent letter.
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