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Canine pyoderma histopathology: Insights and findings

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Pyoderma in dogs is a bacterial skin infection influenced by a combination of factors such as the immune system, genetics and the environment (Guardabassi *et al.* 2008). It can manifest as focal, regional, or generalized lesions on the skin. Pyoderma can be either superficial, affecting the epidermis, dermis and hair follicles, or deep, causing cellulitis, fistulous tracts or subcutaneous tissue involvement, respectively (Scott *et al.* 2001). When the primary cause of pyoderma is not properly identified and resolved, it can lead to recurrent infections (Bajwa 2006). Recent years have shown an increase in the incidence of methicillin and multidrug-resistant *Staphylococcus pseudintermedius* (MRSP) in canine pyoderma, making therapy more difficult (Bryan *et al.* 2012; Miller *et al.* 2013). Additionally, *Staphylococcus aureus*, *Proteus* spp., *Pseudomonas* spp. and occasionally *Escherichia coli* also cause pyoderma. Pyoderma can also develop as a result of localized trauma, parasite infestations, hormonal abnormalities, or contact with irritants or allergens. Managing canine pyoderma have become complicated due to the emergence of multidrug-resistant, methicillin-resistant *staphylococci* (MRS) (Loeffler and Lloyd 2010). Understanding the subtleties of skin changes in pyoderma requires a thorough understanding of dermatopathology. Hence, dermatopathology plays a critical role in understanding skin changes in pyoderma (Bexfield and Lee 2014). Treatment typically spans 2-4 weeks, regardless of the administration route (Bajwa 2016). The aim of the present study was to investigate dermatopathological changes and antibiotic sensitivity pattern in canine pyoderma. The study also focused to identify various causative agents of canine pyoderma and their prevalence across different dog breeds.

Different dog breeds exhibiting symptoms consistent with pyoderma, such as itching, widespread hair loss, pustules, circular skin lesions and dry skin, detection of bacterial cocci or rods during cytological examination, the growth in bacteriological cultures and the development of colonies that matched *Staphylococcus* spp. on mannitol salt agar plates, *E. coli* on Eosin-Methylene Blue (EMB) agar were taken for the study. Skin scraping examination and tape impression smears were conducted to eliminate the possibility of parasitic and fungal infections, respectively (Bexfield and Lee 2014). Additionally, serum biochemical tests, including cholesterol, triglycerides, glucose and cortisol, as well as thyroid profile assessments and urine analysis, were performed to rule out the presence of hypothyroidism and hyperadrenocorticism (Borio *et al.* 2015). Dogs testing positive for conditions other than pyoderma were excluded from the study. Dogs showed skin lesions of bacterial origin were included in the study and were administered with antimicrobial treatment based on the antibiogram results. The protocol was approved by a local Ethics Commission (27/26/CVSc, Hyd, IAEC).

A total of 34 cytological samples that yielded positive results for cocci when subjected to giemsa staining were further studied thoroughly. Sterile swab samples from the skin of these 34 dogs were then cultured on mannitol salt agar plates at 37°C for 18 to 24 h as per the standard procedure (Schick *et al.* 2007; Borio *et al.* 2015). Antimicrobial profiles of isolated *Staphylococcus* spp. were determined using the Kirby-Bauer method on colonies grown in Mueller-Hinton agar. Antimicrobials were chosen for their application in dermatological treatment within the vicinity of the research area and concentrations as follows: Oxacillin (5 µg), amoxicillin clavulanate (30 µg), ampicillin sulbactam (20 µg), enrofloxacin (5 µg), ciprofloxacin (5 µg), doxycycline (30 µg), azithromycin (15 µg), gentamicin (10 µg), ceftiofur (30 µg), and vancomycin (5 µg). Skin punch biopsies were carried out using a sterile 3.5 mm punch biopsy needle from the hot spots. These samples were then preserved in 10% neutral buffered formalin (NBF) until further processing (Punia *et al.,* 2018). Haematoxylin and Eosin (H&E) staining were performed to visualize and evaluate the dermatopathology of skin samples.

In the present study, the highest incidence of pyoderma was recorded in Labradors (26.47%), followed by

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Table 1. Breed predilection and dermatopathological assessment in canine pyoderma

Breed	No. of dogs	Gender	Isolated organism (no of dogs)	Nature of lesion
Labrador	9	M -5, F- 4	*Staphylococcus* spp. (8) and *Proteus* spp. (1)	Erythyma, ulcers, papules, pustules, scales and crusts.
Spitz	4	M -2, F- 2	*Staphylococcus* spp. (4)	Erythematous papules, hemorrhagic crusts.
German Shepherd	4	M -3, F- 1	*Staphylococcus* spp. (3) and *Pseudomonas* spp. (1)	Erythematous lesions, generalized lesions with pustules and collarets.
Pug	5	M -5	*Staphylococcus* spp.(5)	Circular crusts, dry or flaky patches of skin, along with generalized alopecia.
Mongrel	2	M -2	*Pseudomonas* spp. (2)	Generalized alopecia with itching.
Great Dane	2	M -1, F- 1	*Staphylococcus* spp. (2)	Hemorrhagic crusts and ulcers with circular crusts, dry scales with hyperkeratoses, and pruritus.
Golden Retriever	2	M - 1, F- 1	*Staphylococcus* spp. (2)	Papular and pustular lesions, generalized crust formation, hyperkeratoses with pruritus.
Doberman Pinscher	2	M -2	*Staphylococcus* spp. (1) and *E. Coli* (1)	Epidermal collarets and pustular lesions along with itching.
Dachshund	2	M -2	*Staphylococcus* spp. (1) and *Pseudomonas* spp. (1)	Generalized crusty lesions and epidermal collarets, hyperkeratoses and hyperpigmentation with pruritus.
Boxer	1	M-1	*Staphylococcus* spp. (1)	Generalized (post-grooming) alopecia, erythema, and raised area with a white pus-filled center.
Beagle	1	F-1	*Staphylococcus* spp. (1)	Papules and pustules with pruritus.

Pugs (14.71%), Spitz (11.76%) and German Shepherds (11.76%). Pyoderma was also observed in Mongrels, Great Danes, and Golden Retrievers (each 5.88%). The lowest prevalence of pyoderma was recorded in Boxers and Beagles (each 2.94%). Out of the 34 dogs in the study, 24 were male (70.58%) and 10 were female (29.41%). *Staphylococcus* spp. was the frequently observed bacterial species, accounting for 28 out of 34 (82.35%) cases, hence ABST was carried out only for *Staphylococcus* spp. Apart from *Staphylococcus* spp. the study also reported *Pseudomonas* spp. (4/34 cases, 11.76%), *Proteus* spp. and *E. coli* (1/34 each, 2.94%) as causative organisms of pyoderma in dogs (Table 1). In the present study, *Staphylococcus* spp. was identified as the primary underlying cause of pyoderma in dogs (Holm *et al.* 2002; Huerta *et al.* 2011). *Staphylococcus epidermidis* (Miller *et al.* 2013) and *S. pseudintermedius* (Fazakerley *et al.* 2009) were also reported. *P. aeruginosa* was solely isolated and recovered from the dog skin (Hillier *et al.* 2006), whereas, presence of *E. coli* (Summers *et al.* 2014) was also reported in canine pyoderma. The noteworthy clinical observations include erythema, papules, pustules, ulcers, epidermal collarets, hyperkeratosis, hyperpigmentation, widespread alopecia and formation of crusts. In the Antibiotic Sensitivity Test (ABST), the most effective antibiotics identified were amoxicillin clavulanate, azithromycin and enrofloxacin with sensitivities of 58.82%, 55.88%, and 52.94%, respectively. Conversely, ciprofloxacin depicted sensitivity of 26.47% and vancomycin showed the lowest sensitivity of 23.52% (Table 2). A few isolates of *Staphylococcus* spp. was also found resistant to enrofloxacin and ampicillin (Vanni *et al.* 2009). Cephalosporins appear to be appropriate for *Staphylococcus* spp. (Rafatpanah *et al.* 2020).

Table 2. Antibiotic sensitivity test for the *Staphylococcus* spp.

ABST disc	Sensitive	Intermediate	Resistant
Amoxicillin Clavulanate	20 (58.82%)	8 (23.52%)	6 (17.65%)
Azithromycin	19 (55.88%)	5 (14.71%)	10 (29.41%)
Enrofloxacin	18 (52.94%)	8 (23.52%)	8 (23.52%)
Gentamicin	15 (44.12%)	4 (11.76%)	15 (44.12%)
Ampicillin Sulbactam	14 (41.17%)	6 (17.65%)	14 (41.17%)
Ceftiofur	14 (41.17%)	9 (26.47%)	11 (32.35%)
Doxycycline	13 (38.24%)	3 (8.82%)	18 (52.94%)
Oxacillin	12 (35.29%)	13 (38.23%)	9 (26.47%)
Ciprofloxacin	9 (26.47%)	5 (14.71%)	20 (58.82%)
Vancomycin	8 (23.52%)	8 (23.52%)	24 (70.59%)

Microscopical examination of H&E-stained tissue revealed severe infiltration of inflammatory cells, including neutrophils, eosinophils, round cells (lymphocytes) and mast cells in the epidermis along with severe necrosis and ballooning degeneration which extended up to the dermis. Inflammation of subcutaneous tissue and blood vessels was also manifested in cases where the deep infection was noticed. Round cell infiltration was also observed around hair follicles and blood vessels. Cellular infiltration was either focal, diffuse, or close aggregates. The extent of infiltration from the epidermis, dermal-epidermal junction, and the whole of the dermis and subcutis depending on severity (Table 3 and Fig. A-H). The current research documented notable dermatopathological changes in canine pyoderma, viz. massive infiltration of inflammatory cells and ballooning degeneration (Arbaga *et al.* 2021).

In the present study, 34 cases of pyoderma were subjected to antibiotic treatment based on ABST results. For superficial pyoderma, treatment was extended for two weeks beyond clinical cure, while deep pyoderma received three weeks treatment. Seventeen dogs were

Table 3. Dermatopathological changes in canine pyoderma

Dermatopathology	Number	Percentage (%)
Massive infiltration of inflammatory cells	34	100
Congested and dilated blood vessels and abundance infiltration of round cells in the dermis	30	88.23
Mast cell (Stellate-like cell) and round cell infiltration with sparse smooth muscle fiber	24	70.59
Focal area of round cell infiltration	18	52.94
Congested and dilated blood vessels with mild infiltration	14	41.18
Ballooning degeneration with few dense nuclei	12	35.29

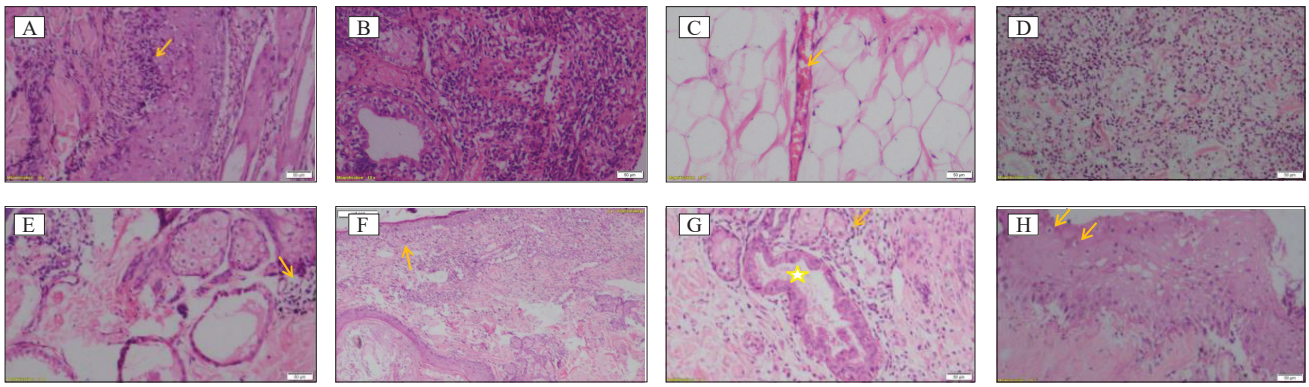

Fig. 1. A. Massive infiltration of cells in the perifollicular area (H&E stain, 10X); B. Abundance infiltration of round cells (small and large lymphocytes) in the dermis (H&E stain, 10X); C. Congested and dilated blood vessels in the sub-cutis area (H&E stain, 10X); D. Mast cell infiltration with sparse smooth muscle fiber (H&E stain, 10X); E. Focal area of round cell infiltration (H&E stain, 10X); F. Round cell infiltration in the dermal area (H&E stain, 4X); G. Congestion of blood vessels (star) with sparse smooth muscle fiber (H&E stain, 10X); H. Ballooning degeneration with few dense nuclei (H&E stain, 10X).

treated with amoxicillin clavulanate (@10 mg/kg body weight orally twice daily), and all animals were fully recovered. Eight cases of superficial pyoderma showed significant improvement within the first week, while five cases achieved excellent response in the second week. In contrast, two deep pyoderma cases and two superficial pyoderma cases completely recovered after three weeks of treatment. Seventeen cases were treated with azithromycin (@ 5 mg/kg body weight orally once daily). Among these, six cases of superficial pyoderma showed recovery within one week, four cases within two weeks, and five cases of deep pyoderma within three weeks. However, two cases of deep pyoderma treated with azithromycin did not exhibit a favourable response by the end of the third week of treatment (Table 4). Antimicrobial resistance was encountered in isolated organisms, all the isolates were showed least resistance to either one or two antibiotics of the present investigation (Rafatpanah *et al.* 2020). Treatment initiated depended up on the depth and extent of cellular infiltration. The cases of focal infiltration of neutrophils and round cells were treated with suitable antibiotic therapy for 7-14 days. Whereas, the dogs with extensive cellular infiltration with congestion of blood vessels were treated with appropriate antibiotic therapy for 21-28 days (3-4 weeks).

Canine pyoderma with extensive inflammatory cell infiltration in skin lesions were primarily caused by *Staphylococcus* bacteria, with occasional isolation of *Pseudomonas* spp. and *Proteus* spp. Labrador breed showed a higher incidence of canine pyoderma. Treatment, tailored to antibiotic susceptibility, lasted 14-28 days, depending on the depth and extent of cellular infiltration emphasizing the importance of understanding skin changes and targeted antibiotic therapy for effective management. The research underscores the significance of dermatopathological alterations in canine pyoderma, highlighting the prevalence of *Staphylococcus* spp. as causative agent and their susceptibility to specific antibiotics, particularly amoxicillin and cephalosporins.

SUMMARY

Pyoderma is a common bacterial skin infection in canines, resulting from a complex interplay between host immunity, environmental factors and genetic predisposition. This study investigated the dermatopathological changes associated with canine pyoderma and its therapeutic management. Thirty-four client-owned dogs with clinical

Table 4. Therapeutic response (n=34)

Antibiotic used	Number of days of recovery in superficial pyoderma (n=17)			Number of days of recovery in deep pyoderma (n=17)		
	1st week	2nd week	3rd week	1st week	2nd week	3rd week
Amoxicillin Clavulanate	8 (47.06%)	5 (29.41%)	2 (11.76%)	-	-	2 (11.76%)
Azithromycin	6 (35.29%)	4 (23.53%)	-	-	-	5 (29.41%)

signs that are suggestive of pyoderma were included. Punch biopsies of skin lesions were subjected to histopathological examination revealed extensive infiltration of inflammatory cells, including neutrophils, eosinophils, round cells and mast cells in the epidermis, dermis and subcutaneous tissue. Bacterial isolation and identification confirmed *Staphylococcus* spp*.,* as the predominant causative agent, with occasional isolation of *Pseudomonas* spp., and *Proteus* spp. A higher incidence of pyoderma was reported in Labrador breeds and therapy was carried out as per ABST. The duration of therapy ranged from 14-28 days, tailored to the depth and extent of cellular infiltration.

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