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Bacteriological examination of the peri- and endo-dontal diseases in dogs

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The study was conducted on 24 clinical cases of dogs with various symptoms related to different peri- and endo-dontal affections. All the cases were evaluated by clinical examination, dental radiography, microbiological evaluation and antibiotic sensitivity testing. On microbial evaluation, bacterial culture like Staphylococcus sp. was isolated from 23 samples and Escherichia coli organisms from 11 samples. Antibiogram of the dental swabs revealed high sensitivity towards ciprofloxacin followed by doxycycline and high resistance towards metronidazole followed by clindamycin. Antimicrobial evaluation and antibiotic sensitivity testing played a major role in treatment of the periodontal and endodontal affections in dogs with good clinical outcome.

Keywords: *Antibiotic sensitivity testing, Dental diseases, Dog, Microbial evaluation*

Dental diseases are common in domesticated animals, of which the two most common conditions affecting the companion animals are periodontal and endodontal diseases. By the time they attain two years of age, 70% of cats and 80% of dogs are affected by some form of periodontal disease (Fadden and Maretta, 2013).

Periodontal disease is a collective term for a number of plaque-induced inflammatory lesions that affect the periodontium. Cementum, alveolar bone, periodontal ligament and gingiva together are considered as Periodontium. Gingivitis is inflammation of the gingiva and is the earliest sign of disease, if it is left untreated it can develop into periodontitis. This leads to destruction of the periodontal ligament and alveolar bone ultimately resulting in exfoliation of the affected tooth (Gorrel, 2004).

Endodontic disease is the disease that affects the internal portion of the tooth, i.e. pulp, and it is the frequently encountered oral problem in dogs and cats. The most common cause of endodontic disease in dogs was reported as tooth fracture with pulp exposure (Niemiec, 2005).

Bacteria are ubiquitous in nature and moist conditions will always favour the growth of the microorganisms. Oral cavity is always moist and flooded with food particles, which can attract a wide variety of microorganisms that are responsible for the disease process to start. Periodontal disease is the inflammatory condition initiated by bacterial plaque. The plaque is a microbial biofilm on teeth surface, which is formed by a well-organized community of microorganisms embedded in a matrix of polymers of bacterial and salivary origin (Schenkein, 2006). Normal bacterial microflora in dog mouth varies from aerobic, facultative to strictly anaerobic bacteria (Pavlica, 2006). In dogs healthy gingivae affected with plaque will be primarily invaded by gram-positive organisms, as plaque matures the bacterial composition shifts to predominantly gram-negative anaerobic flora (Roudebush *et al.*, 2005). This study was aimed in determining the bacteria present in the periodontal and endodontal diseases of dogs and the antibiotic sensitivity was determined to follow the appropriate treatment regimen.

During the study period of 12 months (from Jan-Dec 2020), 896 dogs that were reported with different systemic diseases were screened for dental problems, of which 268 dogs (29.91%) were diagnosed for having dental affections. Among them, 24 dogs affected with peri- and endo-dontal diseases were further examined. Data regarding the age, breed, gender, type of food offered, duration of the diseases were collected (1). Signalment and detailed anamnesis was obtained and the animals were subjected for the detailed physical examination followed by oral cavity examination both in conscious and anaesthetised patient. Based on the clinical signs and complete oral cavity examination, they were staged into different dental affections. The animals diagnosed with peri- and endo-dontal diseases were further subjected for radiological and microbiological examination.

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For the microbiological examination, sterile cotton swabs were used for the collection of samples from the gums of the affected tooth on the buccal surface. The swabs were stored at 4! and were inoculated into sterile Tryptic SoyBroth (TSB) and incubated aerobically at 37! for 24-36 hr. The enriched broth was streaked onto Mannitol salt agar (MSA) and Eosin methylene blue agar (EMB) media. *Escherichia coli* shows metallic sheen on EMB agar and on MSA, while *Staphylococcus* was differentiated into two types based on fermentation of mannitol. *Staphylococcus aureus* species was identified by production of yellow colour mucoid colonies on Mannitol Salt Agar media, whereas other *Staphylococcus* species did not show this character. Later, the cultures were transferred to Muller Hinton Agar and 6 antibiotic discs namely Clindamycin, Amoxicillin - clavulanate, Ceftriaxone, Doxycycline, Ciprofloxacin and Metronidazole were placed on the media and incubated at 37! for 24-36 hr. After incubation, the area of inhibition was measured for all the antibiotic discs.

The swabs collected from affected teeth of all the 24 cases were subjected to bacterial isolation. Analysis of the mouth swabs of the affected dogs showed the presence of both Gram positive and Gram negative cocci and rods. Roudebush *et al.* (2005) and Zambori *et al.* (2012) also reported the presence of both Gram positive and Gram negative organisms in oral cavity. The samples were inoculated on both Mannitol salt agar and Eosin methylene blue agar plates. After 24 hr of incubation, 11 (45.83%) samples were found positive for *Escherichia coli* organisms, which showed colony formation with metallic shean on Eosin methylene blue agar. However, in a similar study Zambori *et al.* (2012) stated that the primary colonizers in dental affections were gram positive bacteria such as *Streptococcus* and *Actinomyces* and the secondary colonizers were gram negative bacteria such as *Fusobacterium*, *Prevotella*, *Capnocytophaga* and the frequent third colonizer as *Porphyromonas*, *Campylobacter* and *Trepanoma.* Tatakis and Kumar (2005) stated that *Porphyromonas spp.* has central role in both human and canine periodontal disease. Out of 24 samples inoculated on Mannitol salt agar, 23 samples (95.83%) were found to be positive for *Staphylococcus* species. Among 23 isolates of *Staphylococcus* species, 15 samples (62.50%) were identified as *S. aureus* based on mannitol fermentation, which converted pink coloured Mannitol Salt Agar to yellow coloured mucoid colonies. In remaining 8 (33.33%) samples, there was colony growth but no colour change was noticed, hence these were categorised under other *Staphylococcus* species.

Antibiotic sensitivity testing was done by disc diffusion method in Mueller Hinton agar using commonly available antibiotic discs such as ciprofloxacin, doxycycline, ceftriaxone, amoxicillin-clavulanate, clindamycin and metronidazole. Out of 24 samples, 8 (33.33%) samples showed high sensitivity towards Ciprofloxacin followed by 4 (16.6%) samples for Doxycycline, 3 (12.5%) for Ceftriaxone, two (8.33%) for Amoxycillin and Clavulanic acid and one (4.16%) for Clindamycin. Antibiogram of the dental swabs revealed high resistance towards Metronidazole followed by Clindamycin. Whereas Cleland (2000), Lobprise (2007b) and Niemiec (2008b), stated that Clindamycin and Metronidazole were effective antimicrobials in the treatment of dental affections. Variations might be due to involvement of various pathogens in these dental conditions.

Table 1: Signalment of dogs presented with dental affections

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Sl. No.	Age (yr)	Breed	Sex	Feeding habits
1	7	Pomeranian	F	Home food
2	3	Shih Tzu	F	Commercial diet
3	8	Cockers spaniel	F	Commercial diet
4	11	Pomeranian	M	Home food and Commercial diet
5	3	Siberian Husky	M	Home food and Commercial diet
6	9	Mixed breed	M	Home food and Commercial diet
7	5	Golden Retriever	F	Home food and Commercial diet
8	7	German Shepherd	F	Home food and Commercial diet
9	6	Labrador Retriever	F	Home food and Commercial diet
10	4	Labrador Retriever	M	Home food and commercial diet
11	9	Pomeranian	M	Home food
12	10	Pomeranian	F	Home food
13	6	German Shepherd	F	Commercial diet
14	5	Mixed breed	F	Home food and Commercial diet
15	7	Mixed breed	F	Home food and Commercial diet
16	8	Mongrel	F	Home food
17	10	Pomeranian	F	Home food
18	5	Labrador Retriever	F	Home food and commercial diet
19	7	Pomeranian	F	Home food
20	8	Pomeranian	M	Home food
21	3	Shih Tzu	F	commercial diet
22	6	Pomeranian	M	Home food and commercial diet
23	11	Pomeranian	F	Home food
24	9	Mongrel	F	Home food
Mean±0.498	**6.95±0.498**			

Table 2: Antibiotic sensitivity/resistance pattern of the samples.

Sl. No.	Antibiotic	S		I		R	
		n	%	n	%	n	%
1	Metronidazole	0	0	0	0	24	100
2	Ciprofloxacin	8	33.3	7	29.16	9	37.5
3	Doxycycline	4	16.6	0	0	20	83.3
4	Ceftriaxone	3	12.5	5	20.8	16	66.6
5	Amoxycillin and Clavulanic acid	2	8.33	4	16.6	18	75.0
6	Clindamycin	1	4.16	1	4.16	22	91.6

S- Sensitive I – Intermediate R- Resistant.

The sensitivity and resistance pattern of these antibiotics and their overall percentages are given in the Table 2. Based on the results obtained, suitable antibiotic was chosen for treatment in a particular case.

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