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CLINICAL STUDIES OF PERIODONTAL DISEASES ON HAEMATO-BIOCHEMICAL, MICROBIOLOGICAL AND CHEMICAL ANALYSIS OF DENTAL TARTAR

Khatariya M. D.[1], Talekar S. H.*[1], Ahlawat AR[2], Dodia V.D.[1] and Kalaria V.A.[3]

[1]Department of Surgery and Radiology, College of Veterinary Science and Animal Husbandry, JAU, Junagadh (Gujarat State).
[2]Department of Department of Animal Genetics and Breeding, College of Veterinary Science and Animal Husbandry, JAU, Junagadh (Gujarat State).
[3]Department of Department of Pathology, College of Veterinary Science and Animal Husbandry, JAU, Junagadh (Gujarat State).

***Corresponding Author: Talekar S. H.**
Department of Surgery and Radiology, College of Veterinary Science and Animal Husbandry, JAU, Junagadh (Gujarat State).

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ABSTRACT

The present work was conducted on 20 dogs suffering from periodontal diseases presented to Department of Veterinary Surgery and Radiology, Junagadh Agricultural University, Junagadh during study period. The present clinical work was carried out to study, haematological and biochemical parameters in relation to periodontal disease, diagnosis of periodontal diseases, and to study oral micro flora, suitable antibiotics therapy and mineral composition of dental tarter. This present study was carried out on 20 dogs in which major complaints from dog owners revealed halitosis, Sticky salivation, anorexia, pawing at mouth and facial swelling in the present study. History further revealed that pet owners ignore any home care and professional dental cleaning for keeping teeth of dogs healthy. A detailed survey regarding food habits of dogs revealed maintained on pure vegetarian diet and both vegetarian and non-vegetarian diet. Majority of dogs were provided homemade food mainly consist of soft food, either pure vegetarian or mixed, also offered commercial available food (vegetarian) in the market in addition to homemade food. Among 20 dogs, oral examination revealed that varying degree of dental plaque, dental calculus, gingival recession, gingival hyperplasia, tooth fracture, persistent deciduous tooth and pulpitis. According to haemato-biochemical analysis on the day of treatment all parameters were recorded within normal range. Whereas there was no significant difference between values of various haematological and biochemical parameters on 0 day of treatment and 10th day post treatment. For purpose of dental scaling premedication with atropine sulphate, diazepam and ketamine hydrochloride were used for anesthesia. During treatment sterile swabs were used for sample collection from mineralized dental plaque of 20 dogs. The result of bacteriological culture revealed the presence of different types of bacterial colonies; viz. *Streptococcus spp.* (40 %), *Staphylococcus spp.* (30 %), *Escherichia spp.* (20 %) were found to be higher growth on culture media. Levofloxacin had the highest in-vitro susceptibility (85 %), followed by Amoxicillin (65 %), Gentamicin (60 %), Ceftriaxone (55 %), Cefotaxime (55 %), Oxytetracycline (50 %) and Chloramphenicol (45 %) Based on this susceptibility test, Levofloxacin was found to be the best antimicrobial drug of choice for periodontal diseases. Dental tarter samples (n=20) were used for chemical analysis. The analyzed dental tarter were composed of Calcium (Ca) 80.07 %, Phosphorus (P) 14.51 %, Magnesium (Mg) 2.83 %, Potassium (K) 1.22 %, Sulphur (S) 0.89 %, Iron (Fe) 0.61 %, Nickel (Ni) 0.44 %, and Zinc (Zn) 0.20 %.

KEYWORDS: Periodontal disease, chemical analysis, ABST and Dental tartar.

INTRODUCTION

A dog is a valuable member of family so deserves proper health attention and care. Forever 10,000 years, the dog has been an integral player in man's social and cultural development. It has hunted alongside humans for centuries and has been hunted by man for food. As eyes for the blind and ears for the deaf, the dog has become an indispensable member of our modern society (Chris, 2004).

Dental health is an extremely important but under rated aspect of companion animal health care. Hence, prevention and treatment of dental diseases are also at most important for general health of companion animals. Dental problems are frequently observed in small

animals and these conditions encompass the whole spectrum of problems as seen in man. The lack of oral hygiene cause plaque deposition and calculus formation, which harbours the bacteria and eventually induces gingival inflammation (Lindhe *et al*., 1975 and Page and Schroeder, 1982). The persistent infection of oral cavity does not only discomfort the affected animal but may also cause disease in other organs and tissues (De Bowes, 1994).

Periodontal disease (PD) refers to a group of inflammatory diseases caused by bacterial plaque in the periodontium. The periodontium contains the supporting structure of the teeth and includes the gingiva, alveolar bone, periodontal ligament and cementum. In veterinary medicine, PD is the most prevalent disease in domestic carnivores and is found in approximately 80% of dogs aged 2 years or older (Carlos *et al.,* 2012). PD is progressive and involves two stages: gingivitis and periodontitis. It is caused by plaque buildup on teeth. The plaque is a smooth membrane, adhesive, contaminated with saliva, bacteria and debris. Bacteria and bacterial products cause inflammation of soft tissue. The plaque becomes mineralized to form calculus, which migrates into gingival sulcus, causing additional inflammation, loss of periodontal ligament, bone loss and ultimately tooth loss (Ford and Mazzaferro, 2007). The ultimate prevention of periodontal disease is directly proportional to the degree of success in the elimination of dental plaque prevention is the key to prevent periodontal diseases (Andrew, 2004). PD is a multifactorial disease that results from the interaction of the host defense mechanisms with the plaque microorganisms. Early detection, diagnosis and treatment are essential in the control of this disease. PD has an enormous impact on human and veterinary medicine due to its high prevalence (Carlos *et al.,* 2012).

The present study was undertaken to note the incidence of dental affections in the dogs presented to TVCC, Junagadh to diagnose various dental affections with precision using available diagnostic tools for maintaining dental hygiene. Further, study was also planned to note the changes in haemato-biochemical parameters in the dogs suffering from various dental affections. To study mineral composition of dental plaque and to identify the oral micro flora and its antibiotics sensitivity test.

MATERIALS AND METHODS

The present clinical work was carried out in Department of Veterinary Surgery and Radiology on dogs presented at TVCC, Veterinary College, J.A.U., Junagadh. With the complaints of halitosis, sticky salivation, facial swelling and others. All the dogs presented with periodontal diseases were diagnosed on the basis of clinical examination and treated under general anesthesia accordingly.

Dogs were prepared for general anaesthesia and subsequent treatment by withholding the food for 24 hours and water for 12 hours before administration of intravenous anesthetics. Further, body weight of animals was recorded and site for intravenous administration of anesthetics was prepared. Atropine sulphate @ 0.05 mg/kg b.wt. was administered subcutaneously as a pre-anesthetic at about 10-15 minutes before administration of general anesthetics. General anesthesia was induced by administering mixture of diazepam @ 0.5 mg/kg b.wt. and ketamine hydrochloride @ 10 mg/kg b.wt. intravenously (I/V). General anaesthesia was maintained by intravenously (I/V) administration as per requirement induction dose of diazepam-ketamine mixture as per the requirement.

Oral examination of affected dogs included general oral and soft tissue examination, supragingival tooth examination, sub gingival tooth examinations and recording the findings like Gingival Index (GI), Plaque Index (PI), Calculus Index (CI), Furcation Exposure (FE) and periodontal pocket depth using periodontal probe as described by Loe and Silness (1963) The affected dogs with periodontal diseases were graded depending upon severity of periodontal disease on four stage i.e. Stage 1 to 4 PD as described by AVDC Nomenclature Committee. Stage 1 (PD1): Gingivitis only, without attachment loss. Stage 2 (PD2): Early periodontitis; less than 25 % of attachment loss, or at most there is a stage 1 furcation involvement. The loss of periodontal attachment is less than 25 % as measured either by probe. Stage 3 (PD3): Moderate periodontitis; 25–50 % of attachment loss. Stage 4 (PD4): Advanced periodontitis; more than 50 % of attachment loss, there is a stage 3 furcation involvement in multi rooted teeth.

Oral lavage with Chlorhexidine gluconate was given before and after scaling to reduce bacterial load in oral cavity.

The dental scaling was done in twenty dogs under general anesthesia. In cases of moderate to severe gingivitis, antibiotic was given Cefotaxime @ 15mg/kg body weight Post operative findings of treated cases were recorded up to 10 days.

On collection of detailed history, all 20 dogs were presented with the complaint of halitosis, sticky salivation, anorexia, facial swelling and pawing at mouth.

A survey during anamnesis of food habits revealed that dogs were maintained on pure vegetarian diet, mixed (vegetarian and non-vegetarian) diet. Homemade food either pure vegetarian or mixed food, commercial food (vegetarian food) available in the market in addition to homemade food. On investigation about dental home care it was found that no client brushed teeth of their pets, lacking unawareness about dental care. The daily addition of a dental hygiene chew to a regimen of tooth brushing every other day reduced the gingivitis scores

and reduced the accumulation of dental deposits (plaque, calculus and stain).

On the day of presentation dogs were restrained without anesthesia for clinical examination of whole buccal cavity with special reference to teeth. The dogs suffering from periodontal diseases were planned for treatment and owners were explained in details regarding the risk of general as well as preparation of patient for anaesthesia.

RESULTS AND DISCUSSION

Haemato-Biochemical Findings

Different haematological parameters like Haemoglobin (Hb), Total Erythrocyte Count (TEC), Total Leukocyte Count (TLC), Differential Leukocyte Count (DLC), Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), Blood Urea Nitrogen (BUN), Serum Creatinine, Total Protein (TP),Calcium (Ca), and Phosphorus (P) of all the dogs on '0' day (n=20) and 10^{th} day were evaluated and the results along with their mean and standard error (S.E.) have been shown in Table 1.

Haemoglobin (Hb)

The mean values of haemoglobin concentration (g %) in 20 dogs suffering from Periodontal diseases was recorded as 12.57±0.35 on '0' day and 12.89 ± 0.26 on 10^{th} post treatment day. Thus, haemoglobin concentration ranged within normal range in dogs suffering from periodontal diseases before and after treatments, and statistically values of Hb on '0' day did not differ from 10^{th} day.

Total Erythrocyte Count (TEC)

The mean value of erythrocyte count ($\times 10^6$/cumm) in all the 20 dogs having periodontal diseases were 6.74 ± 0.21 on '0' day and 7.32 ± 0.18 on 10^{th} post treatment. The values of TEC did not differ significantly.

Total Leukocyte Count (TLC)

The mean value of TLC ($\times 10^3$/cumm) ranged within normal limits in dogs suffering from periodontal diseases before treatment ('0' day) the values were recorded were 14.51 ± 0.46 and 15.05 ± 0.47 for 0^{th} and 10^{th} day respectively. The values of TLC not show significant difference between '0' and 10^{th} day.

Neutrophils (N)

The mean value of Neutrophils (%) ranged within normal limits on haematological examination in dogs suffering from periodontal diseases before treatment ('0' day) 64.55 ± 1.26 and after treatment (10^{th} day) 64.06 ± 1.03. Thus values of Neutrophils count not show any significant difference between '0' and 10^{th} day observation of Neutrophils count (%).

Lymphocytes (L)

The mean value of lymphocytes (%) count in all 20 dogs suffering from periodontal diseases was recorded as 34.05 ± 0.94 on '0' day and 33.85 ± 0.99on 10^{th} post treatment day. The values of lymphocyte (%) did not show any significant difference between '0' day and 10^{th} day.

Eosinophils (E)

The mean value of eosinophils (%) ranged within normal limits on haematological examination in dogs suffering from periodontal diseases before treatment '0' day 1.50 ± 0.18 and after treatment 10^{th} day 1.16 ± 0.08. Thus values of eosinophils count not show any significant different between '0' and 10^{th} day.

Alanine Amino Transferase (ALT)

The mean ± SE value for ALT (U/L) was observed to be 56.22 ± 1.33 on the day of presentation and 55.77 ± 1.21 on 10^{th} day post treatment day in dogs suffering from periodontal diseases. Thus values of ALT did not show any significant difference between on '0' and 10^{th} day.

Aspartate Amino Transferase (AST)

The mean ± SE value of AST (U/L) came to be 55.66 ± 0.96, which was found to be higher than the normal range (8.9 - 49 U/L) and after treatment 10^{th} day55.42 ± 0.92 Thus values of AST count did not show any significant difference between on '0' and 10^{th} day.

Blood urea nitrogen (BUN)

The mean value of BUN (mg/dl) in dogs suffering from periodontal diseases was 24.97 ± 0.91 in normal range '0' day and 25.70 ± 0.91 on 10^{th} post treatment which not was significant changes.

Serum Creatinine

The mean value of serum Creatinine (mg/dl) ranged within normal limits in dogs suffering from periodontal diseases before treatment '0' day 0.93 ± 0.12 and after treatment 10^{th} days 0.77 ± 0.10. The values of serum Creatinine count did not show any significant difference between '0' and 10^{th} day.

Total protein (TP)

The mean ± SE value for total protein (g/dl) observed to be 6.36 ± 0.15 on the day of presentation and 6.42 ± 0.15 on 10^{th} post operative day in dogs suffering from periodontal diseases. Thus values of total protein count not show any significant difference between on '0' and 10^{th} day.

Calcium (Ca)

The mean ± SE value for Calcium (mg/dl) was observed to be 9.94±0.31 on the day of presentation and 9.55±0.25 on 10^{th} post operative day in dogs suffering from periodontal diseases. Thus values of total protein count did not show any significant difference between on '0' and 10^{th} day.

Phosphorus (P)

The mean ± SE value for Phosphorus (mg/dl) observed to be 3.22±0.25 on the day of presentation and 3.66±0.25 on 10^{th} post operative day in dogs suffering from periodontal diseases. Thus values of total protein count

did not show any significant difference between on '0' and 10th day.

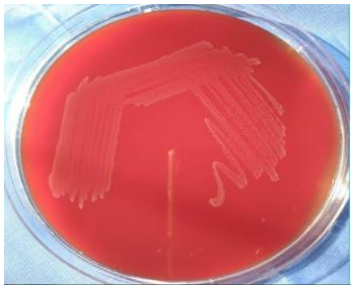
Table 1: Details of haematological and biochemical parameters.

Sr. No.	Parameter	0 day(n=20) (Mean ± SE)	10th day(n=20) (Mean ± SE)
1	Haemoglobin (gm %)	12.57±0.35	12.89 ± 0.26
2	Total Erythrocyte Count (×10^6/cumm)	6.74 ± 0.21	7.32 ± 0.18
3	Total Leukocyte Count (×10^3/cumm)	14.51 ± 0.46	15.05 ± 0.47
4	Neutrophils (%)	64.55 ± 1.26	64.06 ± 1.03
5	Lymphocytes (%)	34.05 ± 0.94	33.85 ± 0.99
6	Eosinophils (%)	1.50 ± 0.18	1.16 ± 0.08
7	ALT (U/L)	56.22 ± 1.33	55.77 ± 1.21
8	AST (U/L)	55.66 ± 0.96	55.42 ± 0.92
9	Blood Urea Nitrogen (mg/dL)	24.97 ± 0.91	25.70 ± 0.91
10	Serum Creatinine (mg/dL)	0.93 ± 0.12	0.77 ± 0.10
11	Total Protein (g/dL)	6.36 ± 0.15	6.42 ± 0.15
12	Calcium(mg/dL)	9.94±0.31	9.55±0.25
13	Phosphorus(mg/dL)	3.22±0.25	3.66±0.25

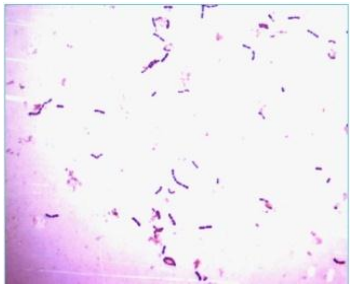
MICROBIOLOGICAL FINDINGS

Identification and Isolation

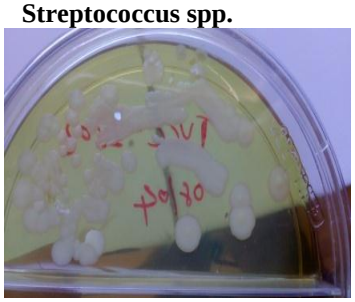
In the present study, sterile swabs were used for sample collection from mineralized dental plaque of 20 dogs and results were presented in table 2. The result of bacteriological culture revealed the presence of different types of bacterial colonies; viz. *Streptococcus* spp. (40 %), *Staphylococcus* spp. (30 %), *Escherichia* spp. (20 %) were found to be higher growth on culture media.



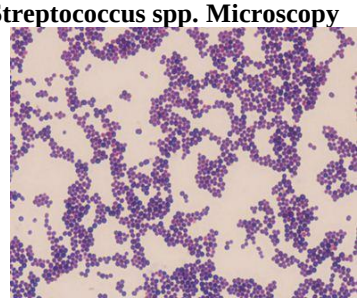
Streptococcus spp.



Streptococcus spp. Microscopy



Staphylococcus spp



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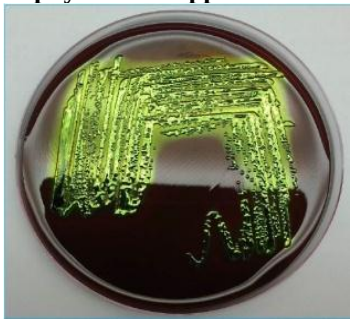
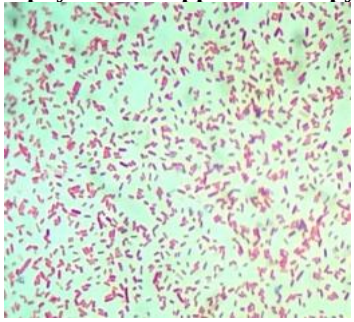


Escherichia spp



Escherichia Microscopy

Table 2: Microbiological analysis.

Organism found	Number of sample	Percentage (%)
Streptococcus spp.	8	40
Staphylococcus	6	30
E.coli	4	20
Bacillus spp.	3	15
Corynebacterium	3	15
Moraxella	2	10
Pseudomonas	2	10
Proteus	2	10
Micrococcus	2	10
Spirochetes	1	5
Neisseria	1	5

In present study, bacteriological culture of aerobic flora from dental swabs showed higher incidence of *Streptococcus* spp. followed by all bacteria spp.

Antimicrobial drug sensitivity test (ABST)

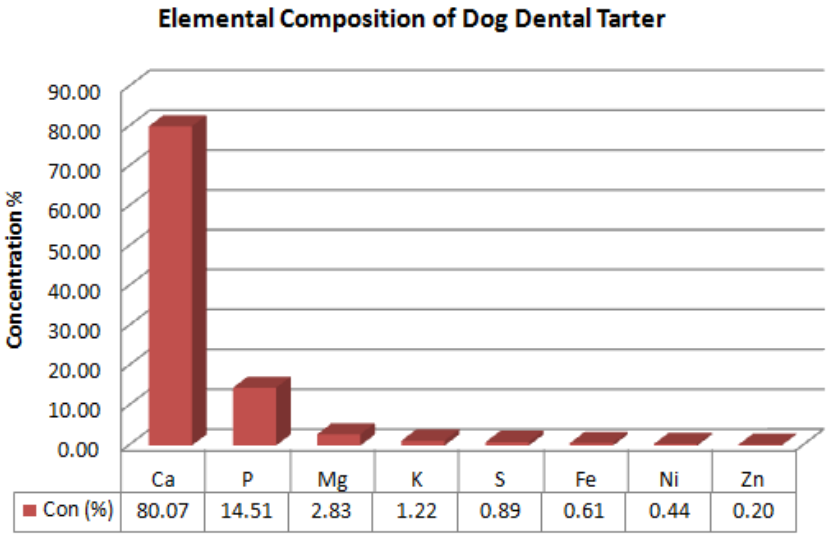
In present study Levofloxacin had the highest in-vitro susceptibility 17 out of 20 dogs (85 %), followed amoxicillin 13 out of 20 dogs (65 %), Gentamicin 12 out of 20 dogs (60 %), Ceftriaxone 11 out of 20 dogs (55 %), Cefotaxime 11 out of 20 dogs (55 %), Oxytetracycline 10 out of 20 dogs (50 %) and Chloramphenicol 9 out of 20 dogs (45 %) Based on this susceptibility test, Levofloxacin was found to be the best antimicrobial drug of choice (table-3).

Table 3: Antimicrobial drugs sensitivity pattern of dental plaque samples.

Antimicrobial drugs	Sensitive Number of samples	Percentages (%)
Levofloxacin	17	85
Amoxicillin	13	65
Gentamicin	12	60
Ceftriaxone	11	55
Cefotaxime	11	55
Oxytetracycline	10	50
Chloramphenicol	9	45

Chemical Analysis Of Dental Tarter

Selected dental tarter samples (n=20) were used for chemical analysis. The analyzed dental tarter were composed of Calcium 80.07 %, Phosphorus 14.51 %, Magnesium 2.83 %, Potassium 1.22 %, Sulphur 0.89 %, Iron 0.61 %, Nickel 0.44 %, and Zinc 0.20 %.



In present study, it was found that principle component of dental tarter was calcium salt followed by others whereas calcium in saliva deposited in the plaque would produce calculus. Calculus would provide rough surface favouring accumulation and maturation of more plaque. Dogs had high salivary pH values would promote the precipitation of calcium salts, leadings to enhanced calculus formation which might lead together with plaque accumulation to gingivitis and periodontal diseases.

CONCLUSIONS

The present work was conducted on 20 dogs suffering from periodontal diseases presented to Department of Veterinary Surgery and Radiology, JAU, Junagadh. The present clinical work was carried out to study haematological and biochemical parameters in relation to periodontal disease, diagnosis of periodontal diseases and to study oral micro flora, suitable antibiotics therapy and mineral composition of dental tarter.

This present study was carried out on 20 dogs in which major complaints from dog owners revealed halitosis, sticky salivation, anorexia, pawing at mouth and facial swelling history, further revealed that pet owners did not follow any home care and professional dental cleaning for keeping teeth of dogs healthy. A detailed survey regarding food habits of dogs were maintained on pure vegetarian diet and non-vegetarian diet. Homemade food mainly consists of soft food, either pure vegetarian or mixed commercial available food (vegetarian) in the market in addition to homemade food. Among 20 dogs, oral examination revealed that varying degree of dental plaque, dental calculus, gingival recession, gingival hyperplasia, tooth fracture, persistent deciduous and pulpitis.

According to haemato-biochemical analysis on the day of treatment all parameters were recorded within normal range. Whereas there was no significant difference between values of various haematological and biochemical parameters on 0 day of treatment and 10th day post treatment. For purpose of dental scaling premedication with atropine sulphate, diazepam and ketamine hydrochloride were used for anesthesia. The depth and duration of anesthesia in the dogs of the present study was found satisfactory to accomplish scaling of affected teeth in dogs.

In the present study, sterile swabs were used for sample collection from mineralized dental plaque of 20 dogs. The result of bacteriological culture revealed the presence of different types of bacterial colonies; viz. *Streptococcus spp*. (40 %), *Staphylococcus spp*. (30 %), *Escherichia spp*. (20 %) were found to be higher growth on culture media. Levofloxacin had the highest in-vitro susceptibility (85 %), followed by Amoxicillin (65 %), Gentamicin (60 %), Ceftriaxone (55 %), Cefotaxime (55 %), Oxytetracycline (50 %) and Chloramphenicol (45 %) Based on this susceptibility test, Levofloxacin was found to be the best antimicrobial drug of choice for periodontal diseases.

Dental tarter samples (n=20) were used for chemical analysis. The analyzed dental tarter were composed of Calcium (Ca) 80.07 %, Phosphorus (P) 14.51 %, Magnesium (Mg) 2.83 %, Potassium (K) 1.22 %, Sulphur (S) 0.89 %, Iron (Fe) 0.61 %, Nickel (Ni) 0.44 %, and Zinc (Zn) 0.20 %. All animal owners were advised to follow daily brushing of teeth, use of mouth wash, provision of bones for chewing and avoiding soft food for keeping teeth of dogs healthy.

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