The Impact of Scaling and Root Planning on *Porphyromonas gingivalis* Load and Periodontal Health in Dogs: A Longitudinal Study

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Running title: Scaling and Root Planing and Dogs Periodontal Health

Abstract

Periodontal disease is a widespread oral health issue in pets, particularly in dogs, linked to plaque accumulation, inflammation, and tissue destruction. Despite the common use of scaling and root planning (SRP) in veterinary dentistry, there is limited research on its effects on bacterial load and periodontal health indicators in pets, particularly in Iran. This study aims to evaluate the impact of SRP on *Porphyromonas gingivalis* load and periodontal health in dogs and emphasize the importance of post-treatment monitoring to prevent disease recurrence. Ten adult dogs with periodontal disease were selected for this split-mouth study. Four teeth from each dog were treated with SRP. Subgingival plaque samples were collected before SRP (baseline) and on days 10 and 30 post-treatment, with bacterial load assessed using real-time PCR targeting *P. gingivalis*. Clinical parameters such as periodontal pocket depth (PPD), gingival index (GI), plaque index (PI), sulcus bleeding, and clinical attachment loss (CAL) were measured at baseline, day 10, and day 90 to monitor the effects of the intervention. The data revealed a significant reduction in PI by day 10, though some plaque reaccumulation occurred by day 90. Bleeding on probing showed mixed results, with some dogs improving by day 90 while others either remained the same or worsened; changes in sulcus bleeding were not statistically significant. GI initially improved by day 10 but returned to baseline in many dogs by day 90, with no statistically significant changes. PPD showed some short-term improvements by day 10, but these were not sustained by day 90. CAL worsened progressively in most dogs by day 90, indicating ongoing periodontal deterioration without intervention, though changes in CAL were not statistically significant. Real-time PCR results showed a sharp increase in *P. gingivalis* load by day 10, peaking at a fold change of 9.45, followed by a slight reduction by day 30, indicating bacterial regrowth post-intervention. This study highlights the importance of ongoing monitoring after SRP to sustain short-term improvements in plaque reduction and bacterial load. Future research should investigate the use of adjunctive therapies, including the application of nanotechnology, to improve long-term periodontal health in pets.

Keywords: Periodontal disease, *Porphyromonas gingivalis*, Scaling and root planning, real-time PCR, Gingival

1. Introduction

Periodontal disease (Periodontitis) is one of the most common oral health issues in pets, particularly in dogs, and is associated with the accumulation of dental plaque, leading to inflammation, tissue destruction, and, if left untreated, systemic effects (1). Considering the global dog population in the millions and the increasing popularity of pet ownership in Iran, this presents a significant issue. The diagnosis of periodontal disease tends to occur late in the disease process (2). With this in mind, veterinarians must be able to diagnose and treat periodontal disease in their early stages, understand the outcomes of traditional treatment methods, and utilize this knowledge to promote preventive strategies (3).

Scaling and root planing (SRP) is a widely used therapeutic intervention in veterinary dentistry to manage periodontal disease by removing subgingival plaque and tartar (4). While SRP is a well-established procedure in human dentistry, relatively few studies have investigated its effects on bacterial load, periodontal indicators, and long-term oral health outcomes in pets, particularly dogs (1). The bacterial load and the response of periodontal health indicators such as periodontal pocket depth (PPD), gingival index (GI), plaque index (PI), sulcus bleeding, and clinical attachment loss (CAL) are critical markers of treatment success in periodontal therapies (5, 6). However, the dynamics of bacterial regrowth and the long-term effectiveness of SRP in pets have been less-researched, and have not yet been specifically studied in Iran. Furthermore, post-intervention monitoring of these parameters is often overlooked, which can lead to the recurrence of periodontal disease and related complications (2).

Chronic inflammation of the periodontium is initiated by complex subgingival biofilms containing several likely periodontal pathogens. The biofilm generally includes a portion of the gram-negative anaerobic commensal microbiota as well as opportunistic pathogens of the oral cavity, including *Porphyromonas gingivalis* (7). *P. gingivalis* is a key periodontal pathogen known for its significant role in developing and progressing periodontal disease in humans and animals. This gram-negative, anaerobic bacterium is frequently found in subgingival plaque and is associated with the destruction of periodontal tissues, leading to tooth loss if untreated (8-12). In dogs, *P. gingivalis* contributes to the chronic inflammatory response observed in periodontal disease, making it a critical target for therapeutic interventions like SRP (13-15). the persistence of *P. gingivalis* post-SRP may lead to recurrent infections and continued tissue destruction if not properly managed. Given its importance in the pathogenesis of periodontal disease, *P. gingivalis* serves as a focal point in evaluating the efficacy of SRP and other therapeutic strategies in maintaining oral health in dogs (13, 16).

Clinicians frequently suggest and carry out SRP treatment for pets; many pet owners view this procedure as sufficient and neglect subsequent follow-up care. Many studies highlight the importance of ongoing monitoring and adjunctive therapies to improve long-term results. However, such insights have not been extensively applied to veterinary dentistry, where monitoring bacterial load, assessing bacteremia, and investigating periodontal healing after SRP could provide valuable insights into animal health (1).

This study aims to address the gap in veterinary research, especially in Iran, by evaluating the effects of SRP on bacterial load and key periodontal health indicators in dogs. The study also emphasizes the importance of post-treatment monitoring to ensure sustained improvements and prevent the recurrence of periodontal disease. Investigating changes in bacterial load and periodontal health over time sets the foundation for future research on the

long-term management of periodontal disease in pets while encouraging the exploration of adjunctive treatments to enhance SRP outcomes.

2. Material and Methods

2.1. Animals

Ten adult dogs diagnosed with periodontal disease were selected from among the clients of the Small Animal Hospital, Faculty of Veterinary Medicine, University of Tehran, Iran. The dogs were between 2 and 6 years of age, with no breed or sex limitations. All dogs exhibited periodontal pockets with depths greater than or equal to 3 mm, which exceeds the normal gingival sulcus depth in healthy dogs. The inclusion criteria required that the dogs had no concurrent oral diseases, no history of antibiotic or other medication use in the past three months, and no systemic conditions such as diabetes or immune-related disorders that could affect periodontal health or healing. Additionally, female dogs included in the study were neither pregnant nor lactating at the time of diagnosis.

2.2. *Study Design*

This study employed a split-mouth design to assess the effectiveness of treatment in dogs diagnosed with periodontal disease. Four teeth were selected from each dog, with one tooth chosen from each quadrant of the mouth: the left maxilla, right maxilla, left mandible, and right mandible. These teeth were then subjected to scaling and root planing (SRP) procedures (**Figure 1**–(**c**)). SRP was performed following established standard procedures (15). Following the interventions, no additional treatments were performed, and the animals were observed at designated time intervals to evaluate the outcomes. Subgingival plaque samples were collected from the deepest periodontal pockets of each selected tooth both before and after the therapeutic intervention, utilizing Roeko Sterile Paper Points (No. 35) (**Figure 1**–(**b**)).

Sample collection was conducted at baseline (day 0) before and after scaling, as well as on days 10 and 30 post-intervention. The paper points were then transferred to tubes containing RTF medium and stored at -20°C until further analysis. The population of *Porphyromonas gingivalis* was quantified using Real-Time PCR.

Periodontal health assessments of the selected teeth were carried out at baseline (day 0) and at follow-up intervals on days 10 and 90. Clinical indicators, including PPD, GI, PI, bleeding on probing, and CAL, were evaluated to monitor the effects of intervention on periodontal tissue healing and disease progression over time. In order to assess each clinical index for each dog, the condition of four selected teeth was averaged and reported. This approach provided a comprehensive evaluation of the intervention's impact on periodontal outcomes.

2.3. Diagnosis Criteria

The criteria used to diagnose periodontal disease in this study were based on book chapters (1, 3, 6), scientific articles (1), and online veterinary education materials (WikiVet, https://en.wikivet.net/Small_Animal_Dentistry). Since the focus of this study is not on the diagnostic process, a brief overview of the methods employed is provided here.

Periodontal disease was diagnosed using a multi-step process. Initial visual inspection in conscious dogs assessed gingival inflammation, plaque accumulation, tooth mobility, and

calculus presence. Periodontal probing was performed under anesthesia to evaluate PPD, bleeding, gingival recession, and CAL (6, 14, 17).

On the baseline (day 0) of the study, several clinical indicators were assessed and recorded to evaluate the periodontal health of the dogs. PPD was measured using a graduated periodontal probe inserted into the gingival sulcus (**Figure 1**–(**a**)). The depth was recorded as the distance between the gingival margin and the bottom of the periodontal pocket, which served as a key measure for assessing the severity of periodontal disease and the extent of attachment loss (3).

Sulcus bleeding during probing was also evaluated by stimulating the gingiva around each tooth with a periodontal probe. The presence or absence of bleeding was recorded, as bleeding indicated active inflammation and gingival irritation, which are critical markers for identifying gingivitis and assessing its severity. The clinical findings were scored based on the amount and location of bleeding. A score of 0 indicated no bleeding, while a score of 1 reflected bleeding in only one spot. A score of 2 indicated several separate bleeding points or a small area of bleeding, and a score of 3 was assigned if the interdental triangle filled with blood after probing. Finally, a score of 4 was given for heavy bleeding during probing, with blood spreading to the gum line (14).

The GI was used to determine the degree of gum inflammation. Scores were assigned based on the visual presence of redness, swelling, and bleeding during probing. A score of 0 indicated natural gums with no signs of inflammation, characterized by a natural color. A score of 1 reflected mild inflammation, with slight changes in color and edema but no bleeding during probing. Moderate inflammation, with redness, hyperemia, swelling, and glossiness, along with bleeding during probing, was given a score of 2. A score of 3 indicated severe inflammation, characterized by clear hyperemia, edema, and the presence of wounds, with the possibility of spontaneous bleeding (14).

In addition, the PI was employed to measure dental plaque accumulation on the tooth surfaces. Each tooth was examined, and a score was assigned based on the extent of plaque coverage. A score of 0 indicated the absence of plaque. A score of 1 was assigned when a thin layer of plaque adhered to the free surface of the gum and the adjacent area of the tooth, which was not visible to the naked eye and could only be detected using a probe or a detector solution. A score of 2 indicated an average accumulation of material in the tooth pocket or along the gingival margin, with or without visible plaque on the tooth surface. A score of 3 reflected significant adhesion of plaque in the soft tissues surrounding the tooth, gums, gum edge, and on the tooth itself. This index is a crucial metric for monitoring the progression of periodontal disease, as plaque buildup is a leading cause of the condition (3).

Lastly, CAL was measured to quantify the total loss of periodontal support around each tooth. This clinical index was calculated by summing the PPD and the extent of gingival recession, offering a quantitative assessment of the degree of attachment loss due to periodontal disease (18).

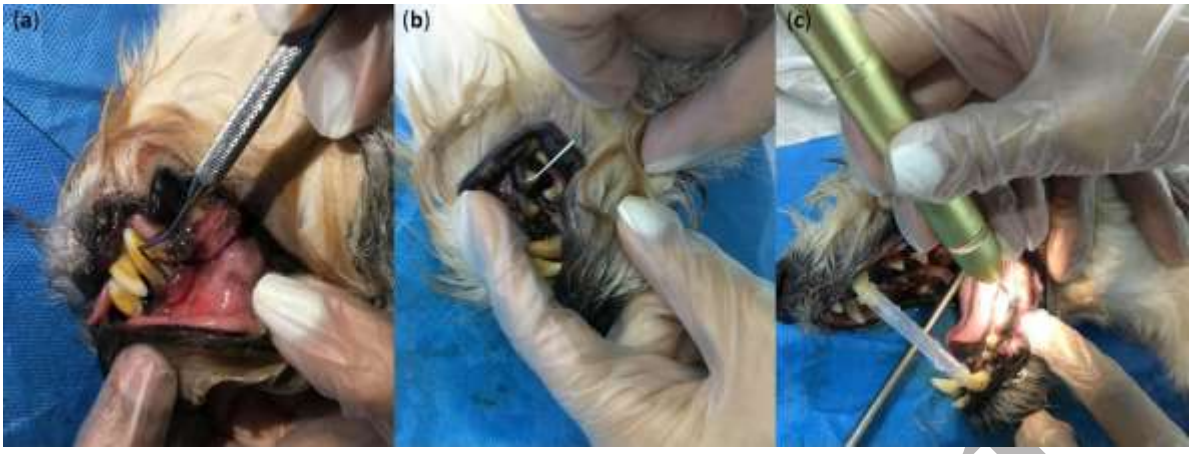

Figure 1. *Clinical procedures used during the study. (**a**) Measurement of PPD using a periodontal probe. (**b**) Subgingival plaque collection with sterile paper points from the deepest periodontal pocket. (**c**) SRP procedure with ultrasonic dental instruments.*

2.4. Quantitative PCR assay

To evaluate the effectiveness of therapeutic intervention on bacterial load, real-time PCR was employed to quantify bacterial DNA before and after intervention. For each sample, bacterial DNA was extracted and purified using the SinaPure DNA extraction kit manufactured by Sinacloon, Iran. Real-time PCR was performed to amplify *P. gingivalis*–specific 16S rRNA using the forward primer (ACCCTTTAAACCCAATAAATC), the reverse primer (ACGAGTATTGCATTGAATG), and a fluorescent-labeled probe (CGCTCGCATCCTCCGTATTAC) (19). The qPCR reaction mixture consisted of 100 ng of extracted DNA per sample, 0.5 µM primers, 0.15 µM probe, and KAPA SYBR® FAST qPCR Kit Master Mix (2X) (Kapa Biosystems). The PCR amplification was carried out using the following cycling conditions: 95°C for 3 minutes, followed by 50 cycles of 95°C for 3 seconds, and 60°C for 30 seconds. Cycle threshold (Ct) values, representing the number of cycles required for the fluorescent signal to surpass the threshold, were recorded as an indicator of bacterial load. Lower Ct values correspond to higher amounts of bacterial DNA.

The bacterial load measured from the pre-intervention sample served as the reference for calculating fold changes. This pre-intervention sample provided the baseline bacterial load before any intervention was applied. The ΔCt value for each post-intervention sample was determined by subtracting the pre-intervention Ct value from the post-intervention Ct value, using the given formula [$\Delta Ct = Ct_{post-treatment} - Ct_{pre-treatment}$]. The relative fold change in bacterial load was calculated according to the formula [Fold Change $= 2^{-\Delta Ct}$]. A fold change greater than 1 indicated an increase in bacterial load, whereas a fold change less than 1 reflected a reduction in bacterial load following intervention. This method facilitated a comprehensive evaluation of the short-term and long-term effects of the intervention on bacterial load.

3. Results

3.1. Clinical Parameters changes

The data suggests a significant reduction in PI by day 10 following the intervention, with most dogs showing complete or near-complete removal of plaque. However, by day 90, some plaque reaccumulation occurred in several dogs, though the levels remained lower than baseline for the majority. This trend indicates that while the treatment was effective in the short term, regular maintenance may be necessary to prevent long-term plaque buildup. The results of the Friedman test, which was used to analyze the differences in PI across the three-time points (day 0, day 10, and day 90), indicate a significant difference between the time intervals. The test yielded a chi-square value of 18.67 with a p-value of 0.000088, which is highly significant ($p < 0.05$). This result confirms that the changes in PI over time are statistically significant, demonstrating that the intervention had a meaningful impact on reducing plaque between the time points, particularly from baseline to day 10, and that there was some reaccumulation by day 90.

While some dogs showed improvement in bleeding on probing scores by day 90 (2 dogs), others either remained at higher bleeding levels or showed signs of worsened conditions (2 dogs). The mixed results suggest that while the intervention may have had positive effects in some dogs, others either did not respond to the treatment or experienced a relapse in bleeding severity. The results suggest that while the intervention had a varying impact on reducing bleeding on probing, long-term improvements may require more consistent or additional interventions for certain cases. The results of the Friedman test for bleeding on probing across the three-time points show a chi-square value of 2.00 with a p-value of 0.3679. Since the p-value is greater than 0.05, this indicates that the changes in bleeding on probing over the time intervals are not statistically significant. In summary, while there were observable variations in bleeding on probing scores among the dogs, these changes were not statistically significant, suggesting that the intervention did not lead to a consistent or meaningful reduction in bleeding on probing across the group.

The analysis shows that the intervention led to an initial improvement in gingival inflammation, with most dogs displaying reduced GI scores by day 10. However, by day 90, many of these improvements were not sustained, and several dogs reverted to their baseline levels of gingival inflammation. These observations suggest that while the intervention had short-term benefits, additional treatments may be required to preserve gingival health. The results of the Friedman test for GI across the three-time points show a chi-square value of 4.57 with a p-value of 0.1017. Since the p-value is greater than 0.05, this indicates that the changes in GI over the time intervals are not statistically significant. In summary, while there were observable changes in GI scores among the dogs, these changes were not statistically significant, suggesting that the intervention did not lead to a consistent or meaningful reduction in gingival inflammation across the group.

The analysis shows that while some dogs experienced short-term improvements in PPD by day 10, most of these improvements were not sustained by day 90. In fact, several dogs showed a worsening in PPD over time (3 dogs). This finding suggests that without intervention, periodontal disease tends to progress, as indicated by the increases in PPD over time. The results of the Friedman test for PPD across the three-time points show a chi-square value of

3.31 with a p-value of 0.1911. Since the p-value is greater than 0.05, this indicates that the changes in PPD over the time intervals are not statistically significant.

The data suggests that while some dogs initially showed slight improvement by day 10, most experienced either no change or further CAL by day 90. The consistent increase in CAL scores from day 10 to day 90, particularly in dogs such as 3 dogs, suggests that CLA is progressive over time without therapeutic intervention. The stability observed in two dogs on day 90 indicates that the rate of progression may vary between individuals, with some dogs stabilizing temporarily. However, overall, the trend points to a gradual deterioration in clinical attachment over time. Changes in CAL over the three-time points were not statistically significant ($p > 0.05$). While there were observable changes in the CAL scores for several dogs, these variations did not demonstrate a statistically significant trend. This matter suggests that the CLA, although progressive in some cases, was not consistent across the group.

3.2. Real-time PCR Results

The results of the fold change analysis using Real-Time PCR provide valuable insights into the bacterial load dynamics in dogs with periodontal disease before and after the intervention of SRP. Overall, the study reveals that after the initial intervention, the bacterial load increases sharply, peaking at around day 10, but begins to decrease slightly by day 30 (**Figure 2**). There was no significant variation in the bacterial load changes among the four selected teeth in each animal. The Ct values, which represent the average cycle threshold of bacterial DNA amplification across the selected teeth, were used to calculate the ΔCt and fold changes at different time points immediately after the intervention, on day 10 and day 30.

Initially, the average pre-intervention Ct value was 28.96, serving as the baseline for the study. After SRP, the average Ct value decreased to 26.43, which corresponds to a ΔCt of -2.53. This negative ΔCt indicates that the bacterial load increased significantly after the intervention, with a fold change of 5.78 (477.57%). This fivefold increase in bacterial load immediately after treatment is expected, as SRP disturbs the bacterial biofilm, causing bacteria to be released into the oral environment. By **day 10**, the average Ct value dropped further to 25.72, resulting in a ΔCt of -3.24 and a fold change of 9.45. This sharp rise in bacterial load suggests that bacterial regrowth or colonization accelerated during the post-intervention recovery phase. Despite the initial increase in bacterial presence due to mechanical intervention, the oral environment might have become conducive to bacterial proliferation during this period. By day 30, the average Ct value slightly increased to 26.13, which gave a ΔCt of -2.83 and a fold change of 7.11. Between day 10 and day 30, the bacterial load decreased by 24.74%.

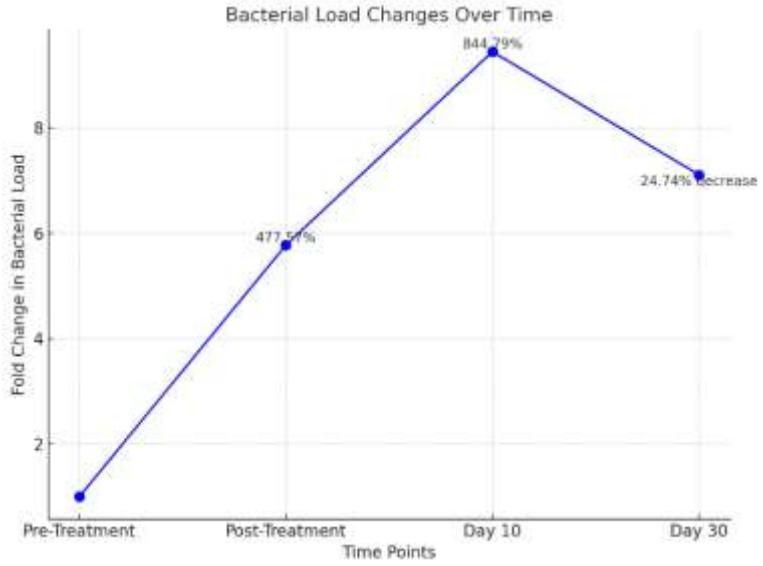

Figure 2. *Bacterial Load Changes Over Time. The graph illustrates the fold change in bacterial load at different time points: pre-treatment (baseline), post-treatment, day 10, and day 30. A significant increase in bacterial load is observed after treatment, peaking on day 10 with an 844.79% rise, followed by a 24.74% decrease by day 30. Despite this reduction, the bacterial load remains higher than the baseline.*

4. Discussion

The present interventional study offers valuable insights into the treatment of periodontal disease and the effects of SRP in managing this condition. The findings demonstrate that while certain aspects of periodontal health, such as PI, responded positively to the treatment, the maintenance of these improvements over time—particularly in more severe clinical conditions like PPD and CAL—remains a challenge. The initial reduction in plaque following the intervention highlights the short-term effectiveness of SRP in disrupting biofilm and reducing bacterial load. However, the rapid bacterial recolonization, as evidenced by the peak in bacterial load by day 10, suggests that the intervention alone may not be sufficient to provide long-term control over bacterial regrowth. By day 30, despite some reduction in bacterial load, it remained substantially higher than baseline, which likely contributed to the limited improvement or worsening of PPD and CAL in several cases.

The present study findings suggest that while SRP is effective in managing plaque and reducing initial bacterial load, maintaining improvements in deeper periodontal tissues, particularly PPD and CAL, may require more aggressive or sustained interventions. For example, repeated scaling sessions, adjunctive therapies such as antimicrobials, or more frequent maintenance visits may be necessary to control bacterial recolonization and prevent long-term deterioration of CAL and PPD. Some studies emphasize that without regular follow-up treatments, the benefits of SRP may diminish, highlighting the importance of a comprehensive periodontal care plan to prevent plaque reaccumulation (20).

In the present investigation, it was observed that bacterial load surged by 477.57% immediately after SRP, which reflects the disturbance of the biofilm and the release of subgingival bacteria. This rise in bacterial load following the procedure is a well-established occurrence (16). Although the initial increase may appear counterintuitive, it is a natural outcome of biofilm disruption caused by the treatment, which ultimately promotes improved periodontal health in the long term (21).

The findings of the present study are in line with those of Maruyama et al., who also demonstrated an increase in bacterial load following SRP. Maruyama et al. utilized quantitative real-time PCR to quantify the bacterial counts of *Porphyromonas gulae*, *Tannerella forsythia*, and *Campylobacter rectus* in dogs. They observed a significant decrease in bacterial numbers immediately after periodontal scaling. However, within 24 weeks, bacterial counts for all three pathogens increased significantly, similar to the bacterial regrowth observed in the current study within the first 30 days. The current study, which examined the *P. gingivalis* load, revealed a significant rise in bacterial load after treatment, reaching its highest point by day 10, followed by a slight decrease by day 30. Both studies highlight the transient effects of SRP, underscoring the need for long-term management to control bacterial recolonization. Maruyama et al.'s use of qRT-PCR further emphasizes its utility in accurately quantifying bacterial load and monitoring periodontal health over time, which complements the findings in the present study (22).

Maruyama et al. did not recognize *P. gingivalis* as a significant bacterial agent in the formation of dental plaque and periodontal disease in dogs. However, studies have indicated that *P. gingivalis* is frequently found in high concentrations within the gum pockets of dogs with periodontitis, with one study identifying it as the most prevalent pathogen at 61%. The presence of *P. gingivalis* in dental plaque is linked to an imbalance in the oral microbiome, triggering inflammatory responses and the destruction of periodontal tissue (23). Its virulence factors, including fimbriae and lipopolysaccharides, make it a key contributor to the onset and progression of periodontal disease (24).

In the study conducted by Polkowska et al., *P. gingivalis* was identified as a key microorganism associated with canine periodontitis, making up the highest percentage of pathogens (61%) among the sampled dogs with periodontal disease. The study involved microbiological analysis of gingival pockets in 36 dogs, where swabs were taken from pockets deeper than 5 mm. Alongside *P. gingivalis*, other significant bacteria, including *Treponema denticola* and *Prevotella intermedia*, were also identified, with the red complex of bacteria being the most prevalent, accounting for 84.26% of the identified microorganisms. The study emphasized the role of *P. gingivalis* as a major contributor to periodontal disease in dogs, potentially acquired through cross-species transmission. The authors also noted that the variability in bacterial profiles across studies could be influenced by factors such as the method of detection, environmental conditions, the host's immune response, and genetic background. This study underscores the importance of *P. gingivalis* in disrupting the oral microbiome and contributing to the progression of periodontal disease in dogs (23).

The findings of the present study can be compared with those of Assaf et al., who evaluated the effects of diode lasers (DLs) combined with ultrasonic scaling on bacteremia and clinical parameters such as PPD, GI, PI, bleeding on probing, and CAL. Both studies assessed similar clinical parameters and demonstrated improvements following treatment, confirming the validity of the methodology used in both studies. In Assaf et al.'s study, while DLs significantly reduced bacteremia associated with ultrasonic scaling, no significant differences were observed in the clinical outcomes between the scaling-only and DL + scaling groups. Despite the different treatment modalities, both studies highlight the short-term clinical improvements in PPD, GI, PI, and CAL after treatment but emphasize the importance of monitoring bacterial dynamics to ensure long-term periodontal stability. The use of diode lasers, while effective at reducing bacteremia, did not provide additional clinical benefits

compared to SRP alone in Assaf et al.'s study, suggesting that the method of SRP remains highly effective in managing gingival health (13).

The current study findings can be compared with the findings of Oteo et al., who evaluated SRP in conjunction with systemic azithromycin in treating *P. gingivalis*-associated chronic periodontitis. While both studies observed reductions in bacterial load and improvements in periodontal health following SRP, Oteo et al.'s study demonstrated significantly greater clinical and microbiological improvements when azithromycin was used as an adjunct. Specifically, Oteo et al. reported a larger reduction in PPD and greater CAL gain in the test group treated with SRP plus azithromycin compared to the placebo group. Additionally, the frequency of *P. gingivalis* detection decreased more substantially in the azithromycin group over time. In contrast, the current study observed a temporary increase in bacterial load following SRP, followed by a gradual reduction, but without the adjunctive use of antibiotics, the reduction in bacterial load and clinical improvements may be less pronounced over time. This comparison underscores the potential benefit of adjunctive antimicrobial therapies, such as azithromycin, to enhance the clinical outcomes of SRP, particularly in cases involving aggressive or chronic periodontitis (25).

Similar to the present study, the work by Shirmohammadi et al. highlights the significant role of *P. gingivalis* in periodontal disease and the potential of novel therapeutic approaches to combat this persistent pathogen. While our study focuses on the impact of SRP on *P. gingivalis* bacterial load and periodontal health in dogs, Shirmohammadi et al. investigated the antimicrobial effects of curcumin-loaded silica nanoparticles on *P. gingivalis* isolated from a human patient. Both studies emphasize the importance of localized therapeutic interventions in managing periodontal disease. Shirmohammadi et al. demonstrated the efficacy of a nanotechnology-based approach in humans, showing significant growth inhibition of *P. gingivalis* using curcumin-loaded nanoparticles, which aligns with our findings that adjunctive treatments, such as nanotechnology, may be crucial for long-term periodontal health. The present study complements this by providing insights into the bacterial dynamics post-SRP in pets, further underscoring that periodontal disease research in both human and veterinary contexts benefits from innovative, targeted therapies (26). Together, these findings suggest that approaches integrating nanotechnology can potentially improve periodontal health outcomes across species (11, 27).

The results of the current study align with some aspects of Eick et al.'s study, particularly in terms of the initial clinical improvements following SRP. Both studies observed significant reductions in clinical parameters such as PPD and CAL after SRP. However, Eick et al. introduced the adjunctive use of hyaluronan gels, which showed additional benefits in reducing PPD and limiting recolonization by certain periodontopathogens like Campylobacter rectus. In contrast, the current study, which did not include any adjunctive treatments, demonstrated a sharp increase in bacterial load post-SRP, peaking by day 10, followed by a modest reduction by day 30. Eick et al. also observed that the counts of *P. gingivalis* and *P. intermedia* increased in the control group (SRP only), similar to the current study's findings where bacterial load increased following SRP. This comparison suggests that while SRP alone can lead to short-term clinical improvements, the use of adjunctive therapies like hyaluronan gels may help further reduce bacterial recolonization and enhance long-term clinical outcomes, particularly in controlling specific pathogens (28).

There is a limited body of research on the changes in dental indicators, oral health parameters, and bacterial load following treatments like SRP in pets, particularly dogs. The present study aims to address this gap and emphasizes the importance of closely monitoring animals after interventions like SRP or similar procedures. Pet owners mustn't assume that treatment ends with the intervention itself; follow-up care and ongoing monitoring are essential for sustained oral health. Using antibiotics combined with nanomaterials as nanocarriers can improve the treatment of these infections. Further research in this field, particularly in relation to periodontal disease, is recommended (12, 27). Furthermore, future studies should focus on a broader range of clinical and microbial indicators, including the incidence of bacteremia in the blood of small animals, which has been underexplored in veterinary medicine (1). The current study lays the foundation for further investigations into periodontal health in small animals and opens the door for similar research in other domestic pets. Expanding research in this area could significantly improve our understanding of how dental treatments affect both short- and long-term health outcomes in these populations.

In particular, studies focusing on the detection and prevention of bacteremia following dental procedures in pets could offer valuable insights, as systemic bacterial infections can have broader health implications beyond oral health. Research like that of Assaf et al. on bacteremia and the use of diode lasers in human dentistry could serve as a valuable reference for future studies focusing on pets (1, 13).

In conclusion, the present study highlights the importance of ongoing periodontal care to sustain improvements in both superficial and deeper periodontal structures after initial interventions such as SRP. The findings suggest that while SRP can effectively manage plaque and reduce initial bacterial load, these improvements are often temporary. To prevent bacterial recolonization and ensure long-term periodontal health, antibacterial treatments should be incorporated following initial interventions to reduce bacterial load further. In this context, the use of effective strategies and newer treatments becomes crucial, particularly in light of the growing challenge of antibiotic resistance. One promising approach is the application of nanotechnology within the field of nanobiotechnology. Nanotechnology-based treatments offer the potential for sustained antibacterial effects, allowing for prolonged control over bacterial populations even in the presence of antibiotic resistance. Such innovative strategies could significantly enhance the long-term success of periodontal treatments by maintaining the antibacterial effects for a longer duration, thereby preventing the progression of periodontal disease and ensuring lasting improvements in patient outcomes.

Declarations

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Ethics approval and consent to participate

This study was conducted in accordance with ethical standards and was approved by the Internal Ethics Committee of the Faculty of Veterinary Medicine, University of Tehran (Protocol number: IR.UT.VETMED.REC.1403.033). Prior to participation, written informed consent was obtained from the owners of the dogs involved in the study. The owners were fully informed about the study procedures, potential risks, and the expected outcomes of the research, ensuring their complete understanding and voluntary participation.

Author Contributions

Study concept and design: Sh.J. and B.N.F.

Acquisition of data: Y.R., A.M., and S.M.J.

Analysis and interpretation of data: S.M.J. and H.A.

Drafting of the manuscript: S.M.J. and Y.R.

Critical manuscript revision for important intellectual content: S.M.J. and B.N.F.

Administrative, technical, and material support: Sh.J. and B.N.F.

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Conflict of interest

The authors declare that they have no conflict of interest.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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