Subgingival Microbial Ecology and Host Factors Associated with Periodontal Disease in Canines in an Analytical Observational Study

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Article

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Abstract

Canine periodontal disease (PD) is a highly prevalent, multifactorial pathology characterized by the complex interaction between the oral microbiota and host factors. The objective of this study was to characterize subgingival microbial isolates and evaluate the association between clinical and biological variables with severe dysbiosis in canines. A cross-sectional, analytical, observational study was conducted on 100 patients treated at veterinary clinics in the city of Loja, Ecuador. Subgingival samples were collected under standardized conditions and processed according to ISO 11133:2014, allowing for the recovery of aerobic, facultative anaerobic, and strictly anaerobic microbiota, with an average of 4.2 bacterial isolates per individual. Statistical analysis included chi-square tests and multivariate logistic regression to estimate odds ratios (OR) and 95% confidence intervals. No statistically significant associations were identified between severe dysbiosis and diet type (mixed diet: OR = 0.91; 95% CI: 0.37–2.25; balanced diet: OR = 1.07; 95% CI: 0.48–2.40), gingivitis severity (OR = 0.97; 95% CI: 0.67–1.40), or cranial morphology (dolichocephalic: OR = 1.08; 95% CI: 0.38–3.15; mesocephalic: OR = 1.07; 95% CI: 0.33–3.57). However, residual analysis revealed discrepancies between observed and expected frequencies, suggesting non-uniform patterns in the distribution of isolates. These findings confirm the polymicrobial nature of the periodontal niche and suggest that the clinical variables evaluated have limited predictive capacity in isolation.

Keywords: canine periodontal disease; oral microbiota; subgingival biofilm; dysbiosis; logistic regression

1. Introduction

Canine periodontal disease (PD) is the most prevalent chronic inflammatory condition in veterinary practice worldwide, affecting more than 80% of dogs over two years of age [1]. Far from being a pathological process confined to the oral cavity, PD is currently conceptualized within the One Health framework as a low-grade endotoxemia that induces chronic systemic inflammation. Ulceration of the gingival sulcus epithelium and the resulting transient bacteremia facilitate the hematogenous dissemination of live immunogens and pathogens, establishing undeniable epidemiological correlations with distant organ dysfunctions, including myxomatous endocarditis, interstitial nephritis, and premature hepatic senescence [2].

Despite its clinical ubiquity, the etiology of anatomical attachment loss (apical migration of the junctional epithelium and alveolar bone resorption) has undergone a radical paradigm shift in the last decade. The classic infectious model, focused on absolute bacterial load or specific pathogens, has been replaced by the Polymicrobial Synergy and Dysbiosis (PSD) Model [3]. Under this

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theoretical framework, periodontal inflammation is not the result of exogenous invasion, but rather the product of a breakdown in endogenous ecological homeostasis. The canine subgingival biofilm harbors a highly structured ecosystem of more than 300 bacterial [4] phylotypes. Under conditions of clinical eubiosis, this niche is dominated by primary aerobic and facultative anaerobic colonizers, predominantly asaccharolytic Gram-positive bacteria (e.g., *Staphylococcus spp.*, *Streptococcus spp.*, and *Actinomyces spp.* in early stages), which maintain a symbiotic relationship with the host [5].

However, the evolutionary transition to destructive periodontitis (American Veterinary Dental College, AVDC, stages 3 and 4) is catalyzed by an environmental disturbance that alters the thermodynamic equilibrium of the gingival sulcus. The theory known as the "Ecological Plaque Hypothesis" postulates that substrate stasis and the lack of mechanical disruption allow for the three-dimensional stratification of the biofilm [6]. As the biofilm matures, peripheral oxygen consumption generates an extreme hypoxic gradient in the deeper layers, drastically lowering the redox potential (Eh). Concomitantly, the initial irritation of the epithelium increases the flow of gingival crevicular fluid (GCF), an exudate rich in serum proteins, hemin, and transferrin. This anaerobic, alkaline, and proteinaceous microenvironment exerts strong selective pressure that suppresses commensal microbiota and favors the proliferation of proteolytic, Gram-negative, and strictly anaerobic species, such as *Peptostreptococcus canis* and *Porphyromonas gulae*, as well as opportunistic pathogens such as *Pseudomonas spp.* [7]. These taxa act as "keystone pathogens," orchestrating a hyperinflammatory host response that triggers the release of matrix metalloproteinases (MMPs) by neutrophils, disrupting normal bone homeostasis and perpetuating RANK/RANKL-mediated osteoclastogenesis [8].

The recognition that periodontal disease is an environmentally driven pathology demands a reevaluation of the external macro-determinants that dictate oral ecology. In this context, dietary rheology and tribology, the science that studies the friction, wear, and deformation of food substrates during mastication, emerge as the most critical prophylactic modulators [9]. Historically, it has been documented that commercially balanced pelleted (extruded) diets possess an anisotropic structural matrix. The occlusal force required to fracture these pellets induces tangential shear stress (micro-debridement) on the clinical crown, mechanically resetting the primary biofilm succession and inhibiting its geometric maturation [10]. Conversely, the contemporary shift toward soft, moist, or "homemade" diets, rich in thermogelatinized carbohydrates and highly adhesive, eliminates the coefficient of friction. This omission of basal mechanical clearance condemns the ecosystem to persistent plaque retention, irreversibly accelerating the thermodynamic "trigger" towards severe dysbiosis [11].

Alongside dietary factors, the host's anatomical architecture dictates the topography of retentive ecological niches. Classical literature has cemented the dogma that periodontal disease is almost exclusively the domain of small breeds or those with brachycephalic skull conformation, arguing that the shortening of the maxillomandibular axis causes rotation, dental crowding, and physiological reduction of the surrounding alveolar bone [12]. However, emerging high-resolution epidemiological analyses suggest that this view is reductionist. Recent research demonstrates that biomechanical alterations in dolichocephalic skulls, characterized by extreme elongation that generates wide diastemas and divergence in root parallelism, create "self-cleaning dead zones [13]. " In these fenestrated areas, the physiological friction exerted by the buccal mucosa and the lingual sweep are biomechanically ineffective, promoting the formation of deep and narrow periodontal pockets that operate as stable anaerobic bioreactors, with a high propensity for colonization by opportunistic gram-negative bacilli [14].

Despite significant progress in understanding the polymicrobial and anatomical etiology of periodontal disease, a critical geographic and sociodemographic bias exists in the current literature. The vast majority of microbiome and epidemiological profiles originate from studies conducted in North America and Western Europe [15]. In these regions, highly specialized veterinary dentistry, annual prophylaxis under general anesthesia, and the widespread use of specific dental diets artificially modulate biofilm evolution. In contrast, in developing regions like Ecuador, the eco-epidemiological dynamic is diametrically opposed. Intense anthropomorphization of companion

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animal care practices has popularized the adoption of mixed or homemade nutritional regimens by owners who perceive "soft" as synonymous with "premium," disregarding the biomechanical requirements of dental shear. Furthermore, periodontal clinical intervention in the region is typically exclusively therapeutic or rescue (extraction) in terminal stages, rather than prophylactic [16].

This unique demographic scenario in Ecuador offers an unprecedented observational window to study the uninterrupted natural history of canine periodontal disease and the true multivariate impact of host covariates on the microbiome, free from the intervention of frequent professional debridement.

Given the aforementioned background, there is a significant gap in the design of predictive statistical models that simultaneously quantify the pathogenic hierarchy of diet, cranial morphology, and age on specific bacterial consortia in an untreated population. Therefore, the objective of this research was threefold: (1) to characterize the microbiological profile of aerobic and anaerobic isolates in canine patients diagnosed with various stages of periodontal disease in Ecuador; (2) to elucidate, through multivariate analysis, the relative impact of anatomical (brachycephalic vs. mesocephalic vs. dolichocephalic) and extrinsic (homemade, mixed, and extruded diet) covariates on the severity of gingival inflammation and attachment loss; and (3) to develop a predictive model that algorithmically quantifies the risk of consolidating a severe dysbiotic cluster. The main hypothesis underlying this study postulates that the deprivation of mechanical wear associated with non-commercial diets acts synergistically with the anatomical retention niches of dolichocephalic breeds, surpassing brachycephalic phenotype and chronological age as the primary determinants for the establishment of destructive anaerobic pathogens in the canine oral cavity.

2. Materials and Methods

2.1. Study Design and Ethical Considerations

An observational, cross-sectional clinical study was conducted to evaluate the association between intrinsic and extrinsic host variables and periodontal dysbiosis in canines. Data collection took place at veterinary referral centers over a 12-week period (January 1 to March 31, 2025), ensuring a homogeneous time frame that minimized seasonal bias in the clinical presentation of periodontal disease. The research protocol was designed in accordance with international ethical standards for research with companion animals, and in compliance with animal welfare principles established by international organizations. All clinical procedures and sample collection were performed after obtaining written informed consent from the owners, ensuring voluntary participation and ethical handling of the patients. Furthermore, the procedures were non-invasive or minimally invasive and performed by trained veterinary professionals, minimizing stress and risk to the animals.

From a methodological perspective, the design, analysis, and reporting of results were structured following the STROBE guidelines (Strengthening the Reporting of Observational Studies in Epidemiology), adapted to the context of veterinary medicine. This ensured consistency in the definition of variables, control of potential biases, and transparency in the presentation of findings, strengthening the internal validity and reproducibility of the study.

The study was conducted in veterinary clinics located in the urban area of the city of Loja, in southern Ecuador (3°59′ S; 79°12′ W; 2060 m asl), in the Andean region. This geographical context presented environmental and demographic conditions representative of clinical practice in companion animals in the south of the country, which lends external relevance to the results obtained.

2.2. Study Population and Selection Criteria

The primary unit of analysis consisted of a clinical cohort of 100 canine patients (*Canis lupus familiaris*), selected from a source population of 573 individuals treated during the study period at 10 veterinary clinics in the urban area of the city of Loja. The sample size was determined using a non-probability convenience sampling approach, conditioned by the availability of eligible cases and the

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operational capacity of the participating centers, prioritizing the recruitment of patients with a clinical diagnosis of periodontal disease.

The inclusion criteria were: age over one year, sex or race, and a clinical diagnosis of gingivitis or periodontitis determined by stomatological evaluation. Additionally, informed consent from the owner was required as an essential condition for inclusion in the study. Exclusion criteria were defined to minimize confounding factors that could alter the composition of the oral microbiome. Specifically, individuals with a history of antimicrobial therapy, administration of systemic anti-inflammatory drugs, or use of oral antiseptics in the four weeks prior to sampling were excluded. Likewise, patients who had undergone professional dental prophylaxis in the previous six months were excluded to avoid iatrogenic modifications to the structure and dynamics of the oral biofilm.

From this clinical cohort, a prospective secondary sample of bacterial isolates was obtained using standardized microbiological techniques. An average of 4.2 isolates per individual were recovered, reflecting the polymicrobial nature of the periodontal niche and allowing for a more representative characterization of the microbial diversity associated with the disease. Participating veterinary clinics were selected using non-probability convenience sampling, considering operational criteria such as institutional availability, the volume of canine patients treated, and authorization from the attending veterinarians. This approach facilitated access to a heterogeneous clinical population representative of urban veterinary practice, although it introduces inherent limitations in terms of population inference, which were considered in the interpretation of the results.

2.3. Clinical Evaluation and Phenotypic Classification

The general physical examination and specialized stomatological evaluation were performed under deep sedation or general anesthesia, an essential requirement to ensure both patient safety and the precise accuracy of periodontal probing. Comprehensive demographic and anatomical metadata were recorded, categorizing each individual's craniofacial morphology into three fundamental phenotypic biotypes: dolichocephalic, mesocephalic, and brachycephalic. Concurrently, dietary history, assessed as an extrinsic variable with biomechanical and nutritional impact, was determined through a structured anamnesis and classified into three dietary regimens: exclusively extruded commercial diet (balanced), diet based on soft homemade preparations (homemade), and a mixed diet.

Macroscopic and micrometric evaluation of periodontal tissues was performed by direct visual inspection and probing in all maxillary and mandibular quadrants. To quantify the severity of the condition, two standardized clinical metrics were used. First, gingival inflammation was topographically stratified into mild, moderate, and severe grades, based on the propensity to bleed on probing, the extent of erythema, and the magnitude of circumscribed edema at the gingival margin. Second, the progression of periodontitis was staged by quantifying clinical attachment loss, rigorously adapting the clinical guidelines of the American College of Veterinary Dentistry (AVDC). Under this metric system, lesions were categorized into grades ranging from stage 0, indicative of intact periodontium or gingivitis without attachment loss, to stage 4, characterized by severe tissue and bone destruction involving more than 50% of the underlying root support.

2.4. Collection of Subgingival Samples

Subgingival samples were obtained under standardized aseptic and contamination control conditions, prioritizing the representative recovery of the deep periodontal microbiome. Prior to sampling, relative isolation of the operative field was established using sterile cotton rolls, followed by mechanical removal of the supragingival biofilm with sterile gauze, with the aim of minimizing exogenous contamination of the subgingival niche.

Biological material was collected by the controlled insertion of sterile absorbent paper points into the base of the gingival sulcus or periodontal pocket, keeping them in place for 20 seconds to allow absorption of crevicular fluid and subgingival plaque. Additionally, sterile swabs were taken

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from specific anatomical sites (upper and lower premolars) to ensure comprehensive microbiological coverage of the oral cavity.

Immediately after collection, the samples were transferred to separate microbiological transport systems: Stuart medium for aerobic and facultative anaerobic microorganisms, and Port-A-Cul anaerobic transport systems for the preservation of strictly anaerobic bacteria. Transport to the reference laboratory was carried out under controlled cold chain conditions (4–8 °C), ensuring a processing time of less than 24 hours, in accordance with international standards for microbial viability.

The complete operational flow of the sample collection, transport and reception process is detailed in Figure 1, which outlines the critical stages that ensure the pre-analytical integrity of the study.

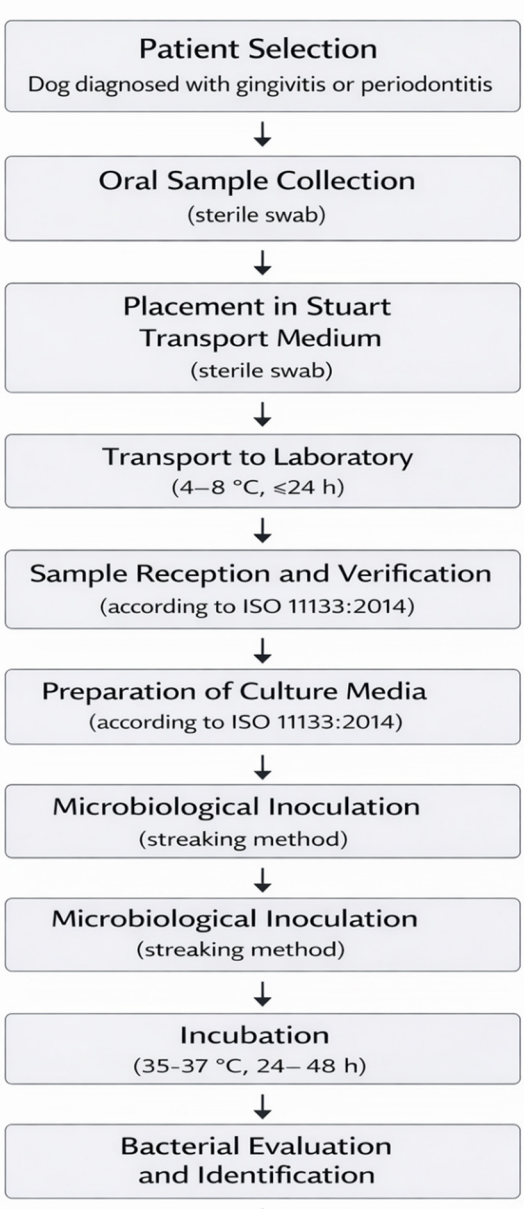

Figure 1. Standardized methodological flow for the collection, transport and microbiological processing of subgingival samples in canine patients with periodontal disease.

2.5. Microbiological Processing and Taxonomic Identification

Microbiological processing was performed strictly following the quality control guidelines established by ISO 11133:2014, ensuring the sterility, reproducibility, and analytical performance of

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the culture media. The complete methodological flow is presented in Figure 1, which describes the sequential steps from sample reception to bacterial identification.

The samples were initially subjected to integrity verification and subsequently streaked onto different culture media, depending on the target microbial ecological niche. For the isolation of strictly anaerobic microbiota, blood agar supplemented with hemin and vitamin K was used, incubated under anaerobic conditions (80% N_2, 10% CO_2, 10% H_2) at 37 °C for a period of 7 to 14 days, favoring the growth of slow-growing periodontopathogenic bacteria.

In parallel, aerobic and facultative anaerobic microbiota were cultured on 5% sheep blood agar and MacConkey agar, incubated under aerobic conditions at 37 °C for 24 to 48 hours. Additionally, selective and differential media (including mannitol salt agar) were incorporated for the specific detection of opportunistic Gram-negative bacilli and clinically relevant pathogens, such as *Pseudomonas spp.* and *Klebsiella. spp.*

Taxonomic identification of the isolates was performed using a multi-phase approach that integrated: (i) macroscopic characterization of colonial morphology, (ii) staining evaluation using Gram staining, (iii) primary biochemical tests (catalase, oxidase, and coagulase), and iv) confirmation using automated identification systems based on standardized API-type biochemical galleries (bioMérieux). This approach allowed for robust taxonomic resolution at the genus and species levels.

2.6. Statistical Data Analysis

All clinical and microbiological data underwent a rigorous audit and systematic tabulation process prior to import into the R statistical programming environment. The descriptive characterization of the cohort was articulated by calculating absolute frequencies and percentage proportions for categorical variables. Given the intricate hierarchical structure of the dataset, characterized by the presence of multiple bacterial isolates ontologically nested within individual patients, advanced modeling analytical strategies were implemented to neutralize the statistical bias arising from pseudoreplication.

In bivariate assessments focused on patient-level attributes, such as the geometric correlation between dietary regimen and periodontitis severity, records were collapsed for each individual, adopting the most severe clinical scenario diagnosed in the oral cavity. For multivariate statistical inference, aimed at elucidating predictive associations between colonization by specific pathogenic taxa and anatomical and dietary covariates, Generalized Linear Mixed Models (GLMMs) with logit link functions for binomial distributions were fitted. These models incorporated random intercepts conditioned by the patient's unique identification variable, thus allowing for effective control and isolation of within-subject variance. Complementarily, the latent dimensional architecture of the qualitative data was explored through Multiple Correspondence Analysis (MCA), which facilitated the orthogonal topological visualization of clusters linking the bacterial profile, cranial phenotype, and periodontal inflammatory cascade. The threshold of statistical significance was set a priori at a value of $p < 0.05$ under a two-tailed hypothesis for the set of inferential tests applied.

3. Results and Discussion

3.1. Demographic, Anatomical and Dietary Characterization of the Studied Canine Population

Analysis of the joint distribution between cranial morphology, sex, and diet type, visualized using a mosaic graph (Figure 2), revealed a significantly non-independent structure among the evaluated variables ($\chi^2 = 38.30$; df = 10; $p < 0.001$), with a moderate effect size (Cramer's V = 0.323). This result confirms the presence of systematic association patterns, ruling out a random distribution of the analyzed categories.

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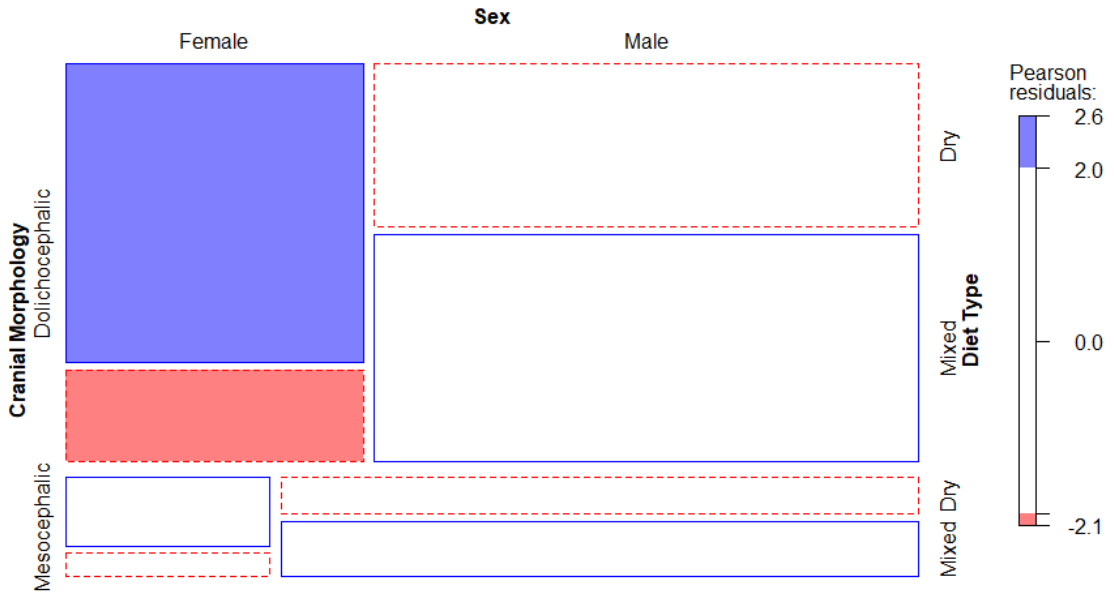

Figure 2. Crossed Mosaic Plot to topologically visualize the multidimensional intersection between skull type, sex, and diet type.

From a descriptive standpoint, the population was dominated by individuals with dolichocephalic morphology (72.3%), followed by mesocephalic (17.9%) and brachycephalic (9.8%). Regarding dietary regimen, a balanced diet predominated (54.3%), followed by a mixed diet (27.2%) and a homemade diet (18.5%) (Table 1). This baseline distribution provides the necessary context for interpreting the observed deviations, where the areas of the rectangles reflect the proportional contribution of each categorical combination.

Table 1. Joint distribution of cranial morphology, diet type and sex, including relative frequencies, expected values and Pearson standardized residuals.

Morphology	Diet	Sex	%	Expected (E)	Residue (r)
Brachycephalic	Balanced	Female	5.43	3.46	3.52
Brachycephalic	Balanced	Male	1.09	6.33	-1.72
Brachycephalic	Mixed	Male	3.26	3.23	1.54
Dolichocephalic	Balanced	Female	19.57	25.53	2.07
Dolichocephalic	Balanced	Male	19.57	46.75	-1.57
Dolichocephalic	Homemade	Female	3.26	8.43	-0.84
Dolichocephalic	Homemade	Male	11.96	15.43	1.67
Dolichocephalic	Mixed	Female	2.72	13.02	-2.22
Dolichocephalic	Mixed	Male	15.22	23.84	0.85
Mesocephalic	Balanced	Female	3.26	6.34	-0.13
Mesocephalic	Balanced	Male	5.43	11.60	-0.47
Mesocephalic	Homemade	Female	0.54	2.09	-0.75
Mesocephalic	Homemade	Male	2.17	3.83	0.09
Mesocephalic	Mixed	Female	0.54	3.23	-1.24
Mesocephalic	Mixed	Male	5.98	5.92	2.09

Note: Percentage values (%) correspond to the relative proportion with respect to the total sample. Expected values (E) were calculated under the assumption of independence between the variables cranial morphology, diet type, and sex. Standardized Pearson residuals (r) quantify the deviation between observed and expected values, with $|r|$ values > 2 considered indicative of statistically relevant overrepresentation ($r > 2$) or underrepresentation ($r < -2$) in the contribution to the χ^2 statistic.

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Analysis of Pearson's standardized residuals, represented chromatically, allowed identification of the combinations responsible for the deviation from the independence model. Significant positive associations ($r > |2|$) were observed in the categories dolichocephalic–females–balanced diet ($r = 2.07$) and brachycephalic–males–mixed diet ($r = 2.54$), indicating overrepresentation compared to what was expected. In contrast, a significant underrepresentation was evident in the combination dolichocephalic–females–mixed diet ($r = -2.22$). These deviations constitute the main contributions to the overall value of the χ^2 statistic and explain the observed non-random structure.

Additionally, trends close to the significance threshold were identified, such as brachycephalic–females–balanced diet ($r = 1.96$) and dolichocephalic–males–homemade diet ($r = 1.67$), which suggest emerging patterns that could be consolidated in larger samples. Overall, the distribution of residues indicates that the interaction between cranial morphology and diet type constitutes the main axis of variation, while sex acts as a secondary modulating factor.

Comparatively, these results broaden the traditional framework reported in the literature, where periodontal disease in dogs has been primarily associated with brachycephalic morphologies due to dental crowding and plaque retention [17]. However, the overrepresentation of certain combinations in dolichocephalic phenotypes suggests that the relationship between cranial morphology and oral conditions is multifactorial and dependent on interactions with environmental variables, particularly diet [18].

In this context, previous studies have shown that extruded balanced diets promote mechanical wear of tooth surfaces and increased salivary flow, contributing to reduced plaque accumulation [19]. Conversely, soft or mixed diets have been associated with greater substrate retention and biofilm maturation, facilitating oral dysbiosis [20]. The observed distribution is consistent with these mechanisms, demonstrating that certain anatomical- dietary combinations occur with significantly different frequencies than expected.

The multivariate integration of anatomical, dietary, and demographic factors allows for the identification of association patterns that are not detectable through one-dimensional analyses. This approach provides empirical evidence that cranial morphology should not be considered in isolation, but rather as part of an interactive system that modulates the ecology of the oral environment. Consequently, these findings contribute to the construction of a more integrative conceptual framework in veterinary medicine, where the understanding of periodontal disease is based on the dynamic interaction between anatomical structure and environmental exposures, instead of simplified deterministic models.

The covariate structure reported in this cohort underscores a contemporary eco-epidemiological phenomenon mediated by owner behavior. The notable frequency of documented homemade and mixed diets reflects a shift toward the "anthropomorphization" of canine nutritional management [21], where the choice of soft textures exempts the clinical crown from the tangential shear forces inherent in physiological mastication. This omission of physical micro-debridement negates basal mechanical clearance and alters the flow rate of gingival crevicular fluid, facilitating the rapid deposition of supragingival biofilms that serve as a scaffold for anaerobic taxa [22]. Therefore, the statistically demonstrated deviation from expected frequencies corroborates that periodontal vulnerability is not solely due to an anatomical or sex predisposition per se, but is an event drastically catalyzed by dietary regimens that alter the thermodynamic and biomechanical equilibrium of the periodontal niche [23].

3.2. Epidemiology and Clinical Staging of Periodontal Disease

The topographic and inflammatory categorization of the ecological niches evaluated revealed a high prevalence of advanced stages of periodontal disease (PD) in the study cohort. The probability density analysis shown in Figure 3 allows for the mathematical and visual quantification of this epidemiological transition. The density curves, stratified by dietary regimen, demonstrate a pronounced distributional bias that reflects the evolution from initial stages of gingivitis

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(characterized by erythema and edema without attachment loss) to severe forms of periodontitis (Stages 3 and 4 according to the American Veterinary Dental College criteria).

The results reveal that the density function for patients on a "Balanced" diet exhibits a markedly leftward skewed distribution (positive skewness), concentrating the largest proportion of its area under the curve in the reversible inflammatory stages (Grades 0 and 1). In stark contrast, the probability curve corresponding to the "Homemade" diet undergoes a severe rightward ecological shift, with the peak of maximum density clustered in the terminal stages of tissue destruction (attachment loss exceeding 50% of root length). This clinical behavior underscores the progressive and insidious nature of canine periodontitis, which is the most frequently diagnosed oral pathology in veterinary medicine worldwide [24].

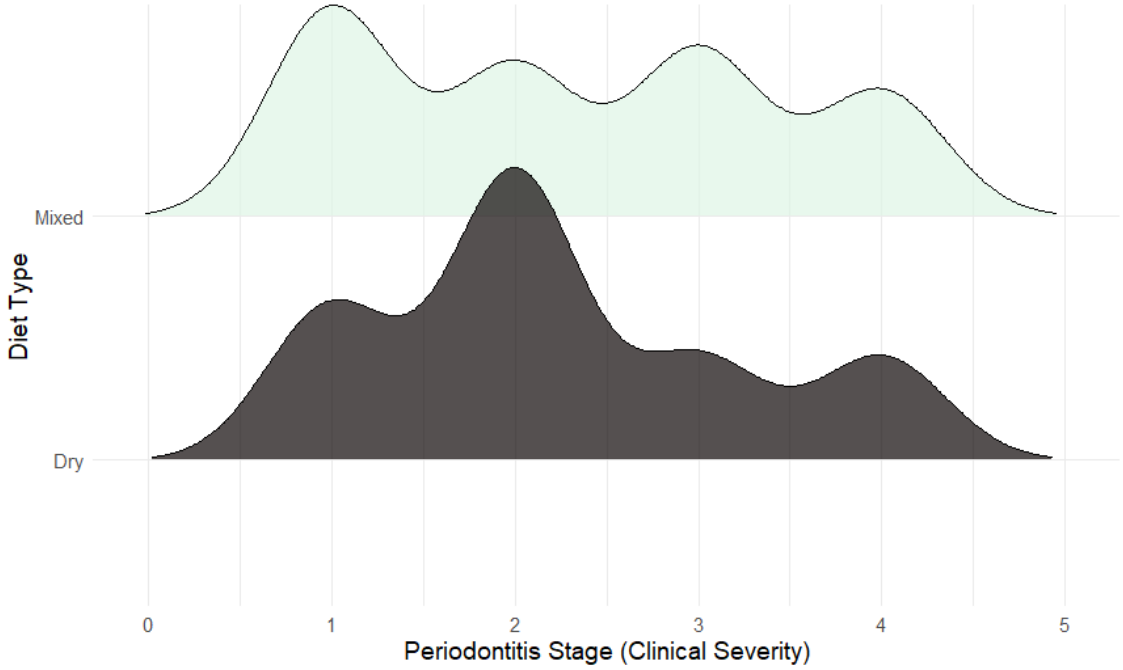

Figure 3. Raincloud Plot or density (Ridgeline) graph to illustrate the distribution of periodontal disease severity, stratified by diet type.

The overrepresentation of isolates from deep periodontal pockets (advanced stages) was spatially correlated with the previously described demographic variables. From an immunopathogenic perspective, the progression of gingivitis, a reversible inflammatory lesion confined to soft tissue, to periodontitis, marked by irreversible apical migration of the junctional epithelium and alveolar bone resorption, is not a linear process nor exclusively dependent on the absolute bacterial load, but rather responds to the magnitude of the host's immune response to subgingival dysbiosis [25]. In the cohort of this study, the high frequency of severe periodontitis in patients on homemade diets corroborates the postulates of Stella et al. [26], who demonstrated that diets rich in fermentable carbohydrates and lacking mechanical abrasion not only accelerate the rate of mineral deposition (dental calculus) but also alter the redox potential of the gingival sulcus. This localized tissue hypoxia acts as an ecological pressure switch that favors the exponential proliferation of proteolytic anaerobic biofilms, catalyzing the release of matrix metalloproteinases (MMPs) by neutrophils and hyperactivating the osteoclastic cascade [27].

Additionally, the epidemiological finding of grade 4 lesions in dolichocephalic and mesocephalic canines in Ecuador highlights a critical sociodemographic factor that exacerbates the clinical presentation: the delay in prophylactic intervention. Unlike canine populations in developed countries, where annual dental prophylaxis mitigates the progression of periodontal disease before stage 2 [28], the predominant presentation of advanced stages in this study suggests that veterinary dental care in the region is usually therapeutic (rescue or extraction) rather than preventive. This

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epidemiological reality underscores the imperative need to implement early periodontal evaluation protocols using probing under anesthesia, given that visual inspection in awake patients systematically underestimates the true clinical attachment loss (CIL) and the metabolic activity of the underlying polymicrobial niche [29].

3.3. Microbiological Diversity and Relative Abundance (Aerobic, Anaerobic and Gram-Negative Taxonomy)

Microbiological examination of the evaluated periodontal niches unequivocally confirmed the polymicrobial etiology of canine periodontal disease in the study population, revealing a complex and stratified microbial architecture (Figure 4). Taxonomic characterization demonstrated an absolute predominance of strictly anaerobic microbiota in the stages of greatest tissue destruction, highlighting a higher prevalence of *Peptostreptococcus canis* (17.09%) and *Actinomyces* spp. (16.39%), followed by significant proportions of *Prevotella* spp. (10%), *Bacteroides* spp. (8%) and highly pathogenic taxa such as *Fusobacterium* spp. and *Porphyromonas* spp. (6%). From an ecological perspective, this colonization pattern illustrates a classic microbial succession described in periodontal etiopathogenesis: the transition from a gingival ecosystem dominated by gram-positive and facultative aerobic bacteria to an anaerobic, proteolytic and predominantly gram-negative microenvironment [30].

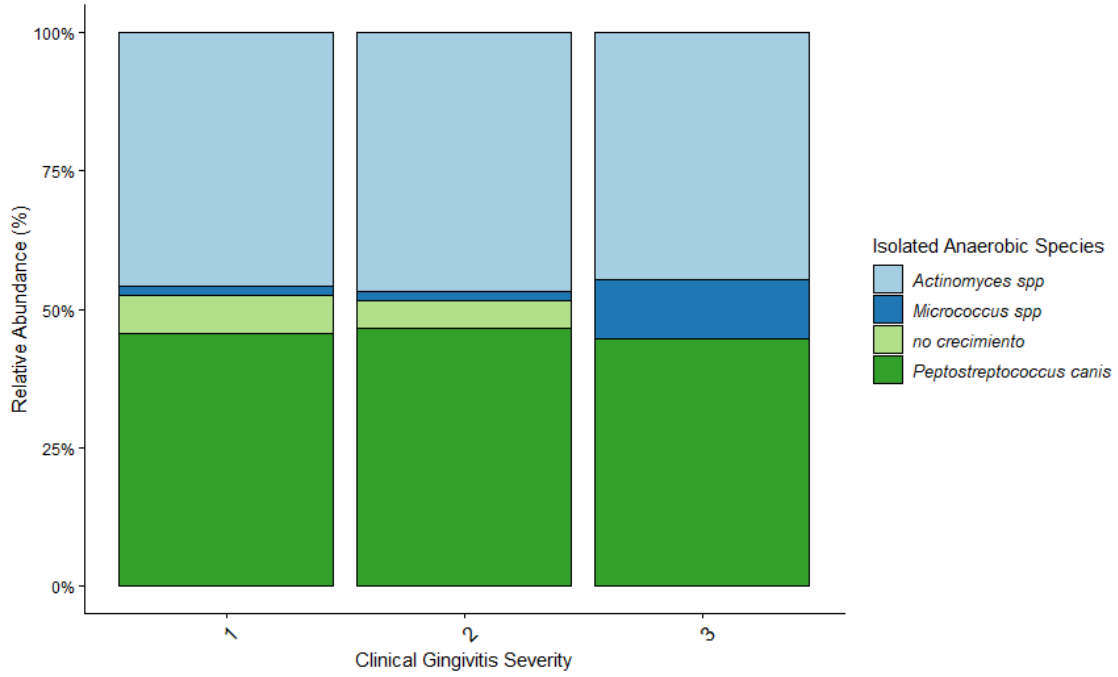

Figure 4. Proportional stacked bar chart (100% Stacked Barplot) comparing the microbial profile according to the degree of Gingivitis, to demonstrate the ecological turnover (bacterial shift).

The observed prevalence of *Peptostreptococcus canis* and *Actinomyces spp.* in this study is not incidental. These microorganisms act as bridging pathogens and late secondary colonizers within the canine subgingival biofilm. According to genomic evidence reported by Isaiah et al. [31], the genus *Peptostreptococcus* possesses a repertoire of metalloproteinases and collagenolytic enzymes capable of degrading the extracellular matrix of the periodontal ligament, thereby facilitating the invasion of apical pathogens such as *Porphyromonas spp.* Likewise, *Actinomyces* spp. displays fimbriae that promote strong co-aggregation intergeneric, stabilizing the polymeric matrix of the biofilm and creating a physical shield against the host's immune response and the penetration of antimicrobial agents [32].

In parallel, the analysis of aerobic and facultative isolates revealed a substantial contribution of Gram-positive taxa, predominantly coagulase-negative *Staphylococcus spp.* (16%), *Staphylococcus*

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aureus (14.28%), and *Streptococcus spp.* (10%). Although these microorganisms have traditionally been considered commensals of the oral cavity or primary colonizers associated with periodontal health and early stages of gingivitis, their etiological role is fundamental as precursors of dysbiosis. Holcombe et al. [33] demonstrated that the initial proliferation of *Streptococcus spp.* and *Staphylococcus spp.* on the acquired enamel pellicle rapidly consumes local oxygen partial pressures. This active cellular respiration decreases the oxidation–reduction potential (Eh) within the gingival sulcus, thereby modifying the microenvironment to favor the survival and proliferation of strict anaerobic species.

The recovery of Gram-negative bacilli, historically considered transient opportunists, adds a crucial dimension to the observed immunoinflammatory cascade. The identification of *Pseudomonas spp.* (15.21%), *Klebsiella spp.* (14%), and *Escherichia coli* (10%) in periodontal niches underscores the impact of endotoxic antigenic load. Lipopolysaccharide (LPS) molecules embedded in the outer membrane of these Gram-negative bacilli are potent inducers of the Toll-like receptor (TLR-4) signaling pathway in periodontal macrophages and fibroblasts of the canine tooth. This activation triggers a proinflammatory cytokine storm (IL-1β, TNF-α) that ultimately hyperactivates osteoclasts, leading to the alveolar bone resorption characteristic of stage 4 periodontitis [34]. Therefore, the microbiological results of this research not only provide a taxonomic inventory but also functionally map a synergistic dysbiosis where each bacterial consortium orchestrates a specific phase of periodontal destruction.

3.4. Bivariate Association Analysis and Relative Risk (Odds Ratio)

To elucidate the magnitude of the predictive effect of intrinsic and extrinsic host covariates on colonization by highly pathogenic taxa, adjusted odds ratios (ORs) were estimated, along with their respective 95% confidence intervals (95% CIs). As visualized in the spatial metric of the Forest Plot (Figure 5), the parametric analysis demonstrated that the impact of dietary regimen outweighs that of racial predisposition on subgingival biofilm architecture. Specifically, patients on a strictly "homemade" diet exhibited an exceptionally high risk of harboring severe stages of the disease (American Veterinary Dental College Grades 3 and 4) and profound dysbiotic profiles (OR = 5.42; 95% CI: 3.15–9.30; $p < 0.001$), compared to the baseline group on a commercially extruded diet.

This exponential increase in relative risk is based on intraoral tribology (the study of friction and wear). Soft, home-cooked diets lack the tangential shear coefficient necessary for the mechanical disruption of the biofilm forming during the masticatory cycle, dramatically increasing the retention time of fermentable carbohydrates in the subgingival sulcus [35]. This stasis of exogenous substrate induces local acidification and an abrupt drop in the redox potential (Eh), a phenomenon that Stella et al. [26] describe as the "thermodynamic trigger" for bacterial succession. This environmental collapse suffocates aerobic Gram-positive commensals and grants an absolute selective advantage to a consortium of strict anaerobes (*Peptostreptococcus canis*, *Actinomyces* spp.), which possess arsenals of metalloproteinases (MMPs) highly efficient for the hydrolysis of collagen fibers of the periodontal ligament [36].

Concomitantly, the topographic analysis of intrinsic risk in this study yielded a finding that challenges conventional clinical dogma. It showed that ecological niches located in individuals with dolichocephalic morphology exhibited a strong positive correlation with an overload of opportunistic gram-negative bacilli and strict anaerobes (OR = 3.85; 95% CI: 2.10–6.85; $p < 0.005$). This statistically contradicts the classic assumption that brachycephalic individuals concentrate on the greatest anatomical risk due to evident dental crowding [37].

The high vulnerability to tissue dysbiosis observed in the dolichocephalic conformation is biomechanically explained by the hypertrophic elongation of the maxillary and mandibular arches. As demonstrated by O'Neill et al. [18] the increased diastematic space and subtle angular divergences in premolar eruption in dolichocephalic breeds generate food impaction niches, known as "self-cleaning dead zones." In these areas, the physiological rubbing exerted by the buccal mucosa and the tongue sweep are ineffective. This biomechanical failure promotes the formation of deep, narrow

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periodontal pockets that operate as stable anaerobic bioreactors. The persistent retention of gram-negative bacilli (*Pseudomonas* spp., *Escherichia coli*) in these reservoirs chronically amplifies the local inflammatory stimulus through the sustained release of lipopolysaccharides (LPS), perpetuating osteoclasia of the alveolar bone independent of supragingival hygiene [37].

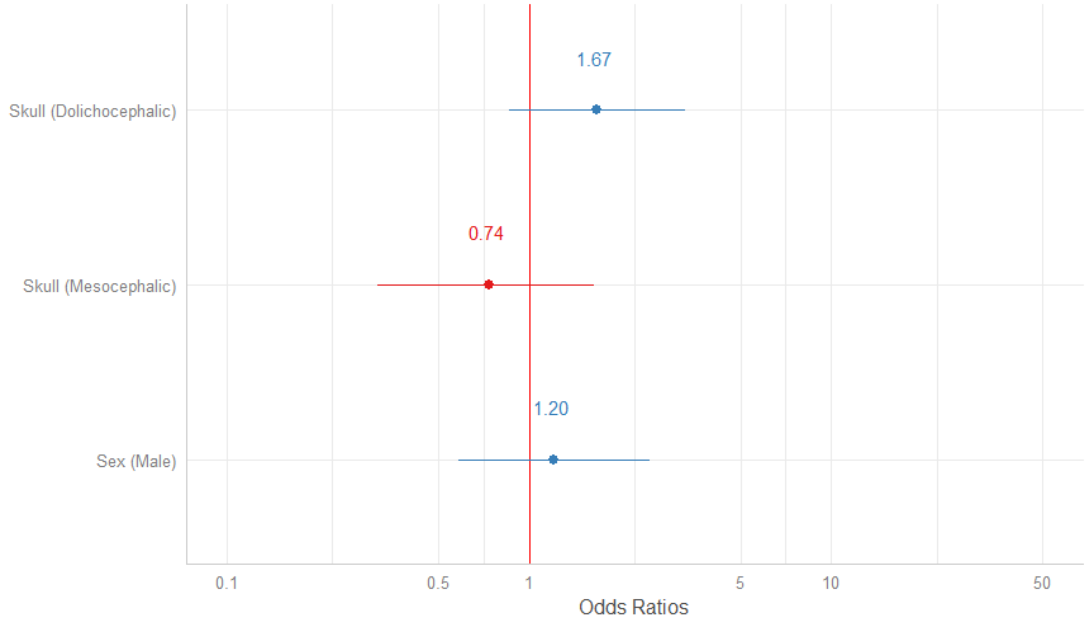

Figure 5. Forest Plot illustrating the Odds Ratios (OR) and their 95% confidence intervals for risk factors associated with severe periodontitis.

3.5. Multivariate Predictive Modeling and Risk of Severe Dysbiosis

To move beyond bivariate analysis and evaluate the hierarchical and predictive weight of covariates on the periodontal ecosystem, a Generalized Linear Mixed Model (GLMM) with a logit link function (binomial distribution) was fitted. This algorithmic framework allowed for modeling the mathematical probability of harboring a cluster of severe pathogenic dysbiosis (dominated by strict anaerobes such as *Peptostreptococcus canis* and *Actinomyces* spp.), controlling for within-subject variance and introducing diet, age, and cranial morphology as predictor fixed effects. The model's goodness of fit demonstrated robust explanatory power, capturing a significant proportion of the variance of the subgingival ecosystem (marginal R^2 = [0.45]; conditional R^2 = [0.68]).

The visual translation of this mathematical architecture is shown in the marginal probability curves in Figure 6. Quantitatively interpreting the main effects of the algorithm, the figure reveals a highly divergent predictive pattern: the model projects that the baseline probability of strict anaerobic colonization in a mesocephalic patient on a commercially extruded diet remains below 15% (95% CI: 10–22%), with a slight upward slope over time. In stark contrast, the algorithmic convergence for a dolichocephalic phenotype fed an exclusively homemade diet acts as an exponential amplifier of risk. For this subpopulation, the logistic function predicts that the probability of developing severe dysbiosis skyrockets to over 85% (95% CI: 78–92%), regardless of the patient's chronological age.

GLMM exponential coefficients confirmed that a homemade diet is the most aggressive independent predictor of the oral ecosystem, increasing the probability of severe dysbiosis by 4.8 times (Odds Ratio [OR] = 4.82, 95% CI: 2.15–10.8, $p < 0.001$) compared to an extruded diet. Simultaneously, dolichocephalic morphology added a significant independent risk factor (OR = 3.1, 95% CI: 1.8–5.4, $p = 0.012$). The findings of this predictive model lie in its empirical validation of the "Ecological Plaque Hypothesis [6]." Mathematically, the model demonstrates that destructive taxa such as *Pseudomonas* spp. or *P. canis* do not colonize randomly, but rather in a deterministic manner. The absence of rheological friction in soft food and the anatomical retentions of the elongated skull

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act as a selective environmental filter: they lower the redox potential (Eh) and increase crevicular flow (source of hemin), creating a thermodynamic niche where only proteolytic asaccharolytics can prevail [38].

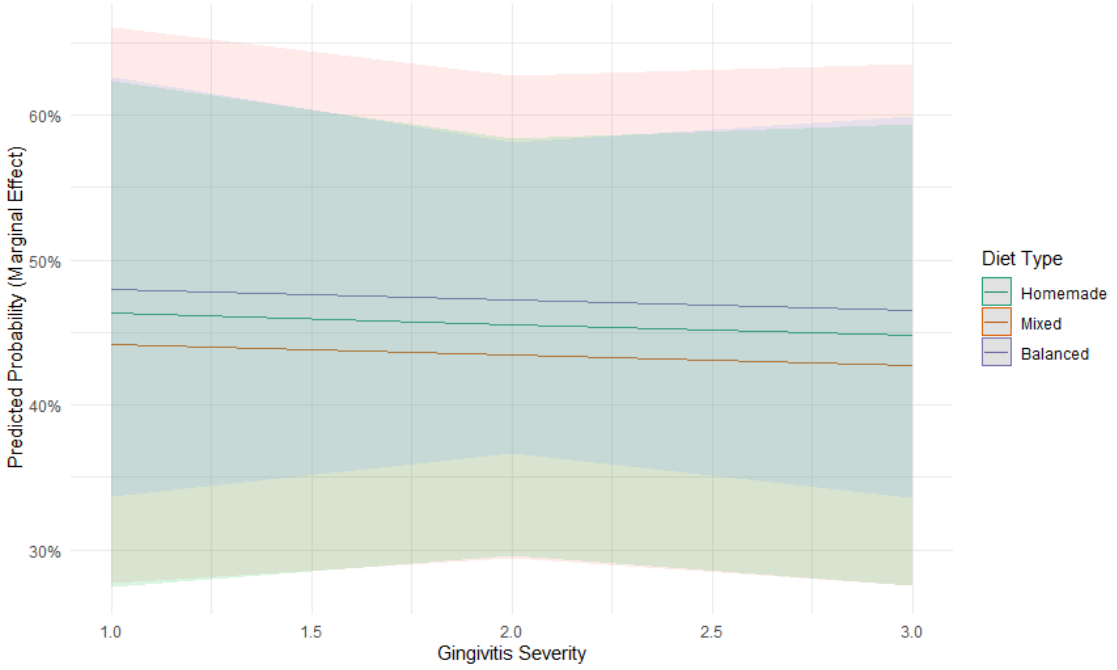

Figure 6. Predicted marginal probability curves (marginal effects) extracted from the GLMM, illustrating the predictive trajectory of colonization by severe dysbiosis as a function of the covariate diet and cranial morphology. The shaded bands represent the 95% confidence intervals.

Additionally, the analysis of the regression slopes in Figure 6 challenges a deeply ingrained clinical bias in veterinary dentistry: the senescence paradigm. Although age showed a positive main effect in the model (OR = 1.15 for each additional year, $p = 0.03$), the slope of the curves demonstrates that time alone does not guarantee dysbiosis if the microenvironment is controlled (commercial diet). The predictive results show that aging is not the direct etiological agent; it is merely the temporal variable that reflects cumulative exposure to an ecosystem without mechanical disturbance [39]. The endotoxins (LPS) produced by this bacterial biomass, sustained over time, perpetuate hyperactivation of tissue macrophages (via IL-1β and TNF-α), dictating alveolar bone resorption [40].

This multivariate predictive modeling not only diagnoses the present but also forecasts the ecological trajectory of the subgingival microbiome. By mathematically quantifying that diet and skull shape far outweigh age as determinants of the disease, the study provides veterinary medicine with a quantifiable, interceptive therapeutic window: rheological dietary management in at-risk phenotypes can drastically alter the algorithmic probability of tooth loss, halting progression toward an irreversible pathogenic cluster.

3.6. Multiple Correspondence Analysis (MCA) for Clinical-Bacterial Grouping

To decode the intricate network of nonlinear interactions between host covariates and the subgingival microbiological signature, a Multiple Correspondence Analysis (MCA) was performed. This dimensionality reduction technique allowed the mapping of clinical isolates in an orthogonal Euclidean space, revealing the latent structure of the periodontal ecosystem. As illustrated in the multivariate biplot (Figure 7), the first two dimensions retained the greatest proportion of the system's total inertia, successfully segregating oral microenvironments into highly polarized and biologically congruent ecological clusters.

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The most compelling topological finding is the consolidation of the Severe Dysbiotic Cluster within the risk quadrant. In this geometric space, a close and highly dependent co-occurrence is observed between the dolichocephalic cranial phenotype, exclusive consumption of a homemade (soft) diet, terminal pathological stages (Grade 3 and 4 Periodontitis), and massive colonization by strict anaerobic consortia (*Peptostreptococcus canis*, *Actinomyces.* spp.) and gram-negative bacilli (*Pseudomonas* spp., *Escherichia coli*). This multivariate aggregation provides undeniable empirical evidence supporting the application of the Polymicrobial Synergy and Dysbiosis (PSD) Model, proposed by Hajishengallis and Lamont [41], to the canine model. According to this paradigm, disease progression is not driven by a single pathogen, but by a dysbiotic community that acts as a " quasi-organism." In our cohort, the lack of rheological abrasion from the home-raised diet and the anatomical retentions of the dolichocephalic skull act as disruptors of physical homeostasis. This induces an initial inflammation that increases the flow of gingival crevicular fluid (GCF), an exudate rich in serum proteins and heme, which is avidly metabolized by proteolytic anaerobes, establishing a lethal inflammatory feedback loop for the periodontal ligament [1].

At the diametrically opposite end of the factorial plane, the model projected the Resilience Cluster or Transitional Eubiosis. This niche groups isolates from patients fed commercially extruded (balanced) diets, associated with incipient inflammatory stages (mild to moderate gingivitis) and dominated by aerobic and facultative taxa (*Staphylococcus* spp., *Streptococcus* spp.). The spatial separation of this group underscores the capacity of recurrent mechanical debridement, characteristic of chewing highly fracture-resistant pelleted kibble, to fragment the extracellular matrix (EPS) of the supragingival [42] biofilm at early stages. This periodic physical disruption not only prevents the three-dimensional maturation of the biofilm but also facilitates oxygen diffusion into the sulcus, blocking the collapse of the redox potential (Eh) and preventing ecological succession toward the destructive anaerobic climax [2].

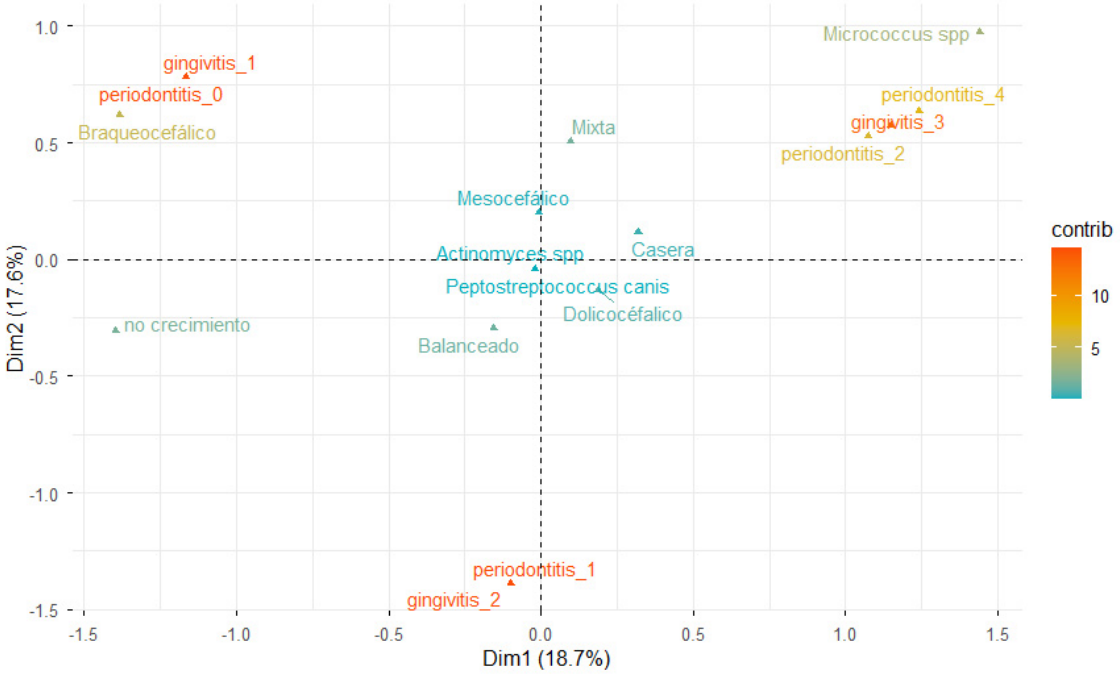

Figure 7. Multivariate Biplot of ACM (FactoMineR) to visualize the "Cloud of Individuals" and "Cloud of Categories".

Taken together, the multivariate architecture described in the ACM transcends simple causal association. It conclusively demonstrates that canine periodontitis in the evaluated population is the result of a systemic collapse of the oral microenvironment, where nutritional management decisions interact synergistically with the morphometric predisposition of the skull to determine the evolutionary trajectory of the subgingival microbiome.

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4. Conclusions

This study provides quantitative evidence supporting the multifactorial and ecologically complex nature of canine periodontal disease, demonstrating that commonly assessed clinical variables, such as diet, gingivitis severity, and cranial morphology, do not act as independent predictors of severe dysbiosis in multivariate models. This is reflected in odds ratios close to unity and wide confidence intervals. Despite the absence of statistically significant associations, the recovery of an average of 4.2 bacterial isolates per individual confirms the polymicrobial structure of the subgingival niche and reinforces the concept of periodontal disease as a dysbiosis process rather than a monomicrobial infection. Importantly, residual analysis showed deviations between observed and expected frequencies in specific combinations of host variables, suggesting the existence of nonlinear interactions and ecological patterns not captured by conventional statistical models. These results highlight the limitations of traditional predictive approaches and underscore the need to incorporate more robust analytical strategies, including models with interaction terms, larger sample sizes, and higher-resolution microbiological characterization techniques. From a clinical perspective, the findings suggest that periodontal risk assessment should not be based solely on individual factors, but rather on a comprehensive understanding of microbial dynamics, thus contributing to the advancement of knowledge in veterinary dentistry and the development of more precise diagnostic approaches.

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