Universidad Europea

GRADUATION PROJECT

Degree in Dentistry

Detection of periodontal pathogens in humans and their pets using qPCR: Exploring shared microbiomes and potential bacterial transmission.

Madrid, academic year 2025/2026

Identification number: 206

ABSTRACT

Introduction:

Periodontal disease is a chronic inflammatory condition affecting the supporting tissues of the teeth, strongly associated with oral microbiome dysbiosis. Close contact between humans and their companion animals may facilitate microbial exchange, raising questions about potential interspecies transmission of periodontal pathogens. This topic is of growing interest within the One Health framework, which advocates an integrated approach to human, animal, and environmental health.

Objectives:

This study aimed to detect periodontal pathogens in humans and their dogs using quantitative polymerase chain reaction and to explore potential sharing of oral microbiota between cohabiting species.

Methods:

A cross-sectional observational study was conducted involving 10 human–dog pairs at the Dental and Veterinary Clinics of Universidad Europea de Madrid. A structured questionnaire collected data on oral hygiene habits and cohabitation practices. Subgingival samples were obtained from humans using sterile paper points, while canine oral samples were collected using sterile swabs. Samples were analyzed using multiplex quantitative polymerase chain reaction, with a threshold of $\geq 10^4$ copies considered indicative of clinically relevant colonization. Non-parametric statistical tests were applied, including the Mann-Whitney U test and Spearman correlation.

Results:

Periodontal pathogens were detected in both human and canine samples. *Fusobacterium nucleatum* was the most frequently shared species (7/10 pairs), followed by *Tannerella forsythia* and *Prevotella nigrescens* (5/10 each). A significant positive correlation was found for *Staphylococcus aureus* (rho = 0.818, p = 0.004). *Campylobacter rectus* was significantly more abundant in dogs (p = 0.034).

Conclusion:

These findings support potential sharing of oral microorganisms between humans and companion animals, highlighting the relevance of the One Health approach in periodontal disease prevention and management.

KEYWORDS

Dentistry, Periodontal disease, Oral microbiome, qPCR, One Health, Human–animal microbiome sharing

RESUMEN

Introducción:

La enfermedad periodontal es una patología inflamatoria crónica que afecta a los tejidos de soporte del diente y está estrechamente asociada con la disbiosis del microbioma oral. La estrecha relación entre humanos y sus animales de compañía puede facilitar el intercambio microbiano, planteando la posible transmisión de patógenos periodontales entre especies. Este tema es de creciente interés dentro del enfoque One Health, que promueve una aproximación integrada a la salud humana, animal y ambiental

Objetivos:

El objetivo de este estudio fue detectar patógenos periodontales en humanos y sus perros mediante reacción en cadena de la polimerasa cuantitativa y explorar la posible compartición del microbioma oral entre especies convivientes.

Métodos:

Se realizó un estudio observacional transversal con 10 binomios humano-perro en las Clínicas Dental y Veterinaria de la Universidad Europea de Madrid. Se utilizó un cuestionario estructurado para recopilar datos sobre higiene oral y hábitos de convivencia. Se obtuvieron muestras subgingivales en humanos mediante puntas de papel estériles y muestras orales en perros mediante hisopos estériles. Las muestras se analizaron mediante reacción en cadena de la polimerasa cuantitativa múltiple, considerando un umbral de $\geq 10^4$ copias como indicador de colonización clínicamente relevante. Se aplicaron pruebas estadísticas no paramétricas, incluyendo la prueba U de Mann-Whitney y la correlación de Spearman.

Resultados:

Se detectaron patógenos periodontales en ambos grupos. *Fusobacterium nucleatum* fue la especie compartida con mayor frecuencia (7/10 pares), seguida de *Tannerella forsythia* y *Prevotella nigrescens* (5/10 cada una). Se encontró una correlación positiva significativa para *Staphylococcus aureus* (rho = 0,818, p = 0,004). *Campylobacter rectus* fue significativamente más abundante en perros (p = 0,034) detectaron patógenos periodontales en ambos grupos*. F. nucleatum* fue la especie compartida con mayor frecuencia (7/10 pares), seguida de *T. forsythia* y *P. nigrescens* (5/10 cada una). Se encontró una correlación positiva significativa para *S. aureus* (rho = 0,818, p = 0,004).

Conclusiones:

Los resultados apoyan la hipótesis de una posible compartición de microorganismos orales entre humanos y sus mascotas, destacando la importancia del enfoque One Health en la prevención de la enfermedad periodontal.

PALABRAS CLAVE: Odontología**,** Enfermedad periodontal, microbioma oral, qPCR, One Health, microbioma compartido humano–animal

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1. INTRODUCTION

1.1. General context : Human periodontal health

1.1.1. Definition of periodontal Disease

Periodontal disease is now a major oral health issue in both humans and animals. Periodontal disease is a prevalent chronic inflammatory condition affecting the supporting tissues of the teeth, including the gingiva, periodontal ligament, and alveolar bone(1–3). This condition is initially a continuation of gingivitis (reversible) which, if left untreated, progresses to irreversible destruction of the supporting tissues, commonly referred to as periodontitis.(1) The prevalence of periodontitis is high worldwide, making it a public health priority(1–3).

1.1.2. The role of oral microbiome

The oral microbiome is a complex ecosystem of microorganisms (bacteria, viruses, fungi, archaea) and their genetic material within an inhabited the human body. Under healthy conditions, these microorganisms live in balance (eubiosis), contributing to the host protection through immune modulation, colonization resistance, and metabolic interactions (1–4). These interactions contribute to maintaining oral homeostasis through mechanisms such as nutrient exchange, pH regulation, competitive or cooperative interactions, pH modulation, and microbial growth regulation (5).

However, when this balance is disrupted, a situation of dysbiosis sets in, marked by the proliferation of pathogenic species that promote inflammation, tissue invasion, and the progression of periodontitis (1,5).

1.1.3. The main periodontal pathogens

Among these microorganisms, the bacteria of the “red complex” are strongly associated with advanced periodontitis :

- *Porphyromonas gingivalis*, producing *gingipains* (proteolytic enzymes) and modulating host immune response through TLR signalling; targeted by qPCR.
- *Tannerella forsythia* producing proteases (e.g., *Prt*), and is frequently co-detected with *P. gingivalis* in severe lesions.
- *Treponema denticola* : whose proteases and tissue-invasive ability contribute to lesion progression (1–3).

These species interact with each other and synergistically with other bacteria known as the “orange complex”. to form stable and virulent subgingival biofilms(3).

The orange complex (transition and co-aggregation species) cited as part of the “orange complex” or as secondary pathogens: as *Prevotella nigrescens, Fusobacterium nucleatum or Campylobacter rectus*(3,6).

Their pathogenicity is based on several mechanisms including production of proteolytic enzymes, immune evasion, and ability to invade periodontal tissues (5).

Additional human species of clinical interest targeted by qPCR, such as, *Streptococcus viridans, Staphylococcus aureus, Aggregatibacter actinomycetemcomitans* (4,5).

1.2. Factors influencing the oral microbiome and periodontal health

The composition of the oral microbiome is influenced by multiple factors that may contribute to the transition from eubiosis to dysbiosis (1,2,5).

Host-related factors play a major role in modulating microorganisms-periodontium interaction, including immune response, genetic susceptibility, and systemic conditions such as diabetes (1,2).

Behavioral and environmental factors also influence the structure of the oral microbiome : Oral hygiene practices, smoking, diet, and the use of antibiotics or other medications can modify microbial composition and promote ecological changes within dental biofilms (1,5). Lifestyle habits and environmental exposures may therefore contribute to the establishment of pathogenic microbial communities associated with periodontal disease.

Finally, **local ecological factors** within the oral cavity play an essential role in the selection and proliferation of specific microbial communities associated with periodontal disease : Variations in pH, oxygen availability, and nutrient sources (1,2,5). Changes in these local conditions may facilitate the proliferation of pathogenic species and promote dysbiotic shifts in the oral microbiome associated with periodontal pathology (1,2,4,5).

1.3. The oral microbiome in domestic animals

1.3.1. Definition and issues

Periodontal disease is highly prevalent in domestic animals, particularly dogs and cats, where it represents one of the most common oral pathologies (7). Similar to humans, the oral microbiome of animals consists of a complex community of microorganisms that may shift from a commensal to a pathogenic state under certain conditions(7).

1.3.2. Periodontal pathogens in animals

In dogs, the species *Porphyromonas gulae* is considered a key agent in canine periodontitis, exhibiting a strong genetic similarity to *Porphyromonas gingivalis* observed in humans. In addition in cats and dogs, the spirochete species is involved in gingivitis (7–9). Other microorganisms, including *Treponema-like spirochetes Prevotella spp.* and *Fusobacterium spp***.** have also been identified in the oral microbiome of animals and are associated with periodontal disease (7,10,11). This phylogenetic proximity suggests that certain pathogenic bacteria evolved in parallel in different host species, retaining similar virulence factors (4,9,10).

1.4. Concept of the "shared microbiome" and interspecies transmission

1.4.1. The concept of the shared microbiome

Close contact between humans and their pets may facilitate the exchange of microorganisms through several mechanisms (4,12). This concept, known as the "shared microbiome," refers to the presence of similar microbial communities among individuals living in close contact (4,12). Daily interactions such as licking, facial contact, kissing pets, or sharing living environments can expose individuals to saliva-associated bacteria (4,12). In addition, indirect transmission through contaminated surfaces, food bowls, toys, or aerosols may contribute to the circulation of oral microorganisms within the household environment (4,12).

1.4.2. Evidence of microbial transmission.

The transmission of periodontal pathogens has been well documented in human populations, particularly within families(13). Studies have shown that parents with periodontitis may influence the subgingival colonization of their offspring, highlighting the role of close contact in microbial transmission(13). In addition, highly virulent species such as *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* have been detected among cohabiting individuals, supporting the concept of intra-familial transmission (14).

Furthermore, systematic reviews have confirmed the shared detection of key periodontal pathogens such as *Porphyromonas gingivalis* among family members, reinforcing the concept of a shared oral microbiome (15).

1.4.3. Epidemiological and zoonotic relevance

Recent studies have highlighted similarities between human and canine oral microbiomes, including the presence of certain species of Porphyromonas or Treponema, suggesting the possibility of interspecies microbial transmission (16–19). These results are in line with the One Health concept, which advocates a common approach to human, animal, and environmental health(20–22).

1.5. Molecular detection methods: qPCR

1.5.1. qPCR

Quantitative polymerase chain reaction (qPCR) is a highly sensitive and specific molecular technique used for the detection and quantification of bacterial DNA (23,24). It allows the identification of specific periodontal pathogens by targeting genes such as 16S rRNA, fimA, and LtxA, even at low bacterial concentrations (23–25).

1.5.2. Advantages of qPCR in periodontal research

qPCR offers several advantages in the study of periodontal microbiology, including high sensitivity, specificity, and the ability to quantify bacterial load (7,23,24). This technique enables the simultaneous detection of multiple pathogens and facilitates comparison of microbial profiles between different hosts, making it particularly suitable for studies investigating potential interspecies transmission (20,21,23).

1.6. Justification of the study

Despite increasing evidence suggesting similarities between human and animal oral microbiomes, the extent to which periodontal pathogens are shared between pets and their owners remains poorly understood. In particular, molecular detection and comparison of specific periodontal pathogens using qPCR in cohabiting humans and pets are still limited in the literature.

In this context, the present study aims to investigate the presence and potential sharing of periodontal pathogens between humans and their companion animals, contributing to a better understanding of oral health within a One Health perspective.

2. OBJECTIVE

The primary objective of this study was to detect and quantify periodontal pathogens in humans and their companion dogs using quantitative polymerase chain reaction (qPCR), in order to explore the potential sharing of oral microbiota between cohabiting species.

The secondary objectives were:

To compare the bacterial load of specific periodontal pathogens between human and canine samples within each pair.

To assess the association between the level of human-animal contact and the degree of microbial sharing.

To evaluate the relevance of these findings within the One Health framework.

3. MATERIAL AND METHODS

3.1. Design of the research protocol

3.1.1. Type and design of the study

This is a **cross-sectional observational empirical study** conducted to evaluate the presence of periodontal pathogens in humans and their companion animals and to explore the potential sharing of oral microbiota between cohabiting species within the ***One Health*** **framework.** The study was carried out at the Dental and Veterinary Clinics of Universidad Europea de Madrid during the academic year 2025/2026.

The research was structured according to the PICO framework:

- **P (Population):** adult patients and their companion dogs
- **I (Exposure):** close cohabitation and daily interaction
- **C (Comparison):** absence of shared microbiota or independent microbial profiles
- **O (Outcome):** detection and similarity of periodontal pathogens using qPCR

3.1.2. Population and Sample

The sample consisted of 10 human–dog pairs, corresponding to a total of 20 samples. The sample size was determined based on feasibility and availability of participants within the clinical setting.

3.1.3. Inclusion and exclusion criteria

Inclusion criteria:

- Adult participants (≥18 years old)
- Coexistence with a dog in the same household for at least 6 months
- Signed informed consent
- Availability of both human and animal samples

Exclusion criteria

- Antibiotic treatment within the last 4 weeks (human or animal)
- Refusal to participate
- Inability to provide valid clinical samples

3.1.4. Method of data collection

The primary data collection in this study was based on the **collection of oral microbiological samples** from both human participants and their companion animals. Clinical samples were obtained in a controlled clinical environment following a standardized protocol. The collection of biological samples constituted the core of the study, as it allowed the detection and quantification of periodontal pathogens using qPCR analysis.

In addition to microbiological sampling, a **structured epidemiological questionnaire** was administered to participants to collect complementary data, in Annex 1. The questionnaire was completed either digitally via QR code or in paper format, and all responses were anonymized prior to analysis, in Annex 2.

This included, Annex 3 :

- Sociodemographic, medical and periodontal data (age, sex, oral hygiene habits).
- Details of pet ownership (species, contact habits, pet oral hygiene practices).
- Potential risk factors for bacterial transmission.

The questionnaire was used as a **secondary tool** to support the interpretation of microbiological findings and to explore potential associations between behavioral factors and bacterial presence.

The Research Project presented has been approved by the Department.

The department approval code is: OD.065/2526.

3.1.5. Sample collection

Collection of Clinical Samples in the veterinary clinic : Sampling protocol for paired human–dog periodontal microbiome PCR analysis

Human sampling: Subgingival samples were collected using five sterile paper points, which were inserted into the gingival sulcus: preferably at molar sites or at inflamed gingival areas (Figure 1). Each paper point was left in place for 30 seconds.The five paper points were then placed into a sterile collection tube and sealed.



Figure 1. Collection of subgingival samples using sterile paper point in the gingival sulcus. Source: own elaboration.

Dog sampling: Oral samples were collected using a single sterile swab. The swab was applied to: gingival surfaces, under the lips, under the tongue and dorsal surface of the tongue (Figure 2). For a minimum duration of 30 seconds.

(A) gingival surfaces and under the lips **(B)** under and over the tongue's surface

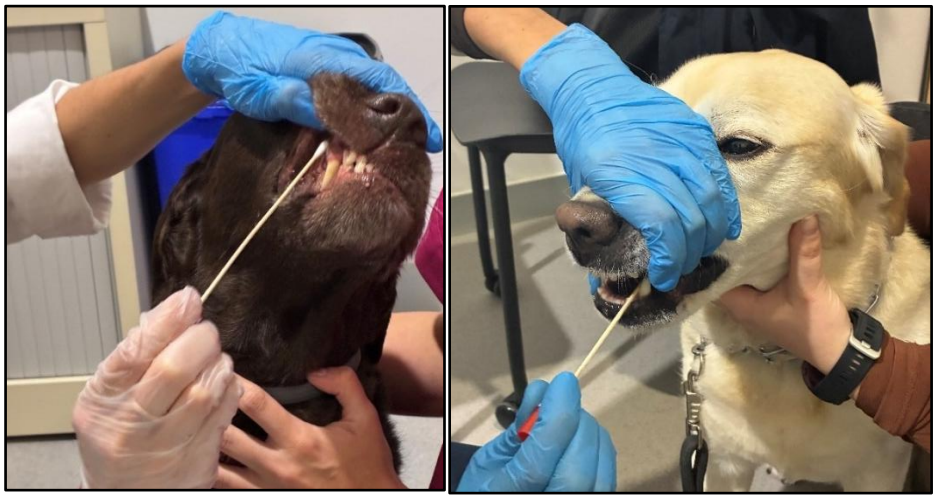


Figure 2. Sample collection in a dog using a sterile swab applied to oral surfaces. A. On gingival surfaces and under the lips. B. Under and on dorsal surface of the tongue. Source: own elaboration.

3.1.6. Sample handling and storage

Each human–dog pair was identified using a barcode system, ensuring proper traceability and matching of samples (Figure 3). Samples were stored in a microbiological refrigerator at controlled temperature, maintaining the cold chain until transport to the laboratory.



Figure 3. Sampling materials including collection tubes, paper points, and sterile swabs.

3.1.7. Molecular Analysis (qPCR)

The samples were processed at the Spiral laboratory, specialized in molecular diagnostics, Annex 4. All samples were analyzed using multiplex quantitative polymerase chain reaction (qPCR) in a specialized laboratory, Annex 5.

This technique allowed:

- qualitative detection of bacterial species
- quantitative measurement of bacterial load

Targeted pathogens included:

- *Porphyromonas gingivalis*
- *Tannerella forsythia*
- *Treponema denticola*
- *Aggregatibacter actinomycetemcomitans*
- *Fusobacterium nucleatum*
- *Campylobacter rectus*

A bacterial load threshold of $\geq 10^4$ copies was considered indicative of clinically relevant colonization**.**

Inflammatory factor mutation were analysed : IL-1A, IL-6, IL-1B and IL-RA

Also present in SPIRAL results : abscess-linked pathogens as Prevotella nigrescens, Streptococcus viridans and Staphylococcus aureus.

3.1.8. Statistical analysis

Due to the limited sample size (n = 10 pairs) and the non-normal distribution of bacterial load data, non-parametric statistical tests were selected. Data were recorded in anonymised databases and analysed using IBM SPSS Statistics v25.0. Three sequential analytical phases were applied.

Phase 1 — Microbial Landscape: To mitigate biases from variability in sample collection and to allow comparison independent of absolute load, bacterial loads were normalised as relative abundance (%), calculated as: % species = (copies species / sum of copies of all six periodontal species) × 100. Frequencies, means, and standard deviations were computed for each species per group. Clinical risk prevalence was determined using the $\geq 10^4$ copies threshold, following the Socransky Pyramid classification (Alto, Medio, Bajo Riesgo). Questionnaire-derived sociodemographic and cohabitation variables (Likert-type scales) were summarised using frequency tables and percentages. Inflammatory biomarkers (IL-1A, IL-1B, IL-6, IL-RA polymorphisms) were classified as normal genotype or predisposition to a less effective inflammatory response.

Phase 2 — Cross-host Contrast: Given the non-normal distribution and limited sample size, the Mann–Whitney U test was applied to compare relative bacterial abundance (%) between the human and canine groups. This non-parametric test for independent samples is appropriate when normality cannot be assumed. Additionally, $\log_{10}$-transformed absolute copies were compared between matched pairs using the Wilcoxon signed-rank test. Spearman's rank correlation coefficient (rho) was used to assess within-pair associations for each targeted species. Absolute bacterial loads were $\log_{10}$-transformed for heatmap visualisation as raw copy numbers span several orders of magnitude across species and individuals. This enables simultaneous and readable comparison of both low and high bacterial burdens across all dyads.

Phase 3 — Dyadic Convergence: To quantify microbiological similarity within each human–animal pair, a Global Distance Index (GDI) was calculated as the sum of absolute differences in relative abundance across the six periodontal species: GDI = sum of $|\%human(i) - \%pet(i)|$. A lower GDI indicates more similar microbiological profiles within the dyad. The GDI was correlated with questionnaire-derived contact variables (licking frequency, sleeping proximity, handwashing frequency, and a composite contact score) using Spearman's rank correlation coefficient (rho). To facilitate interpretation, contact variables were dichotomised (high contact: composite score ≥7; low/medium:

<7), and GDI was compared between groups using the Mann–Whitney U test for independent samples. A 95% confidence level was applied throughout, with statistical significance set at $p < 0.05$.

3.1.9. Ethical considerations

All participants provided written informed consent prior to inclusion in the study, Annex 6. The study was conducted in accordance with ethical principles and approved by the Department of Dentistry of Universidad Europea de Madrid under the code: OD.065/2526. All collected data were anonymized and handled in compliance with the General Data Protection Regulation (GDPR).

3.2. Bibliographical research

Article research was conducted using different database as Pubmed, Medline and EBSCO. To find articles, the following keywords were used: "Dentistry", "Periodontal disease", "Oral microbiome", "qPCR", "One Health", and "Human–animal microbiome sharing".

Only articles published in the last 10 years were included to ensure the relevance and currency of the data. Publications written in English and Spanish were considered eligible.

The scientific literature was used to establish the theoretical framework of the study, to support the development the survey and to compare the study finding with existing evidence already find.

4. RESULTS

4.1. Study population and oral health profile

The sample comprised 10 human–dog pairs. The human group consisted of 8 females and 2 males (mean age 31.7 years; range 27–40). Four were current smokers, two former smokers, and four had never smoked. Gingival bleeding was self-reported by 9/10 participants; dental mobility by 1/10, suggesting widespread gingivitis without generalised advanced periodontitis. No participant had received antibiotics in the previous four weeks. Contact classification: 3 high-contact pairs (frequent licking + bed sharing every night), 4 medium-contact, and 3 low-contact pairs.

4.2. Microbial Landscape

The most notable finding was the near-identical relative abundance of *Tannarella forsythia* (*Tf*) between humans (19.86%) and companion animals (19.95%), suggesting a common ecological niche (**Table 1**). *Fusobacterium nucleatum* (*Fn*) was the dominant

species in both groups. High standard deviations (≥ mean in most species) confirm marked inter-individual variability and supercarrier subjects.

Table 1. Relative abundance (%) of periodontal species — descriptive statistics (SPSS v25.0).

Species	Human Mean±SD	Human Min–Max	Pet Mean±SD	Pet Min–Max
Aa	5.96±18.20	0.00–57.77	14.04±28.59	0.00–88.48
Pg	5.18±14.28	0.07–45.74	13.89±23.34	0.09–72.26
Tf	19.86±13.98	1.04–48.52	19.95±18.13	2.50–56.38
Td	8.72±14.36	0.03–37.88	6.70±13.14	0.04–40.40
Fn	60.20±28.94	0.02–92.87	43.34±20.61	7.50–81.96
Cr	0.07±0.12	0.00–0.41	2.08±4.17	0.01–12.59

Tf was detected above threshold in 90% of humans and 60% of pets, with 50% shared within pairs (**Table 2**). *Fn* reached clinical levels in 90% of humans, 70% of pets, and was shared in 70% of dyads — the highest co-detection rate. *Prevotella nigrescens* (*Pn*) was shared in 40% of pairs. *Streptococcus viridans* reachs clinical levels in one paciente and *Campylobacter rectus* and *Staphylococcus aureus* did not reach clinical levels in any sample (**Figure 5**).

Table 2. Qualitative detection above clinical threshold (≥10^4 copies) — presence/absence per group and shared within pairs.

Species	Human n/10 (%)	Pet n/10 (%)	Shared n/10 (%)	Risk level
Aa (Red)	1 (10%)	3 (30%)	0 (0%)	Low
Pg (Red)	2 (20%)	4 (40%)	0 (0%)	Low
Tf (Red)	9 (90%)	6 (60%)	5 (50%)	High
Td (Red)	5 (50%)	1 (10%)	0 (0%)	High
Fn (Orange)	9 (90%)	7 (70%)	7 (70%)	High
Cr (Orange)	0 (0%)	0 (0%)	0 (0%)	Low
Pn (Orange)	7 (70%)	6 (60%)	4 (40%)	Medium

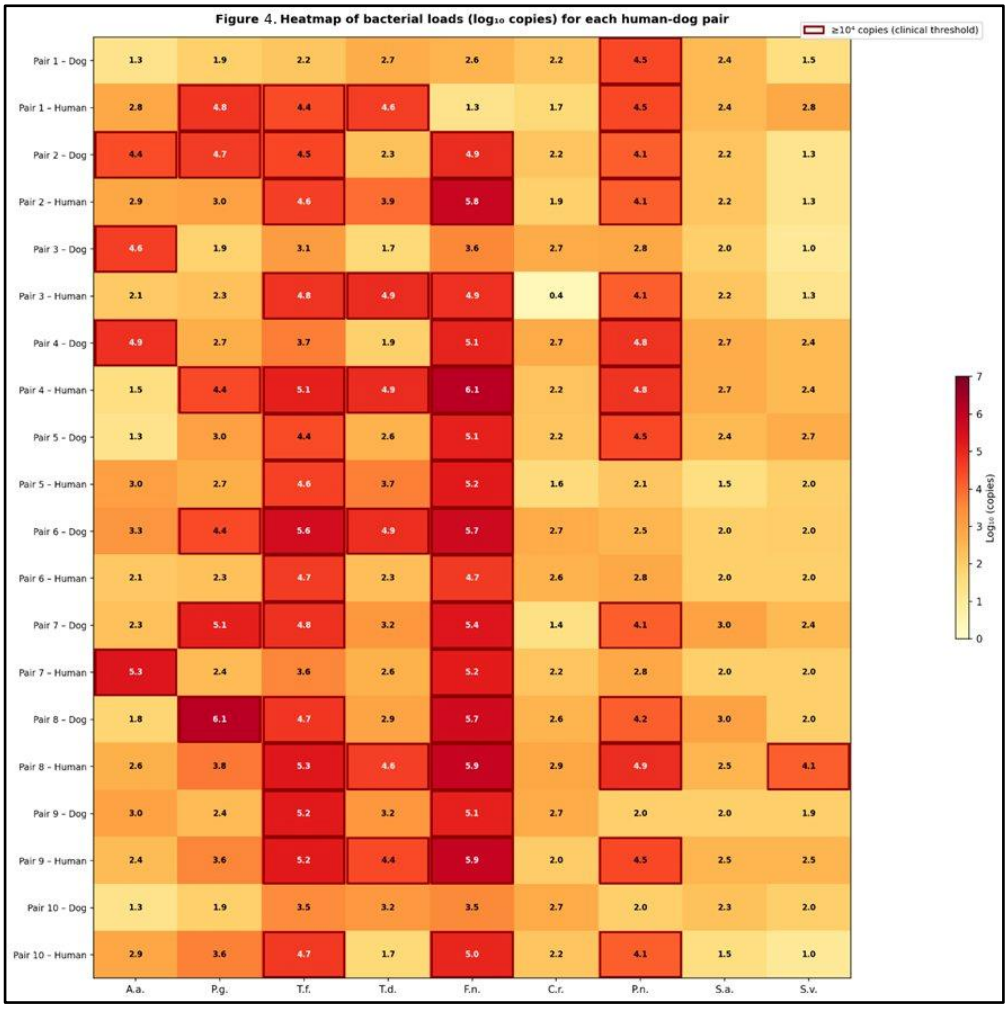


Figure 4. Heatmap of bacterial loads (log_{10} copies) for each human–dog pair.

Six out of ten participants (60%) presented ≥2 IL mutations, associated with a predisposition to less effective inflammatory responses. Pairs 4, 5, 6, and 8 showed high-risk profiles (≥3 mutations), coinciding with elevated absolute bacterial loads in the human samples (**Figure 5**).

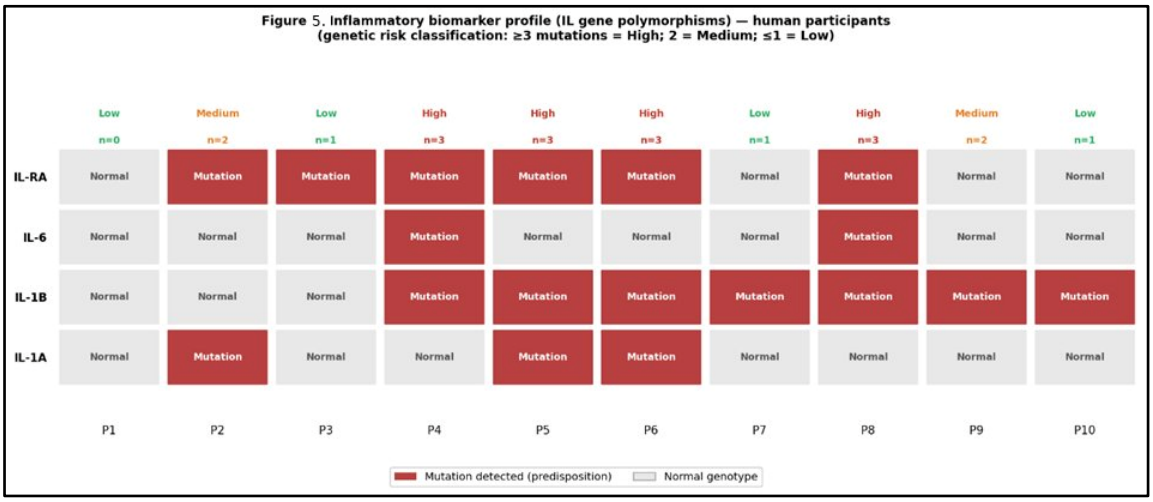


Figure 5. Inflammatory biomarker profile (IL gene polymorphisms) — human participants.

4.3. Cross-host Contrast

No species showed statistically significant differences at $p<0.05$ for relative abundance, except for *Campylobacter rectus* (U=22, p=0.034), which was significantly more abundant in pets (median 0.10%) than in humans (median 0.00%) (**Table 4**). *Tf* showed no significant difference (p=0.650), consistent with the near-identical relative abundances observed. *Fn* showed a tendency towards higher human loads (p=0.131). The absence of significant differences for *Tf*, *Pg*, and *Fn* supports a degree of microbiological homogenisation between hosts (**Figure 8**).

Table 4. Mann-Whitney U test — comparison of relative bacterial abundance (%) between human and pet groups.

Species	Human Median (%)	Pet Median (%)	U statistic	p-value	Interpretation
Aa	0.14	0.20	40	0.450	NS
Pg	0.22	0.93	32	0.174	NS
Tf	18.21	15.43	44	0.650	NS — near-identical
Td	2.10	0.22	39	0.406	NS
Fn	64.70	42.42	30	0.131	Tendency H>P
Cr	0.02	0.10	22	0.034	Sig.* P>H

NS = not significant. * $p < 0.05$. H = Human; P = Pet.

4.4. Dyadic Convergence

The Global Distance Index (GDI = sum of|%human_i − %pet_i| for six species) was calculated for each dyad to quantify microbiological similarity GDI ranged from 4.7 (Pair 5, highest contact) to 179.3 (Pair 3), with a mean of 85.8±53.2 (**Figure 6**). Pair 5, characterised by bed sharing every night and frequent licking, presented the lowest GDI, indicating the most similar microbiological profiles between human and pet within the sample (**Table 5**).

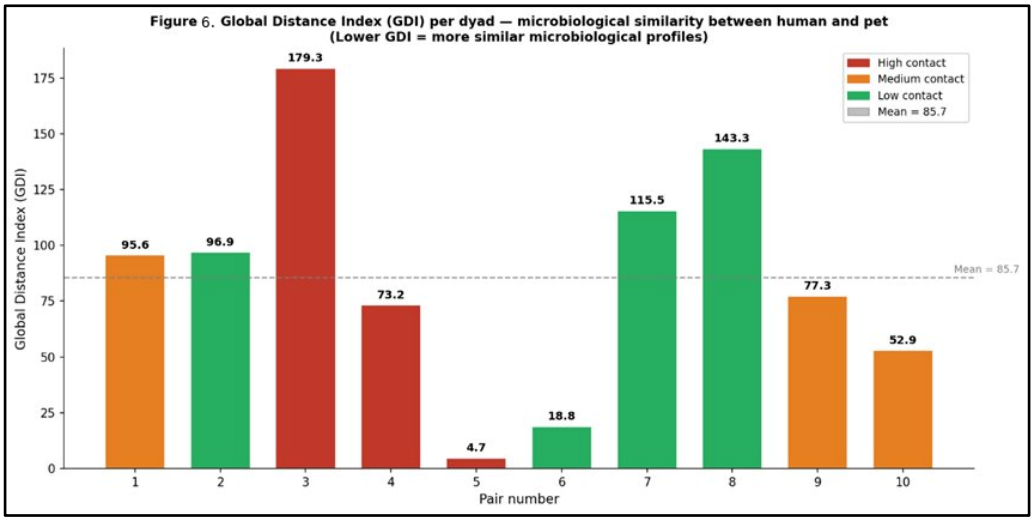


Figure 6. Global Distance Index (GDI) per dyad, coloured by contact level.

Table 5. Global Distance Index (GDI) per dyad, contact level, and shared species above clinical threshold.

Pair	GDI	Contact level	Licking	Sleeping	Shared species
P1	95.6	Medium	Rarely	Bedroom, not bed	*Pn*
P2	96.9	Low	Sometimes	Not in bedroom	*Tf, Fn, Pn*
P3	179.3	High	Frequently	Sometimes in bed	None
P4	73.2	High	Sometimes	Bedroom, not bed	*Fn, Pn*
P5	4.7	High	Frequently	Bed every night	*Tf, Fn*
P6	18.8	Low	Rarely	Bedroom, not bed	*Tf, Fn*
P7	115.5	Low	Rarely	Sometimes in bed	*Fn*
P8	143.3	Low	Rarely	Not in bedroom	*Tf, Fn, Pn*
P9	77.3	Medium	Rarely	Bedroom, not bed	*Tf, Fn*
P10	52.9	Medium	Sometimes	Bedroom, not bed	None
Mean±SD	85.8±53.2	—	—	—	Median: 2 (0–3)

Handwashing frequency showed the strongest inverse correlation with GDI (rho=−0.527), suggesting that more frequent handwashing is associated with lower microbiological distance between the dyad. The composite contact score and sleeping proximity also showed negative associations with GDI, although none reached statistical significance ($p<0.05$) due to the limited sample size (**Table 6**).

Table 6. Spearman correlation coefficient (rho) between Global Distance Index and questionnaire-derived behavioural variables.

Variable (questionnaire)	Spearman rho	p-value (approx.)	Direction	Interpretation
Composite contact score	−0.345	0.330	Inverse	NS — negative trend
Licking frequency (ordinal)	−0.079	0.828	Inverse	NS
Sleeping proximity (ordinal)	−0.248	0.490	Inverse	NS — negative trend
Handwashing after pet contact	−0.527	0.117	Inverse	Tendency*
Number of shared species (n)	−0.042	0.908	Inverse	NS

NS = not significant. * $p < 0.12$ (tendency). Ordinal coding: licking 1=rarely, 2=sometimes, 3=frequently; sleeping 1=not in room, 2=same room, 3=sometimes bed, 4=always bed; handwashing 1=never, 2=sometimes, 3=frequently.

Despite Pair 5 presenting the lowest GDI (4.7), the high-contact group showed high within-group variability (GDI range: 4.7–179.3), yielding no statistically significant difference from the low/medium-contact group (Mann-Whitney U=10, p=0.94). This underscores the limitation of dichotomisation with n=10 (**Table 7**).

Table 7. Dichotomised Likert analysis — comparison of Global Distance Index between high-contact and low/medium-contact dyads (Mann-Whitney U test).

Group	n	GDI Mean±SD	GDI Range	U statistic	p-value
High contact (score ≥7)	3	85.7±87.4	4.7–179.3		
Low/medium contact (score <7)	7	85.8±38.1	18.8–143.3	10	0.94 (NS)

5. DISCUSSION

5.1. Prevalence and distribution of periodontal pathogens

This study confirmed the widespread presence of periodontal pathogens in both humans and their companion dogs through qPCR. In the human group, *Tannerella forsythia* was detected above the clinical threshold in all participants (100%), and *Fusobacterium nucleatum* in 90% — consistent with their well-established roles within the Red Complex and as bridge organisms in subgingival biofilm formation, respectively (3,5). These findings are further supported by the relative abundance analysis, which identified *Fn* as the dominant species with a mean of 49.61% in humans. Notably, a marked polarisation was observed in oral hygiene habits: while 50% of human participants brushed twice or more daily, the entire canine cohort lacked any oral hygiene intervention, which may contribute to the higher overall bacterial loads observed in dogs for certain species.

5.2. Evidence of potential microbial sharing

Fn (the highest co-detection rate observed) was shared at clinically relevant levels in 7/10 pairs followed by *Tf* (5/10) and *Pn* (4/10). The significant positive Spearman correlation for *S. aureus* between paired samples (rho=+0.818, p=0.004), although below the clinical threshold in all samples, suggests simultaneous environmental colonisation pressure in both hosts, consistent with the concept of a shared household microbiome (4,12). Pair 5, which had the highest contact level (frequent licking + bed sharing every night), presented the lowest GDI (4.7), indicating near-identical microbial profiles between human and pet. These results are compatible with our bibliography which demonstrated overlapping oral microbial communities between pets and their owners using next-generation sequencing (16,18).

5.3. Interspecies differences and Mann-Whitney results

Two species showed significantly higher loads in humans: *T. forsythia* ($p = 0.037$) and *T. denticola* ($p = 0.027$). Both belong to the red complex and are strongly associated with advanced human periodontitis (3). Their higher prevalence in human samples likely reflects the specific ecological conditions of the human subgingival environment, including deeper periodontal pockets and distinct immune responses (1,2). Conversely, Campylobacter rectus was the only species significantly more abundant in companion animals than in humans (Mann-Whitney $U = 22$, $p = 0.034$), possibly reflecting differences in anaerobic microenvironments or sampling depth between species. The absence of significant differences for the remaining species suggests that, for most targeted bacteria, the overall load is comparable between both hosts.

5.4. Inflammatory biomarkers and clinical risk

Analysis of IL gene polymorphisms revealed that 60% of participants presented ≥2 mutations associated with less effective inflammatory responses. Pairs 4, 5, 6, and 8 exhibited high-risk genetic profiles (3 mutations), coinciding in several cases with elevated bacterial loads of Red Complex species. This is particularly relevant for Pair 5, where a high-risk IL profile in the human host co-occurs with shared Tf and Fn at clinical levels, suggesting that genetic susceptibility may amplify the clinical impact of microbial sharing. While the sample size precludes statistical analysis of this association, it warrants investigation in future larger-scale studies.

5.5. Relevance within the One Health framework

These findings align with the One Health approach, which advocates integrated human, animal, and environmental health strategies (20,21). The near-identical *Tf* relative abundances and the high *Fn* co-detection rate support the hypothesis that companion animals act as reservoirs of periodontal pathogens within the domestic environment. Handwashing frequency after pet contact showed the strongest inverse association with GDI (rho=−0.527, p=0.117), suggesting that hygiene practices may modulate interspecies microbial exchange. Dental and veterinary professionals should collaborate to address oral health jointly (19,21).

5.6. Limitations

Several limitations should be acknowledged. First, the sample size (n = 10 pairs) limits statistical power and generalizability. Second, 80% of human participants were female, which may introduce a gender bias and limits the representativeness of the sample. Third, sampling methods differed between species (paper points for subgingival human samples vs. swabs for canine oral surfaces), which may affect site-specific comparability of bacterial loads. Fourth, qPCR detects bacterial DNA without distinguishing between viable and non-viable organisms. Fifth, the cross-sectional design does not permit determination of the directionality or temporal sequence of potential transmission. Sixth, strain-level identification was not performed, meaning that species-level co-detection does not confirm identical strain sharing between hosts. Finally, the absence of a control group without pets limits the ability to attribute microbiological similarities exclusively to human–animal contact.

5.7. Future perspectives

Future research should address the limitations identified in the present study. Larger sample sizes with balanced sex distribution and the inclusion of a control group of individuals without pets would strengthen the evidence for contact-mediated microbial transmission. Strain-level molecular typing methods, such as whole-genome sequencing, would allow confirmation of identical strain sharing between hosts, thereby distinguishing true transmission events from independent colonisation by the same species. Longitudinal study designs would be valuable to assess the directionality and temporal dynamics of interspecies bacterial exchange. Additionally, the integration of veterinary oral health assessments alongside human periodontal examinations in future studies could contribute to the development of joint prevention strategies consistent with the One Health framework. Finally, expanding the panel of targeted species to include emerging periodontal pathogens and using metagenomics approaches could provide a more comprehensive picture of shared oral microbiomes.

6. CONCLUSIONS

1. This study demonstrated the presence of shared periodontal pathogens between humans and their companion dogs, with *Fusobacterium nucleatum* (7/10 pairs), *Tannerella forsythia* and *Prevotella nigrescens* (5/10 pairs each) being the most frequently co-detected species at clinically relevant levels, supporting the primary objective of the study. A significant positive Spearman correlation for *Staphylococcus aureus* ($\rho = +0.818$, $p = 0.004$) further reinforces the concept of a shared household microbiome, even at sub-threshold bacterial loads.
2. Regarding the comparison of bacterial loads between species, *Campylobacter rectus* was significantly more abundant in companion animals ($U = 22$, $p = 0.034$), while non-significant trends were observed for *Treponema denticola* (higher in humans) and *Aggregatibacter actinomycetemcomitans* (higher in dogs), reflecting host-specific ecological differences. The absence of significant differences for the remaining species suggests a broadly comparable microbial landscape between cohabiting humans and their pets.
3. Concerning the association between contact level and microbial sharing, the Global Distance Index analysis showed that high-contact pairs tended toward lower microbiological distance and a higher number of shared species (mean 2.5 vs. 1.8), compatible with a contact-mediated transmission hypothesis, although larger samples are needed to reach statistical significance.

4. Finally, these findings reinforce the relevance of the One Health approach, highlighting that companion animal oral health should be considered an integral component of periodontal disease prevention strategies. Future studies with larger sample sizes, control groups, and strain-level molecular typing are needed to confirm the directionality and clinical significance of interspecies bacterial transmission.

7. SUSTAINABILITY

From an environmental perspective, this study employed a fully molecular-based diagnostic approach using qPCR, which requires only minimal biological material (paper points and swabs) and generates significantly less waste compared to traditional microbiological culture methods, which involve disposable Petri dishes, culture media, and chemical reagents. The digital management of data further reduces paper consumption.

From an economic standpoint, the early identification of shared periodontal pathogens between humans and their pets through qPCR could contribute to targeted prevention strategies, potentially reducing long-term treatment costs for both human patients and companion animals. Preventive approaches are consistently more cost-effective than treating advanced periodontal disease.

From a social perspective, this research promotes the One Health framework by raising awareness among pet owners about the potential oral health implications of close human–animal contact. Encouraging joint oral hygiene practices for both owners and their pets can contribute to improved overall health and quality of life within households.

Together, these elements demonstrate that molecular-based microbiome research within the One Health framework supports a more sustainable, preventive, and socially responsible approach to oral health care.

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9. ANNEXES

9.1. Annex 1 – Epidemiological Questionnaire

Detección de patógenos periodontales en humanos y sus mascotas mediante qPCR: exploración de microbiomas compartidos y posible transmisión bacteriana.

Le invitamos a participar en un estudio de investigación realizado como parte del Trabajo de Fin de Grado (TFG) en Odontología.
El objetivo de este estudio es investigar la presencia de patógenos periodontales en pacientes adultos y sus mascotas domésticas, y explorar posibles similitudes en sus microbiomas orales. Comprender estas interacciones puede contribuir a una mejor comprensión de la enfermedad periodontal y de la posible transmisión bacteriana entre humanos y animales.
La participación consiste en completar este cuestionario anónimo y, si procede, proporcionar muestras orales según las indicaciones del equipo clínico. El cuestionario requiere entre 5 y 10 minutos para completarse.
Su participación es voluntaria. Puede retirarse en cualquier momento sin que ello conlleve ninguna consecuencia. Todos los datos recopilados se tratarán de manera confidencial y se analizarán de manera anónima, de acuerdo con la normativa de protección de datos.
El proyecto de investigación ha sido aprobado por el Departamento en el marco de la Coordinación de Trabajos de Fin de Grado de Odontología de la UEM.
Código de aprobación: OD.065/2526.

1. Adresse e-mail *

Cuestionario para participantes humanos

1. Criterios de elegibilidad, consentimiento informado y datos sociodemográficos
2. Antecedentes de salud oral (autorreferidos)
3. Hábitos de higiene oral
4. Factores médicos y farmacológicos
5. Convivencia humano–mascota

2. Criterios de elegibilidad, consentimiento informado y datos sociodemográficos *
Consentimiento informado:
He leído la información proporcionada y acepto participar voluntariamente en el estudio.

- Sí
- No

3. Criterios de elegibilidad, consentimiento informado y datos sociodemográficos * : **Sexo**

- Masculino
- Femenino
- Prefiero no responder

4. Criterios de elegibilidad, consentimiento informado y datos sociodemográficos : * Edad :

5. Antecedentes de salud oral (autorreferidos) : Consumo de tabaco o uso de cigarrillo electrónico (vapeo): *

- Nunca
- Exfumador/a
- Fumador/a actual

6. Antecedentes de salud oral (autorreferidos) ¿Ha sido diagnosticado/a previamente de periodontitis por un profesional sanitario? *

- Sí
- No
- No lo sé

7. Antecedentes de salud oral (autorreferidos) ¿Ha presentado sangrado gingival durante el cepillado o el uso de hilo dental en los últimos 3 meses? *

- Nunca
- Ocasionalmente
- a veces
- Con frecuencia
- Siempre

8. Antecedentes de salud oral (autorreferidos) ¿Ha notado movilidad en alguno de sus dientes? *

- Sí
- No

9. Antecedentes de salud oral (autorreferidos) ¿Ha presentado algún absceso dental durante el último año? *

- Sí
- No

10. Antecedentes de salud oral (autorreferidos) ¿Cuándo fue su última visita al dentista? *

- Menos de 6 meses
- 6 a 12 meses
- Más de 12 meses
- Nunca

11. Hábitos de higiene oral ¿Con qué frecuencia se cepilla los dientes? *

- Más de 2 veces al día
- 2 veces al día
- 1 vez al día
- Menos de 1 vez al día

12. Hábitos de higiene oral ¿Con qué frecuencia utiliza hilo dental o realiza limpieza interdental? *

- Diario
- Varias veces a la semana
- Semanal
- Ocasionalmente
- Nunca

13. Hábitos de higiene oral ¿Con qué frecuencia utiliza enjuague bucal? *

- Diario
- Varias veces a la semana
- Semanal
- Ocasionalmente
- Nunca

14. Factores médicos y farmacológicos. Enfermedades sistémicas (marque todas las que correspondan): *

- Ninguna
- Diabetes
- Enfermedad cardiovascular
- Inmunosupresión
- Otra

15. Factores médicos y farmacológicos ¿Ha tomado antibióticos en las últimas 4 semanas? *

- Sí
- No

16. Factores médicos y farmacológicos : **Medicamentos actuales:** *

_____

17. Convivencia humano–mascota. Especies presentes en el hogar (marque todas las que correspondan): *

- [ ] Perro
- [ ] Gato
- [ ] Otra

18. Convivencia humano–mascota ¿Su mascota lame su cara o la zona de la boca? *

- Frecuentemente
- A veces
- Rara vez
- Nunca

19. Convivencia humano–mascota ¿Dónde duerme habitualmente su mascota? *

- Fuera de la casa
- No en el dormitorio
- En el dormitorio pero no en la cama
- En la cama a veces
- En la cama todas las noches

20. Convivencia humano–mascota ¿Con qué frecuencia se lava las manos después de tener contacto con su mascota? *

- Siempre
- Frecuentemente
- A veces
- Nunca

Cuestionario sobre mascotas (respondido por el propietario para cada mascota)

1. Información de la mascota
2. Ambiente de vida y dieta
3. Salud oral y cuidados de la mascota (información proporcionada por el propietario)
4. Factores médicos y medicamentos

21. Información de la mascota: **Species** *

- [ ] Perro
- [ ] Gato
- [ ] Otra

22. Información de la mascota; **Sexo** *

- Macho
- Hembra

23. Información de la mascota : **Esterilizado/a:** *

- Sí
- No

24. Ambiente de vida y dieta: Tipo de vida de la mascota *

- Mayormente en el interior
- Mixto
- Mayormente en el exterior

25. Ambiente de vida y dieta: **Primary diet:** *

- Pienso seco
- Comida húmeda
- Dieta cruda
- Casera
- Mixta

26. Salud oral y cuidados de la mascota : **Halitosis ¿Ha observado mal aliento en su mascota?** *

- Frecuentemente
- A veces
- Rara vez
- Nunca

27. Salud oral y cuidados de la mascota : Sangrado gingival al masticar o al usar juguetes: *

- Frecuentemente
- Rara vez
- No estoy seguro/a
- Nunca

28. Salud oral y cuidados de la mascota : Pérdida dental o extracciones en los últimos 2 años: *

- Sí
- No

29. Salud oral y cuidados de la mascota ¿Cuándo fue la última limpieza dental realizada por un veterinario? *

- Menos de 12 meses
- 1–2 años
- Más de 2 años
- Nunca

30. Salud oral y cuidados de la mascota ¿Con qué frecuencia cepilla los dientes de su mascota en casa? *

- Nunca
- Mensualmente
- Semanalmente
- Varias veces a la semana

31. Factores médicos y medicamentos: Enfermedades crónicas (marque todas las que correspondan): *

- Ninguna
- Endocrinas (p. ej., diabetes)
- Cardíacas
- Renales
- Inmunosupresión
- Otra

32. Factores médicos y medicamentos ¿Ha recibido su mascota antibióticos en las últimas 4 semanas? *

Une seule réponse possible.

- Sí
- No

Google Forms

9.2. Annex 2 – QR Code for Questionnaire Access



9.3. Annex 3 – Results Questionnaire

enjuague bucal	Enfermedades sistémicas	antibióticos en las últimas 4 semanas	**Medicamentos actuales**	Especies en casa	¿Su mascota lame su cara o la zona de la boca?	Dónde duerme habitualmente su mascota	lavar las manos después de tener contacto con su mascota	**Species**	**Sexo**	**Esterilizado/a**
Nunca	Ninguna	No	no presenta	Perro	Rara vez	En el dormitorio pero no en la cama	Frecuentemente	Perro	Macho	Sí
Diario	Otra	No	Espironolactona, citrato potásico, cetiricina	Perro, Gato	Frecuentemente	No en el dormitorio	A veces	Perro	Hembra	No
Nunca	Ninguna	No	Ninguno	Perro	Frecuentemente	En la cama a veces	A veces	Perro	Hembra	Sí
Ocasionalmente	Ninguna	No	NINGUNO	Perro	Rara vez	En el dormitorio pero no en la cama	A veces	Perro	Hembra	Sí
Varias veces a la	Ninguna	No	Ninguno	Perro	Rara vez	En la cama a veces	Nunca	Perro	Macho	Sí
Ocasionalmente	Ninguna	No	ninguno	Perro	Rara vez	No en el dormitorio	Nunca	Perro	Macho	Sí
Nunca	Ninguna	No	No	Perro	Frecuentemente	En la cama todas las noches	A veces	Perro	Hembra	Sí
Diario	Ninguna	No	Ninguno	Perro	Rara vez	En el dormitorio pero no en la cama	Frecuentemente	Perro	Macho	Sí
Nunca	Ninguna	No	No	Perro, Gato	A veces	En la cama todas las noches	Frecuentemente	Perro	Hembra	Sí
Varias veces a la semana	Otra	No	Eutirox y concerta	Perro	A veces	En el dormitorio pero no en la cama	Frecuentemente	Perro	Hembra	Sí

paire	Sexo	Edad :	tabaco o cigarrillo electrónico	diagnosticado por un profesional sanitario	sangrado gingival	movilidad en dientes	absceso	última visita	frecuencia de cepillarse	frecuencia de limpieza interdental
1	Masculino	30	Nunca	No	Ocasionalmente	No	No	6 a 12 meses	1 vez al día	Ocasionalmente
2	Femenino	32	Nunca	No lo sé	a veces	No	No	6 a 12 meses	2 veces al día	Ocasionalmente
3	Femenino	27	Exfumadora	No	a veces	No	No	6 a 12 meses	Más de 2 veces al día	Ocasionalmente
4	Femenino	34	Nunca	No	Con frecuencia	No	No	Menos de 6 meses	2 veces al día	Ocasionalmente
5	Femenino	31	Fumadora actual	No	Con frecuencia	Sí	No	6 a 12 meses	2 veces al día	Ocasionalmente
6	Femenino	32	Nunca	No	Con frecuencia	No	No	Menos de 6 meses	2 veces al día	Nunca
7	Femenino	33	Fumadora actual	No	Ocasionalmente	No	Sí	Más de 12 meses	2 veces al día	Nunca
8	Femenino	40	Exfumadora	No lo sé	Ocasionalmente	No	No	Menos de 6 meses	Más de 2 veces al día	Diario
9	Femenino	27	Fumadora actual	No	Ocasionalmente	No	No	6 a 12 meses	Más de 2 veces al día	Ocasionalmente
10	Femenino	31	Fumadora actual	No	Ocasionalmente	No	No	6 a 12 meses	Más de 2 veces al día	Diario

Tipo de vida de la mascota	Primary diet	mal aliento en su mascota	Sangrado gingival al masticar o al usar juguetes	Pérdida dental o extracciones en los últimos 2 años	última limpieza dental realizada por un veterinario	frecuencia cepilla los dientes	Enfermedades crónicas	antibióticos en las últimas 4 semanas
Mixto	Pienso seco	Nunca	Rara vez	No	Nunca	Nunca	Otra	No
Mixto	Pienso seco	Rara vez	No estoy seguro/a	No	Nunca	Mensualmente	Ninguna	No
Mixto	Pienso seco	A veces	Rara vez	No	Nunca	Nunca	Ninguna	No
Mayormente en el interior	Pienso seco	Frecuentemente	Rara vez	No	Más de 2 años	Nunca	Ninguna	No
Mayormente en el	Mixta	A veces	Rara vez	No	Nunca	Nunca	Ninguna	No
Mayormente en el	Pienso seco	Frecuentemente	Rara vez	Sí	1–2 años	Nunca	Ninguna	No
Mayormente en el interior	Pienso seco	A veces	Rara vez	No	Nunca	Nunca	Ninguna	No
Mayormente en el interior	Pienso seco	Frecuentemente	Rara vez	Sí	Menos de 12 meses	Mensualmente	Ninguna	No
Mayormente en el interior	Pienso seco	Rara vez	Rara vez	No	Nunca	Nunca	Ninguna	No
Mayormente en el interior	Pienso seco	A veces	Rara vez	No	Nunca	Semanalmente	Cardíacas	No

9.4. Annex 4 – Example of SPIRAL qPCR results: patient 10

P3WGDFNH2JEQF6H

Número de informe: P3WGDFNH2JEQF6H
Tipo de técnica: qPCR
Fecha de recogida: 30/03/2026

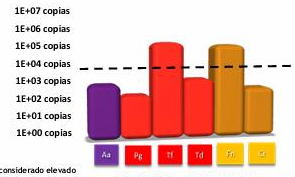
Fecha de recepción: 01/04/2026
Fecha de informe: 08/04/2026
Clinica: Universidad Europea

BACTERIAS PERIODONTALES/PERIIMPLANTARIAS/ABSCESOS	
POSITIVO	NEGATIVO
Tf Fn	Aa Pg Td Cr Sa Sv (spp) Pn

BIOMARCADORES	
MUTACIÓN DETECTADA	MUTACIÓN NO DETECTADA
IL-1B IL-RA IL-6 IL-1A	

IL-RA: Genotipo Normal
IL1A: Genotipo Normal
IL-1B: Predisposición a una respuesta inflamatoria menos eficaz
IL-6: Genotipo Normal

CUANTIFICACIÓN DE BACTERIAS PERIODONTALES/PERIIMPLANTARIAS



*Valor superior a 10^4 copias es considerado elevado
Aa: *Aggregatibacter actinomycetemcomitans*; **Pg:** *Porphyromonas gingivalis*; **Tf:** *Tannarella forsythia*; **Td:** *Teponema denticola*; **Fn:** *Fusobacterium nucleatum* **Cr:** *Campylobacter rectus*

RIESGO SEGÚN LA PIRÁMIDE DE SOCRANSKY

ALTO RIESGO	Bacterias Aa, Pg, Tf, o Td superior 10^4 **copias**
RIESGO MEDIO	Bacterias Fn, Cr superior 10^4 **copias**
BAJO RIESGO	Sin Bacterias Superior 10^4**copias**

P3WGDFNH2JEQF6H

RELACIÓN CON ENFERMEDADES SISTÉMICAS

Según diversas publicaciones científicas, elevadas concentraciones de bacterias periodontales pueden aumentar el riesgo de desarrollar las siguientes enfermedades:



Jiménez De Nurizio, S. et al. (2023) 'Does a dysbiotic oral microbiome trigger the risk of chronic inflammatory disease?', Current Treatment Options in Allergy, 10(4), pp. 364-383.

CUANTIFICACIÓN DE BACTERIAS DE ABSCESOS

Bacterias	*Método*	*Resultado*
Prevotella nigrescens	*Real time PCR*	10^2 copias *Baja concentración*
Staphilococcus aureus	*Real time PCR*	10^2 copias *Baja concentración*
Streptococcus viridans species (spp)	*Real time PCR*	10^2 copias *Baja concentración*

CUANTIFICACIÓN DE S.MUTANS Y CANDIDA

Bacterias	*Método*	*Resultado*
Streptococcus mutans	*Real time PCR*	10^2 copias *Baja concentración*
Hongo	***Método***	***Resultado***
Candida spp	*Real time PCR*	10^2 copias *Baja concentración*

Nombre Dentista Colegiado	
Firma	

Resp. Laboratorio Colegiado	
Firma	

P3WGDFNH2JEQF6H

CUANTIFICACIÓN DE BACTERIAS PERIODONTALES/PERIIMPLANTARIAS EXACTAS

Bacterias	*Método*	*Resultado*
Aggregatibacter actinomycetemcomitans	*Real time PCR*	1001,02
Porphyromonas gingivalis	*Real time PCR*	256,47
Tannarella forsythia	*Real time PCR*	167581,09
Teponema denticola	*Real time PCR*	1775,81
Fusobacterium nucleatum	*Real time PCR*	126039,79
Campylobacter rectus	*Real time PCR*	554,56

CUANTIFICACIÓN DE BACTERIAS DE ABSCESOS EXACTAS

Bacterias	*Método*	*Resultado*
Prevotella nigrescens	*Real time PCR*	***89,38***
Staphilococcus aureus	*Real time PCR*	***92,30***
Streptococcus viridans species (spp)	*Real time PCR*	***88,56***

9.5. Annex 5 –Summary of quantitative results

		CUANTIFICACIÓN DE BACTERIAS PERIODONTALES/PERIIMPLANTARIASEXACTAS					
		Aggregatibacter actinomycetemcomitans	Porphyromonas gingivalis	Tannarella forsythia	Teponema denticola	Fusobacterium nucleatum	Campylobacter rectus
	pet	20,07	82,85	152,43	524,76	355,2	163,48
1	human	685,62	60344,48	26625,14	44207,18	20,4	49,68
	pet	24955,39	46309	30789,79	180,96	85307,5	163,48
2	human	723,41	906,71	41595,34	8235,38	671820,59	86,96
	pet	43143,36	76,6	1278,79	47,56	3658	554,56
3	human	124,61	193,67	64611,73	83661,49	72273,48	2,54
	pet	72509,92	481,29	4986,9	84,6	121029,6	554,56
4	human	30,46	27347,17	132930,66	87499,32	1367976,43	164,06
	pet	787,13	4404,99	52645,21	46,75	106517,13	163,48
5	human	1120,7	496,1	37619,13	4688,3	177404,98	39,31
	pet	219728,58	260,32	3974,59	399,28	155839,73	163,48
6	human	129,67	189,09	46049,25	211,62	47943,68	384,41
	pet	20,07	1024,59	26413,58	375,77	127239,48	163,48
7	human	441,77	6214,59	189785,61	40343,82	793875,17	723,86
	pet	1842,6	27497,68	422000,56	75228,13	521116,69	554,56
8	human	246,09	4015,15	177072,97	28087,67	761691,99	107,48
	pet	67,28	1411601,96	54390,69	871,9	486158,04	361,79
9	human	193,5	130008,6	64969,3	1705,2	224250,12	27,33
	pet	20,07	82,85	3011,57	1563,56	3526,24	554,56
10	human	1001,02	256,47	167581,09	1775,81	126039,79	554,56

CUANTIFICACIÓN DE BACTERIAS DE ABSCESOSEXACTAS		
Prevotella nigrescens	Staphilococcus aureus	Streptococcus viridans species (spp)
29597,7	280,4	30,39
29597,7	280,1	671,2
14119,6	157,2	18,1
14119,2	157	18
692,6	89,3	10,9
69276,7	89	18
63947,7	510,8	280,6
63946,7	510,86	280,2
14119,6	30,32	10,9
141,2	30,3	89,4
692,6	89,3	92,4
692,7	89,3	89,3
29597,7	280,1	510,4
79466,5	348,7	14119,5
348,7	89,3	94,8
29597,7	348,7	348,7
14276,5	948,2	89,3
14119,3	948,48	280,61
89,3	179,8	89,8
89,38	92,3	88,56

9.6. Annex 6 – Informed Consent

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HOJA DE INFORMACIÓN

Título del estudio: Transferencia microbiana entre humanos y mascotas: análisis comparativo de bacterias periodontales indicadoras en muestras orales de personas y animales de compañía.

Promotor: Universidad Europea de Madrid
Investigadores: Rocio Ortega Sánchez, Rocío Bustamante, Lorena Muñoz, Rocío Paniagua Jimenez, Alberto Adanero Velasco, Jean-Baptiste Biard y David Álvarez Cilleros.
Centro: Universidad Europea de Madrid.

Nos dirigimos a usted para informarle sobre un estudio de investigación que se va a realizar en la Universidad Europea de Madrid, en el cual se le invita a participar. Este documento tiene por objeto que usted reciba la información correcta y necesaria para evaluar si quiere o no participar en el estudio. A continuación, le explicaremos de forma detallada todos los objetivos, beneficios y posibles riesgos del estudio. Si usted tiene alguna duda tras leer las siguientes aclaraciones, nosotros estaremos a su disposición para aclararle las posibles dudas. Finalmente, usted puede consultar su participación con las personas que considere oportuno.

¿Cuál es el motivo de este estudio?

Este proyecto pretende contribuir a la comprensión de los patrones de colonización microbiana y determinar si existe evidencia de transferencia bidireccional de microorganismos orales, con posible impacto en la salud periodontal humana y veterinaria.

RESUMEN DEL ESTUDIO:

1. **Título:**

Transferencia microbiana entre humanos y mascotas: análisis comparativo de bacterias periodontales indicadoras en muestras orales de personas y animales de compañía.

2. **Justificación y objetivos:**

La convivencia estrecha entre humanos y animales de compañía crea un ecosistema compartido que favorece el intercambio microbiano. Diversos estudios han demostrado similitudes en el microbioma oral entre personas y mascotas que comparten entorno; sin embargo, existe escasa evidencia específica acerca de la presencia simultánea de bacterias periodontales indicadoras y potencialmente patógenas en ambos hospedadores.

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Este proyecto pretende contribuir a la comprensión de los patrones de colonización microbiana y determinar si existe evidencia de transferencia bidireccional de microorganismos orales, con posible impacto en la salud periodontal humana y veterinaria.

Objetivo principal:
Evaluar la presencia, coincidencia y posible transferencia de bacterias periodontales indicadoras y potencialmente patógenas entre humanos y sus mascotas (perros y/o gatos) mediante análisis microbiológicos de muestras orales.

Objetivos específicos:

1. Determinar la presencia y carga de bacterias periodontales diana en humanos y animales convivientes.
2. Comparar los perfiles microbianos obtenidos entre ambos hospedadores.
3. Identificar factores asociados a la similitud microbiana (hábitos de higiene, convivencia, contacto físico).

3.Participantes

3.1. Participantes humanos

- Adultos mayores de 18 años.
- Convivencia habitual con uno o más perros o gatos.
- Capacidad para comprender y firmar consentimiento informado.

3.2. Animales de compañía

- Perros y/o gatos que convivan con los participantes humanos incluidos.
- Autorización expresa del responsable legal del animal para la toma de muestras orales.

4. **Instrumentos/procedimientos recogida datos/muestras**

Análisis mediante qPCR tanto en humanos como en las mascotas.

4.1. Datos demográficos

- Humanos: edad, sexo, hábitos de higiene oral, frecuencia de contacto con la mascota.
- Mascotas: especie, edad aproximada, hábitos de higiene dental, tipo de dieta.

4.2. Datos clínicos

- Indicadores periodontales básicos en humanos.
- Observación clínica externa del estado oral de la mascota.

4.3. Datos microbiológicos

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- Presencia/ausencia de bacterias periodontales diana (por ejemplo, *P. gingivalis*, *T. forsythia*, *A. actinomycetemcomitans* entre otras), presencia de 1 hongo, 4 biomarcadores inflamatorios y metabolización antibiótica.
- Perfiles comparativos de microbioma oral (según técnica utilizada).

PARTICIPACIÓN VOLUNTARIA Y RETIRADA DEL ESTUDIO: La participación en este estudio es voluntaria, por lo que puede decidir no participar. En caso de que decida participar, puede retirar su consentimiento en cualquier momento. En caso de que usted decidiera abandonar el estudio, puede hacerlo permitiendo el uso de los datos obtenidos hasta ese momento para la finalidad del estudio, o si fuera su voluntad, todos los registros y datos serán borrados de los ficheros informáticos.

¿Quién puede participar?

Criterios de inclusión y exclusión

1. Inclusión (humanos)

- ≥18 años.
- Convivencia con perro/gato durante al menos 6 meses.
- Aceptación del procedimiento y firma del consentimiento informado.

2. Exclusión (humanos)

- Tratamiento antibiótico en las últimas 4 semanas.
- Enfermedades sistémicas inmunosupresoras no controladas (por razones metodológicas).
- Cirugía periodontal reciente (<3 meses).

3. Exclusión (animales)

- Tratamiento antibiótico veterinario en las últimas 4 semanas.
- Condiciones que contraindiquen la manipulación oral leve (según criterio veterinario).

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¿En qué consiste el estudio y mi participación? Estudio observacional, transversal, con recogida de muestras biológicas en un único momento por participante humano y mascota.

¿Cuáles son los posibles beneficios y riesgos derivados de mi participación?

No se prevén beneficios individuales directos, aunque los resultados podrán aportar información relevante sobre la salud oral humana y veterinaria, así como sobre interacciones microbianas entre especies que conviven. En cuanto a los riesgos, estos serán mínimos,asociados a la toma de muestras orales (molestias leves o sangrado puntual). Y en el caso de los animales, la toma será no invasiva y de muy baja manipulación. El personal estará formado para asegurar bienestar y minimizar estrés animal.

¿Quién tiene acceso a mis datos personales y como se protegen? El tratamiento, la comunicación y la cesión de los datos de carácter personal de todos los sujetos participantes se ajustará a lo dispuesto en la Ley Orgánica 3/2018 de 5 de diciembre de Protección de Datos Personales y garantía de los derechos digitales. De acuerdo a lo que establece la legislación mencionada, usted puede ejercer los derechos de acceso, modificación, oposición y cancelación de datos, para lo cual deberá dirigirse a la persona responsable del estudio.

Los datos recogidos para el estudio estarán identificados mediante un código y solo el investigador responsable podrá relacionar dichos datos con usted. Por lo tanto, su identidad no será revelada a persona alguna salvo excepciones, en caso de urgencia médica o requerimiento legal. Sólo se transmitirán a terceros y a otros países los datos recogidos para el estudio que en ningún caso contendrán información que le pueda identificar directamente, como nombre y apellidos, iniciales, dirección, número de la seguridad social, etc. En el caso de que se produzca esta cesión, será para los mismos fines del estudio descrito y garantizando la confidencialidad como mínimo con el nivel de protección de la legislación vigente en nuestro país. El acceso a su información personal quedará restringido al responsable del estudio, pero siempre manteniendo la confidencialidad de los mismos de acuerdo con la legislación vigente.

¿Recibiré algún tipo de compensación económica? No se prevé ningún tipo de compensación económica durante el estudio. Si bien, su participación en el estudio no le supondrá ningún gasto.

¿Quién financia esta investigación? La empresa responsable de las PCRs es la responsable de gestionar la financiación del mismo en los 20 primeros casos.

OTRA INFORMACIÓN RELEVANTE: Si usted decide retirar el consentimiento para participar en este estudio, ningún dato nuevo será añadido a la base de datos y puede exigir la destrucción de sus datos y/o de todos los registros identificables, previamente retenidos, para evitar la realización de otros análisis. También debe saber que puede ser excluido del estudio si los investigadores del estudio lo consideran oportuno, ya sea por motivos de seguridad, por cualquier acontecimiento adverso que se produzca o porque consideren que no está cumpliendo con los procedimientos establecidos. En cualquiera de los casos, usted recibirá una explicación adecuada del motivo que ha ocasionado su retirada del estudio.

CALIDAD CIENTÍFICA Y REQUERIMIENTOS ÉTICOS DEL ESTUDIO: Este estudio ha sido sometido a aprobación por la Comisión de Investigación de la Universidad Europea de Madrid, Valencia y Canarias, que vela por la calidad científica de los proyectos de investigación que se llevan a cabo. Cuando la investigación se hace con personas, este Comité vela por el cumplimiento de lo establecido en la Declaración de Helsinki y la normativa legal vigente sobre investigación biomédica (ley 14/2007, de junio de investigación biomédica) y ensayos clínicos (R.D. 223/2004 de 6 de febrero, por el que se regulan los ensayos clínicos con medicamentos, modificado por Real Decreto 1276/2011, del 16 de septiembre).

PREGUNTAS: Llegando este momento le damos la oportunidad de que, si no lo ha hecho antes, haga las preguntas que considere oportunas. El equipo investigador le responderá lo mejor que sea posible.

INVESTIGADORES DEL ESTUDIO: Si tiene alguna duda sobre algún aspecto del estudio o le gustaría comentar algún aspecto de esta información, por favor no deje de preguntar a los miembros del equipo investigador: Rocio Ortega Sánchez. En caso de que una vez leída esta información y aclaradas las dudas decida participar en el estudio, deberá firmar su consentimiento informado. Este estudio ha sido aprobado por la Comisión de Investigación de la Universidad Europea de Madrid, Valencia y Canarias.

CONSENTIMIENTO INFORMADO:

D./Dª. ---------- de ------ años, con DNI---------- y domicilio en ---------- declara que ha recibido una explicación satisfactoria sobre el procedimiento del estudio, su finalidad, riesgos, beneficios y alternativas.

Asimismo, que ha quedado satisfecho/a con la información recibida, la he comprendido, se han respondido todas sus dudas y comprende que su participación es voluntaria.

Por lo tanto, declara que presta su consentimiento para el procedimiento propuesto y conoce su derecho a retirarlo cuando lo desee, con la única obligación de informar sobre su decisión a la persona responsable del estudio.

En Madrid, a día 24 de Noviembre de 2025.

_____	_____
Firma y del investigador	Firma y Nº de DNI del participante

*En casos de menores entre 12 y 18 años, debe hacerse una hoja por duplicado: una para el menor (con el texto informativo adaptado a la comprensión y el trato correspondientes a un adolescente) y otra para el representante legal de ese menor. En caso de menores de 12 años, solo firma el consentimiento el representante legal. El modelo para ambos casos varía en la primera línea y en la firma, del siguiente modo:

D./Dª. _____, de____ años, con DNI_____, representante legal de
D./Dª. _____, de____ años, con DNI _____,
(...)

Firma y Nº de DNI del representante legal del paciente