Odovtos-International Journal of Dental Sciences (Odovtos-Int. J. Dent. Sc.), Online First, 2026. ISSN: 2215-3411
https://doi.org/10.15517/mg02sz23

ODOVTOS
International Journal of Dental Sciences

UNIVERSIDAD DE COSTA RICA FOd Facultad de Odontología

https://revistas.ucr.ac.cr/index.php/Odontos

CLINICAL RESEARCH:

Identification of *Streptococcus mutans* and *Lactobacillus spp*. in a Community on the Amazon Riviera: a Cross-Sectional Study

Identificación de *Streptococcus mutans* y *Lactobacillus spp*. en una comunidad de la ribera amazónica: un estudio de corte transversal

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Received: 3-IX-2025 Accepted: 14-XI-2025

ABSTRACT: Dental caries is a chronic and infectious disease of high global prevalence, particularly affecting vulnerable populations such as riverine communities in the Peruvian Amazon. Its etiology is associated with an imbalance in the oral microbiome, where *Streptococcus mutans* and *Lactobacillus spp*. play a key role. This study aimed to determine the prevalence of these bacteria and their association with the DMFT index, salivary pH, and sociodemographic variables. A cross-sectional study was conducted among 65 adults aged 18 to 65 years from Amazon River communities, evaluated through the Medical Program Esperanza Amazónica – Vine Trust. Sociodemographic data were collected, the DMFT index was calculated, and saliva samples were analyzed to identify *S. mutans* and *Lactobacillus spp*. using selective culture media. Salivary pH was measured digitally. Non-parametric statistical tests (Chi-square and Spearman) were applied, with a significance level set at $p<0.05$. *Lactobacillus spp*. was detected in 63.1% of participants and *S. mutans* in 24.6%. A significant positive correlation was found between both bacteria and the DMFT index (S. mutans: rho=0.638; *Lactobacillus spp*.: rho=0.484; $p<0.001$). Coinfection was associated with a significantly higher DMFT score ($p=0.03$). Although no significant correlations were found with salivary pH or sex, a trend toward greater bacterial colonization was observed in women and in more acidic environments. The presence of *S. mutans* and *Lactobacillus spp*. was associated with greater caries severity, with coinfection representing a potential additional risk factor. These findings highlight the need for targeted preventive interventions in Amazonian communities.

KEYWORDS: Oral microbiome; Dental caries; *Streptococcus mutans*; *Lactobacillus spp*.; Peruvian Amazon.

RESUMEN: La caries dental es una enfermedad crónica e infecciosa de alta prevalencia a nivel mundial, que afecta de manera particular a poblaciones vulnerables, como las comunidades ribereñas de la Amazonía peruana. Su etiología se asocia a un desequilibrio del microbioma oral, en el cual *Streptococcus mutans* y *Lactobacillus spp*. desempeñan un papel fundamental. El objetivo de este estudio fue determinar la prevalencia de estas bacterias y su asociación con el índice CPOD, el pH salival y variables sociodemográficas. Se realizó un estudio transversal en 65 adultos de 18 a 65 años pertenecientes a comunidades ribereñas del río Amazonas, evaluados a través del Programa Médico Esperanza Amazónica – Vine Trust. Se recolectaron datos sociodemográficos, se calculó el índice CPOD y se analizaron muestras de saliva para la identificación de *S. mutans* y *Lactobacillus spp*. mediante medios de cultivo selectivos. El pH salival se midió de forma digital. Se aplicaron pruebas estadísticas no paramétricas (chi cuadrado y correlación de Spearman), estableciendo un nivel de significancia de $p<0,05$. *Lactobacillus spp*. se detectó en el 63,1 % de los participantes y *S. mutans* en el 24,6 %. Se encontró una correlación positiva significativa entre ambas bacterias y el índice CPOD (*S. mutans*: rho=0,638; *Lactobacillus spp*.: rho=0,484; $p<0,001$). La coinfección se asoció con un puntaje CPOD significativamente mayor ($p=0,03$). Aunque no se observaron asociaciones significativas con el pH salival ni con el sexo, se evidenció una tendencia a una mayor colonización bacteriana en mujeres y en ambientes más ácidos. La presencia de *S. mutans* y *Lactobacillus spp*. se asoció con mayor severidad de caries, siendo la coinfección un posible factor de riesgo adicional. Estos hallazgos resaltan la necesidad de implementar intervenciones preventivas focalizadas en comunidades amazónicas.

PALABRAS CLAVE: Microbioma oral; Caries dental; *Streptococcus mutans*; *Lactobacillus spp*.; Amazonía peruana.

INTRODUCTION

Oral health constitutes a fundamental pillar of overall well-being and quality of life, as it is closely related to the general health status of individuals, as described by Doughty *et al*. and De Paiva Buischi *et al*. (1,2). Among the main oral pathologies, such as dental caries and periodontal diseases, these are defined as chronic, multifactorial, and infectious conditions, and are considered a global public health challenge due to their high prevalence and complex etiopathogenic process, in which the oral microbiota plays a decisive role, according to Spatafora *et al*. and Liu *et al*. (3,4).

The microbial ecosystem, established early in life, may undergo imbalances or dysbiosis that promote the onset of various oral diseases, including caries and periodontal pathologies, as noted by Tuganbaev *et al*. (5). In this context, *Streptococcus mutans* and *Lactobacillus spp*. have been identified as the main cariogenic agents, due to their ability to metabolize carbohydrates, produce acids, and form biofilms that facilitate bacterial adhesion and the progression of the aforementioned diseases, consistent with findings by Tandon *et al*. (6).

The Peruvian Amazon region presents particular conditions that exacerbate this problem: limited access to health services, sugar-rich dietary habits, and socioeconomic factors that increase the population's vulnerability to dental caries, as observed by Chen *et al*. (7). Within this scenario, the Medical Program Esperanza Amazónica del Perú – Vine Trust constitutes an ideal platform for the investigation of the oral microbiota in riverine communities.

The present study aimed to determine the prevalence of *Streptococcus mutans* and *Lactobacillus spp*. in riverine communities along the Amazon River and to analyze their relationship with the DMFT index, salivary pH, and sociodemographic variables (age and sex), in order to identify factors associated with the severity of dental caries in the Ucayali River basin.

METHODS

ETHICAL DECLARATION

This study was approved by the Institutional Research Ethics Committee of Universidad Científica del Sur (code 903-CIEI-CIENTÍFICA-2025). All participants signed informed consent in accordance with the principles of the Declaration of Helsinki.

POPULATION AND ELIGIBILITY CRITERIA

This research employed a cross-sectional study design. The study population consisted of residents of riverine communities along the Amazon River (Barrio Florido, Santa Clara, Santa María de Ojeal, Sinchicuy, San Rafael, Santa Victoria, Timicuro, Manco Cápac, Nuevo Triunfo, and Timicuro Grande) in Iquitos, Peru. Individuals aged 18 to 65 years who were attended by the Medical Program Esperanza Amazónica – Vine Trust were included.

Patients with systemic or immunological comorbidities, recent antibiotic treatment, or dental interventions within the last three months were excluded. Incomplete records were eliminated, and a non-probabilistic convenience sampling approach was employed. The sample size was calculated using the formula for estimating a proportion, assuming a 95% confidence level, an expected prevalence of 5%, and a precision of 6%. These parameters yielded a minimum required sample size of approximately 51 participants.

DATA COLLECTION PROCESS

Data collection was carried out directly in the communities using a boat as a mobile working unit to facilitate access to dispersed populations and to ensure controlled conditions during sample collection. The boat was equipped with portable worktables, refrigerators for temporary storage of samples, and all necessary biosafety materials, including gloves, masks, disposable gowns, and hand sanitizer.

Participants were recruited in coordination with local authorities and community health personnel. Individuals who voluntarily approached for information were invited to participate; the study's purpose was explained, and informed consent was obtained. They were then directed to the dental area, where participants were seated in a dental unit, administered a sociodemographic questionnaire (age, sex, and place of residence), underwent intraoral examination to determine the DMFT index, and subsequently provided a saliva sample. At the end of the process, participants received oral health counseling and their participation was acknowledged.

DENTAL CARIES EXPERIENCE ASSESSMENT

Dental caries experience was evaluated using the DMFT index (Decayed, Missing, and Filled Teeth). Each permanent tooth was clinically examined with a mirror and explorer, recording teeth with active caries (D), teeth lost due to caries (M), and those restored as a result of caries (F). The individual DMFT was calculated as the sum of D + M + F, providing a quantitative score that reflects the cumulative burden of caries for each participant. This allowed for comparisons among individuals and analyses of its relationship with microbiological and clinical variables. Finally, DMFT values were categorized as none, low, moderate, or high caries experience.

Two examiners (G.A.P.-S. and C.P.-P.), underwent standardized training and calibration for the assessment of the DMFT index, following the diagnostic criteria established by the World Health Organization (WHO). Calibration was conducted on a pilot sample of seven adult volunteers who were not included in the main study. Each examiner performed independent DMFT assessments on the same participants, and re-examinations were carried out one week later to determine intra-examiner reproducibility. Agreement was evaluated using Cohen's kappa (κ) and percentage concordance for the presence or absence of decayed, missing, and filled teeth at the tooth level. The inter-examiner agreement yielded a κ value of 0.82 (92.3% agreement), while the intra-examiner reliability was κ=0.88 (94.1% agreement).

SAMPLE PROCESSING

Saliva samples were individually collected in sterile 5 mL tubes. Participants were instructed to refrain from food or drink consumption for at least one hour prior to collection to minimize microbiota variation. Each participant chewed sugar-free gum and discarded the initial saliva to eliminate food debris. Subsequently, 2 mL of saliva were collected in sterile cryovials (ALMED, Peru) and preserved at 4 °C until processing in the mobile laboratory on the boat. Each sample was labeled with indelible marker using a unique code, recording age, sex, and community of origin.

Samples were homogenized with a vortex mixer (DLab, China) for 30 seconds at 10,000 RPM. Serial dilutions (1:1000) were prepared in 0.9% saline solution, and 50 µL of the final dilution was inoculated onto Mitis Salivarius agar (HiMedia, India), supplemented with 1% potassium tellurite and 0.04 U bacitracin, selective for *Streptococcus mutans*, and 50 µL onto Rogosa agar (HiMedia, India), selective for *Lactobacillus spp*. Plates were incubated at 37 °C for 24-48 hours under CO_2 atmosphere or in anaerobic conditions using the candle jar method, depending on the microorganism studied. Colony counts were performed using a colony counter (ISOLAB, Germany), and bacterial identification was confirmed through standard biochemical tests.

Salivary pH of each sample was measured using a compact digital pH meter (Horiba, Japan). All results were recorded in standardized forms and digitized daily to ensure traceability. At all times, personnel maintained biosafety level II precautions and disposed of biological waste according to safe protocols, ensuring bacterial viability and data quality for determining the prevalence of *S. mutans* and *Lactobacillus spp*. in the study population.

DATA ANALYSIS PLAN

All data were processed using SPSS v.29 (IBM, USA). Normality of quantitative variables was assessed with the Shapiro-Wilk test. Variables that did not meet normality assumptions were analyzed with non-parametric tests, including Kruskal-Wallis, Mann-Whitney U, and Spearman correlation. Variables with normal distribution were analyzed with parametric tests, using a significance threshold of $p<0.05$. To explore the relationship between the DMFT index and the presence of the studied bacteria, the Chi-square test was applied. Results were presented in frequency and percentage tables for categorical variables, and in measures of central tendency and dispersion for quantitative variables.

Additionally, a simple multivariate analysis was performed using binary logistic regression to explore the independent association between the presence of *Streptococcus mutans* and *Lactobacillus spp*. with high caries experience (DMFT categorized as high *vs*. low/moderate), adjusting for age and sex. Odds ratios (OR) with 95% confidence intervals (CI) were calculated.

RESULTS

The study included a total of 65 participants. The detailed distribution by age, sex, and place of residence is presented in Table 1. Regarding the presence of cariogenic bacteria, a prevalence of *Lactobacillus spp*. was observed in 63.1% of individuals, while *Streptococcus mutans* was detected in 24.6%. The mean salivary pH was 6.02, indicating a tendency toward a slightly acidic environment. These findings were significantly associated with the DMFT index, showing a moderate positive correlation between *S. mutans* and DMFT (rho=0.638; p<0.001), as well as between *Lactobacillus spp*. and DMFT (rho=0.484; p<0.001).

With respect to salivary pH, although no statistically significant correlations were found with the individual presence of *S. mutans* (rho=–0.063; p=0.615) or *Lactobacillus spp*. (rho=–0.076; p=0.547), a trend toward more acidic pH values was observed in cases with higher bacterial colonization and greater DMFT scores (rho=–0.189; p=0.131) (Figure 1).

Sex-based analysis revealed no statistically significant differences either in the DMFT index (rho=–0.085; p=0.500) or in bacterial levels (*S. mutans*: rho=0.034; p=0.790; *Lactobacillus spp.*: rho=–0.144; p=0.251). Nonetheless, a slight trend toward higher colonization was recorded among women, although this difference did not reach statistical significance (Table 2 and Table 3). In addition, 20% of participants presented coinfection with *Streptococcus mutans* and *Lactobacillus spp.*, which was associated with a significantly higher DMFT index ($\chi^2 = 6.985$; $p = 0.03$) (Table 4).

In the multivariate logistic regression analysis adjusted for age and sex, the presence of *Streptococcus mutans* was independently associated with a higher likelihood of high caries experience (OR=3.41; 95% CI: 1.18-9.85; p=0.024). The association between Lactobacillus spp. and high DMFT did not reach statistical significance (OR=2.12; 95% CI: 0.84-5.34; p=0.11). Participants coinfected with both bacteria showed the strongest association with severe caries experience (OR=4.78; 95% CI: 1.32-17.29; p=0.018). Age and sex were not significant predictors in any model (Table 5).

Table 1. Sociodemographic profile of the population.

Variable	Frequency (n)	Percentage (%)
Sex		
Male	33	50.8
Female	32	49.2
Age		
18-25	15	23.1
26-35	18	27.7
36-45	14	21.5
46-55	10	15.4
56 or older	8	12.3
Community		
Barrio Florido	5	7.7
Santa Clara	8	12.3
Santa María de Ojeal	6	9.2
Sinchicuy	7	10.8
San Rafael	4	6.2
Santa Victoria	9	13.8
Timicuro	6	9.2
Manco Cápac	5	7.7
Nuevo Triunfo	10	15.4
Timicuro Grande	5	7.7

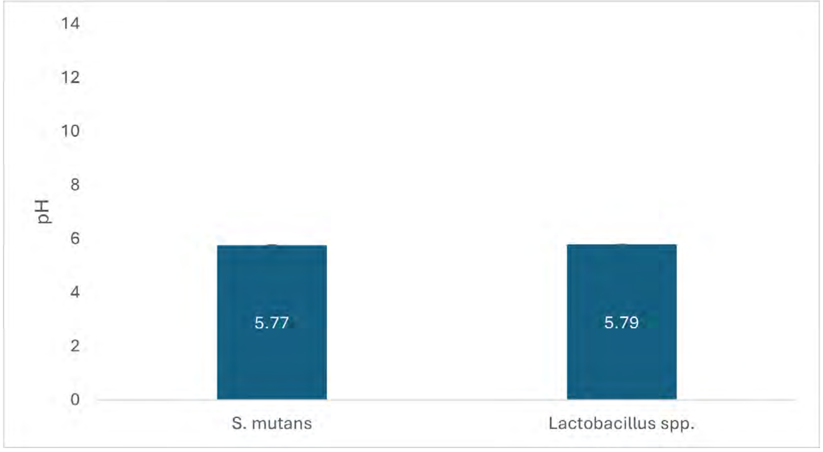

Figure 1. Distribution of pH levels according to bacterial presence. Shows the distribution of pH levels in the presence of *S. mutans* and *Lactobacillus spp*. Data reveal that the mean pH in samples with *S. mutans* was 5.7650 (SD = 1.2478; n=16), while samples with *Lactobacillus spp*. had a mean pH of 5.7920 (SD=1.1577; n=41).

Table 2. Prevalence of *S. mutans* and *Lactobacillus spp*. by sex.

Variable		Woman	Man	Test χ2	
		n (%)	n (%)	χ2	p-value
Presence of *S. mutans*	No	23 (35.4 %)	26 (40.0 %)	0.049	0.824
	Yes	7 (10.8 %)	9 (13.8 %)		
Presence o *Lactobacillus spp.*	No	10 (15.4 %)	14 (21.5 %)	0.308	0.579
	Yes	20 (30.8 %)	21 (32.3 %)		
Presence of bacteria	No bacteria	9 (13.8 %)	12 (18.5 %)	0.154	0.926
	One bacterium	15 (23.1 %)	16 (24.6 %)		
	Two bacteria	6 (9.2 %)	7 (10.8 %)		
Caries experience (DMFT)	None	2 (3.1 %)	0 (0.0 %)	4.356	0.225
	Low	7 (10.8 %)	14 (21.5 %)		
	Moderate	10 (15.4 %)	12 (18.5 %)		
	High	11 (16.9 %)	9 (13.8 %)		

Table 3. Distribution of pH levels according to bacterial presence.

Bacterial presence	Mean	Std. Deviation	N	Minimum	Maximum
No *S. mutans*	6.0994	1.08165	49	3.57	7.72
Yes *S. mutans*	5.7650	1.24780	16	3.46	7.52
No *Lactobacillus spp.*	6.4017	0.96955	24	4.19	7.72
Yes *Lactobacillus spp.*	5.7920	1.15773	41	3.46	7.52

Table 4. Prevalence of *S. mutans* and *Lactobacillus spp*. according to DMFT levels.

Caries experience (DMFT)	*S. mutans*[a]	*Lactobacillus spp.*[a]	Test χ2	
	n (%)	n (%)	χ2	p-value
None	0 (0.0 %)	0 (0.0 %)		
Low	0 (0.0 %)	6 (10.5 %)	6.985	0.03
Moderate	3 (5.3 %)	17 (29.8 %)		
High	13 (22.8 %)	18 (31.6 %)		

Sample size: n=65 patients. Only samples (patients) with a positive diagnosis for the bacteria: 57 (100%) positive samples.

Table 5. Multivariate logistic regression models adjusted for age and sex.

Variable	Model 1: *S. mutans*	Model 2: *Lactobacillus spp.*	Model 3: Coinfection (*S. mutans* + *Lactobacillus spp.*)
Presence of bacteria	OR=3.41 (1.18-9.85), p=0.024	OR=2.12 (0.84-5.34), p=0.11	OR=4.78 (1.32-17.29), p=0.018
Age (years)	OR=1.02 (0.97-1.08), p=0.36	OR=1.03 (0.98-1.08), p=0.29	OR=1.01 (0.96-1.07), p=0.64
Sex (woman vs. man)	OR=1.29 (0.54-3.07), p=0.56	OR=1.34 (0.57-3.15), p=0.49	OR=1.11 (0.46-2.71), p=0.81

DISCUSSION

This study revealed a high prevalence of cariogenic bacteria in riverine communities of the Peruvian Amazon, demonstrating a significant association between the presence of *Streptococcus mutans*, *Lactobacillus spp*., and elevated DMFT scores, particularly in cases of coinfection, within the context of slightly acidic salivary pH and without significant differences by sex.

These results confirm the active colonization of the oral microbiota by acidogenic and aciduric bacteria, which play a determining role in the progression of dental caries, consistent with previous reports by Martignon *et al*., Jalal Hussein *et al*., and Wen *et al*. (8,9,10).

The positive correlation between the DMFT index and the presence of *S. mutans* (rho=0.638), as well as the moderate association with *Lactobacillus spp*. (rho=0.484), reinforces the complementary etiopathogenic role of both bacteria. While *S. mutans* is recognized as the main initiator of the carious process, *Lactobacillus spp*. contributes to the progression of lesions under low pH conditions, consistent with the findings of Yu e*t al*., Steier *et al*. and Chianca *et al*. (11,12,13). The fact that 20% of coinfected patients presented the highest DMFT values suggests a synergistic effect between the two microorganisms, as also described by Bahurupi *et al*., Danaei *et al*. and Lin *et al*. (14,15,16).

Regarding sex-based analysis, although no statistically significant differences were identified ($p>0.05$), a trend toward greater colonization in women was observed. This finding partially aligns with the observations of Pecci-Lloret *et al*. and Cerutis *et al*., who suggest that hormonal factors may influence susceptibility to bacterial colonization, although they recognize that differences do not always reach statistical significance in studies with reduced sample sizes (17,18).

Salivary pH emerged as a key modulator of bacterial colonization and DMFT severity. Patients with acidic pH exhibited higher levels of *S. mutans* and *Lactobacillus spp*., as well as greater DMFT scores. This is consistent with findings by Zhou *et al*. and Szkaradkiewicz-Karpinska *et al*., who reported that acidic environments favor the metabolism and proliferation of cariogenic microorganisms (19,20).

Methodologically, the use of non-parametric tests (Chi-square and Spearman) was justified by the absence of normality in the data according to the Shapiro-Wilk test ($p<0.05$). This choice is consistent with the recommendations of Soundaram *et al*., ensuring the validity of the analyses (21). Factor analysis confirmed the pertinence of the statistical constructs by explaining 40.77% of the variance, this was performed to evaluate the internal consistency and underlying structure of the numerical variables included in the model (DMFT, salivary pH, and bacterial counts).

Finally, this study provides relevant evidence on oral health in Amazonian communities, a historically vulnerable group. The information obtained constitutes a solid scientific basis for designing more effective preventive and control programs against dental caries, as highlighted by Rai *et al*., Gracco *et al*., and Evaristo-Chiyong *et al*., who emphasize the importance of population-based studies to inform public health policies in regional and national contexts (22,23,24).

STRENGTHS OF THE STUDY

One of the main strengths of this study lies in its community-based and contextualized approach, focusing on riverine populations of the

Peruvian Amazon, which have traditionally been underrepresented in scientific research. The use of a mobile unit equipped with clinical and laboratory facilities enabled access to geographically challenging areas while ensuring standardized conditions for sample collection and clinical evaluations.

Furthermore, the application of rigorous biosafety protocols and specific microbiological methods enhanced the reliability of the data obtained. The methodological design, which integrated clinical, microbiological, and sociodemographic variables, provided a comprehensive perspective of the phenomenon, further strengthened by the use of robust and validated statistical analyses for non-parametric data.

LIMITATIONS OF THE STUDY

Among the main limitations, the relatively small sample size (n=65) stands out, which may restrict the generalizability of the findings to other Amazonian or national populations. The cross-sectional design prevents the establishment of causal relationships among the variables analyzed.

Additionally, the use of a non-probabilistic convenience sampling approach represents a methodological limitation, as it may introduce selection bias and limit representativeness. The study also lacked behavioral and socioeconomic variables, such as oral hygiene frequency, sugar intake, fluoride exposure, and educational level, which could have enriched the interpretation of caries determinants.

From a microbiological perspective, only culture-based methods were employed for bacterial identification. While effective for detecting *S. mutans* and *Lactobacillus spp.*, this approach excludes other potentially relevant species. The absence of molecular techniques (e.g., qPCR or 16S rRNA sequencing) restricts the ability to characterize the full complexity of the oral microbiome.

Finally, the calibration process for the DMFT index was performed on a small pilot sample (n=7), which may limit the external validity of reliability estimates, although κ values indicated strong agreement. Therefore, the results should be interpreted with caution, emphasizing the exploratory nature of the study and the need for replication with larger and more diverse samples.

APPLICATIONS FOR PUBLIC POLICY AND FUTURE RESEARCH DIRECTIONS

The findings of the present study provide key evidence for the formulation of public health policies aimed at preventing dental caries in vulnerable Amazonian communities. The implementation of mobile oral health programs, educational campaigns on oral hygiene and sugar reduction, and the strengthening of access to dental services in rural areas is recommended.

Future research should expand the sample size and incorporate longitudinal analyses to evaluate the evolution of the oral microbiome in relation to environmental, dietary, and behavioral factors. Moreover, the application of molecular techniques would allow for a more precise characterization of microbial diversity, facilitating the development of more personalized and effective preventive interventions.

CONCLUSION

Streptococcus mutans and *Lactobacillus spp*. were directly associated with elevated DMFT scores, and their coinfection was linked to the highest levels of dental caries, suggesting a synergistic effect in the pathogenesis of the disease. Likewise, acidic salivary pH was confirmed as a determining factor in bacterial proliferation and in the increased severity of the DMFT index, highlighting the importance of the oral environment in caries progression.

Although no statistically significant sex-related differences were observed, the trend toward greater bacterial colonization among women warrants further exploration in future studies incorporating hygiene, dietary, and physiological factors.

From a public health perspective, these findings underscore the urgent need to implement targeted preventive strategies in Amazonian riverine communities—such as community-based oral hygiene education, fluoride and dietary interventions, and the integration of dental care into existing primary health programs. This study provides essential baseline evidence to support policy-oriented actions and the development of sustainable oral health programs tailored to the socio-environmental realities of the Peruvian Amazon.

AUTHOR CONTRIBUTION STATEMENT: Conceptualization and design: G.A.P.-S., C.P.-P.; Literature review: G.A.P.-S.; Methodology and validation: M.A.C.-M.; M.A.G.-R.,Formal analysis: G.A.P.-S.; Investigation and data collection: G.A.P.-S.; Resources: G.C.-V.; Data Analysis and interpretation: G.A.P.-S.; G.C.-V.; Writin-original draft preparation: G.A.P.-S.; Writing-review & editing: M.A.G.-R., M.A.C.-M.; Supervision: G.C.-V.; Project administration: G.A.P.-S.; Funding acquisition: M.A.C.-M.

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