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Subgingival microbiota from *Cebus apella* (capuchin monkey) with different periodontal conditions

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ABSTRACT

This present study evaluated the subgingival microbiota of the *Cebus apella* with different periodontal conditions kept by the Tufted Capuchin Monkey Procreation Center (São PauloState University – UNESP) or free-ranging monkeys. For this purpose, clinical specimens of subgingival biofilm were collected from 52 monkeys, of both genders, 40 kept in captivity and 12 free-ranging monkeys. The primates were submitted to periodontal evaluation and biofilm samples were transferred to VMGA III transport medium and ultrapure water. The microbiota was cultivated in selective and non-selective culture media and microbial DNA was extracted and the presence of periodontal pathogens was evaluated using PCR and real-time PCR. The actinomycetes, fusobacteria, *Campylobacter rectus*, *Eikenella corrodens*, black-pigmented Gram-negative anaerobic rods, *Tannerella forsythia*, staphylococci and streptococci represent the predominantly detected microorganisms. *Aggregatibacter actinomycetemcomitans*, *Dialister pneumosintes* and *Prevotella nigrescens* were rarely observed, whereas *Treponema denticola* was not found. Populations of *C. rectus*, *E. corrodens, Fusobacterium nucleatum, Porphyromonas gingivalis, Prevotella intermedia, T. forsythia* and the total microbial load were significantly higher in animals with bone loss and, in smaller extension, in animals with gingival bleeding.

1. Introduction

Morphological, biochemical and genetic similarities between human and non-human primates have always made the latter the most relevant animal model in biological research, particularly infectious diseases, such as periodontitis [1,2]. However, limited access to Old World monkeys due to their elevated cost makes their use difficult. In addition, several species of New World monkeys are submitted to severe depopulation, with possible risk of extinction in the next decades, making its use as animal models impracticable in many situations.

In order to provide specimen of non-human primates for biological studies, the School of Dentistry and Veterinary Medicine of Araçatuba (UNESP), under legal authorization of the Brazilian Institute of Environment and Renewable Natural Resources – IBAMA, created the Tufted Capuchin Monkey Procreation Center, the only center of South America capable to provide specimens of these primates for biological research, also providing suitable conditions for maintenance of a healthy and monitored population of 200 free-ranging *Cebus apella* in remnants of Brazilian tropical rain forest.

C. apella is an adequate model for biological research, including dental and medical research [3,4]. However, very little is known about the oral microbiota of these primates, but these primates develop naturally gingivitis and, at times, periodontitis. In humans, different bacterial species are involved in the pathogenesis of periodontitis, particularly *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella intermedia, Prevotella nigrescens, Tannerella forsythia, Treponema denticola* and *Aggregatibacter actinomycetemcomitans,* generally in synergistic associations with other microorganisms [5–7].

The present study evaluated the occurrence of major microorganisms associated with periodontal diseases in both enclosed and

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free-ranging *C. apella* primates as well as the existence of a possible correlations between microbiological and clinical aspects of periodontal diseases in these non-human primates.

2. Materials and methods

2.1. Selection of non-human primates

This study was approved by the Institutional Animal Care Committee of the School of Dentistry and Veterinary Medicine of Araçatuba–UNESP (Proc 0063/2000 and 136/2007), SP, Brazil. Forty monkeys of the species *C. apella,* weighing from 2.5 to 3.5 kg, 4–20 years old (average age of 8.05 ± 4.1 years), with no gender distinction (18 males and 22 females), were selected. These non-human primates were enclosed at the Tufted Capuchin Monkey Procreation Center - UNESP, and received periodic health check-ups and were fed with cereal, fruits and water *ad libidum,* in a humidity and temperature controlled environment.

In addition, out of these primates, 12 young males were captured in the remnants of Atlantic tropical rain forest surrounding the Procreation Center, as they had been excluded from their original groups. The capture occurred in a period of 2–4 weeks prior to the beginning of the experiment.

The animals studied showed neither history of systemic disorder, nor received any antimicrobial drug during the four months preceding the study. All of the procedures related to clinical examination of the animals, as well as the specimen collection for microbiological analysis were performed after the primates had been anesthetized with intramuscular injection of ketamine chloridrate (15 mg/kg).

2.2. Clinical and radiographic evaluation and specimen collection

For periodontal evaluation, the plaque and gingival bleeding indices, as well as probing depth and the clinical attachment level (distance between the amelocementary junction to the base of the gingival sulcus or periodontal pocket) were determined, using methodology previously adapted to non-human primates [8]. The tests were performed in six sites for each dental element (mesiovestibular, vestibular, distovestibular, mesiolingual, lingual and distolingual sites).

In animals with clinical evidence of bone loss, radiographic characteristics of the periodontal tissues were assessed through periapical radiographs (Ultra-speed DF58, Eastman Kodak Co., Rochester, NY). Periodontitis was considered when bone loss was observed in at least three periodontal sites not situated in the same tooth; animals with gingivitis showed no clinical or radiographic evidence of bone loss and evidenced bleeding on probing, while periodontally healthy animals showed no morphological gingival alteration, or bleeding on probing.

Collection of the subgingival biofilm was performed after removal of the supra-gingival biofilm. In order to prevent contamination of clinical samples by saliva, cotton rolls were placed close to the main excretory ducts of the salivary glands. In animals with periodontitis, clinical samples were collected from two non-contiguous periodontal sites with the deepest probing depths, radiographic evidences of bone loss and bleeding on probing. In animals with gingivitis, clinical samples were collected from two non-contiguous periodontal sites showing the most noticeable characteristics of gingival inflammation; whereas in healthy animals, the clinical samples were collected from the distofacial and mesiofacial sites of the lower right first molar.

The specimens were obtained using two sterilized paper points which were inserted into the gingival sulcus or periodontal pockets for 30 s; one paper point was transferred to vials containing VMGA III medium [9], in order to minimize contact with atmospheric oxygen, and the other was transferred to tubes with 300 μl sterile ultrapure water that were stored at −196 °C, in liquid nitrogen, until DNA extraction.

2.3. Isolation and identification of microorganisms

The samples were processed within 4 h. Samples in VMGA III were submitted to serial 10-fold dilutions and plated onto fastidious anaerobe agar (FAA) enriched with 5 μg/mL hemin, 1 μg/mL menadione, 0.5% yeast extract, 10 μg/mL N-acetyl-muramic acid and 5% horse blood. Plates were incubated in anaerobiosis (90% N_2 + 10% CO_2) at 37 °C, during 7 and 14 days, for strict anaerobes. Additionally, samples were also inoculated onto tryptic soy agar (TSA) supplemented with 5% horse blood, 0.5% yeast extract and incubated at 37 °C during 48 h, for aerobic and facultative bacteria [10].

For isolation of enteric bacteria, clinical specimens were also inoculated in peptone water and ethyl violet azide broth (EVA broth, Difco) and incubated at both room temperature and 37 °C, for 3–7 days. After that, from microbial growth observed in peptone water, aliquots of 0.1 ml were transferred to Eosin Methilene Blue agar, Salmonella-Shigella agar, MacConkey agar and Brilliant Green agar. From tubes containing EVA broth, 0.1 ml was transferred to Bile Esculin agar. These agar plates were incubated in aerobiosis, at 37 °C, for 48 h [11].

Isolates were identified by Gram staining, colony morphology on blood agar plates, and by using identification kits Rapid ID 32 A (BioMérieux SA, Marcy-l'Etoile, France) for strict anaerobic rods; RapIDANA II System (Innovative Diagnostic Systems Inc., Atlanta, GA, USA) for strict anaerobic Gram-positive cocci; API Staph (BioMérieux) for staphylococci (Gram-positive cocci, catalase-positive); Rapid ID 32Strep (BioMérieux) for streptococci (Gram-positive cocci, catalase-negative); and API 20E (BioMérieux) for facultative enteric rods [10].

2.4. Detection of pathogens by PCR and real-time PCR

Bacterial DNA from each sample in sterile ultrapure water was extracted by mean of QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). DNA concentrations were determined with a spectrophotometer at A_{260}nm. The presence of *Actinomyces israelii*, *Actinomyces naeslundii*, *Actinomyces viscosus, Dialister pneumosintes, Eikenella corrodens*, family *Enterobacteriaceae*, *Enterococcus* spp., *Enterococcus faecalis*, *Enterococcus faecium*, *Eubacterium* spp., *Eubacterium saphenum, Mogibacterium timidum*, *Slackia exigua*, *Porphyromonas endodontalis*, and *Selenomonas sputigena* was evaluated by PCR using specific primers [10,12–14].

PCR amplification was performed in volumes of 25 μL containing 1X PCR/Mg^{++} buffer (Boehring Mannheim, Indianapolis, IN, USA), 0.2 Mm of each Dntp (Pharmacia Biotech, Piscataway, NJ, USA), 0.5 U *Taq* DNA polymerase (Invitrogen do Brasil, São Paulo, SP, Brazil), 0.4 μM of each primer pair (Invitrogen) and 10 ng of template. Amplification was performed in a DNA Thermal Cycler (Perkin Elmer, GeneAmp PCR System 9700, Norwalk, CT, USA) programmed for 94 °C (5 min), followed by 35 cycles at 94 °C for 30 s, annealing temperature adequate for each primer pair for 30 s to 1 min, 72 °C for 1–2 min; then 72 °C for 5 min to allow the final DNA extension. Amplification products were compared by electrophoresis in 1% agarose gel in 1X TBE (1 M Tris, 0.9 M boric acid, 0.01 M EDTA, Ph 8.4) buffer, stained with 0.5 mg/Ml of ethidium bromide and photographed on a UV light transilluminator (Eastman Kodak Co., NY, USA).

Quantitative detections of *Campylobacter rectus*, *F. nucleatum*, *Parvimonas micra*, *A. actinomycetemcomitans*, *P. intermedia*,

P. nigrescens, *P. gingivalis*, *T. forsythia*, *T. denticola*, and total microbial load were performed by real-time PCR using specific primers and probes [15,16]. All the primers and probes were directed to 16S rRNA gene. The Taqman 5′ nuclease assay PCR method was used. The probes were double labeled with a reporter dye (FAM: 6-carboxyfluorescein) covalently attached at the 5′ end, and a quencher dye (TAMRA: 6-carboxytetramethylrhodamine) covalently attached at the 3′ end.

For amplification reactions, duplicate samples were routinely used and assays were performed in a total volume of 25 μl containing 12.5 μl Taqman Universal Master Mix (Applied Biosystems), 0.2 μl each forward and reverse primer (final concentration 200 nM each), 0.1 μl Taqman probe (final concentration 100 nM), 2 μl template DNA solution and 10 μl of sterilized DNase- and RNase free water. Amplification reactions were performed in a thermocycler programmed as follows: initial denaturation step at 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s and annealing temperature specific for each primer pair for 1 min. The critical threshold cycle (Ct) was defined as the cycle at which the fluorescence becomes detectable above background and is inversely proportional to the logarithm of the initial number of template molecules. The negative control was a PCR TaqMan Master Mix without DNA. Assay conditions for all primer/probe sets consisted of an initial denaturation at 95 °C for 10 min was followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min. The correlation coefficients for all standard curves was >0.99.

In DNA amplification by PCR or real-time PCR, DNA from *A. israelii* ATCC 29322, *A. naeslundii* ATCC 12104, *A. viscosus* ATCC 19246, *Acinetobacter haemolyticus* ATCC 19002, *A. actinomycetemcomitans* ATCC 29523, *C. rectus* ATCC 33238, *D. pneumosintes* ATCC 33048, *E. corrodens* ATCC 23834, *E. faecalis* ATCC 29212, *E. faecium* ATCC 35667, *Escherichia coli* ATCC 25922, *E. saphenum* ATCC 49989, *F. nucleatum* ATCC 10953, *M. timidum* ATCC 33093, *P. micra* ATCC 33270, *P. endodontalis* ATCC 35406, *P. gingivalis* ATCC 33277, *P. intermedia* ATCC 25611, *P. nigrescens* ATCC 33563, *S. sputigena* ATCC 33150, *S. exigua* ATCC 700122, *T. forsythia* ATCC 43037 and *T. denticola* ATCC 33521 were used as positive-controls, and ultrapure water instead DNA were used as negative-controls.

3. Statistical analysis

Statistical analyses were carried out using the software Statistical Package for the Social Sciences (SPSS Incs v.13, Chicago, IL, USA). Differences in clinical parameters were evaluated using Mann–Whitney and Chi-square tests. The frequency of detection of pathogens was computed for each primate. Differences in the prevalence of selected pathogens between different groups of primates were tested using the Mann–Whitney test, Fisher's exact test or Chi-square test using Bonferroni correction. *p*-values were adjusted for multiple comparisons. The results of quantitative detection performed by real-time PCR were analyzed by ANOVA.

4. Results

Clinical-radiographic evaluations of non-human primates demonstrated that 25 (48.1%) were periodontally healthy, 14 (26.9%) had gingivitis and 13 (25%) had periodontitis. No significant differences related to gender were observed. All primates with periodontitis were adults over 8 years old. Clinical parameters of the animals studied are shown in Table 1. The results obtained by culture, PCR and real-time PCR demonstrated that the microbiota of these animals is complex, irrespective of their periodontal condition.

By culture, the actinomycetes and fusobacteria, especially *F. nucleatum*, as well as *C. rectus*, *Eubacterium* sp., *E. corrodens*, *P. micra*, *P. gingivalis*, *Prevotella* sp., *P. intermedia* and *P. melaninogenica*, staphylococci, and viridans streptococci were the most prevalent microorganisms (Fig. 1). Species of the family Enterobacteriaceae were also frequently isolated, mainly *Citrobacter freundii*, *Enterobacter cloacae, E. intermedius*, *E. sakazakii*, *Klebsiella oxytoca, Morganella morganii*, *Pantoea agglomerans*, *Proteus mirabilis*, *P. vulgaris* and *Providencia alcalifaciens*. Results obtained by using molecular methods were similar to those obtained by culture, except for *A. actinomycetemcomitans*, *D. pneumosintes*, *P. nigrescens* and *T. forsythia* which were not detected by culture.

Table 1
Periodontal conditions of *C. apella*.

Clinical parameters	Mean ± standard deviations		
	PHP[a] ($N = 25$)	PG[b] ($N = 14$)	PP[c] ($N = 13$)
Plaque index	1.3 ± 0.3	2.5 ± 0.5	2.8 ± 0.4
Clinical probing depth (mm)	1.5 ± 0.2	2.1 ± 0.3	2.9 ± 0.3
Bleeding index	0.5 ± 0.3	1.4 ± 0.2	2.4 ± 0.2
Attachment loss (mm)	1.4 ± 0.4	1.6 ± 0.4	2.5 ± 0.3

[a] Periodontally healthy non-human primates.
[b] Non-human primates with gingivitis.
[c] Non-human primates with periodontitis.

The most common periodontal bacteria in healthy primates belong to the genus *Prevotella* and, in a lesser extension, *Fusobacterium*. In animals with periodontitis almost all selected microbial groups evidenced a significant increase in frequency (Fig. 2) and populations (Fig. 3), except *A. actinomycetemcomitans* and *P. nigrescens*. It was observed a statistically significant correlation among *C. rectus* (Mann–Whitney, $p = 0.04$), *E. corrodens* (Mann–Whitney, $p = 0.03$), *P. micra* (Mann–Whitney, $p = 0.012$), *P. gingivalis* (Mann–Whitney, $p = 0.017$), *Prevotella* sp. (Mann–Whitney, $p = 0.029$), *P. intermedia* (Mann–Whitney, $p = 0.022$), family *Enterobacteriaceae* (Mann–Whitney, $p = 0.037$), and *T. forsythia* (Mann–Whitney, $p = 0.019$) with bone loss.

In addition, *C. rectus* (Mann–Whitney, $p = 0.023$), *E. corrodens* (Mann–Whitney, $p = 0.023$), *Staphylococcus* sp. (Mann–Whitney, $p = 0.039$), *S. cohnii* (Mann–Whitney, $p = 0.031$), *Streptococcus* sp. and *S. salivarius* (Mann–Whitney, $p = 0.038$), *F. nucleatum* (Mann–Whitney, $p = 0.011$), *T. forsythia* (Mann–Whitney, $p = 0.025$), and family *Enterobacteriaceae* (Mann–Whitney, $p = 0.045$) showed correlation to gingival inflammation and biofilm accumulation.

Certain microorganisms associated with periodontitis in humans, such as *D. pneumosintes*, *P. nigrescens*, and *A. actinomycetemcomitans*, were rarely detected in the studied animals, independent of the detection method employed. Moreover, *T. denticola* was not detected. The composition of the microbiota from the primates enclosed at the Procreation Center and those who initially were free-ranging primates was similar, but *A. actinomycetemcomitans* and *P. nigrescens* were detected just in the enclosed primates.

The population of almost all targeted bacterial species evidenced a significant elevation in primates with gingivitis and particularly periodontitis, except for *A. actinomycetemcomitans* and *P. nigrescens*, that were rarely detected and when present the numbers were low. In periodontally healthy monkeys and primates with gingivitis, the DNA of these selected periodontal bacteria comprised 11.1% and 23.6% of the total DNA detected by real-time PCR, respectively; while in animals with periodontitis, this group of microorganisms represented 19.9% of the bacterial DNA in the samples.

The occurrence of selected species in the subgingival biofilm evidenced associations among microbial species. Then, the

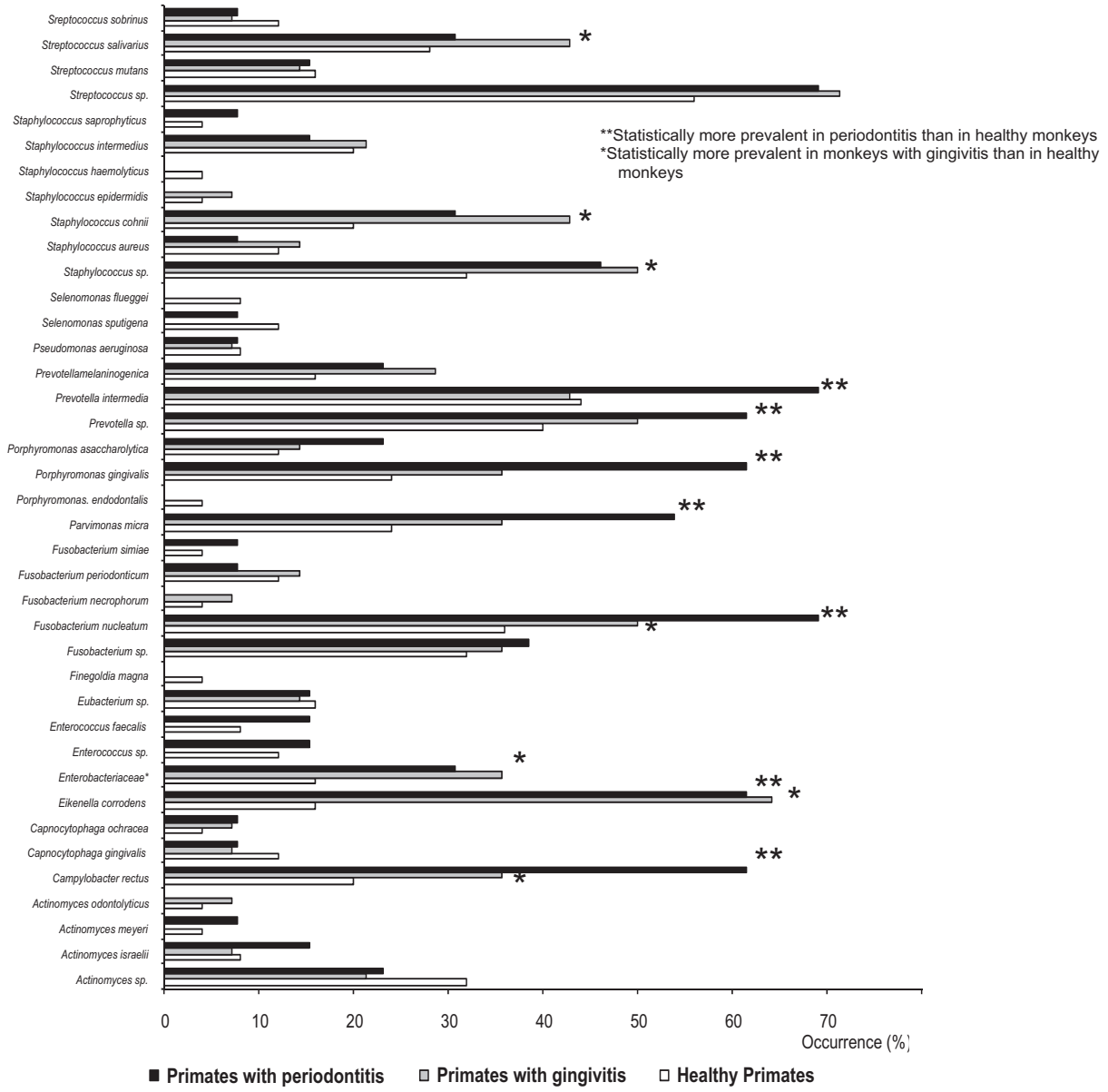

Fig. 1. Subgingival microbiota cultivated from *C. apella* with different periodontal conditions.

occurrence of *E. corrodens* seems positively affected by the presence of *C. rectus* (Chi-Square test, $p = 0.021$), *T. forsythia* (Chi-Square test, $p = 0.013$), and *F. nucleatum* (Chi-Square test, $p = 0.034$), whereas *C. rectus* was positively affected by the presence of *T. forsythia* (Chi-Square test, $p = 0.022$), *P. intermedia* (Chi-Square test, $P = 0.043$), and *P. gingivalis* (Chi-Square test = 0.032). The detection of black-pigmented Gram-negative rods suggests positive association between genera *Porphyromonas* and *Prevotella* in most of non-human primates, irrespective of primates' periodontal conditions. This showed to be particularly significant for *P. endodontalis* and *P. intermedia* (Chi-Square test, $P = 0.012$), *P. gingivalis* and *P. intermedia* (Chi-Square test, $P < 0.001$).

5. Discussion

The capuchin monkey has several advantages over other non-human primates regarding its use as animal model: biological response, easy reproduction in captivity, rapid sexual maturity among others. However, the lack of data about its microbiota has limited its use in biological research, particularly in studies involving dental biofilms. In addition, very little is known about the evolution of infectious oral diseases in this species, particularly gingivitis and periodontitis. In addition, studies about oral microbiota of monkeys may contribute to elucidate the evolution process of endogenous infections in humans, since certain pathogens such as *P. gingivalis*, may have originated from microorganisms that colonize non-human primates [17].

In the present investigation, considering the clinical parameters studied, a correlation among plaque index, clinical probing depth, bleeding index and clinical attachment level was verified, as also widely described in humans [18]. Besides this aspect, the distribution of different levels of periodontal health in the population of *C. apella* studied reinforces the idea that individual susceptibility plays a key role in the pathogenesis of the periodontitis in primates, and it has been demonstrated that a higher individual susceptibility derives from different factors, such

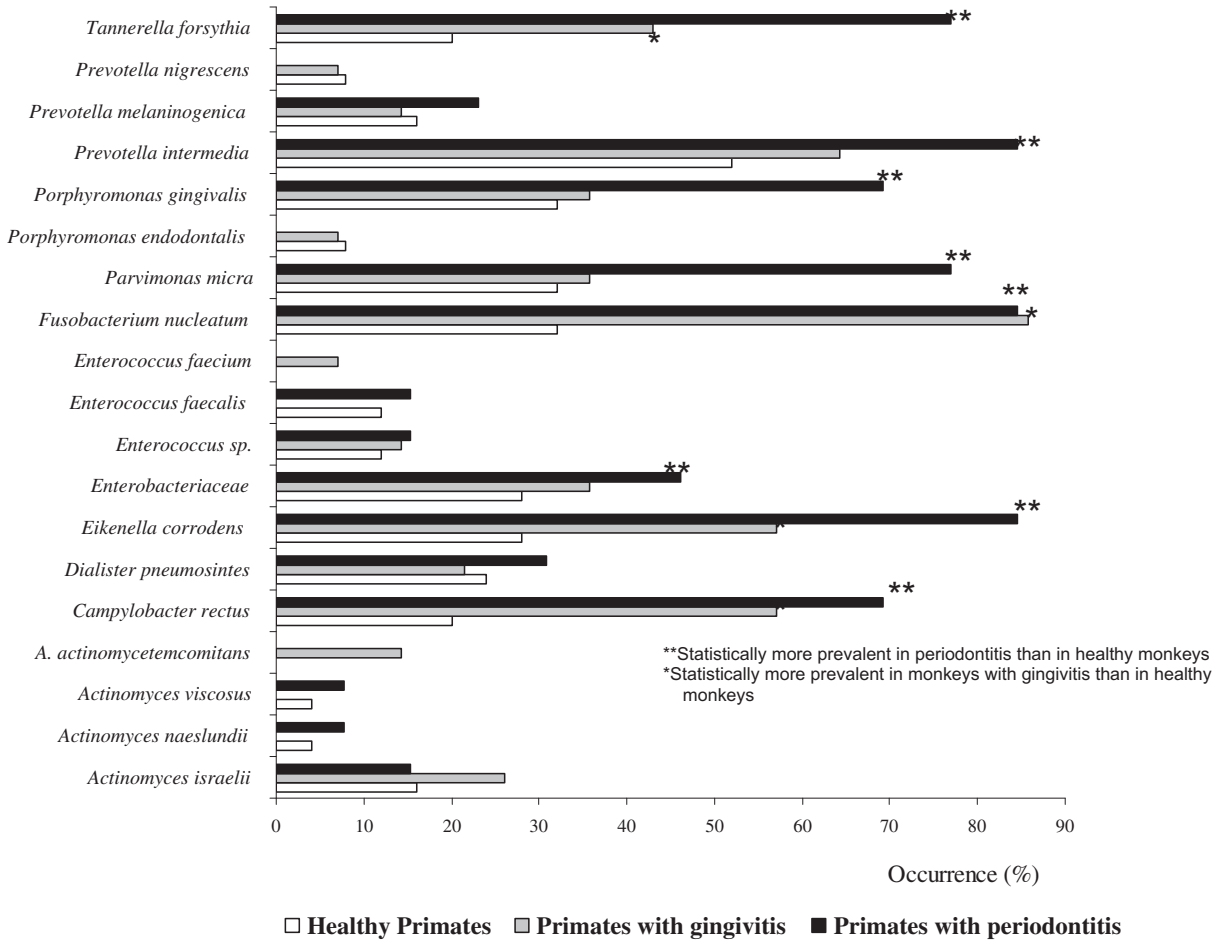

Fig. 2. Subgingival microbiota from non-human primates with different periodontal conditions. Data obtained by PCR and real-time PCR.

as genetic and environmental aspects, as well as levels of exposure to microorganisms typically associated with periodontitis [19,20].

Moreover, taking into consideration the complexity of the oral microbiota of primates, the present study prioritized the detection of pathogens frequently isolated from oral infections, especially found in periodontitis as previously suggested [21], given that the oral microbiota composition of this primate species is unknown. Another aspect that limits the discussion of the results is the fact that the microbiota of New World monkeys has hardly been

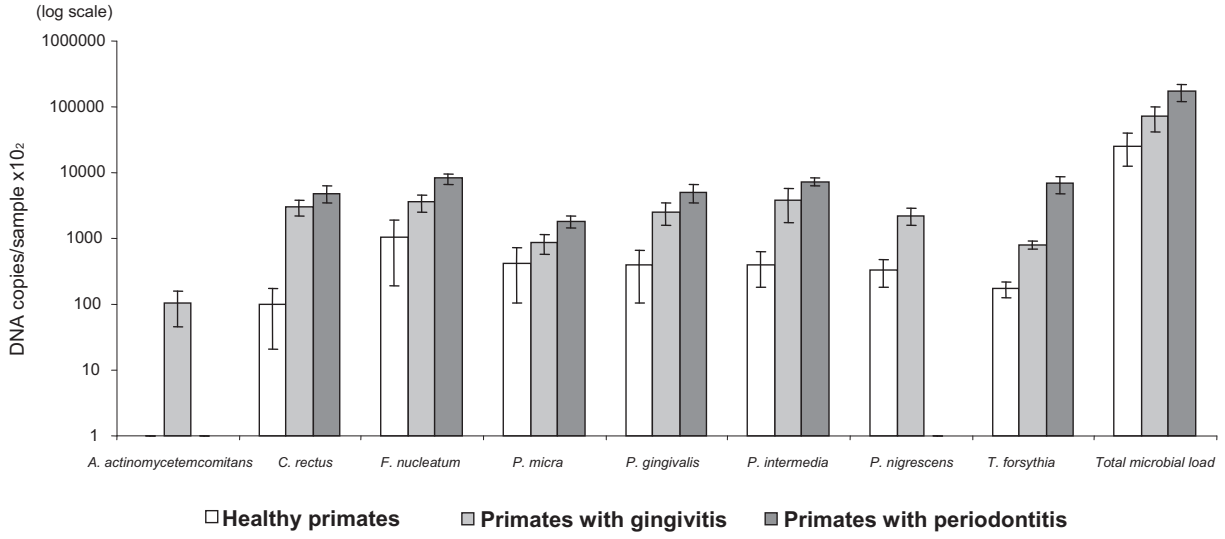

Fig. 3. Number of DNA copies (mean ± standard deviations) of selected bacteria in subgingival samples from *C. apella*. Data obtained by real-time PCR.

evaluated, which contrasts with the abundance of studies on African and Asian primates. Besides, the vast majority of earlier studies were accomplished before the development of detection methods involving DNA technologies, which, at times, were limited to evaluating the presence of certain bacterial morphotypes in the microbiota [22,23] or culture [18,21,24,25], and the taxonomy of the main oral microbial genera has suffered profound changes since then. Furthermore, earlier studies employed a soft diet or a mechanic devices to induce gingival inflammation and attachment loss in the animals, consequently interfering with their microbiota [8,21,26]. However, the present study evaluated the microbiota of monkeys exhibiting gingivitis or periodontitis without any diet change or employing force or mechanic method to facilitate microbial proliferation and tissue damage.

Black-pigmented Gram-negative anaerobes have been associated with periodontal disease and tooth loss [27], and the results demonstrated that the development of gingivitis and periodontitis is associated to a significant increase in the frequency of black-pigmented Gram-negative anaerobes of the genera *Porphyromonas* and *Prevotella,* as well as microorganisms related to them, such as *T. forsythia*. Other species that can establish ecological interrelation with these anaerobes, such as fusobacteria, *C. rectus*, *E. corrodens* and *P. micra* were also frequently cultivated or detected by PCR. Thus, the microbiota composition associated with periodontal health, gingivitis or periodontitis in capuchin monkeys does not seem to differ significantly from the microbiota observed in humans, considering certain particularities, such as low prevalence of *P. nigrescens* and *A. actinomycetemcomitans*, as well as absence of *T. denticola*, and presence of certain microorganisms more rarely observed in the human oral cavity, such as *S. cohnii*.

In humans, the detection of enteric bacteria in periodontal pockets seems to be inversely proportional to the presence of anaerobic periodontopathogens, and these microorganisms are frequently isolated from periodontal sites with significant reduction in the population of anaerobes [28]. In the present investigation, this phenomenon was not observed and no correlation was found between enteric bacteria, particularly family *Enterobacteriaceae*, and oral anaerobes. It is possible that gingival bleeding and periodontal inflammation may provide suitable conditions for the establishment of enteric microorganisms in the oral environment.

Although the total population of major periodontopathogens presented an increase in animals with periodontitis and gingivitis, the participation of these microorganisms in the microbiota showed a proportional decrease. This event had already been described for genera *Porphyromonas* and *Prevotella* in monkeys of the species *Macaca fascicularis*, with gingivitis or ligature-induced periodontitis, where the population of these anaerobes increased in the onset of the periodontal inflammation and decreased with the periodontitis evolution [18].

Among the peculiarities observed in the microbiota of these primates is the low frequency and modest populations of some pathogens such as *A. actinomycetemcomitans* and *D. pneumosintes*, besides the absence of *T. denticola*, microorganisms frequently associated with aggressive and chronic periodontitis [19,20]. The data in the present study referring to the population of *A. actinomycetemcomitans* in New World monkeys evidenced that *A. actinomycetemcomitans* comprises a modest portion of the subgingival microbiota in non-human primates, as previously observed [25,29].

Ebersole et al. [18] demonstrated that the total of microorganisms detected in non-human primates with different periodontal status increased as the periodontal tissues were affected by inflammation, but these animals were fed with soft diet in order to increase development of the oral biofilm. Conversely, in the present investigation the animals received a balanced diet with granulated food, cereals *in natura*, vegetables and fruits, resulted in a lower ability to form biofilm and, consequently, lower plaque index than that reported by those researchers. However, even in such conditions, an increase in the total of microorganisms detected in animals with periodontitis was also observed, in comparison with healthy primates or primates with gingivitis, which may be connected to the fact that along with the development of connective attachment loss, the microbiota present inside the periodontal pocket tends to be less influenced by diet or cleaning through tongue and food movement.

The implantation of microorganisms such as *P. gingivalis,* in non-human primates, seems to be associated with the deterioration of their periodontal status [30,31], being that the immunization of primates using surface proteins from this anaerobe results in the development of an immune response which provides protection to the primates [8,32]. The elevated frequency of microorganisms, such as *P. intermedia*, *F. nucleatum* and other fusobacteria, independent of the periodontal status, demonstrates that they are part of the resident periodontal microbiota, regardless of sex or age. The role *P. intermedia* plays in human periodontal diseases is controversial and its recurrence usually takes place at the same anatomic site as *P. nigrescens*, being that the latter microorganism was not detected by culture and rarely observed by real-time PCR, this way its role in these non-human primates microbiota needs further clarification.

The colonization of black-pigmented anaerobic rods is significantly increased in captive-born monkeys, which had no free contact with other members of the same species [25]. However, the results of the present study, from *C. apella* population recently captured for assisted breeding, show similar microbiota to that observed in captive-born monkeys.

In these primates, microorganisms considered putative periodontopathogens for humans may have their role in the etiology of periodontitis substituted by other microorganisms. Therefore, the small occurrence and proportion of *A. actinomycetemcomitans* in the microbiota of the studied monkeys may reflect specificities in the adhesion properties of these microaerophilics, which would show higher affinity for human and Old World non-human oral epithelial cells than New World primates' receptors [33].

The highest frequency of *T. forsythia* in animals with gingivitis and periodontitis was important, as this event has also been described in humans [34] and in mice [35]. Among all periodontopathogenic microorganisms quantified by real-time PCR, *T. forsythia* showed the highest differences in population of these anaerobes in animals with periodontitis in relation to animals with gingivitis (Fig. 3). The results of the present study were similar to data obtained by Kirakodu et al. [36], in rhesus monkeys with ligature-induced periodontitis.

Other microorganisms, such as facultative anaerobic gram-positive cocci, need further evaluation, since a significant part of isolated microorganisms could not be identified at species level, and the literature shows no data justifying a more active role of these microorganisms in periodontal disease pathogenesis of humans and non-humans.

6. Conclusions

In *C. apella*, gingivitis and periodontitis is associated to a significant increase in the frequency of black-pigmented Gram-negative anaerobes (*Porphyromonas* and *Prevotella*), *T. forsythia*, fusobacteria, *C. rectus*, *E. corrodens* and *P. micra*.In addition, a low prevalence of *P. nigrescens* and *A. actinomycetemcomitans*, as well as absence of *T. denticola* constituted significant data.

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