

# Incidence of *Entamoeba gingivalis* and *Trichomonas tenax* in samples of dental biofilm and saliva from patients with periodontal disease

## Incidência de *Entamoeba gingivalis* e *Trichomonas tenax* em amostras de biofilme e saliva de pacientes com doença periodontal

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### ABSTRACT

#### Objective

This study analyzed the incidence of *Entamoeba gingivalis* and *Trichomonas tenax* in samples of dental biofilm and saliva from patients with gingivitis / periodontitis and in healthy subjects.

#### Methods

Biofilm and saliva samples were taken from 20 patients with gingivitis, 22 with periodontitis and 9 healthy individuals. They were spread on sterile Petri dishes, diluted with saline and examined with a light microscope. Salivary pH was determined by universal pH indicators strips. The chi-square test was used to determine significance ( $p < 0.05$ ).

#### Results

Almost one-third (31.37%) (50% from gingivitis and 50% from periodontitis) of the biofilm samples and 35.29% (39% from gingivitis and 61% from periodontitis) of the saliva samples were positive for *Entamoeba gingivalis*. *Trichomonas tenax* was found in 22.53% of the biofilm samples (16.66% from gingivitis, 41.67% from periodontitis and 41.67% from healthy patients) and 9.81% of the saliva samples (20% from gingivitis, 40% from periodontitis and 40% from healthy patients). The presence of these microorganisms was related to the type of periodontal disease ( $p = 0.001$ ), but not with age ( $p = 0.178$ ) or risk factors ( $p = 0.194$ ).

#### Conclusion

These findings suggest that *Entamoeba gingivalis* more common in the early stages of periodontitis, while *Trichomonas tenax* is considered a protozoan of the gingival sulcus. However, further studies are needed to determine the relationship between these species and periodontitis.

**Indexing terms:** Gingivitis. Parasitology. Periodontitis.

### RESUMO

#### Objetivo

Analisar a incidência de *Entamoeba gingivalis* e *Trichomonas tenax* em amostras de biofilme dental e saliva de pacientes com gengivite/periodontite e de indivíduos saudáveis.

#### Métodos

Amostras de saliva e biofilme foram obtidas de 20 pacientes com gengivite, 22 com periodontite e 9 indivíduos saudáveis. O material foi depositado em placas de Petri e diluído em soro fisiológico para posterior observação. O pH das amostras de saliva foi determinado com fitas indicadoras de pH. Os dados foram tratados por teste qui-quadrado ( $p < 0,05$ ).

#### Resultados

Foi observada positividade para *Entamoeba gingivalis* em 31,37% das amostras de biofilme (50,00% com gengivite e 50,00% com periodontite) e 35,29% de saliva (39,00% gengivite e 61,00% periodontite). Foi observado o *Trichomonas tenax* em 22,53% das amostras de biofilme (16,66% gengivite, 41,67% periodontite, e 41,67% saudáveis) e 9,81% de saliva (20,00% gengivite, 40,00% periodontite, e 40,00% saudáveis). A presença de parasitas esteve relacionada ao tipo de doença periodontal ( $p = 0,001$ ), mas não a idade ( $p = 0,178$ ) e a fatores de risco ( $p = 0,194$ ).

#### Conclusão

Estes achados sugerem que a *Entamoeba gingivalis* aparece mais em estágios iniciais da periodontite, enquanto que o *Trichomonas tenax* é considerado um protozoário do sulco gengival. Contudo, outros estudos são necessários para determinar a relação entre essas espécies e a periodontite.

**Termos de indexação:** Gengivite. Parasitologia. Periodontite.

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## INTRODUCTION

The human oral cavity is home to numerous microorganisms<sup>1</sup>. Since mouth infection symptoms derive from the interaction between pathogenic microbiota and the host's defense mechanisms, it is extremely important to study the microorganisms that cause periodontal disease in adults. Hence, local irritating factors, particularly the dental bacterial biofilm, seem to have a critical role in the susceptibility to, and onset and progression of, periodontal disease<sup>2</sup>.

Microorganisms capable of colonizing the oral cavity are rare. *Entamoeba gingivalis* was the first commensal found in the human oral cavity, and it is found in gingival tissues, particularly in suppurative, inflammatory processes, due to its preference for anaerobic environments<sup>3</sup>. The high incidence of *Entamoeba gingivalis* in individuals with periodontal disease suggests that these protozoa might have an important role in the etiology of this condition<sup>4-5</sup>. However, since it is also found in the oral cavity of healthy individuals, some authors believe that this commensal could be opportunistic, that is, capable of proliferating in a gingival environment modified by periodontal disease<sup>3,6</sup>.

*Trichomonas tenax*, another protozoan which has been isolated from samples of dental calculi, is a member of the subgingival dental biofilm microbiota<sup>7</sup>. The presence of *Trichomonas tenax* in the human oral cavity is considered an indication of poor oral hygiene, since its incidence is about three or four times greater in patients with periodontal disease than in healthy individuals<sup>2,8-9</sup>.

The goal this study was to analyze the incidence of *Entamoeba gingivalis* and *Trichomonas tenax* in samples of dental calculi/biofilm and saliva from patients with periodontal disease and healthy patients and to determine if there is a relationship between periodontal disease and these protozoa.

## METHODS

### Patients

The periodontiums of 51 patients were clinically examined, and diagnosis and classification of the periodontium was done according to the Periodontal

Screening and Recording (PSR) I, in agreement with the American Academy of Periodontology and American Dental Association criteria. Twenty patients were diagnosed with gingivitis (EG1), 22 with periodontitis (EG2) and 9 presented a healthy periodontium (CG). The patients were also asked about the use of medications and systemic conditions which might predispose them to the development of periodontal disease.

### Sample collection

Samples of saliva and dental biofilm/calculi were collected from all patients in the morning, before any oral hygiene. After determining the frontal mandibular area most affected by periodontal disease (by means of PSR), dental biofilm/calculi samples were collected by scraping the area with sterile periodontal curettes. Unstimulated saliva samples were collected as recommended by Navazesh<sup>10</sup>. All samples were placed in sterile Petri dishes and diluted with saline at room temperature (25 to 28°C). Immediately after dilution, the samples were examined under a light microscope.

### Microbiological analysis of the samples

Sterile pipettes were used to put droplets of the samples on microscope slides, which were then examined under x100, x200 and x400 magnification. Strains of *Entamoeba gingivalis* were identified by their shape and presence of vacuoles containing inclusion bodies of the nuclear remains of lymphocytes, bacteria and erythrocytes. *Trichomonas tenax* was identified by its flagella and characteristic locomotion. Giemsa stain was used to facilitate *Trichomonas tenax* identification.

### Determination of salivary pH

Universal indicator strips (pH 0-14, Merck®) were used to determine salivary pH and a possible relationship between pH and presence of the study microorganisms.

### Statistical analyses

The data were analyzed by the non parametric chi-square test. The significance level was set at 5% ( $p < 0.05$ ).

### Ethical and legal issues

This study followed the ethical principles in the Declaration of Helsinki (2000) and was approved by the local ethics committee, protocol number 131004.

## RESULTS

Analysis of the fresh microscope slides revealed that 16 biofilm samples (31.37%) were positive for *Entamoeba gingivalis*, where 50,00% (8 cases) were from patients with periodontitis and 50,00% were from patients with gingivitis. *Entamoeba gingivalis* was not found in the control group. Meanwhile, 12 (23.53%) biofilm samples were positive for *Trichomonas tenax*, where 41.67% (5 cases) were from patients with periodontitis, 16.66% (2 cases) were from patients with gingivitis and 41.67% (5 cases) were from the control group. As for the salivary samples, 18 (35.29%) were positive for *Entamoeba gingivalis*, where 61,00% (11 cases) were from patients with periodontitis and 39,00% (7 cases) were from patients with gingivitis. Once again, *Entamoeba gingivalis* was not found in the control group. *Trichomonas tenax* was identified in only 5 saliva samples (9.81%), where one (20,00%) was from a patient with gingivitis, 2 (40,00%) were from patients with periodontitis and 2 (40,00%) were from the control group (Table 1). There was a significant correlation between presence of microorganisms and type of periodontal disease (chi-square = 22.045,  $p=0.001$ ).

The salivary pH of the participants ranged from 6.0 and 8.0, and the likelihood of both microorganisms being present in the saliva decreased as salivary alkalinity increased (Figure 1).

Regarding the relationship between patient's age and presence of microorganisms, *Entamoeba gingivalis* was more common in individuals aged 31 to 50 years, whereas *Trichomonas tenax* was more common in patients aged 11 to 30 years (Table 2). However, both commensals were found in a wide range of ages, so no statistically significant correlation was found between age and presence of microorganisms (chi-square = 8.915;  $p=0.178$ ).

Only 8 (15.6%) of the 51 individuals examined in this study presented risk factors for periodontal disease, such as alcoholism (2 cases), smoking (5 cases), hypertension (2 cases) and diabetes (1 case) (Table 3). There was no correlation between the incidence of protozoa and these risk factors (chi-square = 9.652;  $p=0.194$ ).

**Table 1.** Rate of *Entamoeba gingivalis* and *Trichomonas tenax* in dental biofilm and salivary samples according to the periodontal status of the patients.

Periodontal status	<i>Entamoeba gingivalis</i>				<i>Trichomonas tenax</i>			
	Biofilm		Saliva		Biofilm		Saliva	
	n	%	n	%	n	%	n	%
Gingivitis (EG1)	8	50.00	7	39.00	2	16.66	1	20.00
Periodontitis (EG2)	8	50.00	11	61.00	5	41.67	2	40.00
Healthy (CG)	0	0	0	0	5	41.67	2	40.00
Total	16	31.37	18	35.29	12	23.53	5	9.81

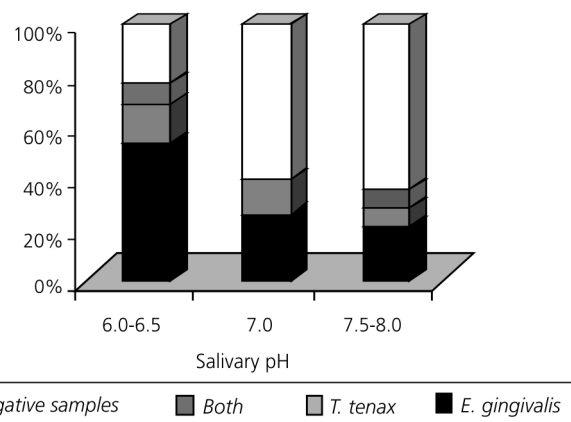
**Table 2.** Rate of *Entamoeba gingivalis* and *Trichomonas tenax* in biofilm and/or salivary samples according to patient's age.

Age	<i>Entamoeba gingivalis</i>		<i>Trichomonas tenax</i>		Both		Negative	
	n	%	n	%	n	%	n	%
0-10	0	0	0	0	0	0	0	0
11-20	7	70	2	20	0	0	1	10
21-30	2	15.4	7	53.8	1	7.7	3	23.1
31-40	8	47.1	3	17.6	2	11.	4	23.5
41-50	3	42.9	1	14.2	0	0	3	42.9
51-60	1	33.3	0	0	0	0	2	66.7
61-70	0	0	0	0	0	0	0	0
71-80	0	0	0	0	1	100	0	0
Total	21	41.2	13	25.5	4	7.8	13	25.5

**Table 3.** Relationship among risk factors, periodontal status and presence of *Entamoeba gingivalis* and *Trichomonas tenax*.

Risk factor	Periodontal status	Presence of microorganisms	
		<i>Entamoeba gingivalis</i>	<i>Trichomonas tenax</i>
Smoking/ alcoholism	Periodontitis	+	-
Smoking	Periodontitis	-	+
Diabetes/ hypertension	Periodontitis	+	+
Alcoholism	Periodontitis	+	-
Smoking	Gingivitis	-	-
Hypertension	Gingivitis	-	-
Smoking	Control	-	-

Note: (+) presence of the microorganism; (-) absence of the microorganism.



**Figure 1.** Distribution of *Entamoeba gingivalis* and *Trichomonas tenax* rates according to salivary pH.

## DISCUSSION

Most studies on oral microorganisms refer to bacteria, and there are only a few reports on the role of oral commensals in the pathogenesis of periodontal disease despite the high incidence of certain protozoa, such as *Trichomonas tenax* and *Entamoeba gingivalis*, in the dental biofilm<sup>11</sup>. Epidemiological studies have showed that the

infection rate of *Entamoeba gingivalis* in patients with periodontitis is much higher than that of healthy patients<sup>12</sup>, so some researchers inferred that this protozoan was related to periodontitis<sup>13</sup>. In addition, there are some reports indicating that the prevalence of people with *Trichomonas tenax* world wide ranges from 4.0 to 53%; besides, some studies have reported a high prevalence of this commensal in patients with chronic marginal periodontitis<sup>5,11</sup>.

Protozoa were found in 74.5% of the patients, and *Entamoeba gingivalis* was almost twice as common as *Trichomonas tenax*. Very similar results were found by other studies<sup>2,14-15</sup>. Additionally, in 10% of the cases (all with periodontitis), both microorganisms were present, corroborating other studies<sup>16-18</sup>.

*Entamoeba gingivalis*, found only in the dental biofilm and saliva of patients with periodontal disease, corroborates the hypothesis that this protozoan might be involved in the development of periodontal disease, as has been suggested by other studies<sup>3,11,14</sup>.

It has been suggested that *Entamoeba gingivalis* could affect the formation of the dental biofilm and contribute to the development and progression of periodontal disease<sup>1</sup>. On the other hand, other researchers state that this microorganism may be opportunistic, since it is capable of proliferating in the microenvironment of the mucobuccal fold affected by periodontal disease<sup>6</sup>. Moreover, some aerobic (*Pseudomonas aeruginosa*) and facultative anaerobic bacteria (*Klebsiella pneumonia*) may cause periodontitis in rats when are given immunosuppressive drugs and *Entamoeba gingivalis* can help the bacteria to cause periodontal disease<sup>3</sup>.

Therefore, if *Entamoeba gingivalis* helps the development and progression of periodontal disease and periodontal disease increasingly facilitates the proliferation of these protozoa, this vicious circle could explain the increased incidence of these microorganisms in the dental biofilm of patients with gingivitis and periodontitis<sup>4</sup>.

Some authors injected *Entamoeba gingivalis* in the base of the gingival pocket of rats, which induced periodontal abscess in 78.9% (30/38) of sample. Live *Entamoeba gingivalis* were found in purulent secretions and cultured from the periodontal abscess. These results strongly suggest that infection by *Entamoeba gingivalis* may destroy the gingival tissues. Furthermore, it has also been proposed that salivary enzymes and free radicals in patients with *Entamoeba gingivalis*-induced periodontitis may be involved in the lysing of the plasmalemma and membranous organelles, damaging the epithelial cells of the gums<sup>19</sup>.

A suspension of *Entamoeba gingivalis* was spread on the gingival margins of rats immunosuppressed with prednisolone, leading to the development of the clinical signs of periodontal disease much faster than that observed in immunocompetent rats<sup>4</sup>. These data indicate that immunosuppression may play a role in the pathogenesis of periodontal disease induced by this commensal.

The prevalence of *Trichomonas tenax* in the present study (23.53%) was in agreement with many other studies that reported prevalences ranging from 12 to 32%<sup>1,11,20-21</sup>.

The role of *Trichomonas tenax* in periodontal disease is still controversial. Although a relationship between the increased occurrence of this protozoan and progression of periodontal disease has been demonstrated recently<sup>5,7</sup>, the precise mechanism of tissue damage is still unknown.

It has been demonstrated that *Trichomonas tenax* presents proteolytic activity mediated by cysteine endopeptidases which hydrolyze different types of collagen molecules (types I, III, IV and V) present in all periodontal tissues<sup>22-23</sup>. Additionally, this protozoan can also lyse human eritrocytes<sup>24</sup>. Acid phosphatase has also been found in vesicles structures of *Trichomonas tenax* in the Golgi apparatus, in primary and secondary lysosomes, in cytoplasmic granules and in the free extremity of the waving membrane<sup>25</sup>. Therefore, these studies seem to suggest a more active role of *Trichomonas tenax* in the progression of periodontal disease. However, further studies are necessary for the complete elucidation of this issue.

Not with standing the above mentioned data, the high proportion of healthy individuals with *Trichomonas tenax* in contrast with the low proportion of patients with gingivitis and *Trichomonas tenax* found by the present study suggests that gingival inflammation may modify the microenvironment of the gingival pockets and disrupt the colonies of this microorganism. On the other hand, the high proportion of patients with periodontitis (inflammation of the periodontium with progressive bone loss) and *Trichomonas tenax* instigates other speculations, such as that the progression of periodontal disease could somehow promote other significant changes in the dental biofilm making it susceptible to new *Trichomonas tenax* colonies.

Salivary pH ranged from 6.0 to 8.0, but the peak incidence of commensals in salivary samples occurred between pH 6.0 and 6.5. As the pH increased becoming more alkaline, the incidence of the study microorganisms decreased, suggesting that a more acidic pH provides a better environment for these microorganisms. These

findings are corroborated by other studies<sup>17,26</sup>. However, still other studies have not found a relationship between salivary pH and the presence of these protozoa<sup>18</sup>.

Regarding gender, both parasites were more common in females, but the difference was not statistically significant. These data are in agreement with those of other studies<sup>27-28</sup>.

Age is also considered a predisposing factor for protozoan infection in patients with periodontal disease. Several authors reported that the incidence of *Entamoeba gingivalis* and *Trichomonas tenax* increases with age<sup>9,16,29</sup>. However, no statistically significant correlation between age and presence of oral commensals was observed in this study. This apparent contradiction may be related to an absence of age group standardization.

Other factors that increase the risk of periodontal disease are diabetes, infection with anaerobic Gram-negative species and smoking<sup>30</sup>.

Tobacco is capable of reducing the synthesis of IgG and IgM by plasma cells, as well as the phagocytic activity and chemotactic response of gingival neutrophils, so the host's defense against bacteria in the gingival pocket is substantially impaired<sup>21</sup>.

There are contradicting reports in the literature regarding diabetes: one study reports a high incidence of these commensals (74%) in adult diabetics<sup>2</sup> and another study reports a low incidence<sup>28</sup>.

## CONCLUSION

The present study did not find a relationship between the presence of *Entamoeba gingivalis* and *Trichomonas tenax* and smoking, alcoholism and diabetes. In fact, risk factors for periodontal disease were present in only a few participants, so any inference or deliberation on this subject should be cautious.

The results of this research suggest that *Entamoeba gingivalis* occurs more frequently in the early stages of periodontal disease, whereas *Trichomonas tenax* sees the oral cavity as its natural habitat given its commonness in individuals with healthy gums. However, further studies are necessary for determining the real nature of the relationship between these species and periodontal disease.

## Collaborators

RLC ALBUQUERQUE JÚNIOR assessed the teeth and gums. CM MELO performed the microbiological analyses. WA SANTANA wrote and reviewed the article. JL RIBEIRO and FA SILVA performed the study.

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