

ENTAMOEBA GINGIVALIS FREQUENCY IN ORAL CAVITY

Casas Pruneda G., PhD
Barron Cruz M., PhD
Mariel Cardenas J., PhD
Gutierrez Cantu F.J., PhD
Oliva Rodriguez R., PhD
Romo Ramirez G.F., MA
Gamez Lopez L.R. PhD

Laboratory of Parasitology. School of Chemical Science,
University of San Luis Potosi, Mexico

Abstract

The parasite *Entamoebagingivalis* is a protozoan from the oral cavity of the human, primarily found in dental plaque which forms on the base of the gums, around and between the teeth and in gingival tissues. It has been studied because of their relationship with dental pathologies. Frequency of *Entamoebagingivalis* in soft dental plaque was studied in 80 samples from three populations with an age range of 4-66 years with a null mouthwash until three times a day. The first population was patients who came to the diagnostic clinic of the faculty of dentistry with a dental pathology such as caries and gingivitis in which a 18.4% frequency of protozoa was found, the second population were students of the Faculty of Chemistry with good oral hygiene in which *Entamoebagingivalis* was found in 18.2%, and the third population of people was taken randomly from the town of Vanegas, San Luis Potosí, México where the frequency of *Entamoebagingivalis* was 5%. Were related variables such as sex and frequency of oral hygiene, without showing an association. In addition to the protozoan, the presence of some germs, such as fungi and bacteria were also found.

Keywords: Entamoebagingivalis, parasite human, oral cavity

Introduction

Protozoan *Entamoebagingivalis* parasite human oral cavity, primarily in dental plaque which forms on the base of the gums, around and between the teeth; and in gingival tissues. It was discovered in 1849. These

microorganisms are present in apparently healthy mouths and the incidence increases significantly in various dental pathologies. In several studies the frequency of these parasites was determined in a population of patients with healthy mouths and was found in 48% (1996)¹; also have been found the presence of some germs such as fungi and bacteria. Continuing the search for this protozoan in patients with oral pathology, *Entamoebagingivalis* was found in 76% (1997)². It is thus considered that the parasite has an important role in diseases such as gingivitis and periodontitis. In Moscow, Museaev et al. in 1978 published results correlating the incidence of oral parasites as pathogens in periodontal disease. It has also been reported that *Entamoebagingivalis* is more frequent in immunocompromised, diabetics and patients with dentures, and has been found in patients infected with the Human Immunodeficiency Virus associated with periodontal disease by 77% (1998)³.

It has been suggested that *Entamoebagingivalis* only exists as trophozoite (mobile form), because cysts have not been described. The trophozoite measures 5 to 35 µm, is ovoid and irregular, their cytoplasm is divided into two regions: ectoplasm, clear and refringent and endoplasm, finely granular. In the latter, there is a spherical nucleus with central karyosome, lysosomes containing hydrolytic enzymes such as acid phosphatase, lacks mitochondria and Golgi apparatus. Presents nutritional vacuoles, which phagocytic capabilities, which presents polymorphonuclear cells, epithelial cells (from the buccal mucosa), bacteria and rarely erythrocytes at various stages of digestion. Trophozoites are mobile when body temperature is favorable due to the emission of several multidirectional pseudopods, which are long and lobulated and sometimes short and blunt, but sometimes the formation of several at once causes the body to remain temporarily in the same site. Pseudopodia may adhere closely from the microvilli of oral epithelial cells.

Transmission is by direct oral-oral contact, the hosts are humans, monkeys, cats, dogs and horses. The laboratory diagnosis is based on finding trophozoites in two types of samples: saliva and soft plaque.

At present have been conducting studies on its relationship to gingival, periodontal and oral hygiene problems and their possible pathogenicity by PCR and RNA probes⁴⁻⁵. For all the above, concern arises to perform this study and to know the frequency of *Entamoebagingivalis* in a population with dental pathology, a student population with good dental hygiene and an open population.

Material and methods

To conduct this project, three groups were studied: the first included patients attending the diagnostic clinic of the faculty of dentistry, the second,

students of the Faculty of Chemical Science (UASLP) and a third group: people from the town of Vanegas, San Luis Potosí, México.

Each patient was administered a questionnaire and the following information was considered: name, age, sex, place of origin, frequency of oral hygiene, oral diseases present, such as periodontitis, gingivitis, caries, diabetes or any chronic illness.

We proceeded to sample soft plaque with a wooden applicator by scraping the teeth, the samples were stored in 0.9% saline (in volumes of 1.5 ml and 0.8 ml).

In the Laboratory of Parasitology identification of *Entamoebagingivalis* trophozoite using methylene blue as contrast dye was performed. Thereafter in the mycology laboratory samples were planted on PDA medium and Biggy for their study.

Results

Were analyzed 80 samples of soft dental plaque of which 22 belong to the population of students of the faculty of chemical sciences, 38 to the diagnostic clinic of the faculty of dentistry and 20 to a population taken randomly from Vanegas, S.L.P. Of all the samples analyzed by staining with methylene blue and observed under a microscope (40X), was found in 12 samples the presence of protozoan *Entamoebagingivalis*, and was considered positive. Fig 1.

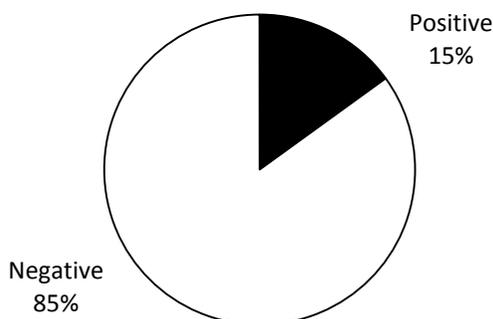


Figure 1. *E. gingivalis* frequency in all samples analyzed.
N=80; positivity was found in 15%

Among the 80 samples, 51 were taken from females and 29 from males. Focusing in each population data were obtained regarding the relationship that might exist between sex and frequency of the parasite.

Table1.Relationship between sex and positive samples.

Sex	Population 1		Population 2		Population 3	
	Sum of persons	Positive samples	Sum of persons	Positive samples	Sum of persons	Positive samples
Female	16	2	22	5	13	0
Male	6	2	16	2	7	1

Population 1.-Students of Faculty of Chemical Science.

Population 2.- Patients from the diagnostic clinic of the Faculty of Stomatology.

Population 3. –People fromVanegas, S.L.P., México.

The total population was divided into three different age ranges and positive results were found: 3/25 positive samples in the range 4-19 years, 6/42 for 20-40 years and 3/13 positive for range 41-66 years. The age range in which the highest percentage of positive samples was observed was in the group of 41-66 years.Fig. 2.

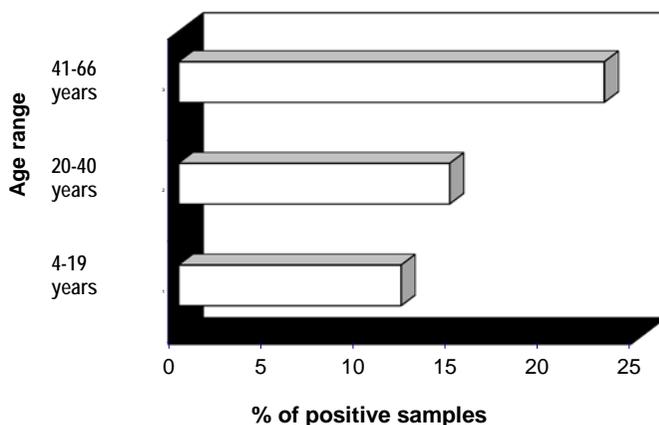


Figure 2. No. of positive samples (n = 12) for *Entamoebagingivalis* according to age range. In which a higher frequency is observed between 41 and 66 years. While in the ranges 4-19 and 20-40 years, is lower.

Taking into account the data previously reported in various publications about the relationship between the frequency of oral hygiene and the presence of the parasite, this variable was considered as a part of the study. Of the 7 people who reported to have no oral hygiene and 11 who do it 1 time a day just 1 positive sample in each group was found. In 22 people with oral hygiene 2 times/day, 6 were positive. Finally the group with frequency of 3 times/day, 4 presented the trophozoite of *Entamoebagingivalis*. Figure 3.

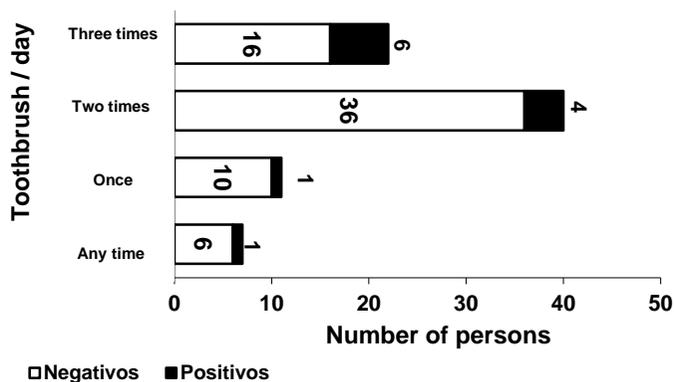


Figure 3.Frequency ratio of oral hygiene and the presence of *Entamoebagingivalis*.

Populations were studied separately, the population of students of the faculty of chemistry were 4/22 samples positive for *Enatomeba. gingivalis*. In the population of patients who attended diagnostic clinic 7/38 samples were positive and about the population from Vanegas, S.L.P. only in 1/20 the parasite was found. Fig.4.

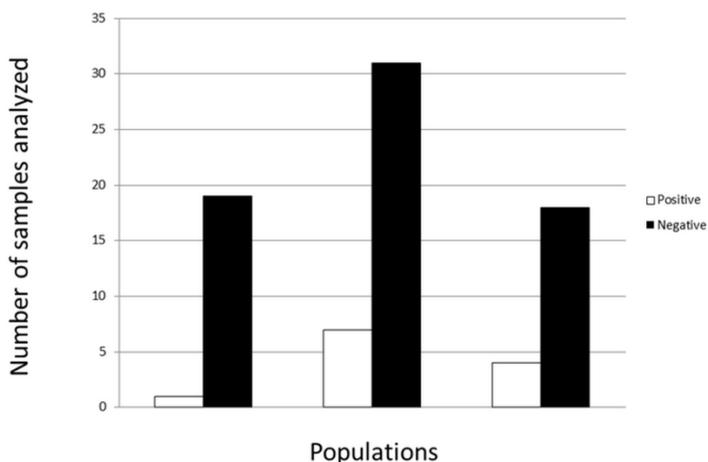


Figure 4.The number of samples analyzed in each population is observed.

Another part of the study was to relate some patients with dental diseases and the presence of trophozoite of *Entamoebagingivalis*. The most common pathologies were gingivitis, caries, dental fluorosis, periodontitis and also diabetes. However, in negative samples this type of health problems also presented; as well as in individuals without any pathology trophozoite was observed.

Besides the protozoan other microorganisms like bacteria and fungi were found, in both positive and negative samples.

The identification of fungi was undertaken, where the most frequent was *Candidasp.* In three of the positive samples, the fungi were *Rhodotorulasp.*, *Aspergillussp.* and *Alternaria*.

Discussion and conclusion

Entamoebagingivalis frequency found in all the samples analyzed was 15%. The percentages of positive samples in the 3 populations analyzed were: student 18.2%, patients of the diagnostic clinic 18.4%, persons belonging to the municipality of Vanegas 5%, being observed a similarity in the percentages of the student population and patients from the diagnosis clinic. Regarding gender, 63.7% of the 3 populations were female, of which only 13.7% were positive; 36.2% were males, with 17.2% positivity for *Entamoebagingivalis*. These data indicate that there is no relationship between gender and the presence of the parasite, since no significant differences were found. Our results in terms of age, showed a higher frequency in the range between 41 and 66 years.

Although *Entamoebagingivalis* has been associated with poor oral hygiene, in this study, no relationship was found between them. Because of people who reported no oral hygiene, 14.2% were positive; those who performed 1 time per day the oral hygiene, only 9% had the parasite; those that performed it 2 times per day, the percentage was 27.2%. And finally, of those who reported hygiene 3 times a day only 5% of the samples had the protozoan. It was determined that in people with good dental hygiene can be found *Entamoebagingivalis*. Furthermore, we found in this study that there was no correlation between the frequencies of parasite as pathogen in dental problems; it could be an opportunistic parasite because has been identified in immunosuppressed individuals, but other authors report it as a parasite pathogen causing various dental diseases or complications.

The presence of the fungi found in the samples from oral cavity can be regarded as part of the normal flora because it has been reported that 300 different types of germs exist and only some of them can cause dental diseases, therefore cannot be stated that fungi found are pathogens, and there regarding the presence of *Entamoebagingivalis*.

Acknowledgements

-Dr. Juan Francisco Reyes Macias. Coordinator of Clinical Diagnosis (Faculty of Stomatology, UASLP)

-QFB.MC. Saldivar Blanca María Ortiz.

Mycology Laboratory Officer (Faculty of Chemistry, UASLP)

References:

- Act of Latin American clinical chemistry, Vol xxx (3); 245-250, 1996
Microbiology Revista Argentina, in Press, 1997
Eric Lucht, Birgitta Evengard, Joakim Skott, Pehr Olov Pehrson, and Carl Erik Nord. *Entamoeba gingivalis* in Human Immunodeficiency Virus Type 1 - Infected Patients with Periodontal Disease. Clinical Infectious Diseases 1998; 27:471-3.
Chen Jinfu, Wangrong Wen, Liu Guangying, Chen Wenlie, Liqun Lin, Hong Hang. Periodontal disease Studies on Caused by *Entamoeba gingivalis* and its pathogenetic mechanism. Chin Med J 2001; 114 (12) 12-15.
Trim RD, Skinner MA, Farone MB, Dubois JD, Newsome AL. Use of PCR to detect *Entamoeba gingivalis* in diseased gingival pockets and demonstrate its absence in healthy gingival sites. Parasitol Res. 2011 Sep; 109(3):857-64.
David Botero, Marcos Restrepo. Human parasitosis. Corporation for Biological Research, Third Edition pp 58.
Act of Latin American clinical chemistry, Vol XXX (3); 245-250, 1996
www.drwebsa.com.ar/fparau/libro01.htm30k