Pathogenic effect of *Entamoeba gingivalis* on gingival tissues of rats

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# ABSTRACT

*Entamoeba gingivalis* was isolated from patients suffering of periodontal problem cultured on a special culture, a suspension of the parasite made and spreaded on the gingival margins of three groups of rats: The first group gives immunosuppressive drugs (prednisolone acetate), while not given to the other two groups.

Results show the rat's gingival tissues of the  $1^{st}$  group became red and swollen within 2 days while a mild inflammation occurs at the gingival tissue of the  $2^{nd}$  group and no lesion or inflammatory process occurs to the third group (control). This experiment revealed that *Entamoeba gingivalis* may become pathogenic in immunocompromised indi-viduals.

**Key Words:** *Entamoeba gingivalis*, gin-giva, rats.

### الخلاصة

عزل طفيلي المتحورات اللثوية من المرضى المصابين بالتهاب اللثة وزرع في مزرعة خاصة. واستخدم محلول المزرعة الحاوي على الطفيلي بمسحه على حافة اللثة لثلاث مجاميع من الفئران. المجموعة الأولى أُعطِيت علاج مثبط للمناعة (prednisolone acetate) بينما لم يعط إلى المجموعتين الأخربين.

أظهرت النتائج أن نسيج اللثة لفئران المجموعة الأولى أصبح أحمر اللون ومنتفخ خلال يومين من الإصابة بينما كان هناك التهاب خفيف لفئران المجموعة الثانية ولم تظهر أي أعراض على فئران المجموعة الثالثة (مجموعة السيطرة). أظهرت التجربة أن طفيلي المتحورات اللثوية قد يكون مرضي خصوصاً في الأشخاص مثبطي المناعة.

#### INTRODUCTION

*Entamoeba gingivalis* is non-patho-genic commensal protozoan colonized on the gingival tissue. Only trophozoite stage present in nature and no cystic form.

The trophozoite measures 10-30  $\mu$ m, actively motile with multiple pseudopodia, the cytoplasm contains food vacuoles with ingested bacteria, leukocytes and epithelial cells. Nucleus is rounded with delicate central karyosome and nuclear membrane

is lined with coarse chromatin granules.<sup>(1)</sup> (Figures 1 and 2)

Nucleur fragments from leukocytes are usually recognizable in stained speci-mens and serve to identify the Amoeba as *Entamoeba gingivalis* as it is the only species that ingests these cells.

Periodontitis is a common disease with complicated factors, difficult treat-ment and serious harm to human beings.<sup>(2)</sup> Grams negative anaerobes are the impor-tant pathogens of periodontitis.<sup>(3)</sup>

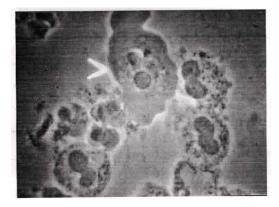


Figure (1): *Entamoeba gingivalis* isolated from pyorrhic gum with surrounding pus cells



Figure (2): *Entamoeba gingivalis* isolated from dental pocket. Note the internal nucleus and other cytoplasmic structures

Some aerobic bacteria (*Pseudomonas aeruginosa*), facultative anaerobic (*Kleb-siella pneumonia*) may cause periodontitis of host under the conditions of giving rats immuno–suppressive drugs and infection of a concentration of bacteria and *Enta-moeba gingivalis* can cooperate with bacteria in causing periodontal disease.<sup>(4)</sup>

Many workers found that the infec-tion rates of *Entamoeba gingivalis* in patients with periodontitis were higher than those of healthy groups through investigation, thus they inferred that it was related to periodontitis.<sup>(5–7)</sup> In the past, it was difficult to culture a large amount of *Entamoeba gingivalis* to do animal test to determine its pathogenicity.

In the current study, we studied the pathogenicity of *Entamoeba gingivalis* in rats with lowered immunity and the possi-bility of this parasite to cause perio-dontitis.

## MATERIALS AND METHODS

*Entamoeba gingivalis* isolated from patients complaining of periodontal prob-lems. The organism cultured on (Cle-veland and collier medium) from Oxoid company in the laboratory for 72 hours, filtered, washed with physiological normal saline for 3 times, then a suspension of the *Entamoeba gingivalis* prepared for experi-mental application by using 9 clean rats about (200–250 g/rat) provided by

animal house of the College of Medicine. The rats had normal teeth and periodontal tissue with average depth of gingival channel of approximately 1mm.

The rats are divided into three groups, (3 rats for each).

The first group injected with predni-solone acetate (0.25 mg/rat) daily for one week before animal test,<sup>(8)</sup> while the other two groups of rats are not given immunosuppressive drug.

*Entamoeba gingivalis* suspension sp-readed on the gingival margin of the rats  $(1^{st} \text{ and } 2^{nd} \text{ groups only})$ . The third group is the control in which their gingival margins are spreaded by normal saline free from *Entamoeba gingivalis*.

# RESULTS

## **First Group**

The rats' gingival tissues have been erythematous and swollen in 2 days after infection, periodontal abscess formed within 5 days, some of the abscesses broken, leading to erosion of the gingiva and necrosis to form ulcer, some of the infected rats died within 7 days.

*Entamoeba gingivalis* along with polymorphonuclear cells were found in purulent secretion from periodontal ab-scess (Table 1).

## Second Group

The rats' gingival tissues have been slightly erythematous and swollen within 2 days after infection. No periodontal pocket formed and no death occurs to the infected rats (Table 1).

#### **Third Group (Control)**

The rats' gingival tissues were normal in appearance and no pathological changes were observed in periodontal tissue (Table 1).

Table (1): Number of infected rats with <i>Entamoeba gir</i>	ıgivalis	
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	No. of Rats	No. of Rats With Infected Gingiva	No. of Dead Rats
<i>Entamoeba gingivalis</i> + Prednisolone	3	3	2
<i>Entamoeba gingivalis</i> + Normal Saline	3	2	0
Control	3	0	0

## DISCUSSION

Epidemiological investigation showed that the infected rate of *Entamoeba* gingi-valis of patients with periodontitis are 67.06% much higher than that of healthy group (38.97%).<sup>(5,7,9)</sup> These results are the same as other reports by workers allover the world, therefore, some workers infer-red that *Entamoeba* gingivalis was related to periodontitis.<sup>(6,10,11)</sup>

Authors injected Entamoeba gingi-valis into the basis of gingival pocket of rat by the method of injection appeared allover the bodies of infected rats. perio-dontal abscess formed and its forming rate reached 78.9% (30/38), living Entamoeba gingivalis were found in purulent secretion and cultured successfully from periodontal abscess.<sup>(12)</sup> This result proves that infec-tion of Entamoeba gingivalis may destroy gingival tissues.

Rats test result prove that Entamoeba periodontitis, gingivalis may cause perio-dontal abscess formation and alveolar bone absorption and so on in different de-gree, in condition of low immunity caused using by immunosuppressive drugs.<sup>(4)</sup>

The rate of periodontitis come on in immunosuppressed group infected with *Entamoeba gingivalis* was higher than that of the control group which have not given immuno–inhibitor drugs. The animal test proved that *Entamoeba gingivalis* in oral cavity can cause periodontitis under the conditions of low immunity of hosts.

The mechanical effect of *Entamoeba gingivalis* of stretching among the epithelial cells of periodontal tissue by their pseudopodia and the cooperation effect of oral symbiotic bacteria, are the major pathogenic mechanisms of perio-dontitis.<sup>(2,12)</sup>

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