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**S.G. Reed · D.E. Lopatin · B. Foxman · B.A. Burt**

# Oral *Chlamydia trachomatis* in patients with established periodontitis

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**Abstract** Periodontitis is considered a consequence of a pathogenic microbial infection at the periodontal site and host susceptibility factors. Periodontal research supports the association of *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Bacteroides forsythus*, and periodontitis; however, causality has not been demonstrated. In pursuit of the etiology of periodontitis, we hypothesized that the intracellular bacteria *Chlamydia trachomatis* may play a role. As a first step, a cross-sectional study of dental school clinic patients with established periodontitis were assessed for the presence of *C. trachomatis* in the oral cavity, and in particular from the lining epithelium of periodontal sites. *C. trachomatis* was detected using a direct fluorescent monoclonal antibody (DFA) in oral specimens from 7% (6/87) of the patients. Four patients tested positive in specimens from the lining epithelium of diseased periodontal sites, one patient tested positive in healthy periodontal sites, and one patient tested positive in the general mucosal specimen. In conclusion, this study provides preliminary evidence of *C. trachomatis* in the periodontal sites. Planned studies include the use of a more precise periodontal epithelial cell collection device, the newer nucleic acid amplification techniques to detect *C. trachomatis*, and additional populations to determine the association of *C. trachomatis* and periodontitis.

**Keywords** *Chlamydia* · *Chlamydia trachomatis* · Fluorescent antibody technique · Periodontal diseases · Periodontitis

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S.G. Reed · B. Foxman · B.A. Burt
Department of Epidemiology, University of Michigan, Ann Arbor, MI 48109, USA

D.E. Lopatin
Department of Biologic and Materials Sciences, University of Michigan, Ann Arbor, MI 48109, USA

S.G. Reed (✉)
Medical University of South Carolina, 173 Ashley Avenue, P.O. Box 250507, Charleston, SC 29425, USA
e-mail: reedsg@musc.edu,
Tel. +1 843 792-1577, Fax: +1 843 792-6626

## Introduction

Periodontitis is characterized by apical migration of the periodontal attachment to the root surface and destruction of the proximal alveolar bone [12]. These diseases are generally considered a consequence of a pathogenic microbial infection at the periodontal site and host susceptibility factors [29]. The prevalence of severe periodontitis is estimated at 7% to 15% of adult populations [11].

In the search for the etiologic agent(s) of periodontitis, research has focused on the microbes of subgingival dental plaque. The relative interest in the microbial dental plaque can be estimated by the large quantity of published research concerning dental plaque and periodontitis. In contrast, there are fewer studies investigating the relationship between periodontitis and bacteria within the periodontal epithelium. In a MEDLINE search of articles from 1966–April 1998, at least 3,815 publications had the keywords “dental plaque” and “periodontitis.” By contrast, the keywords of “bacteria” and “epithelium” and “periodontitis” appeared in only 35 publications.

The Consensus Report, “Periodontal Diseases: Pathogenesis and Microbial Factors,” from the 1996 World Workshop on Periodontics lists evidence for the associations of specific dental plaque microbes with various forms of periodontal disease [16]. The evidence for three microbes, *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Bacteroides forsythus*, as etiologic agents is considered strong. The current viewpoint is that a

"periodontopathic bacterial flora is 'necessary but not sufficient for disease' or that periodontal diseases are 'specific mixed infections which cause periodontal destruction in the appropriately susceptible host'" [52].

Our preliminary study pursued the etiology of periodontitis from the perspective of infection of the periodontal lining epithelium. "Periodontal lining epithelium" is used to describe the superficial epithelium lining the sulcus or pocket, and the junctional epithelium. This study targeted *Chlamydia trachomatis* which is known for infections of the epithelial cell linings of the eyelids (conjunctivitis and trachoma), respiratory tract (neonatal pneumonia) and uro-genital tracts [7, 48, 49, 51, 62, 63]. Through review of the literature, similarities were noted between the natural history of chlamydial cervicitis and periodontitis. First, *C. trachomatis* preferentially infects the columnar or transitional epithelial cells lining the endocervix, and these were considered similar to the cuboidal or junctional epithelial cells lining the periodontal sulcus [8, 62, 73]. Second, both infections are characterized as chronic, usually asymptomatic, with probable bursts of activity, and with the tissue damage due in part to the host immune response [35, 45, 59, 69, 73]. Also, both infections are affected by treatment with tetracycline (especially doxycycline) [12, 32], though the treatment of *C. trachomatis* cervicitis includes concurrent treatment of sex partners to prevent re-infection [13].

Because *C. trachomatis* is an obligate parasite and infects the superficial lining epithelial cells for multiplication by binary fission, the periodontal lining epithelial cells were the target for specimen collection [62, 69]. The distinctive life cycle of chlamydia alternates between intracellular reticulate bodies that are contained within a membrane-bound vacuole of the host cell, and spore-like extracellular elementary bodies (EBs) [35]. Morphologically the EB is a small, spherical cell approximately 0.3 µm (300 nanometers) in diameter [35].

The primary objective of this study was to determine whether *C. trachomatis* could be detected in the periodontal lining epithelium of diseased periodontal sites using the cell collection methods and detection techniques that are commonly used to detect *C. trachomatis* in cervical specimens. The secondary objective of this study was to identify methodological issues when applying *C. trachomatis* detection techniques commonly used for cervical specimens to oral specimens.

## Materials and methods

The study design was cross-sectional to compare the presence of *C. trachomatis* in diseased and healthy periodontal sites in patients with established periodontitis and who also had three periodontally healthy teeth. "Established periodontitis" is defined as the presence of interproximal periodontal clinical attachment level ≥6 mm in two or more teeth and one or more interproximal periodontal sites with probed pocket depth ≥5 mm [46]. For 1 year (19 December 1994–15 December 1995), patients who presented for diagnosis and treatment planning appointments at the dental school clinic were screened for eligibility into the study. The inclusion criteria were: (1) 18–50 years of age at time of dental clinic visit, and (2) at least three teeth that met the case definition of established periodontitis [46], and (3) at least three teeth without periodontitis or gingivitis, i.e., healthy. Four additional criteria for exclusion from the study were applied to help control for possible confounding: (1) a history of specific antibiotics for treatment of *C. trachomatis* in the 3 months prior to the dental clinic visit, or (2) a history of periodontal curettage or surgery, or (3) a history of systemic disease characterized by neutrophil disorders (e.g., diabetes mellitus, Crohn's disease, systemic lupus erythematosus, and ulcerative colitis), or (4) the inability to obtain informed consent

Data were from the patient's dental chart, an investigator administered questionnaire, and periodontal and mucosal cell specimens. Initial periodontal measurements were made by a dental student, confirmed by clinical faculty, and recorded in the patient chart. A single investigator (S.G.R.) screened all patients, confirmed periodontal measurements, performed informed consent, and collected all specimens.

The presence of *C. trachomatis* was assessed in cell specimens originating from three distinct locations in each patient's mouth: (1) the lining epithelium of diseased periodontal sites, (2) the lining epithelium of healthy periodontal sites, and (3) a general collection of mucosal epithelium from the lining of the cheeks, floor of mouth, and tongue. Algorithms were used for the specific tooth selection. For the specimen from the diseased periodontal sites, cells were collected from the linings of the three periodontal sites with the most severe destruction measured (those used to diagnose established periodontitis) and pooled onto one microslide. Likewise, the specimen from the healthy periodontal sites was comprised of cells from the linings of the three periodontal sites with the smallest values of measured disease. The third microslide contained the pooled specimen of mucosal cells.

The periodontal measurements and cell collections were made using sterile periodontal probes (Michigan O) with millimeter demarcations and read to the lesser value. One probe was used for collecting from the pooled diseased sites and another from the pooled healthy sites. The periodontal probe was inserted to the base of the pocket, read, and then wiped along the lining epithelium in the area of the designated interproximal site. The probe was removed from the periodontal pocket and the cell specimen transferred onto the microslide using a rolling action of the probe on the microslide. The wiping action along the lining epithelium was repeated twice at each interproximal periodontal site for cell collection. The surface area of the lining epithelium sampled reflected approximately one-sixth of the tooth circumference.

The general mucosal cell collection was made using a cytobrush. Two strokes were made on each of the fol-

lowing areas of oral tissue: buccal mucosa, floor of mouth, and sides and dorsum of the tongue. Then the brush was pressed and rotated one full turn on the microslide to transfer the pooled cell specimen. All specimens were fixed with methanol and stored at five degrees centigrade for staining and reading within two weeks' time.

A species-specific monoclonal antibody (Behring Diagnostic MicroTrak® *Chlamydia trachomatis* Direct Specimen Test and Direct Specimen Test Control Slide Pack) was used with the direct immunofluorescence technique (DFA) to detect *C. trachomatis.* All microslides were read using an epifluorescence-equipped microscope (Leitz-Wetzlar Dialux 20) for the detection of fluorescein-isothiocyanate (FITC). Each microslide had a designated nine-millimeter diameter field which was methodically scanned under 500× magnification. Any questionable fluorescence was again read under 1000× magnification. Positive and negative control microslides were employed for each run.

Microslides were read for the presence of red blood cells (RBC), white blood cells (WBC), cuboidal epithelial cells, squamous epithelial cells, mucus, interference, and the count of *C. trachomatis* elementary bodies (EBs). A cut off of one or more EBs was used for testing positive for *C. trachomatis*.

All microslides were read by one microscopist experienced (at least 8 years) with the Behring product for the DFA technique. Intra-examiner reliability was evaluated using the four cell types (RBC, WBC, cuboidal and squamous).

The DFA test has not been previously used for oral specimens. As a preliminary investigation of possible cross-reactivity of the commercial monoclonal antibody with oral microbes, the reagents were tested with microbes common to periodontitis and microbes reported in the literature to possibly cross-react with the DFA products (Table 1) [15, 41, 57, 64]. The microbes were obtained from laboratories at the University of Michigan Schools of Dentistry and Public Health, and the Pathology Laboratory of the University of Michigan Hospital.

Data were managed and analyzed using EpiInfo [18]. Group means and standard deviations were used to summarize the descriptive data. For intra-examiner reliability both percent agreement and the Kappa statistic were employed.

**Table 1** Microbes tested for cross-reactivity with MicroTrak® *Chlamydia trachomatis* Direct Specimen Test (all tested negative)

| |
|---|
| *Actinobacillus actinomycetemcomitans* |
| *Bacteroides fragilis* |
| *Bacteroides melaninogenicus* |
| *Bacteroides forsythus* |
| *Eubacterium* |
| *Fusobacterium nucleatum* |
| *Lactobacillus* |
| *Mycoplasma* |
| *Neisseria gonorrhoeae* |
| *Peptostreptococcus micros* |
| *Peptostreptococcus (not species specific)* |
| *Porphyromonas gingivalis* |
| *Propionibacterium acnes* |
| *Selenomonas* |
| *Staphylococcus aureus* |
| *Streptococcus sanguis* |
| *Streptococcus mutans* |
| *Streptococcus intermedius* |
| *Treponema vincentii* |
| *Treponema denticola* |

## Results

Using the methods developed by Aday, the response rate was 82% (87/106) of those eligible for the study [2]. Of the 1432 patients screened, 164 patients were eligible and interviewed, and 50 were excluded because of: (1) a history of specific antibiotics for treatment of *C. trachomatis* in the 3 months prior to dental clinic visit ($n$=28); (2) a history of periodontal curettage or surgery ($n$=4); (3) a history of systemic disease characterized by neutrophil disorders ($n$=17); and (4) the inability to obtain informed consent ($n$=1). Another eight patients were excluded because of the need for a physician consultation prior to dental examination. Only 19 patients considered eligible for the study were not interviewed because of scheduling difficulties ($n$=13), and refusals or indecision ($n$=6).

The study group of 87 consisted of 43 females and 44 males with a mean age of 38±6 years. Seven were in the 20–29 years age group, 43 were 30–39 years old, and 37 were 40–50 years of age. Sixty percent had a history of smoking tobacco with a range of pack-years from less than 1 to 58, and a mean of 18±13 pack-years. One patient reported a history of treatment for *C. trachomatis* (without diagnosis) because of an infected sexual partner.

*C. trachomatis* was detected in oral cell specimens from 7% (6/87) of the study patients. In four patients *C. trachomatis* was detected from the diseased periodonta sites' specimens. Another patient tested positive in the healthy periodontal sites' specimen and another tested positive for *C. trachomatis* in the general mucosal specimen. The study information for the six patients who tested positive for *C. trachomatis* is listed in Table 2.

The diagnostic quality of each specimen was assessed by three criteria: adequacy, interference, and mucous [4]. For endocervical specimens "adequate" is the presence of whole columnar cells, and for the oral specimens cuboidal cells were used [39, 54]. The majority of periodontal specimens, and in particular specimens from the diseased periodontal sites (93%, 81/87) were adequate. Most (69%, 60/87) of the microslide preparations from the healthy periodontal sites were adequate. There were few (18%, 16/87) general mucosal microslides which contained cuboidal cells. Interference was identified on one microslide and presence of mucous was identified in 5% (12/261) of the microslides.

Intra-examiner reliability was evaluated using the four cell types (RBC, WBC, cuboidal and squamous). The test–retest percent agreement was 98.5% (161/168)

**Table 2** Characteristics of six patients with established periodontitis and who tested positive for *C. trachomatis* in the oral cavity

| Patient | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Age (years) | 31 | 42 | 39 | 47 | 43 | 49 |
| Gender | Male | Female | Male | Female | Male | Male |
| Tobacco (pack-years) | 20 | 0 | 5 | 40 | 20 | 29 |
| Location of C. trachomatis | Diseased perio sites | Diseased perio sites | Diseased perio sites | Diseased perio sites | Healthy perio sites | General mucosal sites |
| Count of EBs | 2 | 1 | 1 | 2 | 3 | 9 |
| Cell collection device | perio probe | perio probe | perio probe | perio probe | perio probe | cytobrush |
| Diagnostic quality | | | | | | |
| Adequate | Yes | Yes | Yes | Yes | Yes | No cuboidal epithelial cells |
| Interference | No | No | No | No | No | No |
| Mucous | Yes | No | No | No | No | No |

for the 42/261 (16%) microslides read twice. A Kappa coefficient was calculated for each of the four cell types (1.00, 1.00, 0.95, and 0.71 respectively).

No cross-reactivity was detected when testing the *C. trachomatis* DFA reagents with each of the 20 microbes listed in Table 1.

## Discussion

The exciting discovery in this study was detection of *C. trachomatis* in the epithelial lining cell specimens from the periodontal pocket or sulcus of five participants. This preliminary evidence of *C. trachomatis* in the periodontal location is a first step in investigating the possibility of *C. trachomatis* periodontitis or of the periodontal location as a possible oral reservoir for *C. trachomatis*. However, this evidence must be interpreted with caution until validated by additional studies that use the newer nucleic acid amplification (NAA) techniques for *C. trachomatis* [14] and also culture technique to demonstrate pathogen viability.

The detection of *C. trachomatis* in the epithelial lining of the periodontal pocket or sulcus is a novel, but not necessarily unexpected finding. A deliberate effort is necessary to identify *C. trachomatis* antigen, which may have precluded previous detection [20, 35, 40, 61, 65, 67, 71]. Furthermore, the search for periodontal pathogens has focused on the microbial dental plaque and, for this, study dental plaque was considered to be more similar to the debris that is removed before epithelial cell specimen collection for *C. trachomatis* at cervical and conjunctival locations.

*C. trachomatis* infections are important because they are the most common sexually transmitted bacterial infection in the United States (estimated at least 3 million cases for 1996) [28], and the sequelae of *C. trachomatis* infections in women represent the most costly outcome (estimated 4 billion annually) of any sexually transmitted infection except HIV/AIDS [34, 60]. *Chlamydia* became a nationally notifiable disease in 1995, and the above numbers for genital *C. trachomatis* are probably conservative, reflecting some of the difficulties of introducing surveillance [19, 28]. In light of these estimates, the possibility of the epithelial lining of the periodontal pocket or sulcus as a reservoir for *C. trachomatis* warrants investigation.

Detecting *C. trachomatis* in the oral cavity is consistent with the evidence that supports detection of most other sexually transmitted infection (STI) microbes in the oral cavity. STI viruses found in the oral cavity include Epstein–Barr virus [47], herpes simplex viruses type 1 and type 2 [72], human papilloma virus [33, 38, 53, 58], hepatitis B [8], and cytomegalovirus [36]. Bacterial infections include syphilis [22, 23], Donovanosis [25, 55], chancroid [30, 50] and oropharyngeal gonorrhea [24, 70]. Primary lesions of lymphogranuloma venereum caused by *C. trachomatis* are also found in the oral cavity [3, 17]. Protozoan infections of the oral cavity have been reported in patients with and without periodontal disease [1, 5, 68]. There is evidence for the transmission of non-viral STIs by oral sex [21].

Some of the strengths and limitations of this study are the ability to generalize the findings, the measurement issues of periodontitis, the challenges of adapting methods used for cell collection for *C. trachomatis* cervicitis for the periodontal space, the use of DFA for *C. trachomatis* detection, and the use of this DFA product with periodontal specimens.

The study participants were purposively selected to increase the likelihood of finding *C. trachomatis* and to decrease possible confounding by factors known to affect *C. trachomatis* infection and/or periodontitis. Because the intent of this study was to find out whether the pathogen could be present, the trade-off for the ability to generalize the findings was accepted. Participant enrollment over the calendar year probably decreased any systematic bias in participation influenced by weather or transportation. The relatively high response rate (82%) enhances the internal validity for the study findings.

The major measurement issues for periodontitis were the inclusion criteria, the controlling for confounding, and the lack of a marker of active disease. The decision to include patients who had both diseased and healthy periodontal sites may have lessened the likelihood to identify those patients hypothesized to have periodontal *C. tra-*

*chomatis*. For example, 75 patients with established periodontitis were not included because they did not have at least three teeth that were periodontally healthy. Furthermore, the choice to exclude those patients with systemic factors (other than tobacco use) usually associated with periodontitis would affect the results in a conservative manner. A limitation of the pursuit of the etiology of periodontitis is the inability to distinguish active or current periodontitis from the scars of a previous disease episode. Without a marker of active disease, it is difficult to estimate what proportion of those with the measurements of established periodontitis actually have active infection(s). It is, however, likely that most of the study patients in this cross-sectional study did not have an active case of periodontitis. In longitudinal studies of patients with periodontitis, only a small proportion (7–11%) of those diagnosed with disease experienced additional loss of periodontal attachment during the follow-up years [6, 44, 45]. This may be interpreted that only 7–11% of those patients had active or current infection at those sites during the follow-up years. This initial study was not designed to determine whether those who tested positive for *C. trachomatis* had active periodontitis as evidenced by additional loss of periodontal attachment.

## Collection method and detection technique

For this 1995–1996 study, the DFA technique was chosen because of the (1) opportunity to examine the quality of the oral specimens collected by periodontal probe and cytobrush, (2) availability of the microscopist with 8 years of experience with the commercial product, (3) relatively common use of this species specific monoclonal antibody for detection of *C. trachomatis* in endocervical specimens, and (4) lower cost compared with culture.

For DFA testing of cervical specimens an adequate specimen contains columnar epithelial cells for these are the host cells preferred by *C. trachomatis* [39, 54]. In 82% of the specimens from the diseased periodontal sites with deep pocket depths, the cuboidal epithelial cells were present. In the healthy periodontal sites with shallow sulcular depth, fewer (69%) specimens contained cuboidal epithelial cells and squamous cells were incorporated into the specimens. In most (82%) of the general mucosal specimens no cuboidal epithelial cells were present. These results were consistent with what was expected from the anatomical locations of the specimens.

Early studies of the presence of *C. trachomatis* in specimens from the tonsillar area and throat secretions were equivocal [10, 27]. A 1985 study of *C. trachomatis* from posterior pharyngeal and tonsillar areas in men and women at risk for genital infection found 3.7% of men and 3.2% of women positive by culture technique [37]. The authors stated that they had difficulty with recovery of *C. trachomatis* from the oropharynx and suggested that perhaps *C. trachomatis* was present in low numbers at that location or that unknown factors of pharyngeal secretions interfered with detection [37].

In the current study, *C. trachomatis* was identified from the general mucosal (buccal mucosa, tongue and floor of mouth) specimen in one patient. During the design stage of this study, a 1992 abstract published by Kuroki et al. indicated the presence of chlamydia (not species specific) in oral mucosal specimens in 3/20 patients [42]. Because of this report the third oral location (general mucosal specimen) was added to the healthy and diseased periodontal sites. A major difference in this study as compared to the Kuroki et al. study was that no mucosal irritation or lesion was apparent, nor specifically sampled, as was the oral condition in the Kuroki et al. study. In discussion with the authors and as is true in this study, the recent introduction of the pathogen into the oral cavity prior to testing can not be ruled out.

In this study, the detection of at least one EB was used for the specimen to test positive. This cut-off was selected based upon the experience of the microscopist with the product and the trade-off was increased sensitivity versus specificity. The use and performance of this DFA product with oral specimens has not been previously reported and the product performance was based upon urogenital, rectal, conjunctival, and nasopharyngeal specimens. In the product circular, sensitivity and specificity data generated in clinical trails were based upon detection of two or more EBs for rectal specimens, and four or more EBs for nasopharyngeal swab specimens and 10 or more EBs in urogenital and conjunctival specimens [66]. The sensitivity (compared with culture) of this product with conjunctival and endocervical specimens ranges from 33% to 81% and the specificity ranges from 88% to 98% [9, 43, 56].

In this study, there were few EBs on the microslides (counts of 1–9 EBs). A suspected limiting factor was the use of the relatively smooth metal surface of the periodontal probe for collecting cells from the lining of the periodontal pocket. This surface did not seem conducive for “catching” or transferring the EBs. The mucosal specimen, made using the cytobrush, contained the nine EBs. A cytobrush or swab is commonly used for cervical specimens and provides more surface area for cell and EB collection than the periodontal probe. Unfortunately, the cytobrush or swab is much too wide in diameter for use in the periodontal space. Additional studies are needed to determine the impact of the cell collection methods on the DFA detection of *C. trachomatis* from periodontal specimens.

For preliminary information the commercial reagent was tested against 20 different microbes for possible cross-reactivity. Five of the microbes tested are commonly found in both periodontal and cervical specimens, and at least twelve of the microbes are often found in dental plaque specimens at sites of periodontitis [26, 31]. All tests for cross-reactivity were negative. These results provide evidence to support that the tested microbes were probably not mistaken for *C. trachomatis* EBs in the oral cell specimens using the DFA technique.

## Conclusions

In conclusion, *C. trachomatis* was detected for the first time in cellular specimens from the lining epithelium of diseased periodontal sites using DFA technique. The findings of this study provide preliminary evidence to support detection of *C. trachomatis* in the oral cavity of some adults with established periodontitis. If the oral cavity (and in particular the periodontal lining epithelial cells) can harbor this obligate parasite, consideration of the oral cavity as a reservoir has implications for both periodontal and *C. trachomatis* research.

The study findings indicate that the methods used for identification of *C. trachomatis* in cervical specimens may be applicable to oral specimens. However the development of better periodontal cell collection methods and employment of the more sensitive and specific *Chlamydia trachomatis* detection techniques, e.g., nucleic acid amplification (NAA) [14] are necessary to confirm these findings and advance the science.

The next steps for this research includes using NAA and culture techniques to detect periodontal *C. trachomatis* in populations hypothesized to be at risk for oral chlamydial infections.

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