Viral-bacterial associations in acute apical abscesses

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Objective. Viral-bacterial and bacterial synergism have been suggested to contribute to the pathogenesis of several human diseases. This study sought to investigate the possible associations between 9 candidate endodontic bacterial pathogens and 9 human viruses in samples from acute apical abscesses.

Study design. DNA extracts from purulent exudate aspirates of 33 cases of acute apical abscess were surveyed for the presence of 9 selected bacterial species using a 16S ribosomal RNA gene-based nested polymerase chain reaction (PCR) approach. Single or nested PCR assays were used for detection of the human papillomavirus (HPV) and herpesviruses types 1 to 8.

Results. Two-thirds of the abscess samples were positive for at least one of the target viruses. Specifically, the most frequently detected viruses were HHV-8 (54.5%); HPV (9%); and varicella zoster virus (VZV), Epstein–Barr virus (EBV), and HHV-6 (6%). Bacterial DNA was present in all cases and the most prevalent bacterial species were Treponema denticola (70%), Tannerella forsythia (67%), Porphyromonas endodontalis (67%), Dialister invisus (61%), and Dialister pneumosintes (57.5%). HHV-8 was positively associated with 7 of the target bacterial species and HPV with 4, but all these associations were weak. Several bacterial pairs showed a moderate positive association. Viral coinfection was found in 6 abscess cases, but no significant viral association could be determined.

Conclusions. Findings demonstrated that bacterial and viral DNA occurred concomitantly in two-thirds of the samples from endodontic abscesses. Although this may suggest a role for viruses in the etiology of apical abscesses, the possibility also exists that the presence of viruses in abscess samples is merely a consequence of the bacterially induced disease process. Further studies are necessary to clarify the role of these viral-bacterial interactions, if any, in the pathogenesis of acute apical abscesses. (Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;112:264-271)

Acute apical abscess is characterized by an acute suppurative inflammatory response of the periradicular tissues to bacteria egressing from the infected root canal system.1 Its clinical manifestation involves pain and swelling of soft tissues, and in more advanced and serious cases, patients may present with fever, regional lymphadenopathy, and malaise, with the possibility of cellulitis formation and other complications.2 Numerous microbiologic studies using culture-dependent and culture-independent techniques have demonstrated that the microbiota associated with acute apical abscesses is mixed and dominated by anaerobic bacteria.3,7 Although the most prevalent bacterial species vary from study to study, which can be a result of the idiosyncrasies of the different identification techniques or a result of geography-related issues,8,9 many species are consistently detected and have been regarded as candidate endodontic pathogens. Examples of these species include Treponema species, Tannerella forsythia, Porphyromonas species, Dialister species, Filifactor alocis, and others, many of them only added to the set of candidate endodontic pathogens after the advent of culture-independent molecular microbiology techniques.10

Although apical periodontitis is recognizably an infectious disease caused by bacteria, it has been recently hypothesized that viral-bacterial coinfection may play a role in the pathogenesis of the different forms of this disease,11 basically the same way as in marginal periodontitis.12 Following this model, an active viral infection causes local immunosuppressive effects, which in turn favors the overgrowth of pathogenic bacteria. This theory has also been suggested for the etiology of periodontal abscesses13 and might well be applicable to acute apical abscesses, with the potential to help explain the development of this symptomatic condition.
arising from previously asymptomatic apical periodontitis lesions. Therefore, virus infection may not have the ability to cause abscesses by its own, but it might serve as a disease modifier or severity factor. The proposed mechanisms involve initial bacterial infection of the root canal causing localized inflammation in the periradicular tissues with consequent attraction of host defense cells infected by herpesviruses. As these cells infiltrate and accumulate in the inflamed tissues, the herpesviruses can be reactivated spontaneously, by concomitant bacterial infection or during periods of reduced host resistance. A consequence of active herpesvirus infection may be local immunosuppression, creating an environment favorable to overgrowth of bacteria in the apical root canal. Virally induced reduced host defenses may also favor invasion of the periradicular tissues by a massive amount of bacteria with maximized tissue damage and abscess formation.

In marginal periodontitis, the subgingival presence of DNA from human cytomegalovirus (HCMV) or Epstein-Barr virus (EBV) has been related to an increased occurrence or levels of the periodontal bacterial pathogens \textit{Porphyromonas gingivalis}, \textit{T. forsythia}, \textit{D. pneumosintes}, \textit{Prevotella intermedia}, \textit{Prevotella nigrescens}, \textit{Campylobacter rectus}, and \textit{Treponema denticola}. The interest in the participation of viruses in the pathogenesis of different forms of apical periodontitis is relatively more recent, and there are not many studies on the subject. Specifically, only a couple of studies have examined the associations between herpesviruses and endodontic bacteria. One of them reported the presence of HCMV, EBV, and bacterial taxa, such as \textit{Fusobacterium} species, \textit{Streptococcus} species, and \textit{Parvimonas micra} occurring concomitantly in samples from apical periodontitis and another one detected herpes simplex virus in association with \textit{T. denticola}, \textit{D. pneumosintes}, and \textit{T. forsythia} in samples from necrotic root canals of teeth with apical periodontitis.

Endodontic abscesses have not been extensively studied for virus presence either. In a study targeting 4 herpesviruses, Chen et al. found HCMV in 29% of the patients with acute abscesses, EBV in 6.5%, HSV-1 in 3%, and varicella zoster virus (VZV) in no one. Our group surveyed abscess samples for the presence of herpesviruses types 1 to 8 and human papillomavirus (HPV), and observed that at least one of the target viruses occurred in 61% of the cases. The most prevalent viruses were human herpesvirus (HHV)-8 (48%), HPV (13%), and VZV and HHV-6 (9%). No study so far has investigated the possible viral-bacterial coinfections in endodontic abscesses. Therefore, the present study sought to investigate the possible associations between 9 candidate endodontic bacterial pathogens and herpesviruses types 1 to 8, as well as HPV in samples from acute apical abscesses using polymerase chain reaction (PCR) assays.

\section*{Material and Methods}

\textbf{Subjects, sample taking, and DNA extraction.} Samples used in this study were the same ones from 23 patients included in a previous investigation with the addition of 10 other samples taken following essentially the same protocol and inclusion parameters. The 33 patients who contributed samples were seeking emergency treatment in the Department of Endodontics, Estácio de Sá University, or in 3 hospitals in Rio de Janeiro. Only single-rooted teeth from adult patients (ages ranging from 17 to 64 years), all of them having carious lesions, necrotic pulps, and periradicular radiolucencies were included in this study. Acute apical abscess was diagnosed on the basis of the presence of pain, exacerbated by mastication, and localized or diffuse swelling, along with fever, lymphadenopathy, or malaise. No fistula connecting the abscess to the oral cavity or skin surface was observed. All teeth showed no significant gingival recession and an absence of periodontal pockets deeper than 4 mm. None of the individuals reported to be HIV-positive. The study protocol was approved by the Ethics Committee of the Estácio de Sá University.

Abscesses were sampled by aspiration of purulent exudate from the swollen mucosa over each abscess. The overlying mucosa was disinfected with 2% chlorhexidine solution, and a sterile disposable syringe was used to aspirate pus, which was immediately injected into cryotubes containing TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.6). Pus samples were frozen at \(-20^\circ\text{C}\). DNA was extracted from samples by using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA), following the protocol recommended by the manufacturer. To improve the performance of PCR assays for virus detection, DNA extracts from abscess aspirates were subjected to multiple displacement amplification (MDA) by using the Illustra GenomiPhi V2 DNA amplification kit (GE Healthcare, Piscataway, NJ) following the manufacturer’s instructions.

\textbf{PCR assays.} All PCR analyses were performed in duplicate. Positive and negative controls were included in all batches of samples analyzed. Positive controls for viruses consisted of DNA extracted from clinical samples (blood or saliva) previously tested positive for each target virus as determined by PCR and sequencing. Positive controls for bacteria consisted of DNA extracted from cultures of the test species (\textit{T. denticola} B1 strain, \textit{T. forsythia} ATCC 43,037, \textit{Porphyromonas endodontalis} ATCC 35,406, \textit{D. pneumosintes} ATCC 33,048, \textit{F. alocis} ATCC 35,896, \textit{P. gingivalis} ATCC...
abscess samples as described by Boulet et al.23 The amplified by means of a single PCR protocol for all /H9252 sis, a 268-bp human -globin gene fragment was am-
plified by means of a single PCR protocol for all
batches of samples analyzed.

To check for availability of DNA for further analy-

sis, a 268-bp human -globin gene fragment was am-
plified by means of a single PCR protocol for all
batches of samples analyzed. A multiplex nested-PCR approach
was used to simultaneously detect HSV-1/2, HCMV, and
EBV according to Tafreshi et al.24 Single PCR
assays were used to confirm findings from the multiplex
nested PCR for HSV-1/2,25 and also to detect VZV26
and HPV.27 Nested PCR assays were used for detection of
HCMV,21 EBV,21 HHV-6,28 HHV-7,29 and HHV-
8.30 Aliquots of 2 µL of MDA products were used as
templates in each individual PCR reaction for virus
detection. All PCR reactions and cycling parameters for
virus detection are summarized in a previous study,22
except for those nested PCR assays targeting HCMV
and EBV, which followed the protocol by Chen et al.21

Nine candidate bacterial pathogens were also targeted
in this study. For the analysis of prevalence of these
species, whole-genomic DNA extracts from clinical samples
were used as templates in a 16S rRNA gene based-
nested PCR protocol. In the first PCR reaction, a prac-
tically full-length 16S rRNA gene fragment was amplified
using a pair of universal bacterial primers using aliquots of
5 µL of the DNA extracts (not amplified by MDA),
followed by a second round of individual species-specific
PCR reactions targeting each species and using 1 µL of
the first universal reaction. PCR primers, reactions, and
cycling conditions were as reported earlier for D. in-
visus, D. pneumosintes, F. alocis, P. endodontalis, P.
gingivalis, T. forsythia and T. denticola, universal
primers, O. uli, and P. piscicola (formerly Synergistes
oral clone BA121).31

Amplicons were separated by electrophoresis in
1.5% agarose gel, stained with ethidium bromide and
viewed under ultraviolet transillumination. A 100-bp
DNA ladder digest (New England Biolabs, Beverly,
MA) served as the molecular size standard.

Representative products from positive PCR reactions
were sequenced to confirm identification. For this, ampli-
cons were purified using a PCR purification system (Wiz-
ard PCR Preps, Promega, Madison, WI) and sequenced
with the forward primers on the ABI 377 automated DNA
sequencer using dye terminator chemistry (Amersham
Biosciences, Little Chalfont, Buckinghamshire, UK). Se-
quenoe data and electropherograms were inspected by
using the BioEdit software.38 Sequences were then com-
pared with those available in GenBank to identify the
closest relatives by using the BLAST algorithm.39

Data analysis. All data were analyzed and the prev-
ence of the target viruses and bacterial species were
recorded as the percentage of samples evaluated. Possible
viral-bacterial associations were evaluated by rel-
ative risk (RR) calculation with 95% confidence inter-
val. Phi coefficient was used to determine the strength of
association using the following criteria: −1.0 to 0,
negative or no association; 0 to +0.3, weak positive
association; +0.3 to +0.7, moderate positive association;
+0.7 to +1.0, strong positive association. Associations
involving only bacteria or viruses were also
recorded. Calculations included only those bacterial
species or viruses that were found in 3 or more cases.

RESULTS

All 33 pus aspirates amplified by MDA yielded pos-
itive results in the PCR assay for -globin gene. All of
these samples were also positive for the presence of
bacteria as revealed by the first round of the nested PCR
using universal 16S rRNA gene primers. These findings
indicated that both human and bacterial DNA were
available in the samples for further detection of the
target viruses and bacteria.

Twenty-two samples (67%) were positive for at least
one of the target viruses. Specifically, the most fre-
cently detected viruses were HHV-8 (18/33 cases,
54.5%), HPV (3/33 cases, 9%) and VZV, EBV and
HHV-6 (2/33 cases, 6%). HCMV was the only virus not
identified in any of the abscess samples (Fig. 1).

Nested PCR demonstrated that the most prevalent
bacterial species were T. denticola (23/33 cases, 70%),
P. endodontalis (22/33 cases, 67%), T. forsythia (22/33
cases, 67%), D. invvisus (20/33 cases, 61%), D. pneu-
mosintes (19/33 cases, 57.5%), and F. alocis (18/33
cases, 54.5%) (Fig. 1). All samples but 1 were positive
for at least 1 of the target bacterial species. This sample
negative for the target bacterial species was also neg-
ative for the target viruses.

Some viral-bacterial associations were observed be-
tween the target bacteria and viruses (RR > 1). HHV-8
was positively associated with 7 of the target bacterial
species and HPV with 4. Only P. gingivalis/HPV as-
sociation showed RR value greater than 2. However,
when these findings were analyzed by Phi coefficient
calculation, only weak positive associations were dis-
closed. Data are displayed in Tables I and II.

Several bacterial positive associations were observed
in this study involving all the species tested (RR > 1)
(Table I). Different pairs of species demonstrated a
moderate positive association with both RR greater
than 2 and Phi coefficient greater than 0.3. They include *T. forsythia* and *D. invisus*; *P. endodontalis* and *F. alocis*, *D. invisus* or *D. pneumosintes*; *D. pneumosintes* and *P. piscolens* or *F. alocis*; *F. alocis* and *P. piscolens*; and *O. uli* and *P. piscolens*. Other positive associations are depicted in Tables I and II.

Viral coinfection was found in 6 abscess cases, with 1 case harboring 3 of the target viruses (VZV, HHV-7, and HPV) and the 5 others contained pairs of HHV-8 with HSV, HPV, EBV, VZV, or HHV-6. The very low prevalence of most individual viruses did not allow reliable statistics to be performed for viral associations.

**DISCUSSION**

The concomitant infection with virus and bacteria and the associations between some bacterial species and virus types may suggest that the viral-bacterial coinfection model may be applicable to the etiology of abscesses. Actually, viral-bacterial cooperation to cause disease has been suggested for a series of disorders, including periodontal diseases, otitis media, acute respiratory tract infections, and sinusitis. The present molecular microbiology study evaluated the viral-bacterial, bacterial and viral associations involving 9 candidate endodontic bacterial pathogens, HPV, and herpesvirus types 1 to 8 in 33 samples of acute apical abscesses. Thus far, it seems to be the first study to investigate such associations in acute apical abscesses.

The present findings revealed that two-thirds of the abscess aspirates were positive for the presence of DNA from at least one of the viruses tested. The presence of most of these viruses in the purulent exudate aspirated from acute apical abscesses may be explained by the influx of host defense cells infected by these viruses in the periradicular tissues in response to bacterial stimuli from the root canal. However, because all these viruses can also be shed in saliva, one cannot discard the possibility of their gaining entry into the pulp and periradicular tissues via pulp exposure in teeth with large crown destruction.

In all abscess samples that were virus-positive, bacteria were also present, including at least one of the target species. Observation of viral-bacterial coinfection in abscesses may have basically 2 interpretations. Either the theory of viruses causing impaired local host defense and then favoring bacterial overgrowth might be true or occurrence of viruses is just an epiphenomenon to bacterial infection that caused inflammation with consequent influx of virus-infected inflammatory cells to the area. In a study like this with a cross-sectional design, it is not possible to define if each interpretation is true. The fact that there were 11 abscess samples that tested negative for all target viruses may suggest that the latter explanation would be more appropriate, i.e., viruses accumulate in the lesion as infected defense cells are attracted during inflammation. These negative cases also indicate that patients who are not infected with these viruses can also develop acute apical abscess, which is also in favor of the “epiphenomenon” argument. However, one might consider the possibility that viruses other than those targeted in this study might have been present or that the
### Table I. Bacterial and viral associations in acute apical abscesses as determined by relative risk calculation (95% confidence interval)

<table>
<thead>
<tr>
<th>Taxa</th>
<th>T. forsythia</th>
<th>P. endodontalis</th>
<th>D. invisus</th>
<th>D. pneumosintes</th>
<th>F. alocis</th>
<th>P. gingivalis</th>
<th>O. uli</th>
<th>P. piscolens</th>
<th>HHV-8</th>
<th>HPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treponema denticola</td>
<td>0.9 (0.6-1.5)</td>
<td><strong>1.4</strong> (0.8-2.5)</td>
<td><strong>1.2</strong> (0.7-1.9)</td>
<td><strong>1.3</strong> (0.8-2.1)</td>
<td><strong>1.5</strong> (0.9-2.2)</td>
<td><strong>1.2</strong> (0.7-1.6)</td>
<td><strong>3.4</strong> (0.7-20.9)</td>
<td><strong>3.4</strong> (0.7-20.9)</td>
<td><strong>1.1</strong> (0.6-2.4)</td>
<td>0.8 (0.1-6.5)</td>
</tr>
<tr>
<td>Tannerella forsythia</td>
<td>—</td>
<td><strong>1.7</strong> (0.9-3.3)</td>
<td><strong>2.2</strong> (1.2-3.7)</td>
<td>1.0 (0.6-1.7)</td>
<td><strong>1.4</strong> (0.8-2.2)</td>
<td>0.9 (0.5-1.4)</td>
<td>0.6 (0.2-1.9)</td>
<td>1.0 (0.3-3.3)</td>
<td><strong>1.3</strong> (0.6-2.8)</td>
<td>0.2 (0.0-1.8)</td>
</tr>
<tr>
<td>Porphyromonas endodontalis</td>
<td>—</td>
<td><strong>2.8</strong> (1.2-7.4)</td>
<td><strong>2.6</strong> (1.1-7.2)</td>
<td><strong>8.5</strong> (2.0-48.0)</td>
<td><strong>1.3</strong> (0.4-4.2)</td>
<td>0.0 (0.8-24.1)</td>
<td><strong>1.7</strong> (0.5-7.0)</td>
<td>0.7 (0.4-1.5)</td>
<td>1.0 (0.1-7.4)</td>
<td></td>
</tr>
<tr>
<td>Dialister invisus</td>
<td>—</td>
<td><strong>1.7</strong> (0.9-3.1)</td>
<td><strong>1.9</strong> (1.0-3.3)</td>
<td>0.8 (0.4-1.4)</td>
<td><strong>2.2</strong> (0.6-9.1)</td>
<td><strong>2.2</strong> (0.6-9.1)</td>
<td>1.0 (0.5-2.0)</td>
<td>0.3 (0.0-2.3)</td>
<td></td>
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</tr>
<tr>
<td>Dialister pneumosintes</td>
<td>—</td>
<td><strong>2.5</strong> (1.2-5.8)</td>
<td>0.9 (0.4-1.5)</td>
<td><strong>2.5</strong> (0.7-10.3)</td>
<td><strong>5.8</strong> (1.1-35.5)</td>
<td><strong>1.4</strong> (0.7-2.9)</td>
<td><strong>1.4</strong> (0.2-11.0)</td>
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<tr>
<td>Filifactor alocis</td>
<td>—</td>
<td><strong>1.6</strong> (0.8-2.4)</td>
<td><strong>2.9</strong> (0.8-11.6)</td>
<td><strong>6.6</strong> (1.3-40.1)</td>
<td><strong>1.6</strong> (0.8-3.2)</td>
<td><strong>1.6</strong> (0.8-12.4)</td>
<td><strong>4.0</strong> (0.5-29.8)</td>
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<tr>
<td>Porphyromonas gingivalis</td>
<td>—</td>
<td><strong>1.6</strong> (0.8-2.4)</td>
<td><strong>2.9</strong> (0.8-11.6)</td>
<td><strong>6.6</strong> (1.3-40.1)</td>
<td><strong>1.6</strong> (0.8-3.2)</td>
<td><strong>1.6</strong> (0.8-12.4)</td>
<td><strong>4.0</strong> (0.5-29.8)</td>
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</tr>
<tr>
<td>Olsenella uli</td>
<td>—</td>
<td><strong>5.3</strong> (1.8-14.0)</td>
<td><strong>1.3</strong> (0.6-2.0)</td>
<td><strong>1.3</strong> (0.1-9.4)</td>
<td><strong>1.3</strong> (0.6-2.0)</td>
<td><strong>1.3</strong> (0.1-9.4)</td>
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</tr>
<tr>
<td>Pyramidobacter piscolens</td>
<td>—</td>
<td><strong>1.6</strong> (0.8-2.3)</td>
<td><strong>1.6</strong> (0.8-2.3)</td>
<td></td>
<td>0.0 (0.0-3.0)</td>
<td></td>
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<tr>
<td>HHV-8</td>
<td>—</td>
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<td></td>
<td></td>
<td></td>
<td>0.4 (0.0-3.0)</td>
</tr>
</tbody>
</table>

Bold face, positive associations (relative risk >1).

### Table II. Strength of association between bacterial species and viral types in acute apical abscesses by Phi coefficient (95% confidence interval)

<table>
<thead>
<tr>
<th>Taxa</th>
<th>T. forsythia</th>
<th>P. endodontalis</th>
<th>D. invisus</th>
<th>D. pneumosintes</th>
<th>F. alocis</th>
<th>P. gingivalis</th>
<th>O. uli</th>
<th>P. piscolens</th>
<th>HHV-8</th>
<th>HPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treponema denticola</td>
<td>-0.04 (0.3-0.2)</td>
<td>0.23 (0.5)</td>
<td>0.14 (0.1-0.4)</td>
<td>0.23 (0.5)</td>
<td><strong>0.32</strong> (0.0-0.5)</td>
<td>0.18 (0.1-0.3)</td>
<td>0.25 (0.0-0.3)</td>
<td>0.25 (0.0-0.3)</td>
<td>0.06 (0.2-0.3)</td>
<td>-0.02 (0.3-0.1)</td>
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<tr>
<td>Tannerella forsythia</td>
<td>—</td>
<td><strong>0.31</strong> (0.0-0.6)</td>
<td><strong>0.48</strong> (0.1-0.7)</td>
<td>0.04 (0.2-0.3)</td>
<td>0.25 (0.5)</td>
<td>-0.04 (0.3-0.2)</td>
<td>-0.14 (0.4-0.1)</td>
<td>0 (0.7-0.3)</td>
<td>0.12 (0.1-0.4)</td>
<td>-0.22 (0.4-0.0)</td>
</tr>
<tr>
<td>Porphyromonas endodontalis</td>
<td>—</td>
<td><strong>0.48</strong> (0.1-0.7)</td>
<td><strong>0.43</strong> (0.0-0.6)</td>
<td><strong>0.64</strong> (0.3-0.7)</td>
<td>0.09 (0.2-0.3)</td>
<td>0.28 (0.0-0.4)</td>
<td>0.14 (0.2-0.3)</td>
<td>-0.12 (0.4-0.2)</td>
<td>0 (0.3-0.1)</td>
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<tr>
<td>Dialister invisus</td>
<td>—</td>
<td><strong>0.31</strong> (0.0-0.5)</td>
<td><strong>0.38</strong> (0.0-0.6)</td>
<td>-0.08 (0.4-0.2)</td>
<td>0.21 (0.1-0.4)</td>
<td>0.21 (0.1-0.4)</td>
<td>0.01 (0.3-0.3)</td>
<td>-0.17 (0.3-0.1)</td>
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<td>Dialister pneumosintes</td>
<td>—</td>
<td><strong>0.44</strong> (0.1-0.6)</td>
<td>-0.04 (0.3-0.2)</td>
<td>0.25 (0.0-0.4)</td>
<td><strong>0.38</strong> (0.0-0.5)</td>
<td>0.20 (0.1-0.4)</td>
<td>0.05 (0.2-0.2)</td>
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<td>Filifactor alocis</td>
<td>—</td>
<td>0.25 (0.0-0.4)</td>
<td>0.28 (0.0-0.4)</td>
<td><strong>0.42</strong> (0.0-0.5)</td>
<td>0.26 (0.0-0.5)</td>
<td>0.26 (0.0-0.5)</td>
<td>0.07 (0.2-0.2)</td>
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<td>Porphyromonas gingivalis</td>
<td>—</td>
<td>0 (0.2-0.3)</td>
<td>0.14 (0.1-0.4)</td>
<td>0.25 (0.0-0.4)</td>
<td>0.25 (0.0-0.4)</td>
<td>0.22 (0.0-0.4)</td>
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<tr>
<td>Olsenella uli</td>
<td>—</td>
<td><strong>0.54</strong> (0.1-0.7)</td>
<td>0.14 (0.1-0.3)</td>
<td>0.04 (0.1-0.3)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Pyramidobacter piscolens</td>
<td>—</td>
<td>0.28 (0.0-0.4)</td>
<td>-0.19 (0.1-0.1)</td>
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<tr>
<td>HHV-8</td>
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<td></td>
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<td>-0.13 (0.3-0.1)</td>
</tr>
</tbody>
</table>

Bold face, moderate/strong positive associations (Phi coefficient >.3).
highly sensitive methods used in this study may have in some way failed to detect the target viruses. Further studies are required to help clarify these important questions.

As for specific viral-bacterial interactions, there were many positive albeit weak associations involving HHV-8 and HPV, the 2 most prevalent viruses in this study, and the target bacterial species. The nature and consequence of these positive associations, if confirmed, requires further elucidation. Some authors have suggested a bidirectional interaction between periodontal bacterial pathogens and herpesviruses, in which bacterial pathogens could promote herpesvirus reactivation, and this active virus infection in turn would impair host defenses and contribute to the increase in numbers and virulence of the bacterial pathogens.22 The low prevalence of the other viruses in the present study does not allow for further comparisons with most findings from periodontal studies in which some viruses, especially HCMV and EBV, have been far more prevalent.

Notably, HHV-8 DNA was detected in more than one-half of the cases (54.5%), and showed a weak positive association with 7 of the target bacterial species. Occurrence of this virus in acute endodontic abscesses has been only recently reported22 and almost nothing is known about its role in the disease process. Also, studies in other areas that verified the possibility of coinfection between HHV-8 and bacterial species are scarce.44 Since its discovery, HHV-8 has been related to the development of different pathologies, such as all subtypes of Kaposi’s sarcoma, multicentric Castleman’s disease, primary effusion lymphoma, and body cavity–based lymphoma.45 Given its high prevalence in endodontic abscesses, future studies focusing on this herpesvirus are warranted.

HCMV, EBV, and HSV-1/2 have been commonly detected in samples from periodontally diseased sites and some studies have also found them in samples from endodontic diseases.19,21,46,47 Association of these herpesviruses with bacterial species in both periodontal and endodontic infections has been reported.16,18–20 However, in this study, EBV was detected in only 2 cases, HSV-1/2 in only 1, and HCMV was not found at all. Absence of both EBV and HCMV was previously observed for some of the samples used in this study using 2 different primer sets.22 In this study, we used the same nested PCR conditions and primers used by Chen et al.,21 who detected HCMV in 29% and EBV in 6.5% of abscesses. Even so, we did not succeed in detecting the former and EBV was only present in 2 samples. The low prevalence or absence of these herpesviruses in abscesses does not allow for further analysis about their associations.

The high prevalence of several candidate bacterial pathogens in abscess samples, including T. denticola, T. forsythia, P. endodontalis, D. invisus, and D. pneumosintes, is in agreement with previous studies.10,48 When pairs of the target species were evaluated, several positive associations were evident. Of the test species, only P. endodontalis and F. alocis showed positive associations with all other target species. The strongest bacterial associations based on RR values and confirmed by the Phi coefficient were observed for the pairs P. endodontalis/F. alocis (RR = 8.5, Phi = 0.64), F. alocis/P. piscolens (RR = 6.6, Phi = 0.42), D. pneumosintes/P. piscolens (RR = 5.8, Phi = 0.38), O. uli/P. piscolens (RR = 5.3, Phi = 0.54), and P. endodontalis/O. uli (RR = 4, Phi = 0.28). Associations between bacterial species have already been disclosed by previous culture49,50 and molecular microbiology studies51 and help elucidate the complex interactions among members of bacterial communities occurring in endodontic infections. Positive associations between 2 species indicate that they have more chances of being found together in coinfection and suggest the existence of interactions, such as coaggregation, food chains, cooperation for nutrient acquisition, and pathogenic synergism.1

In conclusion, our findings demonstrated that bacterial and viral DNA occurred concomitantly in two-thirds of the samples from endodontic abscesses. This raises some important questions and may suggest the role of viruses in the etiology of apical abscesses. However, analyses of specific viral-bacterial positive associations were compromised by the low prevalence of most viruses targeted. In addition, the few viral-bacterial positive associations observed were rather weak. The possibility also exists that the presence of viruses in abscess samples is only a consequence of the bacterially induced disease process and may not have a pathogenetic effect whatsoever. Further studies are necessary to clarify the role of these viral-bacterial interactions, if any, in the pathogenesis of acute apical abscesses.

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