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Short communication

Treponema species associated with abscesses of endodontic origin

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Spirochetes have been frequently observed in abscesses of endodontic origin, but they have rarely been identified. This study sought to investigate the prevalence of eight oral treponemes in acute periradicular abscesses using a species-specific nested polymerase chain reaction assay. Purulent exudate was collected by aspiration from 19 cases diagnosed as acute periradicular abscesses and DNA extracted from the samples was initially amplified using universal 16S rDNA primers. A second round of amplification used the first polymerase chain reaction products to detect a specific fragment of the 16S rDNA of each *Treponema* species. The species-specific nPCR assay used in this study allowed the detection of *Treponema denticola* in 79%(15 of 19), *Treponema socranskii* in 26%(5 of 19), *Treponema pectinovorum* in 21% (4 of 19), *Treponema amylovorum* in 16% (3 of 19), and *Treponema medium* in 5% (1 of 19) of the cases. Spirochetal DNA was found in 89% of the cases (17 of 19). The number of *Treponema* species per case ranged from 1 to 3 (mean, 1.5). *Treponema vincentii*, *Treponema lecithinolyticum* and *Treponema maltophilum* were not detected in any pus sample. The present data lend support to the assertion that *Treponema* species, particularly *T. denticola* and *T. socranskii*, may be involved in the pathogenesis of acute periradicular abscesses.

Key words: acute periradicular abscess; endodontic infection; human epidemiology; oral treponemes

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Spirochetes were initially observed in humans by Antonie van Leeuwenhoek at the end of the 17th century (3) and then initially cultivated by Noguchi in 1912 (10), using a medium containing sheep serum, water and a piece of sterile testicle or kidney tissue from rabbit or sheep. Despite other culture media and techniques developed in the ensuing years, until now most of the spirochetes remain difficult or even impossible to cultivate. Novel spirochetal species, or phylotypes, that can not be presently cultivated *in vitro*, have been identified from the human oral cavity, the termite gut, and other host-associated or free-living sources through the use of molecular technology (11). There are now over 200 spirochetal species or phylotypes, of which more than half cannot be cultivated at present (11).

Microscopic studies have long disclosed spirochetes in infections of endodontic origin, including abscessed teeth (8, 21, 22). However, it was not until the advent of molecular genetic methods for microbial identification that spirochetes present in acute periradicular abscesses could be identified. We reported for the first time the occurrence of *Treponema denticola* in cases of acute periradicular abscesses using the single polymerase chain reaction (PCR) method (18). This species was detected in half of the pus aspirates taken from acute periradicular abscesses. When we used the checkerboard DNA–DNA hybridization method to survey samples from abscesses of endodontic origin, we were able to detect both *T. denticola* and *Treponema socranskii* in about 4% of the cases (19). Because difficulties in isolating and identifying these microorganisms may have obscured the participation of *Treponema* species in endodontic infections, the present study was undertaken to check for the prevalence of eight oral treponemes in acute abscesses of endodontic origin using a 16S rDNA-based nested PCR assay.

The examined material was taken from adult patients (aged 18–45 years) who were seeking emergency treatment at three hospitals in Rio de Janeiro, Brazil. Samples were collected from 19 teeth, all of which had carious lesions, necrotic pulps, and radiographic evidence of periradicular bone loss. Pain and swelling were present in all cases, which were diagnosed as acute periradicular abscesses according to Torabinejad & Walton (20). Cases showed

localized or diffuse swelling along with fever, lymphadenopathy, or malaise. No apparent communication from the abscess to the oral cavity or the skin surface was observed. Two patients reported that they had taken systemic antibiotics. Selected teeth showed no significant gingival recession and an absence of periodontal pockets deeper than 4 mm.

Samples were taken by aspiration of purulent exudate from swollen mucosa over each abscess. After disinfection of the overlying mucosa with 2% chlorhexidine, a sterile disposable syringe was used to aspirate pus, which was immediately injected into cryotubes containing 5% dimethyl sulfoxide in trypticase-soy broth (Difco, Detroit, MI). Samples were then immediately frozen at −20°C.

Pus samples were thawed to 37°C for 10 min and vortexed for 30 s. Microbial suspension was washed 3 times with 100 µl of ultrapure water by centrifugation for 2 min at 2500 × *g*. Pellets were then resuspended in 100 µl of ultrapure water, boiled for 10 min and chilled on ice. After centrifugation to remove cell debris for 10 s at 9000 × *g* at 4°C, the supernatant was collected and used as the template for PCR amplification. Reference DNA from *T. denticola* B1 (Forsyth Dental Institute, Boston, MA), *Treponema pectinovorum* ATCC 33768, *T. socranskii* S1 (Forsyth Dental Institute), *Treponema vincentii* ATCC 35580, *Treponema maltophilum* ATCC 51939, *Treponema amylovorum* ATCC 700288, *Treponema lecithinolyticum* ATCC 700332, and *Treponema medium* ATCC 700293 was also extracted to serve as positive control for the primers used.

The primer sequences used are listed in Table 1. Universal bacterial primers anneal at conserved regions near the 5′- and 3′-ends of 16S rDNA, generating a practically full-length 16S rDNA product. Sequences for *T. denticola, T. socranskii*, and *T. lecithinolyticum* consisted of specific forward and reverse primers. Primers for *T. pectinovorum*, *T. vincentii*, *T. maltophilum, T. amylovorum* and *T. medium* utilized a universal forward sequence (position 8–27 of *Escherichia coli* 16S rDNA) along with a species-specific reverse sequence. A spirochetal selective reverse primer C90 was also used along with the forward universal bacterial primer (2). The specificity of the primers has been previously determined by the proponent studies (1, 17, 23), but cross-hybridization between the target species was checked anew in this study.

The nested PCR protocol used in this study to identify treponemes was followed as described previously (12, 17). PCR amplicons were identified in agarose electrophoretic gel and visualized for size using ultraviolet light at 320-nm wavelength. Positive reactions were assigned based on the presence of clearly visible bands of the expected molecular size (Table 1).

The species-specific nPCR assay used in this study allowed the detection of *T. denticola* in 79% (15 of 19), *T. socranskii* in 26% (5 of 19), *T. pectinovorum* in 21% (4 of 19), *T. amylovorum* in 16% (3 of 19), and *T. medium* in 5%(1 of 19) of the cases. Amplification using the spirochetal selective primer C90 revealed that spirochetes were found in 89% of the cases (17 of 19). This finding was confirmed by the fact that at least one of the target species was detected in those 17 cases. The number of *Treponema* species per case ranged from 1 to 3 (mean, 1.5). *T. denticola* was found in coinfection with *T. socranskii* in four cases, with *T. pectinovorum* in three cases, with *T. amylovorum* in two cases, and with *T. medium* in one case (Table 2). Other associations can be viewed in Table 2. Nevertheless, data analysis using Fisher's Exact test failed to show any positive association between the *Treponema* species targeted in this study ($P > 0.05$). *T. vincentii, T. lecithinolyticum* and *T. maltophilum* were not detected in any pus sample.

The species-specific primers yielded a single amplicon with strains of the target species. No detectable bands were observed with the nontarget species. Reference DNA and clinical samples that were positive for the bacteria tested showed only one band of the predicted size. All samples were positive for the universal bacterial primers, demonstrating that bacteria were present in all cases sampled. Such results also indicated that components of the purulent exudate did not inhibit the DNA amplification reaction.

We have previously reported the occurrence of the same eight *Treponema* species in root canal infections associated with either asymptomatic periradicular lesions or acute apical periodontitis (12, 17). The most prevalent species detected were *T. denticola*, *T. socranskii* and *T. maltophilum.* In the present study, five of the eight target species occurred in pus aspirates from acute periradicular abscesses. *T. denticola* was by far the most prevalent species detected (79% of the cases),

Table 1. PCR primer pairs used for detection of *Treponema* species in abscesses of endodontic origin

Target	Primer sequences (5′-3′)	Position (bp)	Amplicon length (bp)	Annealing temperature (°C)	Reference
T. amylovorum	AGA GTT TGA TCC TGG CTC AG CTC ACG CCT TTA TTC CGT GAG	8–155	148	54	23
T. denticola	TAA TAC CGA ATG TGC TCA TTT ACA T TCA AAG AAG CAT TCC CTC TTC TTC TTA	193–508	316	60	1
T. lecithinolyticum	CTT GCT CCT TTC TGA GAG TGG CGG ACG CAT CCG TAT CTC TAC GAA CTT	54–1003	950	65	17
T. maltophilum	AGA GTT TGA TCC TGG CTC AG CCT ATT GTG CTT ATT CAT CAG GC	8–443	436	54	23
T. medium	AGA GTT TGA TCC TGG CTC AG CCT TAT GAA GCA CTG AGT GTA TTC	8–163	156	54	23
T. pectinovorum	AGA GTT TGA TCC TGG CTC AG ATA TAT CTC CAA CTT ATA TGA CCT	8–200	193	53	23
T. socranskii	GAT CAC TGT ATA CGG AAG GTA GAC A TAC ACT TAT TCC TCG GAC AG	148–435	288	53	23
T. vincentii	AGA GTT TGA TCC TGG CTC AG AAT ACT TCT TAT GAA CAT TGA GAC	8–203	196	56	23
Spirochetal C90	AGA GTT TGA TCC TGG CTC AG GTT ACG ACT TCA CCC TCC T	8–1503	1496	60	2
Universal 16S rDNA	AGA GTT TGA TCC TGG CTC AG ACG GCT ACC TTG TTA CGA CTT	8–1513	1506	55	23

Table 2. Occurrence of eight *Treponema* species in abscesses of endodontic origin as assessed by nested PCR

Cases	Clinical features	No. of target species	PCR results									
			Universal	Spirochetal	*T. denticola*	*T. socranskii*	*T. pectinovorum*	*T. amylovorum*	*T. medium*	*T. vincentii*	*T. maltophilum*	*T. lecithinolyticum*
C1	Pain, swelling	1	+	+	+	–	–	–	–	–	–	–
C2	Pain, swelling	2	+	+	+	+	–	–	–	–	–	–
C3	Pain, swelling	1	+	+	+	–	–	–	–	–	–	–
C4	Pain, swelling	1	+	+	+	–	–	–	–	–	–	–
C5	Pain, swelling Fever, tetra cycline	2	+	+	–	+	+	–	–	–	–	–
C6	Pain, swelling Fever, ampi cillin	1	+	+	+	–	–	–	–	–	–	–
C7	Pain, swelling	0	+	–	–	–	–	–	–	–	–	–
C8	Pain, swelling	2	+	+	+	–	–	+	–	–	–	–
C9	Pain, swelling	2	+	+	+	+	–	–	–	–	–	–
C10	Pain, swelling	2	+	+	+	+	–	–	–	–	–	–
C11	Pain, swelling	2	+	+	+	–	+	–	–	–	–	–
C12	Pain, swelling	1	+	+	+	–	–	–	–	–	–	–
C13	Pain, swelling	2	+	+	+	+	–	–	–	–	–	–
C14	Pain, swelling	0	+	–	–	–	–	–	–	–	–	–
C15	Pain, swelling	1	+	+	+	–	–	–	–	–	–	–
C16	Pain, swelling	3	+	+	+	–	+	–	+	–	–	–
C17	Pain, swelling	3	+	+	+	–	+	+	–	–	–	–
C18	Pain, swelling	1	+	+	–	–	–	+	–	–	–	–
C19	Pain, swelling	1	+	+	+	–	–	–	–	–	–	–
Percent			100	89	79	26	21	16	5	0	0	0

followed by *T. socranskii* (26%). These figures are higher than those previously reported for these species, using either single PCR (18) or the checkerboard DNA–DNA hybridization method (19). Such a discrepancy in prevalence values may have been due to the different detection limits of the techniques used, with nested PCR being more sensitive than the other two methods (16). Nevertheless, in addition to confirming that these two treponemes can be found in cases of endodontic abscess, the high prevalence of both *T. denticola* and *T. socranskii* found in the present study seems to support their association with that disease.

To our knowledge, this is the first study to report the occurrence of *T. pectinovorum*, *T. amylovorum*, and *T. medium* in samples taken from acute periradicular abscesses. These species have been only recently found in root canal infections (12, 17) and their occurrence in the purulent exudate associated with periradicular abscesses raises the suspicion that they may participate in the pathogenesis of this disease. Other treponemes previously found in root canal infections, such as *T. vincentii, T. lecithinolyticum* and *T. maltophilum*, were not detected in the abscessed samples examined herein.

Amplification using the spirochetal selective primer revealed that spirochetes were found in 89% of the cases. All these cases were also positive for at least one of species targeted. However, the possibility exists that other *Treponema* species (including unknown species) might have been present in coinfection with the known target species. This could only have been checked by cloning and sequencing of the PCR products generated by the spirochetal selective primer, which was not performed in this study. Ongoing studies from our laboratory are trying to investigate the diversity of spirochetes in different types of endodontic infections.

The polymicrobial nature of the endodontic microbiota suggests that bacteria are interacting with one another and such interaction can play an important role for both survival and virulence (15). In a mixed bacterial community, it is likely that *T. denticola* has its virulence enhanced or it can enhance the virulence of other species in the consortium (4). In the present study, nine out 19 cases harbored more than one of the target *Treponema* species. Although statistical analysis failed to show any positive association between the *Treponema* species, the sample size was not large enough for a consistent conclusion to be drawn. Whether there is some cooperation between *Treponema* species to induce harmful effects to the periradicular tissues is still unknown. Associations involving treponemes and other bacteria in endodontic infections remain to be investigated.

Oral treponemes can cause abscesses when inoculated in experimental animals (5). These microorganisms are reported to possess an array of putative virulence traits that may be involved in the pathogenesis of endodontic abscesses by wreaking havoc on host tissues and/or by allowing the microorganism to evade host defense mechanisms. Such factors include proteolytic enzymes (5), collagenase (7), fibrinolytic enzymes (9), iminopeptidases and phospholipase C (14), hyaluronic acid and chondroitin sulfate-degrading enzymes (13), and locomotor ability (6).

Oral treponemes are pathogenic bacteria that are involved with a variety of oral diseases. The microscopic observation of these microorganisms in pus samples by other studies and their high prevalence as reported by the present study indicate that treponemes are also members of the microbiota associated with acute periradicular abscess and suggest that they can participate in the pathogenesis of this disease. Efforts should be made to cultivate oral treponemes associated with acute periradicular abscesses in order to establish a proper antimicrobial approach, based on antibiotic susceptibility tests, to treat diffuse and life-threatening forms of the disease.

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