Detection of *Treponema denticola* in root canal systems in primary and secondary endodontic infections. A correlation with clinical symptoms

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SUMMARY

Aim. The aim of the study was to investigate the presence of *Treponema denticola* in primary and secondary root-infected canal systems with periapical pathology and correlations with clinical signs and symptoms.
Methodology. Endodontic samples were obtained from canals of 102 teeth: 79 had primary endodontic disease and 23 secondary endodontic disease. For each tooth, clinical data including symptoms and X-ray appearance were examined. The presence of *T. denticola* biological samples from the root canal space was detected by a PCR assay.
Results. T. denticola was detected in 24 out of the 79 teeth with primary infection and in 8 out of the 23 teeth with secondary infection. Teeth with specific clinical symptoms were frequently associated with *T. denticola* presence inside the root canal system.
Conclusions. The presence of *T. denticola* in root canal system in association with specific clinical signs and symptoms of endodontic disease strongly suggests that this spirochete might play a critical role in the pathogenesis of the acute infection and rapid bone tissue alterations in both primary and secondary endodontic infections.

KEY WORDS: Endodontic pathogens, Endodontic infection, Polymerase Chain Reaction (PCR), Apical periodontitis, *Treponema denticola*

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INTRODUCTION

Dental carious lesion is still the most common cause of endodontic disease (Baumgartner, 2004). In addition, pathways for the entry of microorganisms into the pulp (endodontic) space include direct exposure of the pulpal chamber (i.e. trauma, caries, dental procedures) dentinal tubules, lateral and accessory channels. Once pulpal tissues are necrotic and collapsed the whole root canal system becomes a *reservoir* for microorganisms and their specific metabolic products (Baumgartner, 2004). Because of the lack of circulation within the necrotic pulpal tissues, the entire root canal system becomes a kind of "sanctuary" for the bacteria that can survive there safe from the systemic and local immune response (Baumgartner, 2004). The final goal of endodontic therapy is to remove all pathogenic bacteria from the root canal system. Unfortunately, most of the endodontic pathogenic bacteria are able to cross the tooth apical foramen and invade the periapical bone tissue. They consequently provoke localised damage that strongly contributes to cre-

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ate a/symptomatic chronic periapical (periradicular) lesions that are associated with primary infections of the root canal system (Jung *et al.*, 2000; Nair *et al.*, 2005).
Infections of the root canal system with facultative and obligate anaerobic bacteria have been associated with different clinical signs and symptoms (Yoshida *et al.*, 1987; Jung *et al.*, 2000; Gomes *et al.*, 2004; Siqueira & Rocas 2004). Several recent investigations showed that molecular methods, such as PCR, will disclose small populations of bacteria inside the root canal system and provide a taxonomically exact identification of the germs.
While the microbial aetiology of the periradicular disease is well known (Haapasalo *et al.*, 2003), it remains unclear whether the same bacterial species responsible for asymptomatic periapical lesions can also contribute to the development of symptomatic infections or whether a unique assemblage of micro-organisms is responsible for the latter. Some species (e.g. *Streptococcus)* have been implicated with the aetiology of most symptomatic lesions (Chàvez de Paz *et al.*, 2003, Sakamoto *et al.*, 2006). However it was later shown that this species is also present with identical epidemiological frequency in asymptomatic periapical lesions (Portenier *et al.*, 2003; Fouad *et al.*, 2002; Sakamoto *et al.*, 2006).
Recent studies performed using molecular biology techniques suggested that *T. denticola* is likely to be one of the microorganisms frequently identified within the root canals and that these spirochetes may be responsible for the pathogenesis of periapical bone lesions (Baumgartner *et al.*, 2003; Siqueira & Rocas 2005). These studies suggested that the clinical signs and symptoms of the periapical lesions may somehow correlate with the bacterial load and the species of micro organisms identified within the root canal system. In other words, different bacterial species originating from the endodontic site of infection are capable of causing different clinical signs and symptoms when evading through the tooth apical foramen and invading the periapical bone.
Secondary infection (mainly caused by a failed root therapy) occurs when a primary endodontic therapy performed to clear the canal system bacterial contamination is inefficient in terms of removal of the germ load consequently generating the pathogenic conditions that preclude periapical bone healing. Primary endodontic infections are very often caused by polymicrobial flora among which the dominant species are the Gram-negative anaerobic rods. On the other hand, the aetiology of secondary infections is very likely attributable to a single or to a very few differently associated bacterial species (Molander *et al.*, 1998; Portenier *et al.*, 2003; Stuart *et al.*, 2006).
The aim of this study was to investigate the presence of *T. denticola* in the root canal system using a PCR assay, and to assess the correlation between the presence of this spirochete and the specific clinical signs and symptoms of periapical lesions associated with teeth affected by primary and secondary endodontic infection.
The null hypothesis was the lack of correlation between the clinical status of periapical (apical) periodontitis (acute, chronic and exacerbate apical periodontitis) and the detection of *T. denticola* within in the root canal system.

MATERIALS AND METHODS

Study population

The population studied in this study comprised ninety-four patients attending the Endodontic Care Unit of the Department of Dental and Oral Sciences of the University of Bologna, Italy. All patients were in good general health conditions showing no major systemic diseases such as diabetes. Patients receiving any kind of antibiotic treatment in the last two months before the root canal therapy were excluded from the study. Written informed consent was obtained from each individual patient before enrolment in the study. The internal review board of the Department of Dental and Oral Sciences approved the research protocol.
Samples of endodontic material were obtained from 102 teeth affected by periapical disease, as demonstrated by clinical and radiographic signs. In all cases enrolled in the study periodontal probing was inferior to 4 mm. When a tooth was unsuccessfully and improperly isolated with a rubber dam the patient was not enrolled in the study. Clinical features were recorded for each individually treated tooth. The following clinical symptoms were recorded: presence of pain on oc-

clusion and spontaneously; swelling; abscess and presence of periapical radiolucency; presence of a previous root canal filling.
The diagnosis of AAP (acute apical periodontitis) was confirmed when a patient suffered from acute clinical symptoms (pain to occlusion, tenderness to percussion or palpation on the periapical area, swelling), but any evidence of periapical radiolucency was lacking. The presence of periapical radiolucency was determined with a paralleling X-ray technique and scored according to the periapical index (PAI) (Orstavik *et al.*, 1986). Periapical bone resorption was considered present any time a PAI score was greater than "2". CAP (chronic apical periodontitis) was defined as the presence of periapical radiolucency, with no other clinical symptom in the 3 months before the endodontic therapy. The concomitant presence of periapical radiolucency and one or more clinical symptoms such as pain, swelling, and tenderness to percussion was diagnosed as EAP (exacerbated apical periodontitis).

Sampling procedure
One hundred and two endodontic samples were obtained from 98 patients (mean age 47.4±12.4, female to male ratio 1:1,17). After anaesthesia, teeth were isolated using a rubber dam. Surface disinfection of intact enamel was carried out using a small cotton swab soaked with 5% NaOCl solution (Niclor 5, Ogna, Maggiò, Italy) in accordance with a previous study (Foschi *et al.*, 2005) and dried with a sterile cotton pellet. No rubber dam leakage was observed during sampling procedures.
Access cavities were made using sterile burs on a high-speed hand-piece. Cooling water spray was supplied by either Logos Junior or Duo type dental units (Castellini S.p.A., Castelmaggiore-Bologna, Italy) equipped with an Autosteril system (Montebugnoli *et al.*, 2004). The patency of each canal was assessed by inserting a #15 K-file (Dentsply-Maillefer, Ballaigues, Switzerland) with the tip at the pre-determined working length. In cases of filled root canals gutta-percha was removed without chemical solvents with the aid of Gates Glidden burs (Dentsply-Maillefer, Ballaigues, Switzerland) and K-files. A small quantity of sterile saline was introduced before sampling if the root canal space was dry. Two or more paper points extra-fine size (Mynol, Milwaukee, WI, USA) were placed at working length and used to soak up the fluid in the canal. Each paper point was retained *in situ* for exactly 40 seconds. The paper points were then transferred to sterile 1.5 ml tubes (Eppendorf AG, Hamburg, Germany) containing 500 µl of sterile phosphate buffered saline (PBS) solution. Samples were frozen immediately at -20°C and stored up to one month before use.

PCR assays
DNA extraction from samples was performed using the QIAamp DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's indications. PCR testing and amplicons analysis were performed as previously reported (Cavrini *et al*., 2005; Foschi *et al.*, 2005).

Data analysis
Data collected for each sample were recorded into an electronic data spreadsheet and analyzed with SPSS 11.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistical analysis was performed using the Pearson Chi-square test or the one-sided Fisher's Exact test, as appropriate.

RESULTS

Table 1 shows the distribution of cases among the diagnostic groups. Symptomatic cases (68/102, 66.7%) were categorized as AAP (acute clinical symptoms but no radiolucency) and EAP (exacerbated apical periodontitis), whereas asymptomatic teeth (34/102, 33.3%) were categorized as CAP.
Overall, *T. denticola* were detected in 34 of 102 cases. Table 2 shows the percentage of positive and negative samples in each group. *T. denticola* was frequently detected in cases of EAP (26/40, 65%); this association was significant (P <0.01). Only 6 samples were positive for *T. denticola* and CAP, while 2 resulted positive in the AAP group. No correlation was observed between *T. denticola* infection and AAP and CAP.
Table 3 illustrates the percentage of positive samples for *T. denticola* among the three clinical diagnoses of AAP, CAP and EAP. Table 5 shows the incidence and distribution of the *T. denticola* with respect to primary and secondary endodontic infection and illustrates the clinical symptoms.

TABLE 1 - *Distribution of cases and percentage of acute, chronic and exacerbated apical periodontitis according to the type of dental elements.*

Diagnosis	*Tooth Distribution*				
	I	*C*	*P*	*M*	*Total (n=102)*
AAP (27.5%)	6	3	9	10	28
CAP (33.3%)	4	4	10	16	34
EAP (39.2%)	4	6	7	23	40

AAP= acute apical periodontitis; CAP= chronic apical periodontitis; EAP= exacerbated periodontitis with abscess; I= incisor, C= canine; P= premolar; M=molar.

TABLE 2 - *Percentage of positive sample for T. denticola distributed among the three types of clinical diagnosis.*

Diagnosis	*T. denticola +*
AAP (n=28)	2 (7.1%)
CAP (n=34)	6 (17.6%)
EAP (n=40)	26 (65%)
Total (n=102)	34 (33.3%)

AAP= acute apical periodontitis, CAP= chronic apical periodontitis; EAP= exacerbated apical periodontitis.

DISCUSSION

To date PCR is considered one of the most sensitive techniques to investigate the composition of endodontic flora (Siqueira & Rocas, 2005) in patients with bacterial infection of the endodontic canals. This method also detects slow growing, strictly anaerobic and uncultivable bacteria. In association with a sequencing technique unidentified species can also be tentatively classified (Fouad *et al.*, 2002) and the role they might play in the aetiology of endodontic infections can be hypothesized.

The aim of the present study was to investigate

TABLE 3 - *Percentage of positive sample for T. denticola distributed among the types of clinical symptoms and between primary or secondary infection.*

Signs and symptoms	*Positive/negative*	*Primary infection (n=79)*		*Secondary infection (n=23)*	
		T. denticola positive (30.4%)	*T. denticola negative (69.6%)*	*T. denticola positive (34.8%)*	*T. denticola negative (65.2%)*
Pain	+	20,3%	19,0%	21,7%	21,7%
	-	10,1%	50,6%	13,0%	43,5%
Periapical radiolucency	+	25,3%	48,1%	30,4%	47,8%
	-	5,1%	21,5%	4,3%	17,4%
Swelling	+	3,8%	11,4%	21,7%	21,7%
	-	26,6%	58,2%	13,0%	43,5%
Tenderness to percussion	+	22,8%	34,2%	21,7%	21,7%
	-	7,6%	35,4%	13,0%	43,5%

the presence of *T. denticola* in the root canal system and to correlate the presence of this spirochete with the clinical signs and symptoms of periapical lesions of teeth affected by primary or secondary endodontic infection. In addition, we also investigated a possible association between the presence of microbial pathogens and selected clinical manifestations. The results of this study confirmed that *T. denticola* infection may be associated with both primary and secondary endodontic lesions. The presence of this pathogen is statistically associated with defined clinical signs such as pain, swelling and radiographic periapical bone alterations.

Previous investigations suggested that the risk for a *T. denticola* infection is higher if a complication of periapical lesions, such as abscesses and cellulites, bone resorption and acute clinical symptoms are present (Baumgartner *et al.*, 2003). The present study supports the concept that *T. denticola* is frequently detected in conditions where specific symptoms such as pain and swelling are present.

A previous study from this group in a smaller population revealed similar findings as regards the association between clinical signs of periapical periodontitis and *T. denticola* (Foschi *et al.*, 2005), but the overall incidence of this spirochete in the root canal space of affected teeth appeared significantly higher (34% vs. 24%). Similar fluctuations in the prevalence of endodontic pathogens might be ascribed to the larger population studied or epidemiological fluctuations.

A recent study demonstrated that *T. denticola* is rarely present in a South Korean population but is a typical finding in Brazilian patients affected by asymptomatic and acute periapical disease (Siqueira *et al.*, 2005). Taken together the results of these studies indicated that some species are significantly more likely to be detected in samples from a given geographic location than from another. It is possible to suppose that a clinical condition such as EAP is frequently associated with *T. denticola* infection only in specific geographic areas such as Italy (Foschi *et al.*, 2005) and USA (Baumgartner *et al.*, 2004). Hence, the present study demonstrated that the presence of pain and other symptoms specific to acute and exacerbate apical periodontitis (periapical lesion) must prompt the detection of *T. denticola* in patients living in selected geographic areas such as Italy.

The present study strongly suggests that *T. denticola* have the capability to colonize the canal system of teeth affected by primary infection with necrotic pulp tissue and root-filled canal systems with secondary infection. Similar results were reported by Hommez *et al.* (2004) for other bacteria. In both conditions *T. denticola*, like other microorganisms (Nair 2004; Tanomaru-Filho *et al.*, 2005), might be able to rapidly cross apical foramen of infected teeth, invade periapical tissues and induce bone resorption as suggested by studies that revealed the presence of this spirochete in periapical lesions (Sunde *et al.*, 2003) and in lateral canals (Nair *et al.*, 2005). At this stage, the well-known proteolytic activity and induction of bone resorption of *T. denticola* play an important role in the pathogenesis of periapical lesion (Choi *et al.*, 2003, Fenno & Mcbride 1998, Sela 2001). Hence, the presence of periapical bone resorption, detected radiographically, is indicative of a complex mechanism which involves large numbers of bacteria (Stashenko *et al.*, 1998) for a sufficient period of time to stimulate periapical bone resorption induced by immune response, which includes IL-1 and possibly TNFγ (Sasaki *et al.*, 2000) or by osteoclasts and MMP expression (Choi *et al.*, 2003) as confirmed by several studies (Ehmke *et al.*, 2004).

T. denticola may survive within the root canal system and inside dentinal tubules and it could also persist for a long time inside dentinal tubules before initiating secondary disease (Peters *et al.*, 2001) or may be considered a late colonizer. *T. denticola* has been detected in periodontal disease (Sela, 2001): it is elevated in dental plaque of patients affected by adult periodontitis, acute pericoronitis and implant failures (Sela, 2001) It is probable that dentin (or dentinal tubules) from patients with active adult periodontal disease may be rapidly infected and play the role of reservoir. Finally, the presence of this pathogen inside the root canal may increase the risk for iatrogenic exacerbations (flare ups) when infected dentin debris is transported into the apical region during incomplete endodontic procedures (Siqueira 2002).

It is important to consider that the proteolytic enzymes and the cytolytic factors of *T. denticola* together with their high mobility contribute to the bacteria invasive capabilities (Sela, 2001; Siqueira *et al.*, 2004; Nair 2004): these data strongly suggest

that spirochetes can evade from uninfected dentinal canals reaching the periapical bone and leaving the oral compartment to spread in the patient. The relevance of oral spirochetes might not be limited to endodontic and periodontal diseases. Literature reports have speculated on the ability of oral treponemes to induce systemic diseases. Recent investigations demonstrated the presence of genomic DNA of *T. denticola* and other pathogens in a great number of arterial samples and oral samples of patients with Buerger disease (Iwai *et al.*, 2005). The available data suggesting a role in atherosclerosis and other cardiovascular diseases pathogenesis are mostly preliminary (Beck *et al.*, 2005) or observatory (Cavrini *et al.*, 2005); nevertheless the oral *exordium* of systemic infection should be considered in future studies regarding chronic systemic disease.

This study indicates a potential role of *T. denticola* in inducing periapical diseases with major clinical symptoms compatible with the diagnosis of EAP. The study demonstrates that invasive and painful lesions of the periapical region are possible when *T. denticola* infection is present in root canal system of both primary endodontic infection with necrotic pulp and in root-filled canals affected by secondary infections.

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