ORIGINAL SCIENTIFIC WORKS | LUCRĂRI ORIGINALE

IDENTIFICATION OF MICROORGANISMS IN THE ROOT CANALS OF THE DOG - A COMPARISON WITH THE HUMAN ENDODONTIC MICROBIOTA

IDENTIFICAREA MICROORGANISMELOR DIN CANALELE RADICULARE ALE CÂINELUI - COMPARAŢIE CU FLORA MICROBIANĂ ENDODONTICĂ LA OM

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ABSTRACT | REZUMAT

The purpose of this study was to identify the bacterial species occupying the infected root canals of dogs, using an automated biochemical identification system, Vitek 2 Compact 15 (Biomerieux, France). In order to recognize a high number of pathogen bacteria, we used the Vitek 2 identification cards.

We compared the bacteria to the previously identified microorganisms in the human endodontic systems. The microorganisms were collected from six infected root canals of two dogs, that were brought by the owners with fractures and abrasions of the dental crowns and opening of the pulp chambers. Cases with dental fractures but no exposure of the dental pulp were eliminated from the study.

Using the automated system Vitek 2 Compact, four predominant bacterial species have been identified, most of them with different characteristics compared to the microorgansms commonly found in the root canals of infected human teeth.

The results of this study suggested that the different diet and characteristcs of the oral hygene in dogs influence the presence of certain microorganisms in the root canals, when compared to humans.

Keywords: root canals, endodontics, microorganisms, dogs, Vitek identification

Scopul acestui studiu a fost de a identifica speciile bacteriene care ocupă canalele radiculare infectate ale câinilor, cu ajutorul unui sistem de identificare biochimic automat, Vitek2Compact15 (Biomerieux, France).

Pentru a recunoaşte un număr cât mai mare de bacterii patogene, am utilizat cardurile de identificare caracteristice pentru fiecare tip de microorganism.

Bacteriile identificate au fost comparate cu microorganismele izolate anterior din sistemele endodontice umane. Probele au fost recoltate din şase canale radiculare infectate, de la doi câini (aduşi de proprietari) cu fracturi şi abrazii ale coroanelor dentare şi deschiderea camerelor pulpare. Cazurile cu fracturi dentare dar fără expunerea camerei pulpare au fost eliminate din studiu. Cu ajutorul sistemului automat Vitek 2 Compact au fost identificate patru specii predominante de bacterii, cele mai multe dintre ele cu particularităţi diferite în raport cu microorganismele frecvent întâlnite în canalele radiculare ale dinţilor umani infectaţi. Rezultatele acestui studiu au sugerat că dieta diferită şi caracteristicile igienei orale la câini influenţează prezenţa anumitor microorganisme în canalele radiculare, în comparaţie cu flora microbiană prezentă în canalele radiculare umane.

Cuvinte cheie: canale radiculare, endodonţie, microorganisme, câini, identificare Vitek

The endodontic pathology covers in case of the canine species almost the same particularities as in case of the human species. The pathology of the dental pulp follows usually the same steps, from hyperemia to necrosis and apical periodontitis, complicated with abscesses or infections of the anatomical surrounding areas. However, research showed that acute periapical inflammation can occur in dogs even during pulpitis, in the first 20 days of pulp exposure (6). Regarding the endodontic anatomy, studies showed that the terminal zone of the canal presents smaller secondary canals, that form a delta, which is unable to be cleaned. Given the lack of subjective signs and the difficulty to perform dental vitality testing procedures, veterinary practitioners diagnose pulp pathologies in advanced stages. Consequently, the microbiological examination finds its relevance for obtaining a treatment based on confirmed available evidence.

The endodontic system represents a suitable environment for bacterial colonization. Furthermore, infected dog's teeth with chronic periodontitis showed a large dissemination of microorganisms in the root canals and also in the periapical lesion. (17)

Microorganisms poses the capacity to adhere to certain surfaces and to survive in extreme conditions

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of nutrient deprivation, with the help of several gene determined, adaptive systems. The formation of biofilms was therefore accepted as a cause for endodontic infections (20) and research were conducted in order to establish the mechanisms that connect the bacterial adherence to the etiology and evolution of the periapical disease (13). Previous studies showed that oral microorganisms of dogs and humans are similar. Bacteria like facultative anaerobes and Gram-positive species, but also strict anaerobes were identified. However, the endodontic systems of dogs and humans display some differences. Canine and human bacterial population vary by approximately 7% in the gene 16S rRNA (5) and regarding the number of bacteria identified in the root canals of dogs, this was proven to be lower than in the case of humans, because of the low isolation rate of anaerobic microorganisms. Also there are certain bacterial species recovered more often from the root canals of dogs than from the endodontic system of humans. These differences may occur due to the anatomical differences of root canals and the characteristics of the oral cavity microbiota in dogs.

MATERIALS AND METHODS

Isolation of the microorganisms

Six infected root canals, from five incisors and one canine, of two different dogs were used as *in vivo* source for the isolation of endodontic microorganisms.

The teeth suffered fractures and abrasions of the crowns, with the exposure of the pulpal tissue.

The pathologic tissue was collected as follows: an endodontic ISO 10 Kerr file was introduced in the root canal and loaded with infected pulpal tissue.The file was immediately deposited in transport tubes with Amies culture media, sealed and carried to the laboratory.

Culture conditions of the microorganisms

We inoculated the samples on blood agar for bacteria and on Sabouraud agar for yeasts. The plates were purchased from Oxoid, UK. We placed the endodontic file on a surface of 2 cm^2 of the culture media and spread the content evenly on the entire plate. We incubated the plates as follows: for 24 h at 37^0 C for bacteria and for 5 days, at 28^0 C for yeasts.

After the incubation, we examined the morphological and cultural characteristics of the resulted colonies on the plates. Consequently, we identified the microorganisms as Gram-positive and Gram-negative and determined their form and group affiliation (cocci, bacilli, streptococci, streptobacili).

Vitek 2 Compact identification

It was performed the identification of the microorganisms on a Vitek 2 Compact automated microbiology system, produced by Biomerieux, France and owned by the University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca. The system works with a Windows based on software, that makes the interpretation of data more accessible.

For the use of Vitek 2 Compact device, we purchased specific identification cards.Every reagent card presents different characteristics in order to recognize a high number of pathogen species in relation to the scanned biochemical samples. The reagent cards poses 64 wells that contain particular test schemes. They are able to measure different metabolic processes such as hydrolysis of the enzyme, alkalization, acidification or proliferation in the presence of inhibitory agents. The cards present a clear pellicle for maintaining a proper level of oxygen level and for limiting the interference with the microorganisms. The cards are equipped with bar codes to connect them with the samples and to inform the user about the lot number, validity or type of the product.

For the identification of Gram-positive bacteria it was used the Vitek 2GP ID card. This device is able to recognize 120 microorganisms' species. The identification of the Gram-negative bacteria was purchased using Vitek 2GN ID card, which has the ability to recognize approximately 150 fermentative and non-fermentative Gram-negative bacterial species. The Vitek 2 ANC ID card is able to identify almost 90 anaerobic and coryneform bacteria and was also used for this study. The identification time ranges between 2 and 18 hours. We processed the inoculum preparation by creating suspensions based on the obtained microbial colonies, while using the optical density McFarland between 0.5 and 0.63 for Vitek 2GP ID card, between 0.5 and 0.6 for Vitek 2GN ID card and between 0.5 and 0.6 for The Vitek 2 ANC ID card. The samples were automatically identified in 3 hours. The McFarland scale was purchased from Biomerieux, France.

RESULTS AND DISCUSSIONS

The microorganisms identified in this study can be found partially in the endodontic system of humans.

The presence of some bacteria in the root canals of

dogs may be the result of the specific living environment of the animals. *Propionibacterium acnes (Fig. 1, Fig. 2)* belongs to the group of microaerophilic, Gram-positive bacteria and is involved in the etiology of acne vulgaris. It is known as a commensal bacteria but its actual role in the process of inflammation is still unknown. Several studies reported the existence of antibiotic resistant *Propionibacterium* to clindamycin, quinolones, rifampin or beta-lactams. The biofilm formation factor of *Propionibacterium acnes* was proven to be responsible for infections associated with implants.

Fig. 1. *Propionibacterium acnes* on blood agar medium

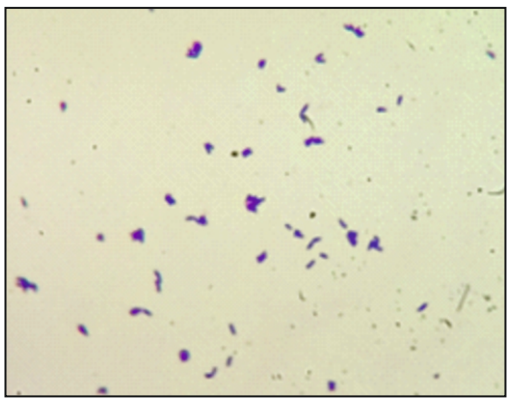

Fig. 2. *Propionibacterium acnes*: Gram stain x1000

Recent studies reported the isolation of *Propionibacterium acnes* from the root canals of the human species. It is considered that the microorganism can enter the root canal system during the endodontic treatment or after the tooth is restored, through micro -leakages, at the limit between tooth and reconstruction. Carious processes or traumatic injuries can also play the role of access gates of *Propionibacterium acnes* into the root canal (10).

Bacillus subtilis (Fig. 3, Fig. 4) is a Gram-positive, facultative anaerobe microorganism, commonly found

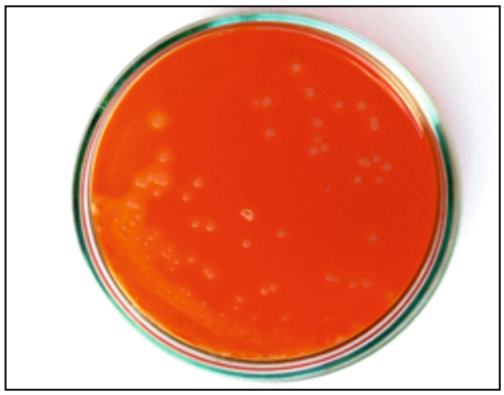

Fig. 3. *Bacillus subtilis* on blood agar medium

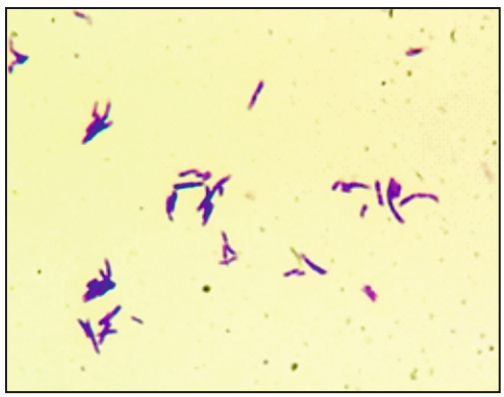

Fig. 4. *Bacillus subtilis*: Gram stain, x1000

in soil and attached to the roots of plants. It has the ability to form biofilms and to protect plants from different diseases. Due to its characteristics, the bacteria are used in agriculture as a fertilizer (4). In human and animal organisms, *Bacillus subtilis* is present in the gastrointestinal tracts (16). *Bacillus subtilis* spores have been developed in order to inhibit the adherence of *Streptococcus mutans* to non-vital tissues (3). In the root canals of the human species, *Bacillus subtilis* was previously identified attached to the canal walls or together with *Enterococcus faecalis*, in contact with the guttapercha cones (18). With the developing of Scanning Electron Microscopy root canal biofilms containing *Bacillus subtilis* were visualized (11).

Pseudomonas putida (Fig. 5, Fig. 6) is a Gram-negative bacteria that is able to survive in different environments, like in the presence of extreme pH, upraised temperatures or in the presence of toxic substances. It is nowadays used in bio-industrial purposes, for the development of polymers or chemical substances (12).

The microorganism is very rare present in the human organism and appears mostly in specific habitats, like in case of hospitalized patients (8). From the genus *Pseudomonas*, the most common bacteria identified in

the root canals of the human species is *P. aeruginosa* (14). The presence of *Pseudomonas putida* in the root canal system of dogs may derive from the particularities of the living environment and dietary content.

Fig. 5. *Pseudomonas putida* on blood agar medium

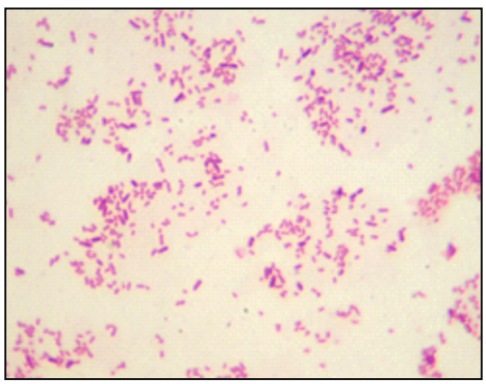

Fig. 6. *Pseudomonas putida*: Gram stain, x 1000

Staphylococcus equorum (Fig. 7, Fig. 8, Fig. 9) is a Gram-positive bacterium, that does not produce coagulase and was first isolated in 1984, together with *Staphylococcus arlettae and Staphylococcus kloosii* (15).

It was identified for the first time on the skin of horses and later in the environments of food processing systems (7). *Staphylococcus equorum* was very rare isolated from human clinical samples (2). In case of the human root canals, the most common isolated member of the genus *Staphylococcus* is *Staphylococcus aureus. Staphylococcus haemolyticus, Staphylococcus sciuri* (19) or *Staphylococcus warneri and Staphylococcus epidermidis* (9) are other microorganisms identified in the endodontic system of the human species.

CONCLUSIONS

By analyzing the results of this study, it can be concluded that the microorganisms identified in the root canals of dogs are different from the bacteria usually identified in the endodontic system of the human species. Most of them appear probably because of the specific environmental factors and alimentation of the animals. The characterization of the various bacterial species present in the root canals of dogs is very important for implementation of a correct treatment based on a reliable scientific background. Further studies are necessary to complete the set of bacteria already identified and to understand the mechanisms that underline the presence of these microorganisms in a biofilm inside the root canals.

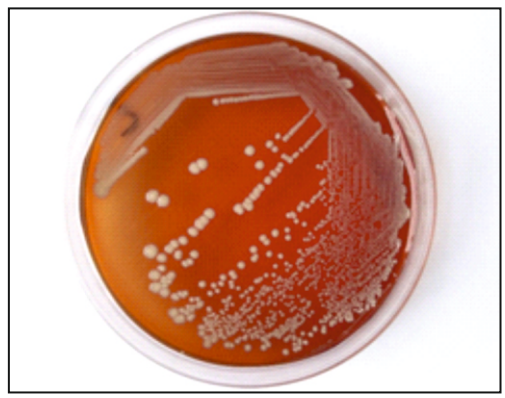

Fig. 7. *Staphylococcus equorum* on blood agar medium

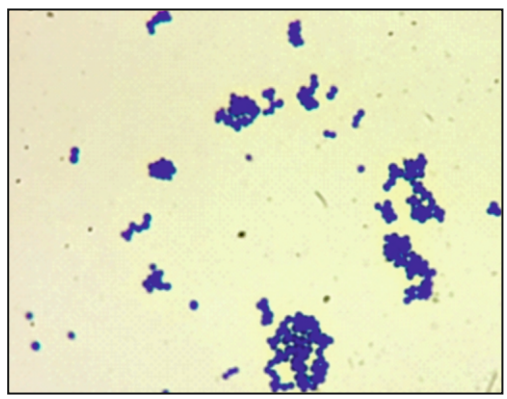

Fig. 8. *Staphylococcus equorum*: Gram stain, X1000

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bioMerieux Customer: **Microbiology Chart Report** Printed Nov 16, 2016 12:23 CST

Patient Name: Patient ID:
Location: Physician:
Lab ID: Cioban 4 Staf Isolate Number: 3
Last Updated:

Organism Quantity:

Source: Collected:

Comments:	

Identification Information	Analysis Time:	6.00 hours	Status:	Final
Selected Organism	94% Probability Bionumber:	Staphylococcus equorum 420046005371031		
ID Analysis Messages				

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	+	8	ADH1	-	9	BGAL	+	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	-	23	ProA	-	24	BGURr	+	25	AGAL	-	26	PyrA	+	27	BGUR	+
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	-	32	POLYB	-	37	dGAL	-
38	dRIB	+	39	ILATk	-	42	LAC	+	44	NAG	+	45	dMAL	+	46	BACI	-
47	NOVO	+	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	-	56	PUL	-
57	dRAF	-	58	O129R	-	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Fig. 9. Data identification system for *Staphylococcus equorum* by Vitek 2

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