

## **UNCONVENTIONAL IMMUNOSTIMULATORY MANAGEMENT OF DOGS' PARODONTITIS WITH AUTOLOGUS STAPHYLOCOCCAL BACTERINE**

**L.B. KÖBÖLKUTI, Z.A. KÖBÖLKUTI, G. CZIRJAK, D. CADAR, MIHAELA NICULAE, A. UNGVARI**

University of Agricultural Sciences and Veterinary Medicine,  
Faculty of Veterinary Medicine, Discipline of Infectious Diseases  
Manastur street 3-5, Cluj Napoca 400372

### **Summary**

Periodontal infection in dogs is one of more important and frequent disease among oral inflammation. Actually sufficient data exist sustaining the intervention of immune mechanisms in evolution of periodontal diseases but the usefulness of immune therapy is unclear. Despite this controversy the utility of diverse immune-modulator products have practical support and popularity in the human stomatology.

These discussed aspects lead to the needful or to the opportunity of rethink in the future the orientation of the periodontal therapy. In this study we analyzed the benefic effect of staphylococcal bacterin on the evolution of periodontal disease on dogs.

Periodontal infection is one of more important and frequent disease among oral inflammation in dogs. (Gorrel, 1998, Harvey, 1998).

Actually sufficient data exist to sustain the intervention of immune mechanisms in evolution of periodontal diseases (Newman & Carranza, 2002, Preshaw et al., 2004) but the usefulness of immune therapy is unclear (Page, 2000). Despite this controversy the utility of diverse immune-modulator products have practical support and popularity in the human stomatology.

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The aim of this study it was to monitoring the clinical effects of the immune-modulating therapy (staphylococcal bacterine) in periodontal disease in dogs.

### **Materials and methods**

The study it was realized in a non-homogeny group formed by 6 dogs: two germane shepherd dogs, Doberman, Akita Inu, Alsacian shepherd, Hungarian vizsla.

For each animal it was determined the Oral Hygiene Index (OHI). For objective evaluation of the immune-modulator therapy effect, the OHI it was determined for the most affected teeth, teeth with most serious lesions. OHI contain the value of the plaque index, the value of the tartar index and the value of

the gingival index; it represents the sum of these indexes (Greene & Vermillion, 1964, Carranza & Newman, 1996, Dumitriu, 1997).

For the evaluation of the affectation rate of the periodontal structures it was determined the Russel Periodontal Index (RPI), Dental Mobility Index (DM), Fuchs Bone Index (FBI), Periodontal Pocket Depth (PPD). It has to be notice that the examination of the teeth it was made using the modified TRIADAM system (Harvey & Emily, 1993).

To evaluate the participation and the importance of the *Staphylococcal spp.* microorganisms in the pathology of the oral diseases, we used the following methodology:

- Collecting the bacteriological samples form the base of the periodontal pockets sounding with sterile periodontal or sub gingival scoop. In the case nr. 2 and 4, we did not collect samples from these sites, because the depth of the periodontal pockets it was normal (1mm).
- The microbiological samples at the level of the most affected teeth it was collected with sterile cotton swabs with Amies transport medium. We collected samples from the biofilm (plaque) of the affected teeth.
- After the sampling, the utilized instruments were washed in 1 ml sterile PBS and the samples were preserved at the -20°C until further analysis.
- To isolate the *Staphylococcus spp.* strains the collected samples were cultured on Chapman broth (Oxoid™). Plates were incubated at 37°C, either in normal atmospheric and microaerophilic (10% CO<sub>2</sub> with Anaerocult C mini (Merck KGaA, Darmstadt, Germany) conditions respectively, for 48h.
- After incubation, all suspect colonies were verified bacterioscopically with Gram stain and tested with KOH 3% solution.
- The *Staphylococcus spp.* colonies were identified as species by using multi-substrate identification stripes API Staph

The immune modulation with staphylococcal antigens it was realized in all six dogs with gingival and/or periodontal inflammation. The inoculation of lyzate is realizable in different ways. To reach the therapeutic success, is required to choice the adequate administration way and to establish the individual dose, the ritm of the antigen inoculation and the period of therapy (Aiden, 2004, Georgescu, 2004). Because the dose, rhythm and period of inoculation depend of the condition of "biological field" the immune stimulation schemes is can not be universal (Georgescu, T., 2004).

Subcutaneous administration being the most accessible, painless and antibody conducive way, in this study we choose this route of administration (Georgescu, 2004).

The administration of staphylococcal bacterine it was effectuate subcutaneously 7 times, in 5-7 days interval (alternating left and right thoracic region) in increasing dose:

**Tabel 1**

**Increasing dose of bacterines administrated in course of immune therapy**

Week 0 Inoculation 1	Week 1 Inoculation 2	Week 2 Inoculation 3	Week 3 Inoculation 4	Week 4 Inoculation 5	Week 5 Inoculation 6	Week 6 Inoculation 7
0.1 ml	0.2 ml	0.3 ml	0.4 ml	0.5 ml	0.8 ml	1 ml

This administration protocol it is applied in human oral medicine (Georgescu, 2004) and it is recommended by Cantacuzino Institute Bucuresti (see <http://www.cantacuzino.ro/ro/Produse/Catalog%202006/Grupa%20A/Subgrupa%20A1/76.doc>).

After each administration the animals it was attentively supervise 30 minutes to prevent the accidental complications. In course of therapy the animals did not received other drugs, just the staphylococcal bacterine and it was recommend to owners to apply daily dental hygiene and to administrate commercial food.

### Results and discussions

From the investigated cases we isolate six *Staphylococcus spp.* strains (*Staphylococcus intermedius* (n=2), *S. epidermidis* (n=2), *S. hycus* (n=1) și *S. hominis* (n=1)). Further, from these strains used an original inactivation method was obtained six various staphylococcal bacterine.

The immune stimulatory effect of staphylococcal bacterine become observable after fifth inoculation (fourth week of therapy) (perfect likeness with results obtained by Georgescu, 2004)

Corroborating the results from all six animals respecting the immune stimulatory therapy we can affirm:

- The oral index in all cases reduced 2-4 points
- The diminution of OHI (oral hygiene index) values it was not realized thanks to decreased plaque and tartar index but because of reduction and disappearing of gingival inflammation demonstrate by: changing in gingival color, decreasing the gingival edema, disappearing the hemorrhages and supuration.
- The cause of decrease Russell periodontal index between 1 to 4 points in the same mode it was the disappearance of gingival inflammation.
- The deepness of periodontal pocket reduced in all cases 1-2 mm
- The immune stimulatory therapy it was inefficient in diminution of dental mobility and to restructure the alveolar dental bone (monitoring by Fuchs bone index).

### **Conclusions**

This results it is in concordance with others (Georgescu, 2004) who apply staphylococcal products in therapy of human periodontal inflammation and suggesting that the immune therapy with staphylococcal lyzate is an efficient, low cost complementary therapeutic method in dogs.

### **References**

1. **Aiden, P. F.**, 2004, Immunomodulation and immunodeficiency, *Veterinary Dermatology* 15, 115–126.
2. **Carranza, F. A, Newman, M. G.**, 1996, *Clinical periodontology*, Ed. Saunders.
3. **Dumitriu, H. T.**, 1997, *Parodontologie*, București, Editura Viața Medicală Românească
4. **Georgescu, T.**, 2004, Modularea răspunsului imun cu vaccin stafilococic în boala parodontală, Ed. Univ., Pitești.
5. **Gorrel, C.**, 1998, Periodontal disease and diet in domestic pets, *The Journal of Nutrition*, 128, p. 2712–2714.
6. **Greene, J. C., Vermillion, J. R.**, 1964, The simplified oral hygiene index, *Journal of the American Dental Association*, 68, p. 7–13.
7. **Harvey, C. E., Emily, P. P.**, 1993, *Small animal dentistry*, St Louis: Mosby.
8. **Harvey, C.E.**, 1998, Periodontal disease in dogs. Etiopathogenesis prevalence, and significance, *The Veterinary Clinics of North America – Small Animal Practice*, 28, p. 1111–1128.
9. **Newman, M.G., Carranza, F.A.**, 2002, Periodontal Pathology, 196–346. In: Newman, M.G., Takei, H., Carranza, F.A. (eds.): *Carranza's Clinical Periodontology*, Saunders, Philadelphia, p. 1056.
10. **Page, R. C.**, 2000, Vaccination and periodontitis: myth or reality, *J. Int. Acad. Periodontol.*, 2, p. 31-43.
11. **Preshaw, P.M., Seymour, R.A., Heasman, P.A.**, 2004, Current concepts in periodontal pathogenesis, *Dental Update*, 10, p. 570–578.