ABSTRACT

Mouth is one of the anatomical segments of the digestive microbiota which is characterized by a marked diversity. Among the multitude of microorganisms that inhabit the oral mucosa at a time, bacteria are the main etiologic factor in producing these conditions followed by yeasts and viruses.

Normal bacterial flora plays an important role in body physiology. It prevents colonization of pathogenic flora, is a nonspecific immune stimulus from birth, producing bacteriocine and toxic degradation products that inhibit pathogen development, promote the maintenance of pH, etc. Under certain conditions, however, may have adverse effects on the body, favoring the onset of infection.

Ignoring an outbreak of oral infection can have serious consequences on the whole body and recognize the primary cause of a systemic disease, may lead to treatment failure. In addition, cohabitation in the the same space with people, diverse diet in relation to the type of food consumed by humans, no specific activities for these carnivores and many other aspects are particular factors that led to the transformation of oral bacterial microflora of dogs and cats and therefore human-like dental problems. Also, the identification in the mouth of dogs and cats of bacteria involved in oral disease in humans, has led to further research the world of these microbiota and trying to overcome the barrier of species shown in some cases.

Thesis entitled *Research on the implications of bacteria in oral disease in dogs and cats* contains 257 being drafted in ten chapters and is structured in two parts according to usage.

The first part (Chapters I, II, III and IV) represents 23,43% and summarizes the main literature data on oral microbiota in dogs and cats, such as bacterial diseases of the mouth of the two species, defense antibiotic mouth and epidemiological data on risk pets to humans.

Second part (cap.V, VI, VII, VIII, IX) representes 76,57% and includes researches made during 2006-2010. Each chapter is divided into chapters that present materials and work methods used, results and their discussion ends with partial conclusions.

In Chapter IX summarizes the main results obtained from research conducted in a brief final conclusions.

The paper has a number of 191 figures, 39 tables and 275 based on titles. The aim of our research was to monitor bacterial microflora involved in oral pathology in dogs and cats, to identify the impact of local and general infection induced by oral and establish the frequency of oral diseases in dogs and cats in terms of age, gender, clinical forms, origin and association with other organic diseases.

Bacteriological investigations were based on the following objectives:

- identify dogs and cats with oral disease;
- identification of bacterial microflora of pathological material taken from dogs and cats with oral diseases;
- pathogenicity tests on some bacterial strains isolated;
- testing the effectiveness of antimicrobials on bacterial strains isolated in pure culture or the total flora;
- Determining the type of bacterial etiology of oral disease in dogs and cats.

Immunological and immunopathological research followed:

- determination of lysozyme in saliva taken from healthy dogs and cats and mouth disease;
- assessment of IgA, IgG, IgM concentrations in saliva and blood serum derived from healthy dogs and cats and mouth disease;
- - determination of serum complement fraction C3a sick animals;
- Assessment of cellular immune effectors in bacterial diseases such as mouth.

Epidemiological data obtained had the following objectives:

- establishing the frequency of oral diseases in dogs and cats according to clinical form, age, gender and association with other disorders;
- evaluating the potential risk of microbiota present oral dogs and cats with oral disease through bite wounds event;
- - Presentation of case studies.

Bacteriological examination of specimens obtained from the oral lesions in dogs and cats led to the isolation of numerous bacterial strains. Of oral pathology specimens were identified in dogs 55,47% pure bacterial strains and 44,53% mixed bacterial strains. In cats, the report of isolates mouth strains has been reversed, isolating predominant mixet bacterial strains 84,5% and 15,5% pure culture.

Identification and classification of bacterial strains, ecological niche criterion, showed that in dogs, most often, indigenous bacterial microbiota was involved in 82,49% cases, whereas incumbent allochthonous microbiota only 17,51% of cases and the cats, 87,9% of the bacterial

microbiota is involved in various oral diseases and only 12,1% domestic origin is composed of allochthonous microorganisms.

Microorganisms that have a device that identifies a pathogenetic complex ecosystem penetrated easily distinguished under the influence of factors, promote or trigger the emergence of clinical entities. Studies in dogs and cats presented to the consultation, but the group of stray dogs, revealed a wide range of oral diseases.

In dogs, the bacteria involved in oral diseases were in **gingivitis:** *Staphylococcus aureus, Staphylococcus intermedius, Staphylococcus epidermidis, Streptococcus pyogenes, Streptococcus canis, Streptococcus salivarius, Arcanobacterium pyogenes, Corynebacterium urealiticum, Corynebacterium species, Listeria monocytogenes, Pasteurella canis, Pseudomonas aeruginosa, Escherichia coli, Actinomyces hardeovulneris;***periodontitis:** *Staphylococcus aureus, Staphylococcus intermedius Streptococcus canis, Arcanobacterium pyogenes, Escherichia coli, Proteus mirabilis, Actinomyces viscosus, Actinomyces haerdeovulneris, Clostridium perfringens, Bacteroides gracilis*, *Bacteroides species*, *Porphyromonas gulae, Porphyromonas gingivalis, Prevotella intermedia, Prevotella bivia, Neisseria zoodegmatis*, *Neisseria animaloris*, *Fusobacterium necrophorum*; **dental caries:** *Streptococcus mutans, Peptostreptococcus species;* **palatinitis:** *Staphylococcus aureus, Streptococcus canis, Listeria monocytogenes, Clostridium perfringens, Escherichia coli, Pseudomonas aeruginosa;* **oral pharyngitis and tonsillitis:** *Staphylococcus aureus, Staphylococcus intermedius, Streptococcus pyogenes, Streptococcus salivarius, Corynebacterium species, Arcanobacterium pyogenes, Listeria monocytogenes, Pasteurella multocida, Escherichia coli, Mannheimia haemolytica;* **mouth abscess:** *Staphylococcus aureus, Corynebacterium species, Arcanobacterium pyogenes, Pasteurella canis, Mannheimia haemolytica, Pseudomonas aeruginosa.* **glossitis:** *Corynebacterium urealiticum, Proteus mirabilis, Escherichia coli, Pseudomonas aeruginosa, Leptospira interrogans* serovar. *canicola;* **oro-sinus fistula:** *Staphylococcus aureus, Streptococcus pyogenes, Actinomyces hardeovulneris, Clostridium perfringens, Pseudomonas aeruginosa, Pasteurella multocida, Bacteroides species, Fusobacterium necrophorum;*

In cats, the bacteria involved in oral diseases were in **gingivitis:** *Staphylococcus intermedius, Staphylococcus aureus, Streptococcus pyogenes, Streptococcus canis, Streptococcus salivarius, Arcanobacterium pyogenes, Corynebacterium species, Enterococcus faecalis, Pseudomonas aeruginosa, Escherichia coli*;**periodontitis:** *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus canis, Streptococcus salivarius, Arcanobacterium pyogenes, Escherichia coli, Actinomyces viscosus, Actinomyces hardeovulneris, Clostridium perfringens, Bacteroides species*, *Bacteroides gracilis*, *Porphyromonas gulae, Prevotella*

intermedia, Prevotella bivia, Neisseria zoodegmatis; **dental caries:** *Streptococcus mutans;* **palatinitis:** *Staphylococcus intermedius, Streptococcus salivarius, Clostridium perfringens, Escherichia coli, Pasteurella multocida, Pseudomonas aeruginosa;* **oral pharyngitis and tonsillitis:** *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus salivarius, Arcanobacterium pyogenes, Listeria monocytogenes, Bacillus cereus, Pasteurella multocida, Escherichia coli, Mannheimia haemolytica, Neisseria zoodegmatis;* **mouth abscess:** *Staphylococcus aureus, Streptococcus pyogenes, Arcanobacterium pyogenes, Pasteurella multocida, Pseudomonas aeruginosa;* **glossitis**: *Escherichia coli, Leptospira interrogans* serovarul *canicola;* **oro-sinus fistula:** *Staphylococcus aureus, Streptococcus pyogenes, Corynebacterium species, Clostridium perfringens, Proteus mirabilis, Fusobacterium necrophorum.*

In our study, bacterial strains identified in oral diseases of dogs and cats, are listed in different specialized studies as cooperating in the production or maintenance of these lesions. However, given the relatively large number of dogs and cats that have been identified with such conditions and diversity of microorganisms, isolated from pathological specimens mouth, was appropriate.

Testing in vitro effectiveness of antibiotics on strains isolated from dogs, showed a sensitivity as we expected range. Thus, **for aerobic Gram-positive strains** were most effective metronidazole-spiramycin (76%), cefadroxil (78,3%), chloramphenicol (76,7%), enrofloxacin (76,7%), cefoperazone (69,8%), amoxicillin and clavulanic acid (68,2%), amoxicillin (61,2%), erythromycin (56,6%), doxycycline (51,9%); **for Gram-negative aerobic stems** were ceftiofur (84,9%), spectinomycin (71,2%), enrofloxacin (65,8%), chloramphenicol (64.4%), amoxicillin and clavulanic acid (64,4 %.), amoxicillin (61,6%), norfloxacin (58,9%), cefoperazone (57,5%); **for Gram-positive anaerobic strains** were metonidazol-spiramycin (80%), amoxicillin-clavulanic acid (70%) and amoxicillin, chloramphenicol and clindamycin (60%) and **for anaerobic strains Gram-negative bacteria** were metronidazole-spiramycin (80%), clindamycin (80%), doxycycline (60%).

Bacterial strains isolated from cats have shown sensitivity to a smaller number of antimicrobene substances. Thus, **aerobic Gram-positive strains**: metronidazole-spiramycin (64%), amoxicillin-clavulanic acid (52,2%), Rovamicine (51%), penicillin (51,2%), cefadroxil (49%), **for aerobic strains Gram negative**: enrofloxacin (77,2%), metronidazole-spiramycin (76%), Rovamicine (72%), norfloxacin (72%), ceftiofur (63,2%), amoxicillin-clavulanic acid (54,3%); **for Gram-positive anaerobic strains**: amoxicillin - clavulanic acid (83,3%), metronidazole-spiramycin (83,3%), chloramphenicol (66,7%), amoxicillin (66,7%) and

clindamycin (58%), **for Gram-negative anaerobic strains**: metronidazole-spiramycin (80%), clindamycin (40%) and chloramphenicol (40%).

Considering the multitude of microorganisms that may act synergically in the production of localized oral disease, but also the relative effectiveness of antimicrobial substances with oral effect, it is absolutely necessary to carry aut microbiological examination and performing sensitivity testing.

Immune response against various infectious antigens is the main way to eliminate and prevent infections by potentially pathogenic microorganisms. Lysozyme is a glycoprotein (mucopeptide) enzyme acting on structural peptidoglicanului bacterial wall. Lysozyme intervention leads eventually to cell lysis. In the end we determined the amount of lysozyme in healthy animals and those with oral disease. Comparing the results obtained in the control group (mean values 118 mg/ml in dogs and 87,9 mg / ml in cats) with reference values of the variables showed differences in dogs and cats.

Thus, in dogs with oral disease was identified a high titer of lysozyme whose average was 145,05 mg / ml. Noticeable differences were identified in cats with oral disease, overall averages were 142,05 mg / ml, indicating an expansion of activity of immune defense forces. Quantitative determinations of IgA, IgG, IgM class antibody in saliva were performed by two methods: enzyme immunoassay method diffusimetrical and ELISA.

Healthy dogs and cats that have formed the control groups, limits of serum IgG (mg/dl), IgM (mg/dl) and IgA (mg/dl) obtained by ELISA method and diffusimetrical method were correlated. However due accuracy and time to obtain results, immunoenzymatic test was used for further investigations.

Determinations on serum and saliva samples from dogs and cats with oral lesions such as bacteria showed a significant increase in all groups of immunoglobulins. We identified a salivary IgA titer increased to 14 dogs (over 70 mg/dL) and 17 cats (over 20 mg/dl) and similar situation was observed in serum IgA in 10 dogs (over 350 mg/dL) and 16 cats (over 300 mg/dL). In 9 cases of all tested dogs was identified a serum (112 mg/dL) and salivary (45 mg/dL) IgA deficiency.

Depending on clinical forms identified in dogs, salivary IgA titre fluctuated from 78 mg/dl (palatinitis, oral abscesses, oral-pharyngitis) to 140 mg/dl in one case of chronic periodontitis and serum IgA titre ranged from 378 m/dL (dental abscess, periodontitis – oro-sinus fistula) to 550 mg/dL (periodontitis, palatinitis, oral-pharyngitis).

In cat salivary IgA titre ranged from 22 to 23 mg/dL (gingivitis, glossitis), up to 50-58 mg/dL (abscess, periodontitis, palatinitis) and serum IgA titer ranged from 330 mg/dL (moderate periodontitis) at 700 mg/dL (chronic periodontitis).

Salivary IgG titre dogs ranged from 3,3 mg/dL (abscess, palatinitis, oral-pharyngitis) and 6,9 mg/dL (chronic periodontitis) and serum IgG titer had values between 2636 mg/dL (tonsillitis, gingivitis) and 5250 mg/dL (chronic periodontitis).

In cat salivary IgG titer was between 2,3 mg/dL (gingivitis, oral abscess) and 13 mg/dL (periodontitis, oral-pharyngitis) and serum IgG values in the range was 2475 mg/dL (periodontitis) and 5250 mg/dL (chronic periodontitis).

IgM titre of saliva in dogs ranged from 6,2 mg/dL (gingivitis with inflammation bleeding) and 11,9 mg/dL (chronic periodontitis) and had serum IgM levels between 305 mg/dL (moderate periodontitis) and 647 mg/dL (chronic periodontitis).

In cat salivary IgM titre ranged from 4,2 mg/dL (gingivitis) and 12 mg/dL (oral abscesses and oral fistulas) and serum IgM titer had levels between 305 mg/dL (moderate periodontitis) and 647 mg/dL (chronic periodontitis).

Evaluations of serum complement C3 fraction in healthy individuals showed values ranging from 4,5 to 5,5 u/L in dogs and 4-5 U/L in cats. The highest values of strength than C3 were identified in chronic periodontitis in dogs (6,5 to 6,8 U/L) and cat (8,9 U/L).

Hypergammaglobulinemia demonstrate an increased activity of local immune system and general pet with mouth diseases. Due to changes in serum and salivary antibody titres and lack of correlation between them shows that each individual reacts private. However the results obtained from serum and salivary IgA, except with IgA deficiency is correlated with different clinical forms located mouth.

In dogs and cats with oral disease was found leukocytosis and lymphocytosis in all cases investigated and erythrocytosis in nine dogs and four cats. Corroborating immunograma values with those of complement C3 and hematological parameters in the study reflect the implications of the immune system to defend both antibiotic local and general.

Periodontitis associated with other oral diseases or single lesion, compared with other oral lesions, oral disease remains the biggest impact on local and general immune system. This is justified and numerous specialized studies addressing common periodontal disease in both humans and pets.

To supplement the bibliographic data, it has attempted a gross report of epidemiological assessment of oral disease frequency (gingivitis, periodontitis, abscesses, oro-pharyngitis, tonsillitis, palatinitis, dental caries, etc.) identified in dogs and cats in a year. Data were

centralized, counseling records of five points veterinarian examination: Clinical Pathology of FMV Medical Science, Veterinary Medical Iasi No.1 Iasi, Veterinary Medical Iasi No.2, Veterinary Medical Bacau No.3. A special situation was Miroslava paddock where 96 dogs were examined existing achievable inside the paddock at a certain date, to identify individuals with oral disease.

After synthesizing the data, found that oral diseases have been identified in cats, with a frequency of 10,81% of 1248 animals examined, while in dogs, 7,26% from 2751 dogs presented to the consulate. Grouping by age showed that oral diseases are most commonly found in dogs aged 5-10 years (30,37%) and cats aged 10 to 15% (47,5%). Also found that females of both species were most affected.

Of the 200 dogs, 91% had plaque and tartar, 61,5% with periodontitis, 27% gingivitis, 5% dental caries, 3,5% glossitis and 3% palatinitis and in cats, 89,6% had plaque and tartar, 85,18% periodontitis, gingivitis 53,3%, 14,81% oro-pharyngitis, tonsillitis, palatinitis 5,18%, 370% caries and 1,48 % glossitis. Some investigated pets presented mouth disease associated associated with other organic diseases.

There were found 53,5% of dogs and 39,25% cats with oral diseases and organic disorders. In dogs, of the 107 cases associated with oral disease, 42 (39,25%) cases were urogenital diseases, 39 (36,45%) cases exhibited skin diseases, 23 (21,5%) cases of otitis, 1 (0,9%) heart syndrome and two (1,8%) cases with liver syndrome.

From the 53 cats with stomatitis associated with other organic diseases 25 (47,5%) cases were urogenital infection, 16 (30%) cases of bacterial dermatitis, 8 (15%) cases associated with otitis, 3 (5%) and cardiac syndrome cases exhibited only 1 (1,8%) cases with liver syndrome.

Theories have been formulated regarding the mechanisms of oral transmission of infection to other organs and, in some cases, demonstrated the origin point of departure mouth of bacteria isolated from other systemic illnesses. However it is difficult to study this issue in a casuistry whose owners do not allow full investigation and sometimes flooded.

The close relationship between dogs, cats and humans may constitute a risk to human health by transmitting across the species barrier of potentially pathogenic bacteria. Making a case studies highlighted the impact of trauma and infections caused by dog and cat bites in humans. Studies were conducted on two groups of people exposed to systematic risk that owners of animals that become aggressive under certain conditions and veterinarians.

Given the identification, in the oral cavity in dogs and cats, of species with risk of cross transmission (*Staphylococcus aureus, Streptococcus pyogenes, Streptococcus mutans, Streptococcus canis, Corynebacterium urealyticum, Enterococcus faecalis, Porphyromonas*

gingivalis, Pasteurella multocida, Leptospira interrogans serovarul *canicola*) is recommended avoiding oral-oral contact between owners and pets, exposure to bites, contact with the skin lesion secretions of the oral cavity.

The microbiological investigations performed on pathological material taken from both wound bitten, but the mouth of animal aggression, made a brief demonstration of clinical effect produced by insemination of potentially pathogenic bacteria in the wound.

Oral microbiota plays a major role in oral pathology. Multitude of microorganisms in the mouth, and pathogenic capacity and local and general mode of action of bacterial strains, induce a permanent risk to animals and humans. All results obtained allow us to suggest the great importance of these seemingly insignificant mouth disease by frequency and clinical impact.