CLINICAL AND MICROBIOLOGICAL ASPECTS IN CATS WITH GINGIVOSTOMATITIS COMPLEX

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Abstract: Introduction. *Gingivostomatitis* complex represents one of the most frequent and frustrating disease in small animal clinics (Healey, 2007). The clinical manifestation of this disease complex is described and classified in four stages (Mihaljevic, 2008) but the microbial flora is different in each stage of the disease (Dolieslager, 2010). Aims. The purpose of this study was to evaluate de clinical and microbiological aspects in cats with *gingivostomatitis*, to correlate the stages of the disease with the bacteria identified from the mouth of each patient. Materials and Methods The study was done between October 2016 - June 2017, in 10 adult cats, mixed breed ,with median age of 7,95 years, from which 8 was male and 2 were female. Each consultation means a careful examination of the oral cavity, which was followed by the sampling from oral cavity. These samples were exanimate microbiologically and we performed antibiogram for each case. Results. Of the ten patients examined, two patients were in the stage I with an average age of 3 years, 7 patients had stage II with an average age of 9 - 12 years and in stage III a single patient was found with the age of 12 years. This aspect shows that the progression of the disease is directly proportional with the ageing. In 3 cases the feline chronic gingivostomatitis was associated with urinary infection (2 cases) and *diabetes mellitus* (1 case). The oral microbiota is changing depending on the resistance of these bacterias to the more acidic environment present within the biofilm, with the appearance of Gram negative *bacili* in the advanced stages of the disease. The most effective antibiotics in the cases presented were Enrofloxacin, Convenia, Doxycyclin, and due to antibiotic resistance to Metronidazole and Clindamycin, these are not indicated in the treatement of oral cavity diseases in cats.

Keywords: cats, clinical aspects, oral microbiome, oral injuries, antibiogram

INTRODUCTION

Chronic feline gingivostomatitis (GSCF)This name is synonymous with: lymphoplasmocytic gingivostomatitis, stomatitis-plasmocellular pharyngitis, feline stomatitis, etc. (Mihaljevic, 2008), recurrent oral ulceration (Kenneth F. Lyon, 2007).

Definition: is the inflammation of the mucosal layer of any of the structures in the mouth. In the clinical field, the term should be reserved to describe widespread oral inflammation (beyond gingivitis and periodontitis), which may extend to submucosal level (e.g., marked caudal mucositis that extends to the submucosa tissues at the caudal portion of the oral cavity may be called caudal stomatitis, the inflammation of the alveolar mucosa extending from the muco-gingival junction to the mouth of the mouth is called alveolar mucositis, sublingual mucositis is inflammation of the sublingual mucosa (AVDC, 1988). Epidemiologically, this disease entity is reported with a prevalence of 0.7% in a study of 12 practitioners on 4858 cats (Healey & col, 2007), and in a recent study there is reported a prevalence of 5 , 5% (Johnston, 2015). Stomatitis syndrome in a cat's life can occur in three

different age categories. The first time during the vaccination of kittens, manifested by mild oral inflammation, which can be explained by a post-vaccinal reaction or by the eruption of the obese teeth (3-6 weeks old). The second period is marked by the eruption of permanent teeth (4-6 months old). The last, also the most common, period of chronic gingivostomatitis is at the age of 7 years (Johnston, 2012).

The current etiology of the disease is considered to be the complex outcome of a reaction involving several factors of various kinds. Thus, environmental conditions, bacterial infection (and the body's response to it) along with viral infection are all influenced by the disease process (Johnston, 2015). Among the factors involved in the aetiology of the disease we mention:

- race (in Johnston's experience (Johnston, 2012), mixed breeds represent most cases, with an important incidence in Main Coon and Siamese),
- environmental conditions (cats in shelters or households are more frequently affected by the disease, it seems that there are high levels of stress but also the cohabitation of cats that allow the transmission of microorganisms), the bacterial plaque (the oral flora incorporated in the bacterial plaque matrix (biofilm) attracts an abnormal immune response from the host (Milella, 2011). The heterogeneity of bacterial species and their inclusion in a biofilm adhering to oral mucosa or tooth explains why systemic antibiotic treatment is generally ineffective in the GSCF (Judy Rochette, 2012). In susceptible individuals, low plateau levels at the tooth surface may trigger an exaggerated reaction (DentalVets, 2015).
- existence of overlapping viral infections
  o Feline Alveolitis (FCV): It is known that the FCV carrier state is a co-factor in the onset and progression of the disease, (Johnston, 2012). It is suspected that the virus destroys cell membranes, allowing the antigens of other biotic agents to penetrate more easily. Another study has shown that caudal stomatitis is a manifestation associated with calicivirus infection (Milella, 2011).
  o Feline Immune Deficiency Virus (FIV): The immunological weakening condition of IVF infection predisposes the cat to secondary infections, possibly including oral ulcers. Both IVF and FeLV contribute to the hyperimmune response to the antigens present in the oral cavity. (Johnston, 2012)
- dental disease: Any kind of pre-existing dental disease can have an exacerbating effect on this syndrome. Paradontal disease, bone resorption or both are important co-factors and contribute to the global hyperimmune reaction (DentalVets, 2015).

Clinical signs. In terms of clinical manifestations, stomatitis depends on the area and the extent of inflammation, the degree of pain and the level of impairment of the normal function of mastication. Thus, it may range from superficial mucosal inflammation to erosions and ulcers, with the possible involvement of the gum, mucous membranes of the buccal, labial or sublingual, palatal tongue and palate (Reiter & Lewis, 2010). Paradoxically, this disease occurs frequently even in the absence of a tooth surface advanced tartar (Johnston, 2012). The main clinical signs are halitosis, hypersalivation, low appetite, dysphagia, mouth dysphagia, pain, lethargy, loss (Reiter & Lewis, 2010), low or moderate bacterial plaque, lack of tooth or root exposure due to associated bone resorption, tooth mobility due to periodontal tissue recession (Milella, 2011). In severe cases, cats neglect body-based pain relief, are sensitive to headaches, and submandibular lymph nodes are

enlarged (DentalVets, 2015). The lesions may include some or all of the soft structures in the oral cavity. The most severe form of stomatitis is evidenced by erythematous, ulcerative and proliferative lesions in the lateral walls of the pharynx (in the area of the palatal-glossal arch), the mucosa in the premolar / molar area, reaching to the oral mucosa, at the tongue, of the lips including the tough and / or soft palace (Thomas & Colab, 2017). Gingival inflammation is present around the teeth, not just the oral or buccal face that is characteristic of gingivitis (Heidi B. Lobprise, 2012). Sublingual mucositis may be present, as well as contact mucositis, where soft tissue lesions appear secondary to dental contact - also known as "kissing ulcers". Lingual, pharyngeal and mandibular salivary glands can be affected in severe conditions (DentalVets, 2015).

MATERIALS AND METHODS

Biological material. The study was conducted at the Faculty of Veterinary Medicine, Clinical and Medical Pathology, USAMV Cluj-Napoca and at SC Clinic Vetplace SRL Cluj-Napoca, which included 10 cats. The cats are European, with an average age of 2.5-13 years (average age is 7.95), of which 8 were males and 2 females. The study was conducted between October 2016 and June 2017.

Non-biological material. The following medical instruments were used in the study: stethoscope (Adion), thermometer (Predictor). Sterile swabs with or without transport medium (Aimes medium) were used to colect the samples depending on the working time of the biological material. The collection method involved the initial contention of the animal, the opening of the oral cavity, and sampling was done from the inflamed areas, upper and lower sides of one's mouth. Thus, they were transported to the laboratory within 24 hours of their sampling (those without culture medium) and 24 hours respectively (in the case of sterile sticks with transport medium). Each patient's label was passed to the label on each tube: name, age, date and harvest area. The place of harvest is the upper and lower gum, the caudal oral mucosa, the alveolar and labial mucosa. Sample processing took place at the Microbiology Laboratory of the Faculty of Veterinary Medicine, Cluj-Napoca, where the following materials were used: 5 Petri dishes with blood agar medium, 5 Petri dishes with simple agar medium, blades, sterile broth, Gram stains, microscope, 10 Müeller-Hinton Petri dishes, sowing, needle-type sowing, antibiotics in the form of micro compresses with a diameter of 6mm containing a preset amount of antibiotic, thermostat, tweezers curved, electronic beacon to measure the inhibition zone.

Perform the general clinical examination and complete the soft tissue evaluation sheet from the mouth. Interpretation of this sheet is made according to the results in table 1, ranking the final score in one of the stages of the disease:

- Stage I: minimal-mild gingivostomatitis: score between 0-6
- Stage II: mild-moderate gingivostomatitis: score between 7-14
- Stage III: moderate-severe gingivostomatitis: score between 15-21
- Stage IV: severe gingivotomatitis: score between 22-28.

Assessment of soft tissues from the oral cavity
Sheet: Stomatitis Activity Index (IAS)
Patient:
Date: Age: Weight:
Diet:
Owner's report (circle the answer number)

1. Appetite: 3 = eat only puree and only by hand; 2 = eating wet food alone, dry food is refused; 1 = eat wet food and dry food but less than normal; 0 = eating normally

2. Level of activity: 3 = no interest in other animals or people, sleeping most of the time; 2 = low activity level, but play if caused by animals or humans; 1 = playing spontaneously but not frequently; 0 = normal activity level (is curious and playful)

3. The dressing behavior: 3 = does not roll; 2 = occasionally, but not as usual; 1 = excessively washable; 0 = normally roll

4. Perceived comfort: on a scale of 0-3, where 0 is the most comfortable state and 3 the most painful state, the current state of the ca t is classed.

Table 1

Stomatitis Activity Index Sheet

The stomatitis activity index	0	1	2	3
Veterinarian evaluation				
Owner's evaluation (appetite / activity / self-toilet)				
Owner's evaluation (comforted received)				
Inflammation of the maxillary buccal mucosa				
Inflammation of the mandible mouth mucosa				
Inflammation of the jaw gum				
Inflammation of the mandibular gum				
Side inflammation of the palatal-glossy arch ("faucitis")				
Inflammation of the molar salivary gland				
Inflammation of the tongue and / or sublingual space				
Score TOTAL (max = 27)				

RESULTS AND DISCUSSION

In this study, 10 cats with clinically different gingivostomatitis diagnosis were clinically evaluated. This condition after examination of the oral cavity is classified in 4 stages of soft tissue inflammation according to the previous blistered sheet, of which only in the study we identified the first three stages. All of the cats were sampled from the oral cavity for the bacteriological and antibiogram examinations, following the technique described above. All but one (which was sterile) samples were compliant for interpretation.

Results of the soft tissue evaluation sheet from the oral cavity. From the assessment of soft tissues in the oral cavity, the most exposed and most frequently affected area is the maxillary gingival mucosa (each cat has upper jaw gum). Severe inflammation occurs in the gums of canines and upper premolars (104, 204, 107, 108, 207, 208). This is followed by the mandibular gingival mucosa, inflammation at this level seemed less severe, and was observed in all cats except one. The inflammation of the maxillary buccal mucosa and the lateral mucosa of the palate-gloss arc had a moderate incidence, 6 of the 10 examined had this lesion. The mandible mouth and tongue is much less affected compared to the rest of the tissues. Another observation was the relatively high incidence of fractures in upper canines, which occurred over the age of 6 years. In three cases, the upper left canine suffered fracture, and one case reported the fracture of the superior canine.

Patients in stage I (mild-mild gingivostomatitis) were aged 2.5-3.5 years, with an average age of 3 years. Class II (mild-moderate gingivosomatitis) and III (moderate-severe

gingivitis) were 4-13 years of age and mean age was 9.12 years. Thus, based on the outcome of the clinical examination, it can be stated that the severity of the lesions is directly proportional to the progression. This result was also noticed by Norman Johnston, 2012 Infections of the oral cavity can be associated with systemic diseases, as supported by De Simoi, 2012. In this study, the history of two cats with chronic feline gingivotrophitis reports urinary infection (Case No 3 and 4) and one case of diabetes (Case 6).

Generally, the agents found in the oral microflora of most cats were: *Streptococcus spp., Staphylococcus spp.*, and Gram negative cocobacilli bacterial species that were observed only once are *Pseudomonas spp., Bacillus cereus*, and in two samples the presence of yeasts, possibly of the genus *Candida*, was noted. Similar results can be found in the study by Bunduţă Cristina Mihaela, 2010.

Oral bacterial flora observed in cats classified in stage I was composed of the following microorganisms: *Streptococcus spp., Staphylococcus spp, Corynebacterium spp.*, and Gram negative bacteria. The bacterial flora observed in cats classified in Stage II and III was composed of the following microorganisms: *Streptococcus spp., Staphylococcus spp., Neisseria spp., Pseudomonas spp., Bacillus cereus*, Gram negative bacilli and coccobacils, yeasts. With some additions, similar results are also described by Bunduţă Cristina Mihaela, 2010.

The results of the study show that as the disease progresses from the initial stage (mild inflammation) to the final stage (severe lesions), the microorganisms in the mouth and plaque / tartar change only partially. Those that resist the more acidic environment resulting inside biofilm remain and those that do not cope with conditions disappear. Thus, the germs that colonize the surface of the tooth and the gum, regardless of the stage of the disease, are streptococci and staphylococci mainly, and Gram negative bacilli persist. Those bacteria that interfere mainly in advanced stages are *Neisseria spp, Pseudomonas spp.*, and other Gram negative coccobacils. These results and conclusions are broadly similar to the studies and research done in the field by Zambori Csilla and colab. in 2012.

Antibiogram results. Their results are very variable, they are different for each patient. This aspect is also suggested by Philippe Hennet, 1997, so it is recommended to perform the individual antibiogram. The resistance of germs to some antibiotic substances has been observed even in the first stage disease patient (case 1), who has never had contact with antibiotics, on any route of administration. These antibiotics are: Clindamycin, Cefazolin and Metronidazole.

It has also been observed that animals in which history reports single or multiple treatment with the commercial product called Stomogryl (s.a. Spiramycin, Metronidazole - cases No. 3, 6, 9), the antibiotic reported absolute resistance to Metronidazole and Clindamycin. Finally, the most effective antibiotics regardless of the stage of the disease are represented by:

I. Enrofloxacin - the bacteria in all samples examined were susceptible to this substance - 100%.

II. Convenia, Doxycycline - a single cat was resistant - 90%

III. Amoxicillin + Clavulanic Acid, Marbofloxacin, Trimethoprim, Penicillin G - Two cats showed resistance - 80%

IV. Flumequin - 3 cats were resistant - 70%

V. Clindamycin - 5 cats showed resistance - 50%

VI. Metronidazole, Cefazolin - resistance of all cats - Efficiency 0%.

Antibiotics most commonly used in oral cats include Doxycycline, Clindamycin, Metronidazole, Amoxicillin, Penicillin G, and Convenia, which are recommended by Fraser A. Hale, 2012 as the most effective in these cases. Our results show that many cats have antibiotic resistance to Metronidazole and Clindamycin, so they are not indicated in the treatment of oral infections in the cat.

CONCLUSIONS AND RECOMMENDATIONS

The age of the examined cats and the degree of severity of the present lesions is in a close relationship, as the age of the patient is higher, the disease is more complex, the lesions stretch to the surrounding tissues both on the surface and in depth, and the incidence of dental diseases increases.

Cats classified in stage II-III of oral inflammatory disease presented in addition to oral lesions and general clinical signs such as deviation, lack of activity and appetite reduction, thus it can be said that chronic untreated inflammation also affects the general condition of the animal.

The effect of the antibiotic on the body is influenced by several factors, including the individual's factor and the previous contact with the substance, thus the efficacy or ineffectiveness of the antibiotic is very variable. Therefore, it is advisable to perform the individual antibiogram prior to starting anti-infective treatment. In our study, antibiotics with the highest efficacy were 100% Enrofloxacin, 90% Convenia, and Doxycycline, while Clindamycin had only 50% efficiency and Metronidazole was not effective at all.

Recommendation:

Careful examination of the oral cavity in a consultation is difficult to do without the owner's involvement, and therefore only a simple visualization of the superior gingival mucosa following lip lift, but we recommend opening the oral cavity during each clinical consultation and to partially evaluate the soft and harsh tissues. This practice can help early detection of inflammation with treatment before the disease progresses in a chronic manner. It is known that frequently untreated gingivitis can lead to severe paradontal diseases.

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