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Gingivostomatitis and Feline Immunodeficiency Virus

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Abstract

Feline chronic gingivostomatitis (FCGS) is a challenge for the veterinary practitioner since its etiology and treatments remains undefined. The aim of this study was to investigate the role of the feline immunodeficiency virus (FIV) in the severity of the FCGS. Oral mucosal biopsies were obtained from 19 cats with FCGS and then divided into two groups according to their FIV serology status. Further, the clinical lesion score was correlated with the histopathological grade of FCGS lesions and the degree of immunostaining in both groups. Most of the animals had significant histological changes, however no correlation with the intensity of immuno staining for FIV was observed. It was concluded that the presence of FIV infection or the seropositive status of the animals does not seem to interfere with the severity of clinical signs nor the degree of histopathological changes when compared to the seronegative group.

Keywords: Feline ulcerative stomatitis; Lymphocytic-plasmacytic stomatitis; Glossitis

Abbrevations: FCGS: Feline Chronic Gingivostomatitis; FIV: Feline Immunodeficiency Virus; FELV: Feline Leukemia Virus; FCV: Feline Calicivirus; FHV-1: Feline Herpes Virus 1; UHV: Veterinary Hospital Unit; UECE: University of Ceará

Introduction

Feline chronic gingivostomatitis (FCGS) or feline chronic lymphocytic plasmacytic stomatitis/gingivitis [1] is characterized by proliferative and ulcerative lesions in the palatoglossal arch and buccal gingiva, affecting areas such as pharynx, tongue and lips [2]. FCGS is a severe, idiopathic oral inflammatory disease without a clear effective treatment [3]. Thirty percent of the cats will be refractory to the current standard treatment, which is full-mouth or near full-mouth tooth extraction [4]. Its pathogenesis is poorly understood but is thought to be due to the host immune system responding inappropriately to chronic oral antigenic stimulation secondary to underlying oral disease such as subclinical viral infections or autoimmune disorders, such as systemic lupus erythematosus and pemphigus [5]. Scientific evidence points to several viral agents as possible in the etiology of FCGS etiology, such as Feline Immunodeficiency Virus (FIV), Feline Leukemia Virus (FeLV) [6,7]; Feline Calicivirus (FCV) and Feline Herpesvirus 1 (FHV-1) [8]. FIV has been reported to be correlated to FCGS in 15% of the cases

FIV is a lentivirus capable of inducing a progressive loss of CD4+ lymphocytes and increased CD8+, due to its tropism by CD4+ T lymphocytes, B lymphocytes and macrophages. This change allows the occurrence of chronic and recurrent infections due to the acquired immunodeficiency syndrome, characterized by a long incubation period, slow clinical evolution and progressive course. Due to the progressive dysfunction of the immune system in FIV+ cats and the possible common association of the virus with FCGS, the objective of this study was to investigate the role of the feline immunodeficiency virus (FIV) in the severity of the FCGS.

Materials and Methods

Animals and clinical examination

This study was conducted with approval of the Ethical Committee for the Use of Animals, State University of Ceará-Brazil, under the number 0119213/2014. All ethical precepts of animal protection were respected and the owners signed an

informed consent. Inclusion criteria included cats affected by FCGS for at least six months before enrollment. If corticosteroids therapy was prescribed, it had to be discontinued for at least 2 months prior to the biopsy. Samples were collected from 19 cats routinely examined at Veterinary Hospital Unit (UHV) of the State University of Ceará (UECE). Six cats that died from trauma or received indication of euthanasia were selected to be used as a control group. None of them presented chronic or degenerative systemic diseases.

All cats were screened for FIV and FELV infection by immune chromatographic test. After, the cats were divided into two groups according to the result: Group FIV+ Group and Group FIV- Group. Patients were submitted to anesthesia using intravenously ketamine hydrochloride (3mg/kg), midazolam (0.4mg/kg) and methadone (0.3mg/kg) intravenously in order to proceed with a complete oral cavity clinical evaluation and collection of biopsies. First, macroscopic lesions were classified and the animals were sorted according to the severity of the lesion, as follows: grade 0, absence of lesion; grade 1, hyperemia of palatoglossal arch, without ulceration, bleeding or proliferative lesions; grade 2, hyperemia and proliferation of palatoglossal arch, without ulceration or bleeding; grade 3, hyperemia, proliferation and ulceration of palatoglossal arch, with bleeding induced by mild digital compression; grade 4, hyperemia, proliferation and ulceration of palatoglossal arch with spontaneous bleeding (Figure 1A-1D).

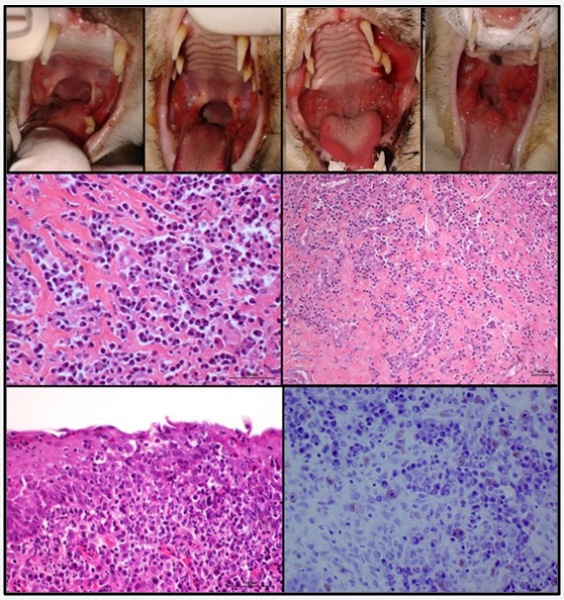

Figure 1: a) Feline with macroscopic lesion 1 score, palatoglossal hyperemia.
b) Feline with score 2 of macroscopic lesion, proliferation of granulomatous tissue in the palatoglossal arch, without ulceration.
c) Feline with macroscopic lesion score 3, intense proliferative tissue, with ulceration, but without spontaneous bleeding.
d) Feline with macroscopic lesion score 4, granulation tissue, ulceration and spontaneous bleeding.
e) Photomicrography of oral mucosa of FIV positive feline with GECF, granulocytic infiltrate (arrow), HE (40x).
f)Photomicrography of feline FIV positive oral mucosa with GECF, Mott cells in the submucosa (arrow), HE (40x).
g) Photomicrography of FIV positive feline oral mucosa with GECF, presence of mast cells in the submucosa (arrow), AT (20x).
h)Motic® BA310 Trinocular Microscope (Motic® 2000 2.0 MP Live Resolution) and Motic Image Plus 2.0 software.

An incisional biopsy of oral mucosal lesions was performed using a 4mm punch. Samples were always obtained in the caudal region of the palatoglossal arch. Oral biopsies were fixed in 10% neutral buffered formalin solution for 24 hours for further histological processing. The sections were made at 3µm thickness and stained with hematoxylin and eosin and Toluidine Blue.

Immuno histochemical processing

Immuno stainings were performed on 5µm thick paraffin sections. After standard deparaffinization and rehydration procedures, tissue sections were subjected to antigen retrieval by 0.005M citric acid buffer, heated under constant pressure for 5 minutes. Endogenous peroxidase activity was blocked by incubating the sections in 6% hydrogen peroxide in PBS solution for 30 minutes. The sections were incubated overnight with 1/200 diluted FIV monoclonal primary antibody (PBS, 1% bovine serum albumin, 0.3% Tween 20 and 0.1% sodium azide). Subsequently washed in PBS and incubated with HRP-polymer. Positive areas for FIV were developed after staining with diaminobenzidine solution and counterstaining with Harris hematoxylin. Negative controls were performed with replacement of the primary antibody by deionized water.

Morphometric analysis

A random quantitative analysis was performed according to the types of inflammatory cells found and later classified on a four-point scale, based on the degree of inflammation, as advocated.

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Where,

0=No inflammation (Normal)

1= Mild inflammation

2=Moderate inflammation

3=Severe inflammation

Further, neutrophils, macrophages, plasma cells, eosinophils, lymphocytes and mast cells were quantitatively analyzed in 10 fields at 400x magnification for both groups. At least 500 cells were counted using Image J digital imager program to conduct and further a descriptive statistics differential in percentage was made. The Leica Qwin version 3.0 image analysis program was used in a conventional optical microscope (Leica - DMR, Germany), with a digital coupled camera (Leica - DFC500, Germany) for the recording of the photomicrographs.

Twenty random fields of the sample in a x400x magnification were selected for quantification of the stained area. Further, the results were transformed in percentage by the following formula: (Σ(A)/20) X 100, where (A) corresponds to the area of immuno labeled cells per field (Motic Image Plus 2.0 software). After that, the animals were classified according to this percentage, they were distributed in degrees of immune blotting, where: the grade 0, corresponds to the absence of immuno staining; grade 1, for a weak immune staining (up to 10%); grade 2, for a moderate immuno staining (from 10.01% to 50%); grade 3, for a strong immune staining (50.01% to 70%); and grade 4 represents for an intense immune staining (above 70.01%).

Statistical analysis

Statistical analyses were performed using Graphpad Prism 6 (GraphPad Software, Inc., CA, USA). Results were checked for normality by D'Agostino & Pearson test. Results were compared using a t-test for parametric results or Mann-Whitney test for non-parametric results. Significance was set at $P<0.05$. The Pearson correlation coefficient test was used to determine the correlation of clinical classification with histological classification and degree of immune staining.

Results

Nineteen cats from 2 two to eight8 years-old were included in this study, of which 57.8% (11/19) were mixed breed, 26.3% were Siamese and 18.8% were Persian. Fifty-eight percent (11/19) were males and 42% (8/19) were females. According to the results of the Immuno chromatographic tests revealed for FIV and FELV, the animals were divided into two distinct groups: eight FIV positive animals and 11 non-positive FIV animals. However, two animals of the FIV negative group (animals 4 and 19) had moderate FIV-immuno labeling.

The main clinical signs presented were: palatoglossal hyperemia 100%(19/19), enlargement of submandibular lymph nodes 95%(18/19), halitosis 90%(17/19), dysphagia 90%(17/19), pain when the mouth was open (10/19), loss of weight 52%(10/19), anorexia 45%(8/19), oral ulceration 45%(8/19), and dental resorption lesions 20%(3/19).

In the clinical classification of the lesion, it was observed that 15.8% (3/19) had grade were grade

1. while grade 2 was 26.3% (5/19), had grade 2; 42.1% had grade 3(8/19) and 15.8% (3/19) had grade 4 (Table1). For the Histopathological classification showed that, 90% of the cats had lesions grade 3 and 10% had lesions grade.

Table 1: Clinical score, degree of histopathological changes and degree of immunostaining in cats with chronic gingivitis stomatitis tested for FIV.

Cat	Fiv State	Clinical Score	Histopathologic Grade	Immunostaining Degree
1	Negative	2	3	0
2	Positive	1	3	2
3	Positive	2	3	3
4	Negative	2	3	2
5	Positive	3	3	4
6	Negative	3	3	0
7	Negative	3	3	0
8	Negative	3	3	0
9	Negative	1	2	0
10	Negative	2	3	0
11	Negative	2	3	2
12	Positive	1	3	1
13	Positive	3	3	2
14	Positive	4	2	1
15	Positive	4	3	4

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16	Positive	3	3	2
17	Negative	3	3	0
18	Negative	4	3	0
19	Negative	3	3	3

2. There were was no significant difference in the clinical and histopathological classification and they presented a correlation of 0.9. Moreover, no differences on the distribution of any grade between FIV positive and FIV negative groups was observed.

The main histopathological changes found in both FIV positive/negative groups were: hyperplasia, parakeratosis, vacuolar degeneration of the epithelium and ulceration. The inflammatory infiltrate composed mainly of plasma cells (Figure 1E), lymphocytes, neutrophils (Figure 1F) and mast cells, affecting the submucosa, perivascular regions and salivary glands. Mott cells (Figure 1G) were found in three animals (two FIV+ and one FIV-).

Peculiarities of these cells help to perpetuate the virus, such as: They produce and release relatively low amounts of infectious HIV-1 and are less sensitive to viral cytotoxicity compared to CD4+T cells, being more resistant [9]. Especially long-lasting macrophages may harbor the virus for long periods of time, thus constituting HIV-1 reservoirs and a major obstacle to the eradication of virus from infected individuals [10], with viral hideouts during the phase chronic infection. Since macrophages secrete cytokines that attract/recruit T lymphocytes to infection sites, they can "support" the establishment of viral infection by increasing the number of primary target cells by transmitting the virus to CD4+ T cells on the mucosal surface via Cell-to-cell contact during antigen presentation.

Identified the infected cell population early in the case of IVF inoculation and highlighted the role played by macrophages in IVF uptake and viral spread, with the targets being most infected by the retrovirus [11]. Some strains of FIV are monocytotrophic in-vivo, which may be related to viral virulence, vertical transmission and infection of tissue macrophages [12].

The histopathological changes found corroborating with other publications [13]. Mast cells were found in lower amounts, and eosinophils were not present in the samples examined (Figure 2). Diverging from Harley et al., where the amount of lymphocytes was considered superior to that of neutrophils. It is known that FIV causes a decrease in the neutrophil population of the affected feline [14]. However, no statistical difference was observed in the neutrophil infiltrate between FIV+ and FIV- groups. The large amount of neutrophils in the mucosa of the animals evaluated in our study probably reflects the bacterial component of the etiopathogenesis of FCGS. The presence of several oral bacteria associated with the disease was previously reported [15].

The marked presence of plasma cells in the inflammatory infiltrate, together with the decrease in lymphocytes can be explained by the immunosuppressive action of FIV [16]. However, in this study, the same histopathological pattern was repeated in seronegative animals for the virus. Plasma cells are differentiated B-lymphocytes, present in chronic oral inflammations, which have a large capacity to produce immunoglobulins, which may explain the increase in IgG and IgM levels in animals with GECF [17]. Mott cells are plasmocytes with multiple immunoglobulin vacuoles, mainly IgM.

These cells were observed in three cats of this study and it might be due to the chronic immune stimulation to produce immunoglobulins. These cats had severe histopathological grade of associated periodontal diseases such as dental calculi and lesions of tooth resorption. GECF may present along with periondontal disease, something common in FIV-positive cats [18].

Mast cells are important sentinel cells that participate in mucosal immunity, and their involvement in feline oral diseases has been reported to play an important role in GECF. The presence of mast cells possibly due to the chronic stage of inflammation, since these cells are generally involved in the acute inflammatory process.

Two discordant results between immune chromatographic and immune staining could be due to the false-negative results of immune chromatographic exams, especially at the initial and the late stages of the infection [19]. This happens since in the initial stage the sero conversion has not been started yet and in the late stage the immunodeficiency has already been developed. Both false positive cats had severe cases of gingivitis stomatitis, with a degree of histopathological lesion and high clinical score, and this may suggest chronic infection and possibly low availability of antibodies, resulting in false-negative results. No animal was positive for FELV, which diverged with high prevalence and association with FELV with FCGS described previously [20].

Similar distribution of the cats according to the clinical and histological scores indicates that there is probably no correlation between FIV and the severity of the lesions. Moreover, no correlation between the degree of immune staining intensity and the clinical score of the animal was observed, demonstrating that possibly the degree of viral infection by FIV does not predict the severity of the oral disease.

Conclusion

FIV infection does not seem to interfere in the severity of the clinical signs nor in the degree of histopathological lesions. Histopathology and immuno pathogenic studies are suggested in the early stage of FCGS in order to investigate the association with different etiological agents. These might contribute to

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the elucidation of the etiology, prevention and treatment of the disease. The inflammatory infiltrate in both groups consisted mainly of plasma cells and neutrophils, followed by lymphocytes. Neutrophils, lymphocytes, plasmocytes and mast cells were distributed similarly between both groups, however macrophages was significantly higher in the FIV+ group (Figure 2). In the present study, the presence of mast cells was moderate and observed mainly in the submucosa in 95% of the animals (Figure 1H).

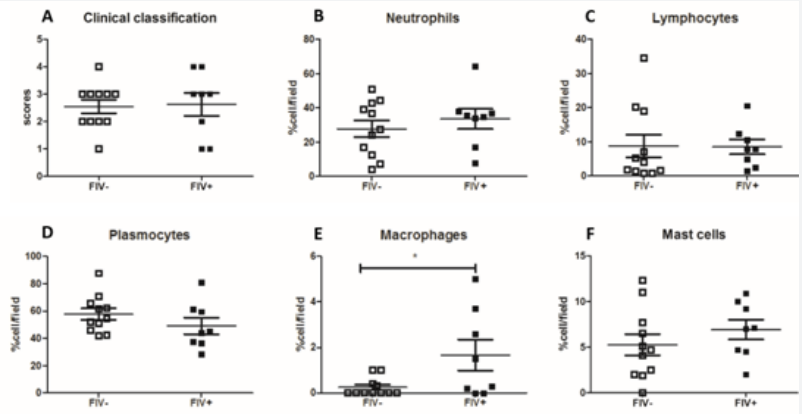

Figure 2: Clinical classification
A. Quantification of histological staining of neutrophils.
B. Lymphocytes.
C. Plasmocytes.
D. Macrophages.
E. Mast cells.
F. Bar heights represent mean values, and brackets indicate SEM. Five fields were counted on two slides of each organ from three mice in each group. FIV+ cats showed significant higher amount of macrophages than FIV - cats ($P<0.05$).

Cytoplasmic immunostaining for FIV was observed mainly in plasma cells, lymphocytes and neutrophils. All FIV+cats had positive immuno staininning for FIV. Moreover, two FIV-cats also presented immuno staining for FIV (Grade 2 and 3). It was observed grade 2 immuno labeling grade 2 in 43% of the cats (5/11), followed by 18% of the other grades (2/11) (Table 2). No correlation between the degree of immunos taining intensity and the clinical score of the animal was observed. Considering IHC results our study presented 52.6% (10/19) positivity for FIV.

Table 2: Quantification of inflammatory infiltrate in the oral mucosa of cats affected by FCGS.

Cat	Fiv State	Clinical Score	Histopathologic Grade	Immunostaining Degree
1	Negative	2	3	0
2	Positive	1	3	2
3	Positive	2	3	3
4	Negative	2	3	2
5	Positive	3	3	4
6	Negative	3	3	0
7	Negative	3	3	0
8	Negative	3	3	0
9	Negative	1	2	0
10	Negative	2	3	0
11	Negative	2	3	2
12	Positive	1	3	1
13	Positive	3	3	2
14	Positive	4	2	1
15	Positive	4	3	4
16	Positive	3	3	2
17	Negative	3	3	0
18	Negative	4	3	0
19	Negative	3	3	3

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Sources of acquisition

A: Anigen Rapid FIV/FeLV Test, Bioeasy, São Paulo-SP

B: Anti-FIV p24 antibody [PAK3-2C1] ab65289, Abcam, USA

C: Super Picture TM HRP Plymer Conjugate Broad Spectrum-Invitrogen Corporation, Frederick, MD 21704, USA.

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