Feline lymphoplasmacytic rhinitis (FLPCR): severity of inflammation correlates with reduced mucosal IgA expression

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Abstract

Feline lymphoplasmacytic rhinitis (FLPCR) is a rare disease with an unclear pathogenesis characterized by lymphoplasmacytic (LPC) inflammation and progressive tissue destruction. Aims were to evaluate specific FLPCR clinical and pathological features to gain insights into disease pathogenesis. Signalment, clinical signs, serology and 47 pinch biopsies were retrospectively collected from 33 FLPCR and 3 normal cats. Microscopical lesions and immunohistochemistry results utilizing anti-CD3, anti-CD20, anti-FOXP3, anti-feline-IgA, IgG, IgE and anti-FeLV (p27 and gp70), FIV, FCV and, FHV were scored and most were analyzed statistically. The majority of cats were domestic short haired (26/31) with median age of 11 years and a 0.35 F/M ratio. Serology evidenced 3/22 FIV and 1/22 FeLV positive cats. Immunohistochemistry evidenced 1/33 FeLV-p27 positive cats. Common clinical signs were sneezing (19/24 [79%]), mucous discharge (13/24 [54%]) and stertor (10/24 [42%]). In normal tissues, IgAs were expressed in mucin, apical and lateral cell membrane of columnar cells and in periglandular plasma cells. IgGs were expressed in 20-30% of columnar cells. Number of clinical signs was statistically significantly higher in female cats (p<0.0001) and was significantly correlated with chronicity (p=0.004), and IgG scores (p=0.01). LPC severity scores correlated positively with infiltration of neutrophils (p=0.015), gland destruction (p=0.019) and angiogenesis (p=0.016) and negatively with fibrosis (p<0.0001). LPC severity scores were also significantly associated to female sex (p=0.01) and to IgA (p=0.03), with higher IgA scores associated to lower LPC scores.

FLPCR associated to disruption of mucosal defense mechanisms generating cycles of tissue inflammation, tissue damage and repair with progressive loss of function independent from viral infections.

Key words: cat; rhinitis; lymphocytes; plasma cells; immunohistochemistry; Immunoglobulins, IgA

Abbreviation list:

FCR = Feline chronic rhinitis

FLPCR = Feline lymphoplasmacytic rhinitis

URT = Upper respiratory tract

FHV-1 = Feline herpesvirus type-1

FCV = Feline calicivirus

FIV = Feline immunodeficiency virus

FeLV = Feline Leukemia virus

NALT = Nasal Associated Lymphoid tissue

DIMEVET = Department of Veterinary Medicine

UNIMI = University of Milan

HE = Hematoxylin and Eosin

LPC = Lymphoplasmacytic

IELs = Mucosa intraepithelial lymphocytes

DSH = Domestic shorthair

PMNs = Neutrophils

ROS = Reactive oxygen species

Introduction

Feline chronic rhinitis (FCR) represents a common disease of domestic cats with unclear pathogenesis that may coexist with variety of systemic diseases or develop independently. Although the definitive etiology is yet undetermined, FCR represents a multifactorial condition likely associated with several different initiating or precipitating factors (Kuehn, 2006). Among the conditions that associate with FCR, primary nasal diseases such as feline lymphoplasmacytic rhinitis (FLPCR), idiopathic chronic rhinosinusitis, nasopharyngeal polyps, nasopharyngeal stenosis, dental diseases and, neoplasms have all been described (Forrester et al., 2002; Kuehn, 2006)In a subset of juvenile cats, acute upper respiratory tract (URT) infections caused by Feline herpesvirus type-1 (FHV-1) and Feline calicivirus (FCV) have been hypothesized to trigger FCR (Kuehn, 2006). Up to 80% of cats that recover from viral rhinitis may become chronic carriers and develop persistent clinical signs and FCR (Scherk, 2010).

Among chronic nasal disorders, FLPCR is considered a rare condition also with unclear pathogenesis (Allen et al., 1999; Kuehn, 2006), that may predispose to FCR. FLPCR has been described as a progressive disease with clinical signs varying from minimal to severe nasal discharge, sneezing, and stertorous breathing that may worsen progressively with cycles of lymphoplasmacytic inflammation, tissue damage and repair. The signs can regress or persist unaltered for years (Kuehn, 2006; Michiels et al., 2003). Contrary to most common FCR, cats developing FLPCR do not have a previous history of acute respiratory infections (Johnson et al., 2005; Kuehn, 2006). Given the wide spectrum of clinical manifestations, early diagnosis, therapy and prognosis of FLPCR are still problematic.

Diagnosis of FLPCR is one of exclusion and is based on the persistence of clinical signs confirmed by gross and microscopical lesions in the absence of any other primary or systemic condition associated with FCR (Kuehn, 2006). Nasal biopsies from cats with FLPCR are initially

characterized by mucosal erosion and ulceration (López and Martinson, 2017), followed by re-epithelization and attenuation of respiratory epithelium with variable development of squamous metaplasia and/or dysplasia. In chronic cases, the most consistent microscopical finding is a variably severe, multifocal to diffuse submucosal to periglandular lymphoplasmacytic inflammation; in chronic cases, fibrosis and glandular atrophy ensue (Caswell and Williams, 2016; Michiels et al., 2003).

In the mucosa of the upper respiratory tract, the local innate and adaptative immune system comprising the Nasal Associated Lymphoid tissue (NALT) monitor antigen entry and modulate immune responses against commensal microorganisms and harmless antigens inhaled from the environment (Faria and Weiner, 2005). IgA and IgG represent the predominant immunoglobulin isotypes in the normal respiratory mucosal lining of the nasal tract of most species. IgA isotype plays a major role in inhibiting tissue penetration of antigens, whereas specific IgG bind harmful antigens penetrating the mucosa (such as bacterial antigens) and induce their clearance by the immune system (Kaneko et al., 1995; Norris et al., 2003).

Persistent or recurrent challenge of NALT by environmental antigens may cause abnormal responses to normal antigens that may induce excessive IgG production with tissue damage and/or sensitization and development of local IgE-mediated type I hypersensitivity contributing to derangements of immune function and development of nasal disease (Snyder, 2017). IgEs are monomeric or dimeric isotypes of immunoglobulins major contributing to allergic diseases. Indeed, recent studies have implicated pollens in the emergence of allergic rhinitis, bronchial asthma and cutaneous reactive patterns in cats (Jensen-Jarolim et al., 2015). As a sequela of persistent allergic rhinitis, a lymphoplasmacytic inflammation ensues in association with variable degrees of eosinophilic infiltration (Reed and Gunn-Moore, 2012). During allergic reactions, local IgE synthesis develops following migration of B-lymphocytes at sites of

inflammation and their subsequent transformation in antigen specific antibody producing plasma cells.

Lymphoplasmacytic rhinitis has been widely recognized as a specific entity in dogs (Lobetti, 2014), and is increasingly reported in cats where still represents a diagnostic and therapeutic challenge. FLPCR is a chronic persistent disease with possibly complex and multifactorial pathogenesis that, due to problems in tissue sampling as previously reported in dogs, is seldom studied microscopically (Little, 2012; Pietra et al., 2010). Thus, a detailed description of lesions affecting different components of the nasal mucosa and the modulation of resident immunoglobulins is needed to better characterize the pathogenesis of FLPCR.

The aim of this study was to perform a comprehensive histopathological evaluation of microscopic lesions and of immunohistochemical mucosal immune cell infiltrates in cats diagnosed with FLPCR and to evaluate the associations between the type and severity of lesions and the type of inflammatory response and derangements in IgA, IgG, and IgE tissue expression.

Materials and methods

Cases

In this retrospective study, nasal pinch biopsies from cases of FLPCR were retrieved from the electronic database of the surgical pathology service of the Department of Veterinary Medicine (DIMEVET) of the University of Milano (UNIMI) collected between 2006 and 2016. Cases that encompassed the keywords "cat", "nasal cavity", and "rhinitis" were searched and selected on the basis of the diagnosis. Slides were reviewed and only cases with lymphoplasmacytic rhinitis were included. Cases where FLPCR was concurrent with neoplasia (e.g. nasal lymphoma or

adenocarcinoma) were excluded from the study. A total of 33 cats met the inclusion criteria. All tissue samples included in the study were collected by anterograde biopsy of the respiratory nasal mucosa performed by the same operator (CMM) at the Small Animal Veterinary Teaching Hospital of the UNIMI for diagnostic purposes following owner's informed consent. Samples came from cats with chronic history of upper respiratory signs. In cats with bilateral clinical signs, both nasal cavities were sampled. Vestibular (anterior) and respiratory mucosal linings were never biopsied. For each cat, information regarding signalment, presenting complaints, clinical signs, FIV/FeLV serologic data, and vaccination status were collected.

To compare and score nasal tissue lesions, respiratory nasal mucosal samples from 3 normal cats were enrolled. These cases were selected according with the following criteria: cats not affected by upper respiratory tract condition (lack of specific clinical signs) confirmed by lack of microscopical tissue lesions. The tissues came from 3 male, Domestic shorthaired (DSH) cats of 3, 7 and 10 years of age that were necropsied (following owner's request) after spontaneous decease and with the definitive diagnoses of hypertrophic cardiomyopathy (2 cats) and end stage renal disease (1 cats). These three cats were serologically FeLV and FIV negative and regularly vaccinated for FeHV, Calicivirus and Panleukopenia virus.

Microscopic Evaluation

Biopsy samples were fixed in 10% neutral-buffered formalin, routinely processed, and stained with hematoxylin and eosin (HE). To reduce inappropriate diagnostic interpretation, all histological samples were blindly and independently submitted to pathologists with different degrees of experience, including 2 residents in anatomic pathology (MT, FG) and 2 board-certified pathologists (ST, PR). Only cases that were diagnosed as FLPCR by all pathologists were included in the study. Lymphoplasmacytic (LPC) inflammation was scored, for statistical

purposes, on the basis of severity inflammation as follows: mild (score 1), moderate (score 2), severe (score 3) according with indications by Johnson et al. (2004) after the setting of their rhinoscopy gross/clinical grading system and summarized in Table 1. Based on the presence/absence of lesions and their severity, additional microscopic lesions observed in the mucosa, lamina propria, submucosal glands, and turbinates were scored from 0 to 3 (Table 1). Lesions evaluated and scored included: mucosal erosion/ulceration; mucosal hyperplasia; squamous metaplasia; mucosal intraepithelial lymphocytes (IELs); lamina propria infiltration of neutrophils, eosinophils, and mast cells; lamina propria angiogenesis; fibrosis; glandular destruction/loss; resorption, microfractures and bone remodeling of the turbinates. Microfractures were identified when small pieces of mature bone with irregular contours were surrounded by fibrous tissue with osteoclast bordering the thin, irregular surface. Bone remodeling was identified by reduction of bone lining cells and presence o reactive plump osteoclasts and/or Howships lacunae.

In the instance of multiple biopsies being available from the same nasal cavity, all samples were included in the microscopic examination; however, for the purpose of statistical analysis, the final score was reported only for the single biopsy characterized by the most severe lesions. In cases where both nasal cavities were sampled, the scores were reported for each cavity and consisted of the biopsies with the most severe lesions.

Primary antibodies specific for feline immunoglobulins were utilized. Viral antigen expression was assessed with antibodies raised against feline specific epitopes. Anti-CD3-ε, anti-CD20 are antibodies specific for the respective human proteins. Anti-CD3 and anti-CD20 antibodies have been characterized as cross-reactive and have been used extensively in veterinary medicine (Affolter and Moore, 2006; Darbès et al., 1997; Gilbert et al., 2004; Jones et al., 1993;

Mason et al., 1989; Swerdlow et al., 2016) while anti-FOXP3 is indicaed as cross reactive by the manufactures. A reactive feline submandibular lymph node was utilized as positive control for CD3, CD20 and FOXP-3. Positive tissue controls for feline antigen viral expression came from paraffin embedded tissues received for diagnostic purposes and included a FeLV-positive cutaneous lymphoma from a previous study (Roccabianca et al., 2016), a FHV-1-positive biopsy taken from the nasal planum, a sample of tongue from a FCV-positive necropsied kitten, and a lymph node from a FIV-positive necropsied cat. For immunoglobulin expression, formalin-fixed specimens from a feline mesenteric lymph node (IgG), duodenum (IgA) and a submandibular lymph node (IgE) from a cat with an oral eosinophilic granuloma were included as positive controls. For each cat, immunoglobulin isotype (IgA, IgE, and IgG) relative expression by the plasma cells was assessed semi-quantitatively in the corresponding biopsy with the most severe inflammation. The percentage of plasma cells positive for IgA, IgG or IgE was determined by one board-certified pathologists (PR) and one resident in training (ST) on a scale ranging from 0% (i.e. lack of expression of a specific marker) to 100% (i.e. all plasma cells expressing a specific marker). Briefly, paraffin-embedded tissue samples were cut in 3-5 μm sections, mounted onto Superfrost Plus slides (Menzel Glasser; Gerhard Menzel, Glasbearbeitungswerk GmbH & Co, Braunschweig, Germany) deparaffinized in xylene and hydrated through graded ethanol solutions. Endogenous peroxidase was quenched with hydrogen peroxide (0.3%) and sodium azide (0.1%) in Tris buffer (0,1-M solution, pH 7,5) for 30 minutes and rinsed in 3 changes of Tris buffer for 5 minutes each. Sections were incubated with primary antibodies for 12 hours at 4°C. Source and conditions of use of primary antibodies are listed in Table 2. Immunoreaction was visualized with amino-9-ethyl-carbazole chromogen (AEC; Vector, Burlingame, CA). Sections were counterstained with Mayer's hematoxylin and mounted. Samples of normal respiratory nasal mucosa from the 3 cats enrolled in the study

were used to establish baseline expression of immunoglobulins. Negative tissue controls consisted in normal samples of feline tongue. Application of an isotype-matched nonspecific Mab anti-canine MHCII (for monoclonal antibodies), anti-bovine papillomavirus (for polyclonal antibodies) served as negative antibody controls.

Statistical Analysis

First, the association between ordinal LPC scores (expressing FLPCR severity) and other observed microscopic lesions was tested through polychoric correlation that allows the analysis of ordinal variables. Subsequently, the effect of sex, age, spayed/intact status, chronicity of FLPCR (i.e. <4 months, 4-12 months, >12 months), IgG and IgA on the probability of having a higher LPC score were explored through ordinal logistic regression. Finally, we examined variation in the number of observed clinical signs through a generalized linear mixed model with Poisson error distribution, testing for an effect of sex, age, spayed/intact status, chronicity, IgG, IgA and LPC score. In both models, we included cat ID as a repeated measure to account for cats where both nasal cavities were sampled. IgE data were not included in the analysis due to the low sample size. All the analyses were carried out using SAS® 9.4 Software (Copyright © 2012 SAS Institute Inc., Cary, NC, USA).

Follow-up

Follow-up information regarding clinical evolution, treatment, and time/cause of death were collected via telephone interviews with the owner or with the referring veterinarian at the end of the study.

Most cats were DSH (26/31 [87%]); other represented breeds were Persian (n=2), Norwegian Forest (n=1), Maine Coon (n=1), and Siamese (n=1). For 2 cats, the breed was not retrieved. Median age was 11 years (range: 0.5-19 years); the age was not available for one cat. The female/male ratio was 0.35 (9 females, 23 males). Of these, 6 females (6/9 [67%]) and 17 males (17/23 [74%]) were neutered. In one cat, the sex was not reported. Serologic data for FeLV and FIV were available in 22/33 cats (67%). Only one cat was FeLV positive; 3 cats were FIV positive. All cats were routinely (once a year) vaccinated against FHV-1 and FCV (Day et al., 2016; Scherk et al., 2013).

Clinical signs associated with FLPCR were recorded for 24/33 cats and included sneezing (19/24 [79%]), nasal mucous discharge (13/24 [54%]), stertor (10/24 [42%]), nasal serous discharge (7/24 [29%]), ocular discharge (7/24 [29%]), epistaxis (7/24 [29%]), reverse sneezing (5/24 [21%]), cough (4/24 [17%]), dyspnea (3/24 [12%]) and frontal sinus swelling (3/24 [12%]).

Histological Evaluation

In the three cats examined, normal respiratory mucosa was characterized by a ciliated columnar simple respiratory epithelial lining, admixed with rare mucous cells and a lamina propria composed of fibrillar to dense collagen, small vessels, and mucous glands surrounded variably by 1-2 layers of mature plasma cells (Fig. 1A).

In 19 cats with FLPCR, only one cavity was biopsied; in 14 cats, tissues were collected from both nasal cavities (Table 3). Multiple biopsies were taken from lesional areas of nasal respiratory mucosa directly visualized during endoscopy with an average of 9.8 biopsies per cavity and, with an average size of 2-6 mm. A total of 47 nasal biopsies collected from 33 cats were examined to evaluate microscopical lesions and inflammation was scored for severity. Microscopic lesions and scores are summarized in table 4. In 8 biopsies from different cats,

lesions including bone resorption could not be scored due to the lack of turbinates in the samples obtained.

In all biopsies, the lamina propria was infiltrated by a prevalence of mature plasma cells with variable percentages of Mott cells (40/47 [85%]); the degree of inflammation varied in severity from interstitial infiltration to band-like accumulation of plasma cells obscuring the submucosal lamina propria. In 7 biopsies, a mixed population of plasma cells and small mature lymphocytes (1/47 [2%]) was observed, or a prevalence of small mature lymphocytes (6/47 [13%]) was detected. The severity of LPC lamina propria infiltration was mild (score 1, Fig. 2A) in 17/47 biopsies (36%); moderate, (score 2 Fig. 3A) in 18/47 biopsies (38%); and severe (score 3 Fig. 4A.) in 12/47 biopsies (26%). Mucosal hyperplasia was present in 30/46 biopsies (65%), mucosal erosion/ulceration was observed in 21/43 samples (49%); whereas IELs infiltration was mild to moderate in 21/46 biopsies (46%). Neutrophils were present in 28/47 biopsies (59%). Eosinophils (8/47 [17%]) and mast cells (7/47 [15%]) were rarely seen and never scored greater than 1.

Glandular loss was observed in 30/43 biopsies (70%). Angiogenesis characterized 28/45 biopsies [62%], fibroplasia to fibrosis was present in 25/47 samples (53%), turbinate lesions with remodeling were observed in 28/38 biopsies (74%).

In cats with lesional severity scored as 1 (13/17 [76%]), fibrosis was the most frequent lesion associated with FLPCR. In cats with lesional severity scored as 2, the predominant lesion associated with FLPCR was mucosal hyperplasia (16/18 [89%]). Contrarily, cats with lesional severity scored as 3 frequently exhibited angiogenesis (9/12 [75%]).

Squamous metaplasia was never observed. Low numbers of aggregated coccoid bacteria were observed admixed with luminal mucus and neutrophils only in one biopsy and were considered secondary.

Feline viral antigen expression

All the immunohistochemical results are listed in Table 4. The expression of viral antigens was evaluated only in those cases where residual tissue was available for further sectioning. For FeLV, the expression of p27 and gp70/85 was examined in 29/33 cats. Only 1/29 cats resulted positive for FeLV p27; its corresponding biopsy had lesional severity scored as 2. The expression of FCV and FIV was assessed in 29/33 cats respectively and all samples tested negative. FHV was examined on 8/33 cats and was negative in all cases.

IgA, IgG, and IgE expression

In normal nasal respiratory mucosa IgA was expressed in the superficial mucous film and in the luminal cell membrane of mucosal columnar epithelial cells (Fig. 2B). IgG positivity was rare and characterized by multifocal positivity observed in the cell membrane of approximately 20-30% of mucosal epithelial cells. IgE expression was never observed in normal mucosal lining. In the lamina propria, one to two layers of IgA-positive plasma cells were observed, mostly surrounding deep mucous glands. Plasma cells surrounding glands and superficial mucous were negative for IgG and IgE expression was consistently negative.

Expression of IgA and IgG was assessed on all pathological samples (47/47), while IgE expression was evaluated on 30 biopsies (30/47 [64%]) obtained from 22 cats due to lack of residual tissue from the other cases. Percentages of plasma cells expressing IgA, IgG and IgE are listed in table 4. In FLPCR, IgA expression (Fig. 2B, 3B, 4B) ranged from 0-90%, whereas IgG expression (Fig. 2C, 3C, 4C) ranged from 0-100%, and IgE expression ranged from 0-10% of the total plasma cell population. Most cases had low IgA expression (10% IgA, 9/47 [19%]), which was more frequently recorded in biopsies with LPC inflammation scored as 1 (5/47

[11%]). In 1 case, the highest percentage of IgA expression was 90% (1/47 [2%]) in the context of lesional LPC infiltration severity of 1. Most cases had moderate to high IgG expression (60% and 80%, 7/47 [15%]), especially in biopsies with LPC inflammation scored as 2 (. In 1 cat with unilateral rhinitis (severe, scored as 3), the IgG expression was 100% (1/47 [2%]); additionally, this cat had no IgA expression.

The majority FLPCR cases were IgE negative (19/30 [63%]). In the positive cases, the expression of IgE was less than 5% of the total plasma cells (9/30 [30%]). The highest IgE expression (range of 5-10%) was observed only in 2 biopsies, both with LPC inflammation scored as 2.

CD3, CD20 and FOXP-3 expression

All the immunohistochemical results are listed in table 4. The expression of CD3 and CD20 was assessed in microscopically normal respiratory nasal mucosal samples obtained from 3 cats and in 21 lesional biopsies (21/47 [45%]) from 17 cats (table 4). In normal tissues T or B cells were not observed. In 2 cases, the number of CD3 positive IELs was mildly increased (2/21 [9%]). In 8 biopsies, increased CD20-positive IELs were present (8/21 [38%]. In 5 cases, CD20-positive lymphocytes were increased in the lamina propria. For all cases examined, plasma cells within the lamina propria were variably CD20 positive.

FOXP3 was subsequently assessed in the same samples where CD20 and CD3 were evaluated and no positive cells were detected.

Statistical analysis

LPC inflammation scores were statistically significantly associated with multiple microscopic lesions. Specifically, the LPC scores correlated positively with infiltration of neutrophils

(correlation coefficient ± SE: 0.39±0.16; p=0.015), gland destruction (0.38 ± 0.16; p=0.019) and angiogenesis (0.39 ± 0.16; p=0.016), whereas a strong, negative correlation with fibrosis was found (-0.55 ± 0.14; p<0.0001).

LPC severity scores were significantly affected by sex (X^2_1=6.66; p=0.01), with female cats showing a higher probability of having a higher lesional score than males (odds ratio: 4.9; 95% CI: 1.5 – 16.3). Concerning immunoglobulin expression, LPC scores were negatively related to IgA (X^2_1=4.63; p=0.03), with higher IgA associated to lower LPC scores (coefficient estimate ± SE: -2.24±1.04). Conversely, IgG scores were not associated with LPC scores (p>0.05).

The number of observed clinical signs was significantly affected by sex (X^2_1=17.7; p<0.0001), by the chronicity class (X^2_2=10.88; p=0.004) and by IgG scores (X^2_1=6.20; p=0.01). Female cats had a higher number of concurrent clinical signs than males (mean ± SE: 4.17±0.54 and 3±0.27, respectively). Cats with a longer FLPCR history (i.e. > 12 months) presented a greater number of clinical signs compared to cats with FLPCR of intermediate duration (i.e. 6-12 months) (mean number of signs ± SE: 3.57±0.41 and 2.87±0.35, respectively; p=0.001), whereas there was no difference in the number of observed signs between cats affected by FLPCR of intermediate and short durations (p>0.05). Additionally, the number of clinical signs significantly increased with mucosal IgG scores (coefficient estimate ± SE: +0.43±0.17).

Follow-up

Follow up information was available for 21 of the 33 cats (64%) and is summarized in Table 4. At the end of the study, 11 cats were alive. Of these, 2 cats had achieved complete remission (lesional severity 2 and 3), and 3 had recurrent rhinitis (lesional severity 1 and 2). Severity and progression of respiratory signs leading to airway obstruction with lack of response to therapy prompted euthanasia in 2 cats. One cat was euthanized 15 days after the diagnosis (lesional

severity was 1), and euthanasia was elected for one cat 12 months after diagnosis (severity 1 and 2 in each nasal cavity). The remaining 6 cats died spontaneously due to causes unrelated to upper respiratory tract diseases.

DISCUSSION

This study is the first to describe normal feline respiratory nasal mucosal immunoglobulin expression and to characterize and correlate clinical, microscopical and immunoglobulin isotype expression in feline lymphoplasmacytic rhinitis (FLPCR). Normal feline nasal respiratory mucosa was preliminarily assessed because descriptions are largely unavailable and was pivotal to estimate and score FLPCR tissue lesions. Normal microscopical aspects paralleled descriptions for other species (Chamanza et al., 2016; Plopper and Adams, 2006) and IgA was the main mucosal immunoglobulin being expressed on ciliated epithelial cell surface similarly to minipigs and humans (Moneret-Vautrin et al., 1991; Yang et al., 2017). On the contrary, IgG on the normal mucosal surface were less represented as described in minipigs (Moneret-Vautrin et al., 1991; Yang et al., 2017) but differing from humans (Moneret-Vautrin et al., 1991; Stiema, 2001) where IgGs are diffusely expressed (Stiema, 2001).

Mucosal surfaces represent physicochemical barriers and immune organs with complex functions aimed at the continuous balance of immune tolerance to harmful antigens and immune defense against pathogens (Smith, Phillip D., Thomas T. MacDonald, 2012). In FLPCR, mucosal ulceration, loss of ciliated columnar cells and epithelial cell hyperplasia were common and contributed to disruption of the mucosal barrier. Also, the consistent reduction of mucous glands in the lamina propria may have hampered barrier functions by reduction of surface mucous coating. This finding differed from previous descriptions (Milner et al., 2004) and may be explained by the patients enrolled in this study that were mostly referral patients

with long history of chronic to recurrent FLPCR. The mucus layer and mucosal barrier preserve resident microflora (Proctor and Relman, 2017) prevent antigen adhesion, and facilitate microbial clearance (López and Martinson, 2017). Microflora prevents infection by occupation of local niches and by production of bacteriocins active against Gram-positive and Gram-negative pathogens (Simons et al., 2020). Thus, dybiosis induced by reduction of mucus and/or prolonged antibiotic therapy in FLPR opens to bacterial infections (Kato et al., 2014; Kuehn, 2006; Lappin et al., 2017) that may be involved in recurrence and progression of FLPR (Kuehn, 2006; Lappin et al., 2017) and may induce autoreactivity by mechanisms such as molecular mimicry (Proal et al., 2017)

Although, viral infections have long been considered major triggers of FLPCR (Kuehn, 2006), in this work serological and immunohistochemical investigations for FIV, FeLV, FCV and FHV resulted negative. Feline herpesvirus may cyclically reactivate and potentially cause recurrent disease and has been implicated in FLPR (Kuehn, 2006). However, in Italy, cats are regularly vaccinated reducing the likelihood of FHV-1 as a potential trigger of FLPCR in this caseload. PCR testing was not performed as no diagnostic microscopical findings (intranuclear inclusion bodies) nor viral FHV-1 protein expression were observed. Noteworthy, false positive PCR results have been reported in cats with corneal and skin disease (Persico et al., 2011; Stiles and Pogranichniy, 2008) underlying that PCR FHV-1 positivity must be interpreted in the context of clinical presentation, microscopical lesions and IHC positivity.

A prevalent infiltration of mature plasma cells and lesser small mature B cells was characteristic of FLPR paralleling previous descriptions (Johnson et al., 2004). In this work, LPC severity scores correlated significantly with mucous gland loss, neutrophil infiltration, and angiogenesis while a negative correlation was identified with fibrosis, suggesting that tissue repair represents the end stage of the disease with regression of signs (nasal discharge,

sneezing) and of inflammation likely due to loss of function. B cells and plasma cells in FLPR lesions expressed CD20. CD20 is a target of several therapeutic monoclonal antibodies used for the treatment of human (Atallah-Yunes et al., 2020; Cortelazzo et al., 2020; Hou et al., 2020; Lue and O'Connor, 2020) and canine (Jain et al., 2016; Mizuno et al., 2020) B cell lymphomas. More interestingly, anti-CD20 therapeutic antibodies are currently applied to the control of human chronic inflammatory disorders mediated by B cells (Afzali et al., 2020; Florou et al., 2020; Terrier et al., 2020). Thus, CD20 may represent a target molecule for the therapy of recurrent and severe cases of FLPR and other chronic lymphoplasmacytic disorders of cats. Unfortunately, at present, no specific anti-CD20 therapeutic antibodies seem to be registered for the cat.

Regarding immunoglobin expression, LPC severity scores correlated positively with reduction of mucosal IgA expression and negatively with gland loss. This finding gains additional significance when considering that serum levels of IgA and IgM are reported to increase in older cats (Campbell et al., 2004). Thus, reduction of mucosal IgA was deemed as a specific finding. In normal conditions, IgAs are produced by plasma cells surrounding mucosal glands in response to commensal bacteria (Van Der Waaij et al., 1996), are transported by mucus (Ali et al., 2002; Fahrbach et al., 2013) to the mucosal surface and preclude bacterial adhesion and clumping by a mechanism termed enchained growth (Moor et al., 2017). Because the main advantage of IgA is to prevent infections without inducing a specific inflammatory response, the significant association between higher IgA and lower LPC scores in cats with FLPCR further supports the existence of this protective mechanism in nasal respiratory mucosa of cats. IgG expression correlated with increased number of clinical sings and with their prolongation over 12 months but not with severity of inflammation. This result leads to the hypothesis that IgG may not have a direct role in FLPCR pathogenesis,

and their increased expression may be triggered by as an example, opportunistic bacterial colonization.

In humans, chronic rhinitis is largely caused by IgE-mediated reactions to inhaled allergens (Bousquet et al., 2020). However, this mechanism seemed not to play a major role in cats enrolled in this study, as IgE expression and mast cells were largely absent. As no viral nor bacterial pathogens were identified in chronic cases, other mechanisms need to be considered for feline LPCR. Favored by failure of local immune defense and tolerance mechanisms, viral, bacterial and/or environmental antigens may facilitates the emergence of autoreactive T-cells driven by mechanism such as epitope spreading similarly to what has been documented in humans (Burastero, 2006).

A last consideration regards signalment of cats with FLPR. Neutered males were overrepresented in this caseload paralleling previous reports (Michiels et al., 2003) however, spayed females developed a significantly higher number of clinical signs and higher LPC scores. As a parallel, bilateral turbinate destruction and abnormal repair have been previously described as more common in spayed cats (Michiels et al., 2003). Differences in nasal mucosa immune responses among sexes have been reported in humans (McClelland and Smith, 2011), rats (De Azevedo et al., 1997) and, mice (Aguilar-Pimentel et al., 2020) and variations in estrogen/testosterone ratios have been linked to changes in B-cell function and immunoglobulin levels in mice (Aguilar-Pimentel et al., 2020) with higher lesional profiles in females.

The negative role of gonadectomy in immune responses has been described in dogs (Sundburg et al., 2016), rats (Azad et al., 1998), and mice (Penhale and Ahmed, 1981). In rats, reduction of estrogen levels following ovariectomy has been linked to reduced hydrogen

peroxide and phagocytosis in macrophages in females (De Azevedo et al., 1997). In women, onset of premature menopause has been linked to increased severity and prevalence of autoimmune diseases (Sammaritano, 2012). Moreover, a significant worst clinical profile for rhinitis and asthma has been identified in 50 to 64 year old Swedish women when compared to men of the same age and/or younger women further supporting the protective role of estrogens in younger women (Larsson et al., 2007). Thus, a higher severity of rhinitis in neutered female cats might derive from loss of the protective anti-inflammatory effects of estrogens, similarly to what occurs for women in menopause (Larsson et al., 2007; Vegeto et al., 2008). Therefore, female neutered status and old age should be considered as a predisposing factor for FLPCR development.

CONCLUSIONS

According to our findings, FLPCR is a chronic progressive/relapsing disease with a multifactorial pathogenesis comprising predisposition for spayed cats and characterized by disruption of mucosal barrier, reduction of surface IgA coating, plasmacytic inflammation, progressive fibrosis and loss of mucous glands. A sequence of events leading to FLPCR may involve an initial ill-defined insult, likely associated with a viral infection, to the mucosal respiratory epithelium that activates cycles of inflammation, tissue damage and repair with chronicity and recurrences possibly associated to emergence of autoreactive mechanisms and leading to scarring and loss of function.

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Table 1. Rhinitis scoring system

	Rhinoscopy grading system (Johnson et al., 2004)			Proposed histological grading system			
	Mild	Moderate	Severe	Absent Score 0	Mild Score 1	Moderate Score 2	Severe Score 3
Hyperemia	Absent	Moderate	Severe	Not evaluated	Not evaluated	Not evaluated	Not evaluated
Mucus accumulation	No or minimal	Patchy to confluent	Abundant	Not evaluated	Not evaluated	Not evaluated	Not evaluated
Turbinate (bone) remodeling	Mildly increased space between turbinate	Moderate increased space between turbinate	Severe increased space between turbinate	Absent	<10%	10-25%	25%-50%
Lymphoplasmacytic infiltration	Not evaluated			Absent	<10%	10-25%	25-50%
Eosinophils	Not evaluated			Absent	<10 HPF	10-20 HPF	> 20 HPF

Mast cells	Not evaluated	Absent	<10 HPF	10-20 HPF	> 20 HPF
Neutrophils	Not evaluated	Absent	<10 HPF	10-20 HPF	> 20 HPF
Intraepithelial lymphocytes	Not evaluated	Absent	<5 HPF	5-10 HPF	> 10 HPF
Mucosal ulceration	Not evaluated	Absent	Minimal mucosal defects (basal membrane preserved) flat edge	Moderate mucosal defects (large erosion, basal membrane preserved, fibrin and exudate-covered)	Severe mucosal defect (deep ulcer, basal membrane damaged) with a slightly raised edge
Mucosal hyperplasia	Not evaluated	Absent	<10%	10-25%	25%-50%
Glandular damage or destruction	Not evaluated	Absent	<10%	10-25%	25%-50%

	Not evaluated	Absent	< 5 vessels/HPF	5-10 vessels/HPF	>10 vessels/HPF
Angiogenesis	Not evaluated	Absent	< 5 vessels/HPF	5-10 vessels/HPF	>10 vessels/HPF
Fibroplasia/Fibrosis	Not evaluated	Absent	<10%	10-25%	25%-50%

Table 2. Panel of Primary Antibodies utilized for the characterization of FLPCR.

Antibody	Clone/Species Specificity	Dilution	Retrieval	Source	House
CD3-ε †	CD3-12 Human†	1:10	Heat[#]	Rabbit polyclonal	Serotec, Oxford, UK
CD20 †	Human†	1:40	Heat[#]	Rabbit polyclonal	Neomarkers, Freemont, CA, U.S.A.
FOXP3	Feline	1:100	Heat	Rat monoclonal	Invitrogen, Thermo Fisher Scientific, U.S.A.
IgA	Feline	1:300	Trypsin*	Goat polyclonal	Biosciences Pharmingen, San Diego, CA, U.S.A.
IgE	Feline	1:10	Trypsin*	Mouse monoclonal	Santa Cruz Biotechnology Inc. Dallas, TX, U.S. A.
IgG	Feline	1:200	Trypsin*	Goat polyclonal	Custom Monoclonals Int., Sacramento, CA, U.S.A.

FCV	αFCVS19 Feline	1:250	No retrieval	Mouse Monoclonal	Custom Monoclonals Int., Sacramento, CA, U.S.A.
FCV	FCV1-43 Feline	1.250	No retrieval	Mouse Monoclonal	Santa Cruz Biotechnology Inc. Dallas, TX, U.S. A.
FeLV gp70/85	C11D8 Feline	1:100	Heat[#]	Mouse Monoclonal	Novus Biologicals, CO, U.S.A.
FeLV p27 core	PF12J-10A Feline	1:100	Heat[#]	Mouse Monoclonal	Custom Monoclonals Int., Sacramento, CA, U.S.A.
FHV-1	FHV-5 Feline	1:250	No retrieval	Mouse Monoclonal	Custom Monoclonals Int., Sacramento, CA, U.S.A.
FIV-gp120	SU1-30 Feline	1:250	Heat[#]	Mouse Monoclonal	Santa Cruz Biotechnology Inc. Dallas, TX, U.S. A.

* enzymatic retrieval was achieved by incubating sections with 0,05% Trypsin (Sigma chemical Co., Darmstadt, Germany) in phosphate-buffered saline solution at pH 7.0 in a humid chamber at 37°C for 15 minutes

[#] heat induced antigen retrieval was performed by incubation of hydrated and quenched tissue sections in 10-mM citrate buffer, pH6 (DAKO, Carpinteria, CA) by heating in a microwave oven at maximum power (1000 Watts) for 1 minute and at 750 W for 3 minutes for two times, and cooled at room temperature for 20 minutes. For FOXP3 antigen retrieval was

performed by incubation in citrate buffer pH 6.0 and heating for 30 min in a microwave oven at 750 W, and cooled at room temperature for 20 minutes.

Table 3: Signalment, FIV and FeLV serological status and major clinical complaints in cats with FLPCR

No#	Nasal Cavity	Sex	Age(years)	Breed	Serology	S	RS	SD	MD	OD	E	SS	D	ST	C
1	bilateral	M	17	DSH	NA	+	-	+	-	-	-	+	-	-	-
2	unilateral	FS	11	DSH	-	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
3	unilateral	FS	2	DSH	-	+	+	-	+	-	+	-	-	+	+
4	unilateral	MN	2	DSH	NA	+	-	-	-	-	+	-	-	-	-
5	unilateral	M	8	DSH	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
6	unilateral	MN	12	DSH	NA	+	-	-	+	-	-	-	-	-	-
7	unilateral	MN	15	Maine Coon	NA	+	-	-	-	+	-	-	-	+	-
8	unilateral	MN	17	Siamese	-	+	+	-	+	-	-	-	-	-	-
9	bilateral	FS	12	DSH	-	-	-	+	+	-	-	-	-	+	+
10	unilateral	MN	19	Persian	NA	-	-	-	+	-	-	-	+	-	-
11	bilateral	M	7,5	DSH	FIV+ FeLV +	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

12	unilateral	MN	11,5	DSH	NA	-	-	-	-	-	-	+	-	-	-
13	unilateral	M	12	DSH	FIV+	+	-	+	+	+	+	+	-	-	-
14	bilateral	MN	7	DSH	-	+	+	-	-	-	-	-	-	-	-
15	unilateral	M	5	NA	-	+	-	-	+	-	-	-	-	-	-
16	bilateral	M	11	DSH	FIV+	+	-	+	-	-	-	-	-	-	+
17	bilateral	M	4,5	Norwegian Forest	-	-	-	+	+	-	-	-	-	+	-
18	unilateral	MN	14,5	DSH	NA	+	-	-	-	-	+	-	-	-	-
19	unilateral	MN	6	DSH	-	+	-	-	+	-	-	-	-	-	-
20	bilateral	MN	15	DSH	-	+	+	-	+	+	+	-	-	+	-
21	unilateral	F	6	DSH	-	+	+	-	-	-	-	-	+	+	+
22	bilateral	MN	14	DSH	-	+	-	-	-	+	-	-	-	+	-
23	unilateral	NA	NA	DSH	-	+	-	+	-	+	+	-	+	+	-
24	unilateral	MN	9	DSH	-	-	-	-	-	-	-	-	-	+	-
25	bilateral	FS	12	DSH	-	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
26	unilateral	FS	2	DSH	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
26	unilateral	FS	2	DSH	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
27	bilateral	FS	4	DSH	-	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

28	bilateral	MN	6	Persian	-	+	-	-	+	+	+	-	-	-	-
29	bilateral	MN	10	DSH	-	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
30	unilateral	MN	11	DSH	-	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
31	bilateral	MN	14	DSH	-	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
32	unilateral	F	13	DSH	NA	+	-	+	+	-	-	-	-	+	-
33	unilateral	F	0,5	NA	NA	+	-	-	-	+	-	-	-	-	-

M= male; MN= male neutered; F= female; FS= female spayed; DSH= Domestic Short Hair; NA= Not available; S= sneeze; RS= reverse sneeze; SD= serous discharge; MD= mucous discharge; OD= ocular discharge; E=epistaxis; SS=sinus swelling; D=dyspnea; ST= stertor; C= cough

Table 4: severity, microscopic findings and scores, immunoglobulin, CD3 (T cells) and CD20 (B cells) expression and follow up in cases of FLPCR

	Microscopic Lesional Score		Immunoglobulin isotype	CD expression	Follow up

No#	Nasal Cavity	MU	MH	IELs	LPCS	N	E	M	GD	A	F	BR	IgA	IgG	IgE	CD3	CD20	Time Days/years	Clinical signs/Cause of death
1	R	0	1	1	1	1	0	0	1	1	1	1	80%	20%	0%	-NA	+NA	4 years	Alive, recurrent rhinitis
	L	2	1	0	2	2	0	0	2	2	1	1	10%	90%	0%				
2	R	3	0	0	3	3	0	0	1	3	0	0	60%	40%	NA	NA	NA	4 years	Alive
3	R	1	1	0	2	2	0	0	2	1	0	2	55%	40%	0-5%	NA	NA	5 years	Alive, recurrent rhinitis,

4	L	1	0	0	1	2	0	1	0	0	1	NA	10%	90%	0%	NA	NA	5 years	Alive
5	R	3	0	0	3	1	0	0	3	2	0	NA	10%	90%	NA	NA	NA	NA	NA
6	L	1	0	1	1	1	0	0	1	1	1	0	30%	70%	0%	-	-	NA	NA
7	R	0	0	0	1	0	0	0	0	0	1	0	10%	0%	0%	NA	NA	5 years	euthanized for chronic renal disease
8	L	2	0	1	2	1	1	0	1	1	0	NA	15%	80%	0-5%	-	+	5 years	Alive, FANS

daily therapy

monthly

therapy

		c1	c2	c3	c4	c5	c6	c7	c8	c9	c10	c11							
9	R	NA	1	2	2	0	0	0	1	1	1	1	30%	70%	NA	-	+	5 years	Alive
	L	1	0	1	2	2	0	0	1	0	1	1	50%	50%	NA	+	NA		
10	L	2	1	0	1	0	0	1	0	2	3	2	10%	0%	NA	NA	NA	1.5 years	Deceased, not related causes
11	R	1	0	0	1	2	0	0	1	0	0	1	20%	0-5%	NA	NA	NA	NA	NA
	L	2	1	0	1	1	0	0	2	0	2	NA	10%	NA	NA	NA	NA		

12	R	0	2	1	1	0	0	0	1	1	1	2	50%	50%	0%	NA	NA	15 days	euthanized due nonresponsive rhinitis
13	R	0	0	0	1	0	0	0	1	1	1	1	30%	70%	0%	NA	NA	NA	NA
14	R	1	1	1	3	0	0	0	0	1	0	NA	0%	50%	0-5%	+	-	NA	Deceased, not related causes (intestinal and renal tumor)
	L	0	0	0	1	0	1	0	0	1	1	0	10%	90%	0%	NA	NA		
15	R	2	1	2	2	1	0	1	1	3	1	1	40%	60%	0%	-	+	6 years	Alive, recurrent rhinitis

		1	2	3	4	5	6	7	8	9	10	11							
1 6	L	0	2	1	1	0	0	0	0	0	3	0	90%	10%	NA	-	+	1 year	euthanized due to rhinitis
	R	1	1	1	2	1	0	0	1	0	1	2	40%	60%	NA	-	+		
1 7	L	NA	NA	0	1	1	0	0	1	2	0	NA	40%	60%	0%	NA	NA	6 years	alive
	R	0	0	0	3	1	1	0	3	0	0	1	20%	80%	0%	NA	NA		
1 8	L	0	0	1	1	0	0	0	0	0	2	1	50%	50%	0%	-	-	NA	NA
1 9	R	0	1	0	2	0	0	0	1	0	1	2	5%	0%	NA	NA	NA	NA	NA

ID	Side	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11							Status
20	R	1	1	0	2	2	0	0	0	0	0	1	40%	60%	0%	NA	-	N	Deceased, not related causes
20	L	NA	1	1	1	NA	NA	0	NA	NA	1	NA	10%	80%	NA	-	-	A	
21	L	NA	0	0	1	0	0	0	3	0	0	1	30%	70%	0%	NA	NA	NA	Deceased, not related causes
22	R	1	0	1	3	2	1	0	3	0	1	2	40%	60%	0%	-NA	-	N	Euthanized, not related causes
22	L	0	1	0	1	0	0	0	1	0	0	1	60%	40%	0%		NA	A	
23	L	1	1	1	2	2	0	0	NA	1	1	2	0%	20%	NA	NA	NA	NA	NA

ID	Side																			Outcome
24	R	0	1	0	2	1	0	0	0	1	0	0	40%	60%	NA	NA	NA	NA	
25	R	1	2	0	3	2	0	1	1	1	0	1	40%	60%	NA	NA	NA	NA	Dead, not related causes (neoplasia) 5 years
25	L	0	0	1	3	3	0	0	0	2	0	0	70%	30%	NA	NA	NA		
26	L	1	1	1	2	1	1	0	0	0	0	2	80%	15%	0-5%	-NA	+	NA	NA
26	R	1	1	0	2	2	1	1	0	1	0	1	20%	75%	0-5%	NA	NA		
27	R	1	1	0	3	2	3	0	1	1	2	0	0%	100%	0%	NA	NA		Alive, recurrent rhinitis 7 years
27	L	0	1	0	2	2	2	0	1	0	2	0	90%	90%	NA	NA	NA		

												0%			NA				(sneezing and ocular discharge)
28	R	0	1	0	3	0	0	0	NA	2	0	NA	10%	90%	0%	NA	NA	7 years	Alive, complete healing
28	L	0	1	0	3	0	0	0	3	1	0	3	20%	75%	0-5%	NA	NA		
29	R	1	1	1	1	1	0	0	1	1	1	2	55%	40%	0-5%	-	+	NA	NA
29	L	1	1	1	2	0	0	1	3	1	1	2	20%	70%	5-10%	-	-		
30	L	0	0	NA	3	0	0	0	3	2	0	0	0%	5%	NA	NA	NA		NA

ID	Side	EU	MH	IELs	LPCS	N	E	M	GD	A	F	BR					Survival	Outcome
31	R	1	1	1	2	0	0	0	3	0	2	2	200%	75%	0-5%	NA	NA	Dead, not related causes
	L	0	1	1	2	0	0	0	0	1	0	1	100%	80%	10%	-		
32	R	1	1	0	3	1	0	0	NA	NA	0	NA	5%	30%	0%	NA	NA	NA
33	R	0	1	2	2	1	0	1	2	1	0	2	10%	10%	NA	-	11 years	Alive, complete healing

EU= erosions and ulcers; MH= mucosal hyperplasia; IELs= intraepithelial lymphocytes; LPC S= lymphoplasmacytic infiltrate/severity; N= neutrophils; E= eosinophils; M= mast cells; GD= glandular destruction; A= angiogenesis; F= fibrosis; BR= bone remodeling; NA= Not available
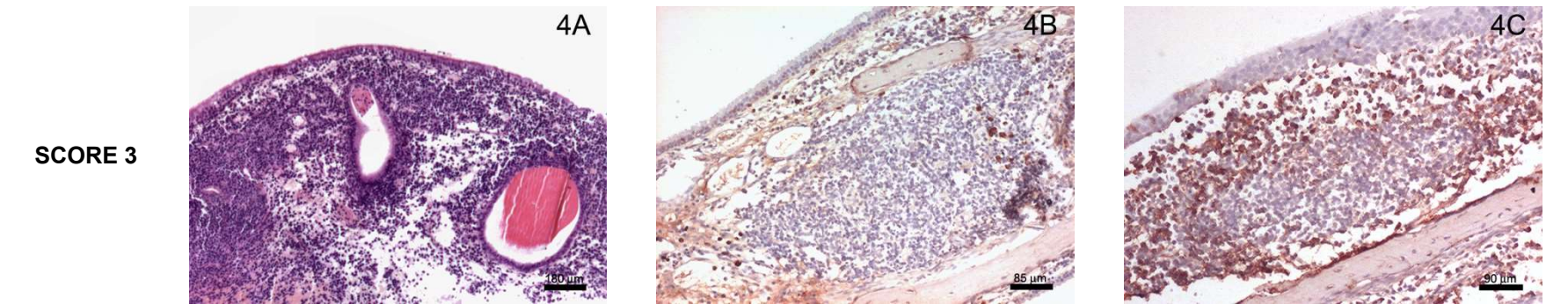
Figures

Hematoxylin and Eosin	IgA Expression	IgG Expression

SCORE 3

4A   4B   4C
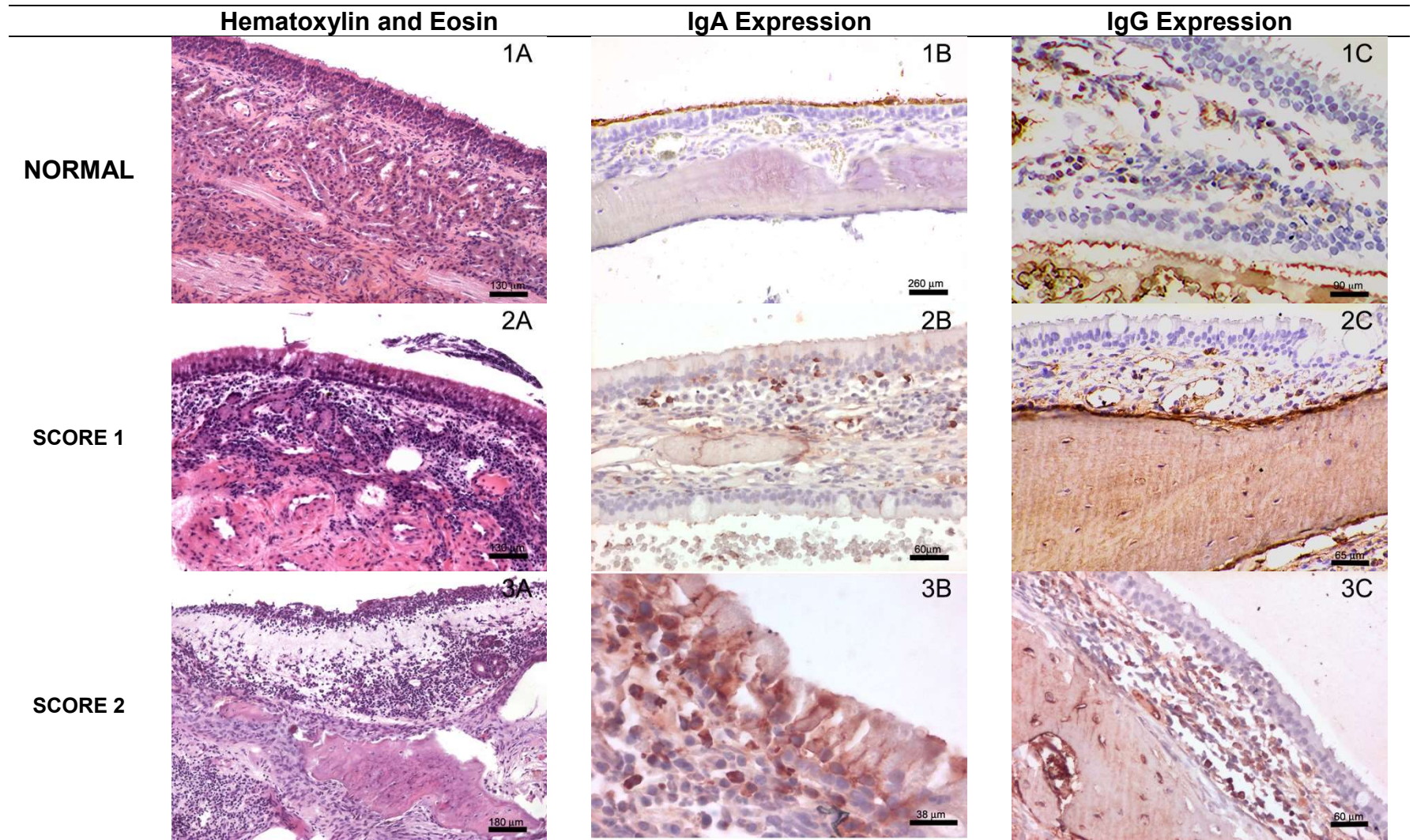
Figure Legends:

Fig.1 A. Normal feline respiratory nasal mucosa lined by columnar ciliated epithelium (arrowhead). Abundant seromucous glands in the lamina propria (arrow). Hematoxylin and eosin. Bar=130 microns

Fig 1B. Normal feline respiratory nasal mucosa. Intense apical IgA positivity of mucosal epithelial cells (arrowheads). AEC chromogen, hematoxylin counterstain. BAR= 260 microns

Fig. 1C. Normal feline respiratory nasal mucosa. Apical IgG multifocal and variable positivity of mucosal epithelial cells. Very rare IgG positive plasmacells (arrowheads). AEC chromogen, hematoxylin counterstain. BAR= 90 microns

Fig. 2A. FLPCR characterized by infiltration of the lamina propria by mature plasmacells (arrow) and small mature lymphocytes (arrowhead) with a severity score 1. Hematoxylin and eosin. Bar=130 microns

Fig. 2B. FLPCR with a severity score 1 characterized by IgA positive plasmacells in the lamina propria (arrowheads). Reduction of IgA expression at luminal surface. AEC chromogen, hematoxylin counterstain. BAR= 60 microns

Fig. 2C. FLPCR characterized by rare IgG positive plasmacells (arrowheads) in the lamina propria. Vascular lumens chow IgG positive serum. AEC chromogen, hematoxylin counterstain. BAR= 65 microns

Fig. 3A. FLPCR characterized by an inflammatory severity score 2. Submucosal fibrosis (asterisk) and reduction of sero-mucous glands is evident. Inflammation can be observed lining both sides of bone (arroheads). The bone is fractured and dissected by fibroplasia (arrow). Hematoxylin and eosin. Bar=180 microns.

Fig. 3B. FLPCR with a severity score 2 characterized by variable, intense IgA epithelial cell membrane expression and IgA positive plasma cells (arrowheads) in the lamina propria. Hematoxylin and eosin. Bar=38 microns.

Fig. 3C. FLPCR with a severity score 2 characterized by numerous IgG positive plasmacells (arrowheads) in the lamina propria. AEC chromogen, hematoxylin counterstain. BAR= 60 microns

Fig. 4A. FLPCR characterized by an inflammatory severity score 3. Severe diffuse infiltration by plasma cell and mature lymphocytes with expansion of the lamina propria. Severe reduction of mucous glands and dilation and stasis of seromucous material in two residual gland lumens (arrows). Bar=180 microns.

Fig. 4B. FLPCR with a severity score 4 characterized by very rare IgA positive plasmacells (arrowheads) in the lamina propria. A large nodular pseudofollicular lymphoid aggregate expanding the lamina propria is evident (arrow). AEC chromogen, hematoxylin counterstain. Bar=85 microns.

Fig. 4C. FLPCR with a severity score 4 characterized by numerous IgG positive plasmacells (arrowheads) in the lamina propria. A large nodular pseudofollicular lymphoid aggregate expanding the lamina propria is evident (arrow). AEC chromogen, hematoxylin counterstain. BAR= 90 microns.