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## Investigation of Serum allergen-specific IgE concentrations in feline asthma: a clinical experience

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**ABSTRACT:** The aim of this study is to investigate allergen agents by allergen-specific serum IgE analysis in domestic cats diagnosed with feline asthma (FA). A total of 21 cats (16 FA and 5 control cats) were included in the study. Allergen-specific serum IgE levels were measured in cats diagnosed with feline asthma and in the control group. Allergen levels were classified as negative, weak, moderate and strong. The difference between the two groups was compared statistically. The most common clinical manifestations in cats with FA were cough, sneezing, wheezing, and acute dyspnea, respectively. Allergen-specific IgE levels tested for 20 different allergens showed that 14 out of 16 cats developed a reaction to more than one allergen. The vast majority of cats with FA had a moderate or strong positive reaction to house dust mites. In conclusion, FA is an important and common chronic lower respiratory tract disease of feline patients and allergen-specific serum IgE levels can be easily used in clinical practice to guide the selection of allergens in the treatment of this disease and adjust for the quality of life and well-being of cats.

**Keywords:** Allergy; Cat; Feline asthma; HDMA; IgE

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## INTRODUCTION

Feline asthma (FA) is a common lower respiratory tract disease with cough, dyspnea and, in advanced cases, wheezing or labor breathing. It is originated by aeroallergens and driven by the stimulation of T helper 2 (TH2) response which produce Immunoglobulin E (IgE) antibodies (Reinero, 2011; Trzil, 2020). Although the exact etiology is unknown, FA is defined as a type I hypersensitivity reaction mediated by IgE antibodies that may lead to many changes in the long term such as airway inflammation and bronchoconstriction (Norris et al, 2003; Grotheer et al., 2020). After the aeroallergens are inhaled, IgE molecules bind to a common antigen particle, attach to the surface of mast cells resulting to degranulation as well as release of histamine and leukotrienes that promote vascular permeability and contraction of smooth muscle (Norris et al., 2003; Venema et al., 2010).

Previous studies indicated that the incidence rate of FA is considerably high in general feline population, varying between 1 to 5% (Trzil and Reinero, 2014). A similar prevalence has been reported in humans (National Center for Health Statistics, 2020). According to the Global Burden of Disease, Injuries and Risk Factors Study, approximately 2,60 million people worldwide have asthma (Vos et al., 2020). Considering the common living space and environmental factors shared by humans and pets, as well as the same etiopathogenesis of FA investigating the etiology of this increasing trend seen in both species is very valuable (Reinero et al., 2009). Although there is ample evidence that aeroallergens cause a human asthma-like hypersensitivity reaction in FA, there are very limited studies focusing on the allergen-induced naturally occurring asthma in cats (Reinero et al., 2009).

The hypothesis of the current study was that commonly identified agents that could potentially act as allergens affect FA. Therefore, it has been suggested to identify possible allergic agents in cats with FA using allergen specific serum IgE test kits.

## MATERIALS AND METHODS

This retrospective study consisted of client-owned cats presented to the Ankara University Veterinary Faculty Clinic of Small Animal Medicine and in a private veterinary clinic in Ankara between February 2018 and December 2021. According to the medical records, 16 cats diagnosed with allergic asthma and

5 healthy cats presented for routine wellness examination were included in the study as a control group. The age, body weight, gender, and sterilization status, breed, living environment, diet, vaccinations, and anti-parasitic treatments and fecal examination results, and all the recorded medical data of the cats were reviewed. Clinical examinations and allergen-specific IgE tests were performed with a written owner consent form (Ankara University Animal Experiments Local Ethics Committee Decision number: 2021-22-200).

### Inclusion & exclusion criteria:

The medical records of cats with history of sneezing, coughing/wheezing, and/or dyspnea were evaluated. Of these patients, only the cats with eosinophils >20% in the bronchoalveolar lavage fluid (BALF) cytology and with an allergy test result were included in the study (Gortheer et al., 2020). The serum samples for allergy tests were collected before the bronchoscopy procedure in the suspected cases and serum allergen-specific IgE tests were performed with the consent of owners to determine the possible underlying etiology of the disease.

Patients with concurrent cardiovascular disease and/or suspected primary or secondary neoplasia based on their laboratory and imaging findings and history of dermatological disease were excluded. In all cats included in the study, allergy testing and bronchoscopy were scheduled after the patient's first visit to the clinic and were performed at least two weeks after discontinuation of any medication, including steroid therapy (Chang et al., 2011). The inclusion criteria of the cats also included regular administration of the vaccinations and antiparasitic treatments as well as normal fecal examination results.

### Diagnostic approach:

Clinical examinations were performed in each case and two later thoracic radiographs were taken at the first presentation to the clinic. The cats suspected to have asthma underwent bronchoscopy and multiple site bronchoalveolar lavage was performed in a standardized way as described previously (Johnson and Drazenovich, 2007; Ybarra et al., 2012). Briefly, the cats were administered with midazolam (0.1 mg/kg) and propofol (4 mg/kg slow infusion and CRI). Pre-oxygenation of the patients was provided before the procedure with various medications and oxygen was supplied by jet ventilation during the procedure. Bronchoscopy was performed with a 2.5- or 4-mm

flexible endoscope (Eickemeyer, Germany). In most cases, 3–5 mL of warm sterile saline (total of 10–15 mL) of individual volume per site of the lungs was used and the fluid was sucked with a syringe. Location, instilled fluid volume and volume recovered were recorded. To perform BALF cytology, the sample was stored in ice, centrifuged within an hour at 1000 rpm for 5 minutes and the sediment was stained with modified Wright's stain. At least 100 cells were counted from each slide under the microscope. The samples were also sent to the laboratory for culture and only the cats with negative BALF culture were included to the study group.

#### Allergy tests:

Polycheck Feline Allergy Test (Polycheck, Allergy test, GmbH, Germany; distributor RDA Group, Istanbul) in vitro test which detects allergen-specific IgE in serum was used to detect underlying etiology as described previously (Ural et al., 2018). This test is based on an immunoassay principle, the relevant allergens are coated separately in lines as well as the calibrators on a carrier which is fixed in the well of the test cassette. Available number of precipitate is linked to the specific IgE levels found in the present serum, which result in a significant/no significant coloring of 20 relevant and different individual allergens (listed in Table 1). To calculate the results, a scanner was used. According to the IgE concentrations (kU/l), it was classified in four levels; < 0.5 Level zero (nega-

tive); 0.5–2.0 Level 1 (Weak), 2.0–20 Level 2 (moderate), and >20 Level 3 (strong).

#### Statistical Analysis:

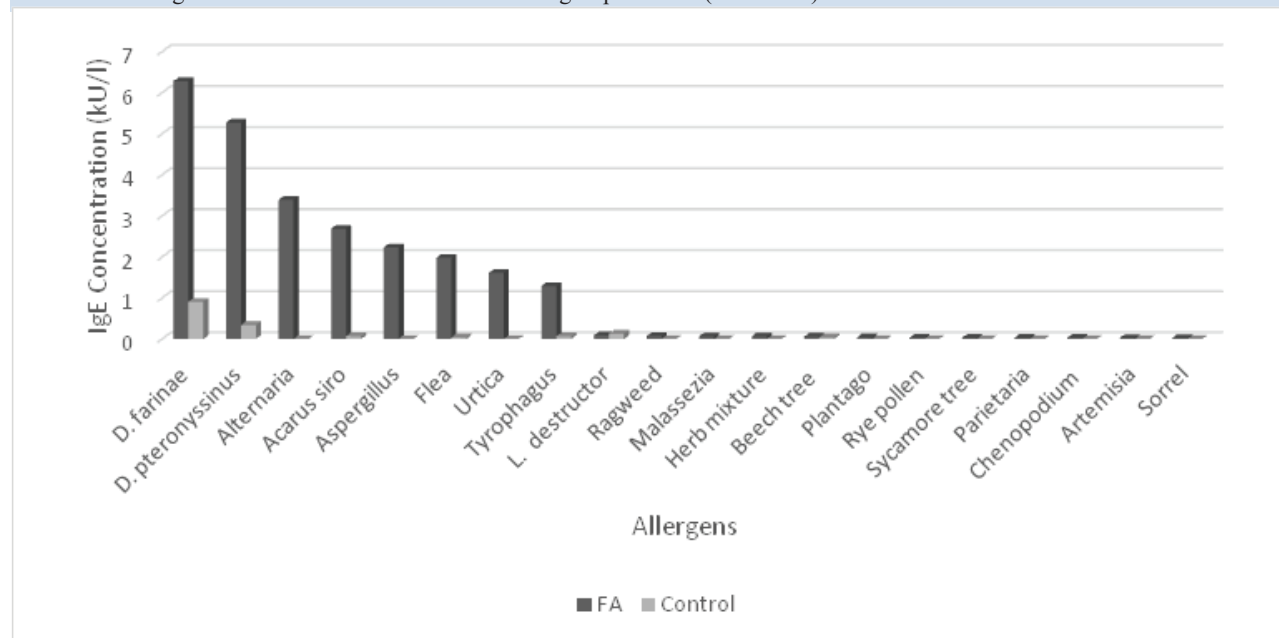
Statistical analysis was performed using SPSS v.18 Chicago: SPSS Inc. To analyze any differences between the FA and the control groups, Mann Whitney U test was used and the significance was defined as  $p \leq 0.05$ .

## RESULTS

#### Demographic data:

Of the 42 cats diagnosed with FA between February 2018 and December 2021, 16 were included in the present study. The mean age of the cats with FA was  $3.9 \pm 2.4$  years ranged from 6 months to 10-year-old. The mean body weight was  $3.8 \text{ kg} \pm 0.97$  ranged from 1.5 to 6 kg. Of the 16 cats, 7 were female (2 intact and 5 spayed) and 9 were male (7 intact and 2 neutered). While 14 of the cats were domestic short-haired, 2 were Himalayan and 1 was a British shorthair. All the cats were living indoor, fed with commercial food and all the vaccinations and the anti-parasitic treatments were completed routinely. The cats did not have any known previous diseases. All the cats included in this study had a history of symptoms of respiratory system disease that persisted for weeks or even months including recurrent episodes. The most common clinical presentations were coughing (93.75%), sneezing (87.5%), wheezing (81.25%) and acute dyspnea (75%).

**Table 1.** Serum IgE concentrations in the FA and control groups in kU/l (mean  $\pm$ std).



The mean age of the cats in the control group was  $3.4 \pm 2.2$  years ranged between 8 months to 8 years-old ( $p > 0.05$ ). The mean body weight was  $3.96 \pm 1.07$  ranging between 2.4 to 5.5 kg ( $p > 0.05$ ). Of the 5 cats, 4 were female (3 intact, 1 spayed) and 1 was an intact male. Control group cats fed with commercial food were indoor, had completed vaccination and antiparasitic treatments and healthy without any history of disease.

### Thoracic imaging and bronchoscopy findings:

While bronchial pattern was determined in 15 out of 16 cats in the FA group, it was recognized that a smaller number of cats had alveolar pattern (5 out of 16). Of 16 cats, one cat showed a normal x-ray. We did not determine any abnormalities related to heart disease in the cats. The control group cats did not have any abnormalities in the auscultation and we did not determine any pathologies related to lung or heart disease in these cats. The most common findings of the bronchoscopy in cats with FA was bronchial mucus accumulation ( $n=14/16$ ), collapse in the airways ( $n=13/16$ ), hyperemia and irregularity in the epithelium ( $n=12/16$ ) and bronchiectasis ( $n=4/16$ ).

### Allergen specific IgE levels:

In the study, the allergen specific IgE levels were tested for 20 different allergens indicating a moderate

or strong positive reaction against to two most common house dust mites (HDMAs), *Dermatophagoides farinae* (*D. farinae*) and *Dermatophagoides pteronyssinus* (*D. pteronyssinus*). We have found a statistically significant difference in the IgE values between the FA and control groups for *D. farinae* ( $p < 0.01$ ) and *D. pteronyssinus* ( $p = 0.05$ ). While the FA cats had moderate or strong positive reaction to fungi [*Alternaria/Cladosporium* ( $n=5$ ) and *Aspergillus/Penicillium* ( $n=4$ )], and to *Acarus siro* ( $n=4$ )], there was not a significant difference ( $p > 0.05$ ) between the FA and control groups against these allergens (Table 1).

Of 16, 14 cats developed a reaction against multiple allergens (Table 2). However, any of the cats did not show a reaction against sycamore tree, *Parietaria*, rye pollen, *Chenopodium*, *Artemisia*, and sorrel.

In the control group, the allergen-specific IgE levels were also tested and only 1 cat showed a weak positive reaction to HDMAs. The other 4 cats did not show any reaction to any of the tested agents.

### DISCUSSION

FA is similar to human asthma in many ways. This similarity gives rise to the idea that cats are a model for human asthma. In this direction, it becomes important to evaluate environmental factors, as well as to study physiological and /or pathological changes

**Table 2.** IgE concentration levels in the cats in the Feline asthma (FA) and control (C) groups.  $< 0.5$  Level zero (negative);  $0.5-2.0$  Level 1 (Weak),  $2.0-20$  Level 2 (moderate), and  $> 20$  Level 3 (strong). The allergens to which no cats reacted are not included in the table. *D. farinae*: *Dermatophagoides farinae*; *D. pteronyssinus*: *Dermatophagoides pteronyssinus*; *L. destructor*: *Lepidoglyphus destructor*.

	D. farinae	D. pteronyssinus	Alternaria	Acarus siro	Aspergillus	Flea	Urtica	Tyrophagus	L. destructor	Ragweed	Malassezia	Herb mixture	Beech Tree	Plantago
FA-1	2	0	0	0	0	0	0	0	0	0	0	0	0	0
FA-2	1	0	0	0	1	0	0	0	0	0	0	0	0	0
FA-3	2	2	0	3	0	0	3	1	1	0	0	1	0	1
FA-4	1	1	3	0	1	3	0	0	0	1	1	0	1	0
FA-5	1	2	3	0	2	0	0	0	0	0	0	0	0	0
FA-6	2	1	2	0	2	0	0	0	0	0	0	0	0	0
FA-7	2	0	0	2	0	0	0	0	0	0	0	0	0	0
FA-8	3	2	0	0	0	0	0	0	0	0	0	0	0	0
FA-9	2	0	1	0	1	0	0	0	0	0	0	0	0	0
FA-10	1	2	0	0	0	0	0	0	0	0	0	0	0	0
FA-11	1	3	0	0	2	0	0	0	0	0	0	0	0	0
FA-12	0	1	0	0		0	0	0	0	0	0	0	0	0
FA-13	1	1	0	0	0	0	1	0	0	0	0	0	0	0
FA-14	3	2	3	2	1	0	0	0	0	0	0	0	0	0
FC-15	2	0	0	0	0	2	0	0	0	0	0	0	0	0
FA-16	2	2	0	2	1	0	0	0	0	0	0	0	0	0
C-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C-2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C-3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C-4	1	1	0	0	0	0	0	0	0	0	0	0	0	0
C-5	0	0	0	0	0	0	0	0	0	0	0	0	0	0

in the process of disease development (Reinero et al., 2009; Reinero, 2014). However, studies showing these allergens and their prevalence in the clinical practice are quite limited. In this study, we sought to understand the allergic etiology of asthma in clinical cases of naturally occurring asthma by measuring IgE levels against specific allergens and that may also shed light to environmental inducers of human asthma disease.

Consistent with previous reports (Adamama-Moraitou et al., 2004; Grotheer et al., 2020), the mean age of onset of symptoms in cats diagnosed with asthma was 3.9-years in the presented study. However, a 6-month-old cat showing severe symptoms of respiratory tract disease was also included in our study. This, again in parallel with previous reports (Reinero and DeClue, 2010; Trzil, 2020), shows that asthma may be presented with more severe and acute symptoms at a much younger age. Therefore, it is important for veterinarians to evaluate asthma as a differential diagnosis in young patients with severe clinical courses.

In contrast to the previous belief in the predisposition of pure-breed and female cats to have FA (Padrid, 2000; Adamama-Moraitou, 2004), no significant breed or gender presentation was determined among the asthma cats in our study. The best plausible explanation for the high numbers of domestic-shorthair cats in the present study is the high prevalence of this breed regionally due to owner preference.

In this study, we did not find a significant difference in the weight of the cats between the study and control groups. In order to reveal the relationship between living conditions and cat asthma several questions were asked to the animal owners within the scope of a questionnaire (Carolino and Duarte, 2020). Within the scope of this study, animal owners reported that their cats were active with ideal weight. Although the average weight of the patients diagnosed with FA was within the normal limits in our study, it was noted in the hospital records that the patient owners noticed weight loss in some of our patients. For this reason, we think that it would be beneficial to conduct more comprehensive studies on the body condition of cats with FA in the long term.

In the diseased group, the most common clinical presentation was coughing. In asthma, variable air-flow obstruction causes symptoms such as wheezing, sneezing, difficulty in breathing, and commonly cough (Niimi, 2011). Cats have the ability to hide

their diseases very well, and these features cause their diseases to be diagnosed when they reach more advanced levels in many cases (Sigrist et al., 2011). Therefore, we realized that the owners of the patient could not fully notice the changes due to respiratory stress but that cough, which is a more obvious symptom, was noticed and the patient was brought for examination. When we went deeper into the patient's story, we also found out that infrequent coughing and/or respiratory stress have actually been existed for a much longer time but the owner did not consider these changes as significant. This is one of the points that clinicians should pay attention to, as the disease may have progressed more than expected. Moreover, if clinical signs of airway hyperactivity are ignored, admissions to the emergency room due to acute severe bronchoconstriction and fatality of the disease may increase (Chalifoux et al., 2021).

Imaging provides very important findings in lower respiratory tract diseases as in many areas. In our study, when chest radiographs were examined in cats with asthma, it was seen that the most common finding was bronchial pattern, which was consistent with other studies (Adamama-Moraitou et al. 2004; Reinero et al. 2011). Mucus accumulation, collapse in the airways, and hyperemic and irregular epithelium findings are thought to be a part of the inflammatory process in the lungs and there are many factors that cause the formation of this response. In more severe cases, chronic bronchoconstriction and excessive mucus production may even lead to bronchial obstruction, as well as infiltration of the airway mucosa by eosinophils. (Lopez and Martinson, 2017).

Allergy to HDMA is a major cause of asthma in humans and pets (Loft and Rosser, 2010; Cao and Liu, 2020). Several studies have investigated the potential allergens in the microenvironments of cats. In a previous study, the sleeping and resting areas of the cats have been shown to contain high levels of *D. farinae* and *D. pteronyssinus* in more than 70% of the houses (Loft and Rosser, 2010). The HDMA can be found in carpets, quilts and couches. For this reason, it may be extremely easy for mites to develop hypersensitivity by entering the respiratory tract of human and pet animals in the home environment (Cao and Liu, 2020). Considering these facts, the significant alterations of the IgE levels against mites between healthy and allergic cats in our study can be explained by continuous exposure in the home environment. Various studies on preventive measures in

human allergies and with regard to HDMA have concluded that avoiding HDMA (HEPA filters, less carpeting, etc.) can significantly improve clinical signs (Götzsche and Johansen, 2008). Therefore, a similar approach should be taken into consideration in pets by warning the pet owners to take preventive actions against mites in the household.

## CONCLUSION

To the best of our knowledge, this is the first study in Turkey on the evaluation of allergic agents in feline asthma. As a result of the study, it was determined that allergens that are thought to have a high impact in the etiology of feline asthma can be detected through in-vitro tests that can be easily applied in

clinical practice. In addition, within the scope of the study, it was revealed that house dust mites are one of the important allergens in feline asthma. During the evaluation of patients, physicians and patient owners should consider the possibility of sensitivity to these allergens, which will help in keeping the disease under control and contribute to the quality of life of cats.

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## CONFLICT OF INTEREST STATEMENT

There is no conflict of interest in this study.

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