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SHORT COMMUNICATION

Allergen Determination in Thoroughbred Stallions via Detecting Serum Specific IgE

Didem PEKMEZCİ [1] Aziz Arda SANCAK [2] Orhan PINAR [3]
Duygu ÇAKIROĞLU [1] Serhat ARSLAN [4]

[1] Department of Internal Medicine, Faculty of Veterinary Medicine, University of Ondokuz Mayis, TR-55139 Kurupelit, Samsun - TURKEY
[2] Department of Internal Medicine, Faculty of Veterinary Medicine, University of Ankara, TR-06130 Diskapı Ankara - TURKEY
[3] The Jockey Club of Ankara, TR-06130 Ankara - TURKEY
[4] Department of Biometry, Faculty of Veterinary Medicine, University of Ondokuz Mayis, TR-55139 Kurupelit, Samsun - TURKEY

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Summary

In horses, allergic disease has been implicated in the pathophysiology of atopic dermatitis, recurrent urticaria, and recurrent airway obstruction. With the present study, it is aimed to give information about allergens which determined in Thoroughbred stallions with detecting serum IgE. 18 Thoroughbred stallions averaged 11 years old were included in the study. Fifteen single and five mix allergens were checked for allergen-specific IgE. English plantain *(Plantago lanceolata)*, Birch/Alder/Hazel and *Micropolyspora faeni/Thermoactinomyces* were detected as quite allergic to six Thoroughbred stallions at total. Consequently, assays detect equine IgE, can be an effortless method in determining allergens in horses.

Keywords: *Allergen, IgE, Stallion, Thoroughbred, Urticaria*

İngiliz Aygırlarında Serum Spesifik IgE Tespiti ile Alerjen Tayini

Özet

Atlarda alerjik hastalıklar atopik dermatitis, tekrarlayan ürtiker ve tekrarlayan havayolu hastalıklarının patofizyolojisinde rol oynamaktadır. Bu çalışma ile İngiliz aygırlarında serum spesifik IgE tespiti ile alerjenlerin tayini hakkında bilgi verilmesi amaçlanmıştır. Yaşları ortalama 11 olan, 18 İngiliz aygır çalışma materyalini oluşturmuştur. On beş tek ve beş karışık alerjene serum spesifik IgE yönünden bakılmıştır. Toplam altı İngiliz aygırda English plantain *(Plantago lanceolata)*, Birch/Alder/Hazel ve *Micropolyspora faeni/Thermoactinomyces* oldukça alerjik olarak tespit edilmiştir. Sonuç olarak, atlara özgü IgE'yi belirleyen serolojik testler alerjenlerin tespiti bakımından zahmetsiz bir yöntem olarak değerlendirilebilir.

Anahtar sözcükler: *Alerjen, Aygır, IgE, İngiliz atı, Ürtiker*

INTRODUCTION

In horses, allergic disease (hypersensitivity to insect and environmental allergens) has been implicated in the pathophysiology of atopic dermatitis (AD), recurrent urticaria (RU), and recurrent airway obstruction (RAO) [1]. Diseases in horses such as AD, RU, and RAO [2] have long been thought to have an allergic etiology due to similarities with human allergies which have been shown to be mediated by IgE [3]. The clinical signs in AD, RU cases can become so severe that the animals injure their skin and become unable to be worked [4] or predisposing to RAO, which leads unable to race. Therefore, it is important to determine whether a horse is allergic or not or the allergens have to be detected. The molecular mechanisms of allergy and the recent proof of the integral role of IgE in mediating equine skin hypersensitivities [5] suggests that serological assays using detection of allergen-specific IgE could be an alternative method in horses. The recent development of highly specific monoclonal antibodies to detect equine IgE [3] allowed us to show the causal role of IgE in skin hypersensitivity and allergies. With the present

İletişim (Correspondence)
+90 505 4779524
dkazanci@omu.edu.tr

study, it is aimed to give information about allergens detected in Thoroughbred stallions via detecting serum specific IgE and take attention the importance of detecting allergens before revealing the symptoms.

MATERIAL and METHODS

Horses

18 Thoroughbred stallions which ranged from 5 to 17 years (mean 11 years) were included in the study. The stallions were kept in The Jockey Club. Four of them showed signs of urticaria with different levels of pruritis, and fourteen of them were free of urticaria. There were several wheals and plaques that elevated, round, flat-topped, and 0.5-4 cm in diameter, mainly presented on the back, flanks, and legs in these four urticaria positive stallions. Based on the skin scraping and culture tests any bacterial or mycotic infections were detected in the uricaria positive stallions in their routine clinical examinations. All stallions had no history of respiratory diseases or any urticaria symptoms before on their medical records. They were stabled and fed hay and had a regular deworming protocol at 3 month intervals with a standard vaccination program. The stallions had received no antihistamine, corticosteroid or other medications including vaccines by any route for at least eight weeks prior to the test. The health statuses of the stallions were also checked routinely by the veterinary surgeons of the Jockey Club.

Test Procedure

Venous blood (10 ml) was obtained from the jugular vein and was centrifuged at 3.000 *g* for 10 min at room temperature. Serum were separated and stored at -25°C until the analysis. Blood samples were collected by the veterinary surgeons of the Jockey Club within their routine clinical examinations in the summer season. The assay procedure was performed according to the manufacturer's recommendations and directions (Polycheck Equss®, Germany). In comparison to the standard curve the amount of allergen-specific IgE for each allergen is given as relative kilo units per liter (kU/l) and results determined based on their concentration of IgE as negative (0), mild (1), marked (2) and strong (3).

Allergy Panels

Fifteen single allergens and five mix (pollens: grass, trees, weed; house dust mites; storage mites, moulds; insects) were checked for allergen-specific IgE. These included with their numbers used in the study *(Table 1)*.

Statistical Analyzes

Zero-inflated Poisson Regression described by Famoye and Singh [6] is used to model count data that has an excess of zero counts. Furthermore, structure of present data as well as many count datasets is the joint presence of excess zero observations and the long right tails, both relative to the Poisson assumption [7]. An odds ratio of 1 indicates that the condition or event under study is equally likely to occur in both groups. An odds ratio greater than 1 indicates that the condition or event is more likely to occur in the first group. An odds ratio less than 1 indicates that the condition or event is less likely to occur in the first group. The odds ratio must be nonnegative if it is defined.

RESULTS

Thoroughbred stallions were tested for specific serum IgE for 20 allergens and the test results with the degrees of reactions were given in *Table 1*. Only 4 stallions were classified as urticaria positive based on their clinical findings while others were described as negative. Contrary to the urticaria positive stallions, number 1 and 13 stallions gave positive reactions to *P. lanceolata* and Birch/Alder/Hazel allergens even they were urticaria negative *(Table 1)*. According to the odds ratios Black fly *(Simulium equinum)* was determined 1.77 times much more allergic in the presence of the other eight allergens in urticaria positive stallions and 1.75 times more allergic in urticaria negative stallions compared to the other eight allergens *(Table 2)*. Biting midges *(Culicoides nubeculosus)*, Horsefly *(Tabanus* spp.), Rape *(Brassica napus*), Mugwort *(Artemisia vulgaris)* and Ragweed *(Ambrosia)* also have the risk of giving positive reactions as 3.57 times greater in the presence of the other eight allergens in urticaria positive stallions while, these allergens have the risk of giving positive reactions as 3.52 times greater in urticaria negative stallions when compared to the other eight allergens *(Table 3)*. On the other hand, *P. lanceolata*, Birch/Alder/Hazel and *Micropolyspora faeni/Thermoactinomyces* were found the most allergic compounds among all the allergens. *P. lanceolata* was determined 7.75 times more allergic in the presence of the other eight allergens in urticaria positive stallions and 7 times more allergic in urticaria negative stallions contrasted with the other eight allergens. With the presence of other six allergens *P. lanceolata*, Birch/Alder/Hazel and *M. faeni/Thermoactinomyces* were detected as quiet allergic substances in stallions even they were urticaria negative when compared to others *(Table 3)*.

DISCUSSION

Allergic disease (hypersensitivity to insect and environmental allergens) has been implicated in the pathophysiology of AD, RU, and RAO [1] and may be associated with IgE-mediated hypersensitivity. They often result in pruritus and self excoriation and on rare occasions may affect athletic performance and even prompt euthanasia [8]. Therefore, determining the allergens in the horses even with different protocols is indisputably very important. Based on a specific monoclonal antibody test

Table 1. *Allergic reactions with varying degrees and urticaria conditions of the stallions (Urticaria positive stallions are marked with upper asterisk*)*
Tablo 1. *Aygırların değişen derecelerdeki alerjik reaksiyonları ile ürtiker olan aygırlar (Ürtiker pozitif aygırlar yukarı asteriks ile işaretlenmişlerdir*)*

Allergens	Stallions																	
	1	2*	3*	4*	5	6*	7	8	9	10	11	12	13	14	15	16	17	18
Tyrophagus spp.																		
Meal mite *(Acarus siro)*																		
Stable fly *(Stomaxys calcitrans)*																		
Black fly *(Simulium equinum)*		1																
Mosquito																		
Biting midges *(Culicoides nubeculosus)*			1															
Horsefly *(Tabanus* spp.)			1															
Rape *(Brassica napus)*			2															
Mugwort *(Artemisia vulgaris)*			2															
English plantain *(Plantago lanceolata)*	2	2	2	1		2							2					
6-grass mix																		
Rye, common *(Lolium perenne)*																		
Platane/Willow *(Salix viminalis)*/Poplar																		
Birch/Alder/Hazel	1	2		1		1							1					
Ragweed *(Ambrosia)*			2															
Micropolyspora faeni/Thermoactinomyces		2		1														
Aspergillus fumigatus/Penicillium notatum																		
Lepidoglyphus destructor																		
Dermatophagoides pteronyssinus																		
D. farinae																		

Table 2. *Likelihoods of nine allergens as percentages*
Tablo 2. *Yüzde olarak dokuz alerjenin olasılıkları*

Allergens No	Allergy Positive	Allergy Negative	Among 9 Allergens		With All Allergens	
			Allergy Positive	Allergy Negative	Odds	Relative Risk
4	0.33	0	0.027	0	1.77	1.75
6	0.33	0	0.027	0	3.57	3.52
7	0.33	0	0.027	0	3.57	3.52
8	0.33	0	0.027	0	3.57	3.52
9	0.33	0	0.027	0	3.57	3.52
10	100	0.14	0.111	0.015	7.75	7
14	0.75	0.14	0.083	0.015	5.63	5.25
15	0.33	0	0.027	0	3.57	3.52
16	0.50	0	0.055	0	7.05	7.41

Table 3. *Risk ratios, odds values and their log.ods in 9 allergens (¹ indicates 95% confidence limits for Lower and Upper)*
Tablo 3. *9 Alerjende risk oranları, odds değerleri ve bunların log.ods değerleri (¹ %95 güven aralığı için Alt ve Üst sınırları ifade eder)*

Sample		Risk Ratio[1]	Odds	Odds Ratio[1]	Log Odds	F. exact
4	1 0	3.5 (0.27-44.35)	0.33 0.07	4.33 (0.206-90.852)	1.46	0.03
6	1 0	3.5 (0.27-44.35)	0.33 0.07	4.33 (0.206-90.852)	1.46	0.03
7	1 0	3.5 (0.27-44.35)	0.33 0.07	4.33 (0.206-90.852)	1.46	0.03
8	1 0	3.5 (0.27-44.35)	0.33 0.07	4.33 (0.206-90.852)	1.46	0.03
9	1 0	3.5 (0.27-44.35)	0.33 0.07	4.33 (0.206-90.852)	1.46	0.03
10	1 0	0.233 (0.105-0.514)	0.25 0.07	0.041 (0.012-0.136)	-3.17	<.0001
14	1 0	0.2917 (0.135-0.626)	0.33 0.07	0.055 (0.016-0.18)	-2.89	<0.001
15	1 0	3.5 (0.27-44.35)	0.33 0.07	4.33 (0.206-90.852)	1.46	0.03
16	1 0	0.5 (0.187-1.332)	1 0.07	0.0714	-2.63	0.03

to detect equine IgE will be useful to get reliable results in horse allergy with its easy and effortless application. Our Thoroughbred horses in the study were all stallions, though Scott and Miller [9] have been reported there is no convincing gender predilection to atopy or urticaria in horses. However, the influence of seasonal factors on serum IgE level is poorly understood, the tests for allergen specific IgE quantification in all eighteen stallions in the present study were made during the summer season. Wilson et al.[4] reported that horses generally, do not develop clinical signs of skin hypersensitivities before the age of 3-4 years of ages. In addition, our stallions were all aged than this pointed years. In current study, surprisingly, *P. lanceolata* was detected as the most allergen rather than the others. Birch/Alder/Hazel and *M. faeni/Thermoactinomyces* were followed as the second and third most important allergens. One of the most common species is *P. lanceolata*, which is distributed in the temperate zones of Europe, Australia and North America [10]. Besides, *P. lanceolata* pollen has already been associated with hay fever since the beginning of this century and this weed has been considered as one of the most important dicotyledons that cause allergic diseases. Our results are also verifies that this plant is also an important allergen in horses. Birch/Alder/Hazel was the second most important allergen even they gave positive reactions in urticaria negative stallions. Over 50 species of fungi and actinomycetes have been identified in stable air, the highest challenge from stable materials and feed being thermophilic and thermotolerant mould species [11]. However, the most important organisms, in terms of their role in chronic RAO, are *Faenia rectivirgula (M. faeni), Thermoactinomyces vulgaris* and *Aspergillus fumigatus* [12]. Exposure to fungal spores is associated with allergic IgE related and non-specific neutrophilic inflammation in lower airways of horses [13], which may result directly from inhalation of fungi or from inhalation of volatile chemicals produced by fungi. As mentioned *F. rectivirgula, T. vulgaris* and *A. fumigatus* could also be allergic in horses. Results of the present study also indicate that these allergens are quite allergic to Thoroughbred horses. Interestingly, beside the summer season our stallions were less allergic to flies especially Stable fly *(Stomaxys calcitrans)*, Black fly *(Simulium equinum)*, Mosquito, Biting midges *(Culicoides nubeculosus)* and Horsefly *(Tabanus* spp.). On other hand, *Culicoides* spp. are the most important inducers of skin hypersensitivities such as summer eczemas, especially in horses [13] which the prevalence of the disease varies between 3-72%. There is only one stallion all over eighteen gave positive reaction to this allergen in the present study, even the tests were performed during summer season.

Consequently, blood sampling is a simpler procedure and provides a convenient diagnostic tool for identifying causal allergens in skin hypersensitivities even in horses. Such assays highly specific monoclonal antibodies to detect equine IgE, can be an alternate method in detecting allergens in horses. Accordingly the results of current study, *P. lanceolata,* Birch/Alder/Hazel and *M. faeni/ Thermoactinomyces* were found quite allergic sources to Thoroughbred stallions even they were urticaria negative. That is why some precautions such as keeping away of these allergens from horses should be taken. For example, the constituents of commercial feeds such as alfalfa cubes and pellets generally contain fewer mites and their faeces, fungi, actinomycetes and fungal spores particularly those incriminated in inducing chronic airway inflammation or RAO than hay and haylage. Horses housed on deep litter wood shavings bedding, or on bedding that is inconsistently changed and cleaned of excretions (as may occur in some poorly managed racetrack stables), may be exposed to higher concentrations of fungi and actinomycetes, which multiply in degrading wood shavings, (particularly pinewood) and stable dung. Further studies evaluating larger numbers of both normal horses and horses with allergic diseases (AD, RU, and ROA) in Thoroughbred horses are required. Therefore, it is important to determine whether a horse is allergic or not or the allergens have to be detected.

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