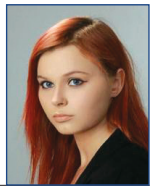
Assessment of serum allergen-specific IgE levels in horses from Siedlce County, Poland



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Abstract

The aim of the present study was to determine the frequency of different allergies in horses from central Poland. Allergic diseases are common in horses all over the world. Common conditions that have been associated with immunoglobulin E (IgE)-mediated allergy in horses include insect bite hypersensitivity (IBH), urticaria and recurrent airway obstruction (RAO). A variety of serological assays are available to measure serum levels of allergen-specific IgE antibodies. They can provide supportive evidence for a diagnosis of equine allergic dermatitis associated with environmental allergens. This study was performed on clinically healthy, Polish noble half-breed horses. In this study, four groups of allergens were identified as commonly inducing allergic reactions: American house dust mites (*D. farinae*), cultivated plants (rape), English plantain (*Plantago lanceolata*) and biting midges (*Culicoides nubeculosus*). More than 50% of horses showed positive reactions to these groups of allergens. The factor that contributed most to allergy occurrence among horses was American house dust mites (*D. farinae*). In recent years, efforts have been made to identify new allergens and to better understand the specific pathogenesis of allergic diseases in animals. The development of allergic disease diagnostics has been facilitated by the availability of diagnostic methods along with enhanced awareness of allergic diseases in veterinary practice. Allergic disorders in horses are ultimately gaining the attention they deserve thanks to the fact that veterinary allergology has become a dedicated veterinary discipline.

Key words: *horse; allergy; allergen*

Introduction

Although some species, such as dogs and horses, may seem to be more prone to allergies than other species, allergic diseases are common in most mammals (Zahradnik et al., 2018; Jania et al., 2020). The interaction of three major factors is involved in allergic diseases: genetic constitution, exposure to allergens, and immune dysregulation. Allergic diseases are common in horses all over the world,

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with prevalence ranging from 0% to 74% depending on the region (Wilkołek et al., 2014; Mueller et al., 2016).

Common conditions associated with immunoglobulin E (IgE)-mediated allergy in horses include insect bite hypersensitivity (IBH), urticaria, recurrent airway obstruction (RAO), and headshaking. The most common allergic skin disease in horses is equine insect bite hypersensitivity (IBH), caused by bites of midges (*Culicoides* spp.) and other insects. The allergens causing IBH are most likely salivary gland proteins from these insects. Roughly 10% of all horses in the world are affected by IBH (Fettelschoss-Gabriel et al., 2018). Lesions are characterized by hyperkeratosis and crust formation. Sick horses may exhibit behavioural disorders. There is currently no long-term effective treatment (Wilkołek et al., 2014).

RAO is a recurring pulmonary disease that typically affects middle-aged horses. It results in coughing and loss of performance (Einhorn et al., 2018). These clinical signs are largely triggered by bronchoconstriction and compromised gas exchange (Herszberg et al., 2006). Airborne fungi, often of the species *Aspergillus*, are important allergens isolated from horses with RAO (Jensen-Jarolim et al., 2015). Hyperreactivity to storage mites and house dust mites can induce the disease. Other factors causing or conducive to disease include toxins and allergens ingested with food, as well as bacterial or viral infections. Present treatments include allergen avoidance, anti-allergic medications, and immunotherapy, known as desensitization (Klier et al., 2017). Allergen-specific immunotherapy (ASIT) involves the use of initially very small but gradually increasing amounts of allergen solutions, determined on the basis of blood tests or skin tests. Urticaria is characterized by bumpy lesions affecting extensive areas of skin. Flat-topped wheals are classical lesions. They are transient, resolving within 24 to 48 hours (Jensen-Jarolim et al., 2015). Behaviours such as headshaking are thought to be manifestations of hypersensitivity in some horses (Newton et al., 2000).

A variety of serological assays are available to measure serum levels of allergen-specific IgE antibodies. They can provide supportive evidence for a diagnosis of equine allergic dermatitis associated with environmental allergens (Marteles et al., 2019). The aim of the present study was to determine the frequency of different types of allergies in horses from central Poland.

Materials and methods

The study was conducted on 28 horses belonging to breeders from Siedlce County. The horses were clinically healthy, with no signs of skin or respiratory diseases or other allergic conditions. This study was performed on Polish noble half-bred horses. During the summer, they were kept at pasture with access to shelter, and during winter they were housed in the stables. Blood was collected from the jugular vein. The analyses were carried out at the facilities of the LAB-WET Veterinary Diagnostic Laboratory in Warsaw.

Serum allergen-specific IgE concentrations were determined using a monoclonal anti-IgE antibody (Polycheck Allergie NF Horse Panel, Biocheck GmbH, Münster, Germany). The test is an immunoassay. First, the allergen-specific IgE attaches to the corresponding allergen. After washing, the biotinylated antibody detects bound IgE. Another specific antibody labels the bound monoclonal antibody and reacts in a further incubation step with streptavidin-alkaline-phosphatase conjugates. Following washing and the addition of 5-bromo-4-chloro-3-indolyl-1-phosphate/nitro blue tetrazolium (BCIP/NBT), the enzymes produce a coloured precipitate. The amount of pre-

cipitate is directly proportionate to the specific IgE level in the serum, resulting in significant or insignificant colouring of each individual allergen. The following allergens were tested: American house dust mite *(Dermatophagoides farinae),* European house dust mite *(Dermatophagoides pteronyssinus),* storage mite *(Lepidoglyphus destructor), Aspergillus fumigatus/ Penicillium notatum, Micropolyspora faeni/ Thermoactinomyces vulgaris,* short ragweed *(Ambrosia artemisiifolia),* birch/alder/hazel, plane/oak/olive trees, English ryegrass (*Lolium perenne*), 6-Grassmixture, English plantain *(Plantago lanceolata),* mugwort (*Artemisia vulgaris*), rape (*Brassica napus*), horsefly *(Tabanus spp.), Culicoides nubeculosus,* mosquito, blackfly *(Simulium spp.),* stablefly *(Stomoxys calcitrans),* flour mite *(Acarus siro) and* mould mite *(Tyrophagus putrescentiae).*

A computer with a scanner and Biocheck Imaging Software was used to evaluate the tests. Cassettes placed on the flat scanner were read, and the program analysed data from the image. The levels of allergen-specific IgE for each allergen were given as relative kilo units per litre (kU/L) and classified according to the guidelines provided by the manufacturer of the test (Table 1).

Table 1. Levels of allergic reactions in accordance to the serum IgE concentrations in horses

Concentration of IgE (kU/L)	Level
< 0.5	0
0.5 – 20	1
2.0 – 20	2
> 20	3-4

Table 2. Frequency of allergies to individual allergens in the horses tested

Allergen	Horses (*n*=28) Number of positive reactions (%)
American house dust mite (*Dermatophagoides farinae*)	18 (64.3)
European house dust mite (*Dermatophagoides pteronyssinus*)	13 (46.4)
Storage mite (*Lepidoglyphus destructor*)	0 (0.0)
Aspergillus fumigatus/Penicillium notatum	0 (0.0)
Micropolyspora faeni/Thermoactinomyces vulgaris	0 (0.0)
Short ragweed (*Ambrosia artemisiifolia*)	12 (42.9)
Birch/Alder/Hazel	7 (25.0)
Plane/Oak/Olive trees	0 (0.0)
English ryegrass (*Lolium perenne*)	7 (25.0)
6-Grassmixture	4 (14.3)
English plantain (*Plantago lanceolata*)	15 (53.6)
Mugwort (*Artemisia vulgaris*)	3 (10.7)
Rape (*Brassica napus*)	16 (57.1)
Horsefly (*Tabanus* spp.)	10 (35.7)
Culicoides nubeculosus	15 (53.6)
Mosquito	1 (3.6)
Blackfly (*Simulium* spp.)	8 (28.6)
Stablefly (*Stomoxys calcitrans*)	3 (10.7)
Flour mite (*Acarus siro*)	5 (17.9)
Mould mite (*Tyrophagus putrescentiae*)	13 (46.4)

Results and discussion

The analysis of results took into account the frequency of individual allergens in the blood serum of the horses, as shown in Table 2.

Four groups of allergens were identified as commonly inducing allergic reactions: mites (*D. farinae*), cultivated plants (rape), weed allergens (*Plantago lanceolata*) and insects (*Culicoides nubeculosus*). More than 50% of horses showed positive reactions to these groups of allergens. The factor that contributed most to the occurrence of allergies among the horses was house dust mites (*D. farinae*), which were diagnosed in 18 animals (64.3%). The high prevalence of mite allergens found in other studies (Tahon et al., 2009; Wilkołek et al., 2014) may be because of the pellets and grains used in horse feed that contains large amounts of forage mites. In the present study, horses had straw bedding and thus had ongoing contact with mites. This frequent exposure placed them at risk of developing subclinical disease. Rape was a common allergen factor, identified in 16 cases (57.1%). An allergic reaction to *Culicoides* was found in 15 horses (53.6%). The same number of individuals were sensitive to *Plantago lanceolata*. The least common allergen, diagnosed in only one individual (3.6%), was the mosquito. Factors for which an allergic reaction was not detected in the group of animals were *Lepidoglyphus destructor*, *Aspergillus fumigatus*/*Penicillium notatum*, plane, oak and olive trees, and *Micropolyspora faeni*/*Thermoactinomyces*. Stepnik et al. (2012) reported that 40% of horses had positive reactions against tree pollens (olive, orange, red cedar and white alder tree), and that 54.5% of horses had a positive reaction to *Penicillium notatum*. In a study conducted in southern England, only 2% of horses showed positive reactions to mixed moulds (Rendle et al., 2010). Positive reactivity to grain mill dust, grasses, mites, horseflies, mosquitoes and moulds was reported by Kalina et al. (2003). Marteles et al. (2019) used the Allercept ELISA test to analyse allergens in serum samples from 73 horses with allergic diseases. Fifty-four horses had allergic dermatitis (AD) with high IgE levels for *Tyrophagus putrescentiae* (51.9%), *Tabanus* (46.3%) and *D. farinae*/*D. pteronyssinus* (40.7%). Seven horses had RAO, with a high incidence of positive reactions to *T. putrescentiae* (85.7%), *Acarus siro* and *D. farinae* (57.1%). Horses with RAO had more positive responses to mites than horses with allergic dermatitis ($P \leq 0.05$).

In the analysis of the results, the incidence of allergic reactions in individual classes was also determined (Table 3). Reactions were interpreted as weak positive (<0.5 kU/L), mild positive (0.5-2.0 kU/L), moderate positive (2.0-20 kU/L) and strongly positive (>20 kU/L).

The highest percentages of weak positive reactions among the horses were detected for *Aspergillus fumigatus*/*Penicillium notatum*, *Micropolyspora faeni*/*Thermoactinomyces*, *Ambrosia* spp., and rye (100%). There was also a high percentage of positive reactions for *Simulium* spp. (96.4%), *Acarus siro* (89.3%), rape (89.3%) and *Plantago lanceolata* (85.7%). Mild reactions were most common for mugwort (28.6%), *Culicoides nubeculosus* (17.8%), mosquito (17.8%) and *Stomoxys calcitrans* (14.3%). For the allergens of *Tabanus* spp. (57.1%), a high percentage of moderate reactions was observed. A high sensitization rate was detected for the house dust mite *D. farinae* (53.6%), *Lepidoglyphus destructor* (50.0%), the storage mite *Tyrophagus putrescentiae* (42.8%), *D. pteronyssinus* (39.3%), and the mixture of birch, alder and hazel tree pollens (39.3%).

Allergies are common diseases in horses that can be diagnosed by clinical examination alone, but definitive diagnosis is only possible using laboratory tests. Performing these tests

Table 3. The levels of allergic reactions in horses from central Poland

Allergen	Levels of allergic reactions in horses			
	0	1	2	3-4
	number (%)			
American house dust mite (*Dermatophagoides farinae*)	10 (35.7)	3 (10.7)	15 (53.6)	0 (0.0)
European house dust mite (*Dermatophagoides pteronyssinus*)	15 (53.6)	2 (7.1)	11 (39.3)	0 (0.0)
Storage mite (*Lepidoglyphus destructor*)	14 (50.0)	0 (0.0)	14 (50.0)	0 (0.0)
Aspergillus fumigatus/Penicillium notatum	28 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Micropolyspora faeni/Thermoactinomyces	28 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Short ragweed (*Ambrosia artemisiifolia*)	28 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Birch/Alder/Hazel	16 (57.1)	1 (3.6)	11 (39.3)	0 (0.0)
Plane/Oak/Olive tree	21 (75.0)	2 (7.1)	5 (17.8)	0 (0.0)
English ryegrass (*Lolium perenne*)	28 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
6-Grassmixture	21 (75.0)	2 (7.1)	5 (17.8)	0 (0.0)
English plantain (*Plantago lanceolata*)	24 (85.7)	3 (10.7)	1 (3.6)	0 (0.0)
Mugwort (*Artemisia vulgaris*)	13 (46.4)	8 (28.6)	7 (25.0)	0 (0.0)
Rape (*Brassica napus*)	25 (89.3)	3 (10.7)	0 (0.0)	0 (0.0)
Horsefly (*Tabanus* spp.)	12 (42.8)	0 (0.0)	16 (57.1)	0 (0.0)
Culicoides nubeculosus	18 (64.3)	5 (17.8)	5 (17.8)	0 (0.0)
Mosquito	13 (46.4)	5 (17.8)	10 (35.7)	0 (0.0)
Blackfly (*Simulium* spp.)	27 (96.4)	1 (3.6)	0 (0.0)	0 (0.0)
Stablefly (*Stomoxys calcitrans*)	20 (71.4)	4 (14.3)	4 (14.3)	0 (0.0)
Flour mite (*Acarus siro*)	25 (89.3)	3 (10.7)	0 (0.0)	0 (0.0)
Mould mite (*Tyrophagus putrescentiae*)	15 (53.6)	1 (3.6)	12 (42.8)	0 (0.0)

and regular updating of allergy panels increases the effectiveness of diagnosis and identification of allergens (Jonsdottir et al., 2016).

In recent years, efforts have been increased to identify new allergens and to broaden the understanding of the specific pathogenesis of allergic diseases in animals. The development of allergic diseases diagnostics has been facilitated by the availability of diagnostic methods along with the enhanced awareness of allergic diseases in veterinary practice. Allergic disorders in horses are ultimately gaining the attention they deserve thanks to the fact that veterinary allergology has become a significant dedicated veterinary discipline.

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Procjena razine alergen specifičnog IgE u krvi u konja iz Siedlce okruga, Poljska

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Cilj ove studije bio je ustvrditi učestalost različitih vrsta alergija u konja iz središnje Poljske. Alergijske bolesti su učestale u konja diljem svijeta. Uobičajena stanja koja su povezana s alergijskom reakcijom posredovanom imunoglobulinom E (IgE) u konja uključuju preosjetljivost na ubode insekata (IBH), urtikariju i povratnu opstrukciju dišnih putova (RAO). Dostupni su različiti serološki testovi za mjerenje razina specifičnog IgE antitijela na alergene u krvi. Oni mogu osigurati potkrepljujuće dokaze za dijagnozu alergijskog dermatitisa konja s alergenima iz okoliša. Ova studija provedena je na poljskim plemenitim, klinički zdravim, križanim pasmina konja. U ovoj studiji identificirane su četiri skupine alergena koje obično prouzroče alergijske reakcije: američke grinje iz kućne prašine (*D. farinae*), kultivirane biljke (repica), uskolisni trputac (*Plantago lanceolata*) i mušice (*Culicoides nubeculosus*). Više od 50 % konja pokazalo je pozitivne reakcije na ove skupine alergena. Čimbenik koji je najviše pridonio pojavnosti alergijskih reakcija među konjima bile su američke grinje iz kućne prašine (*D. farinae*). U posljednjih nekoliko godina, pojačanje napora u suzbijanju alergijskih bolesti dovelo je do identifikacije nekih novih alergena, kao i dubljeg razumijevanja patogeneze životinja specifične za alergijske bolesti. Razvoj u dijagnozi alergijskih bolesti olakšan je dostupnošću dijagnostičkih metoda uz povećanu svijest o alergijskim bolestima u veterinarskoj praksi. Alergijski poremećaji u konja konačno dobivaju pozornost koju zaslužuju zahvaljujući činjenici da je alergologija postala značajna specijalizirana veterinarska disciplina.

Ključne riječi: *konj, alergija, alergen*