Polish Journal of Veterinary Sciences Vol. 20, No. 4 (2017), 789–794

DOI 10.1515/pjvs-2017-0099

Original article

Comparison of serum concentrations of environmental allergen-specific IgE in atopic and healthy (nonatopic) horses

P. Wilkołek[1], W. Sitkowski[1], M. Szczepanik[1], Ł. Adamek[1], M. Pluta[2], I. Taszkun[1], M. Gołyński[3], A. Malinowska[1]

[1] Sub-Department of Clinical Diagnostics and Veterinary Dermatology, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Głęboka 30, 20-612 Lublin, Poland
[2] Equine Breeding and Management Unit, Department of Biology and Animal Breeding, University of Life Sciences in Lublin, Akademicka St. 13, 20-950 Lublin, Poland
[3] Department of Internal Diseases, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Głęboka 30, 20-612 Lublin, Poland

Abstract

Allergic responses in humans, horses and other species are mediated by immunoglobulin E (IgE) antibodies. Serum testing to detect allergen-specific IgE antibodies has been developed for dogs, cats and horses; this allows for the identification of allergens and determination of appropriate allergen-specific immunotherapies. This study compared serum allergen-specific IgE concentrations in atopic and healthy horses. The study was performed on Malopolski breed atopic (n=21) and nonatopic (n=21) clinically healthy horses. Allergen-specific IgE serum concentrations were measured in summer seasons of 2008-2015 using a monoclonal anti-IgE antibody. A Northern and Central European allergen panel containing mite, insect, mould and plant pollen allergens, including 15 tests of individual allergens and 5 tests of allergen mixtures was used. The mean allergen-specific IgE concentrations in the atopic and normal horse populations were compared. Among the atopic horses, the strongest positive reactions occurred against the storage mites *Tyrophagus putrescentiae* and the domestic mite *Dermatophagoides farinae*. The atopic horses also demonstrated high IgE concentrations against insects, particularly *Tabanus* sp., the plant pollens colza, cultivated rye and the mould pollen mixture *Aspergillus*/*Penicillium*. No horses in the atopic group were IgE-negative. Among all mite, insect, mould and some plant allergen groups the differences in mean specific IgE concentrations between allergic and healthy horses were significant. The mean IgE concentrations for most allergen groups were significantly higher in the atopic horses than in the healthy animals. However, a high incidence of positive reactions was observed in both healthy and allergic horses. Our results showed a high frequency of polysensitization in atopic horses.

Key words: IgE, equine atopic dermatitis, healthy horses, immunology, allergens

Correspondence to: P. Wilkołek, e-mail: pwilku@poczta.onet.pl

Introduction

Atopic skin disease is less well-characterized in horses than in other species. Equine atopy (EA) is an inflammatory condition characterized by chronic relapsing pruritus with or without non-pruritic urticaria. Affected individuals are genetically predisposed to develop IgE-mediated allergies to environmental allergens (Stepnik et al. 2012, Wagner 2016). The diagnosis of equine atopic skin disease is primarily based on history, clinical presentation and exclusion of other pruritic and/or urticarial skin diseases (Stepnik et al 2012). Horses diagnosed with atopic dermatitis commonly present pruritus during seasons lacking insect activity or year-round; however, EA and insect bite hypersensitivity (IBH) can have overlapping clinical presentations. Furthermore, in horses, IBH is initially seasonal, however, it can progress to year-round dermatitis [Frey et al. 2008]. Clinical signs of atopic dermatitis include pruritus with secondary intense self-trauma crusting and alopecia with chronic lesions, including lichenification and hyperpigmentation. Commonly affected areas include ears, face, abdomen and legs. The clinical signs of atopic dermatitis are similar to those of IBH, and it is extremely common for a horse to have both diseases concurrently (Jensen-Jarolim et al. 2015).

Although the use of intradermal tests remains the "gold standard" diagnostic method for causative allergens, the use of serum allergy tests, which measure the concentrations of circulating allergen-specific IgE, serves as an alternative method for identifying allergens to which an individual is hypersensitive (Wagner 2016, Lorch et al. 2001, Marti et al. 2008). Serological assays have limitations that have been demonstrated in multiple comparisons between allergic and healthy dogs, cats and horses (Wagner et al. 2006, Diesel and DeBoer 2011, Lauber et al. 2012). The frequency of detection of allergen-specific IgE by serological assays is not significantly different between atopic and healthy dogs or cats for any allergen groups. However, to date, no comparative studies with a broad panel of allergens have been performed in horses. The aim of this study was to compare serum concentrations of allergen-specific IgE using an equine monoclonal antibody panel test in atopic horses (those with the year-round form of the disease) and healthy horses.

Materials and Methods

This study was performed on Malopolski atopic (n=21, AH) and nonatopic clinically healthy horses (n=21, HH). Some animals were privately owned, and some were patients of the Department of Clinical Diagnostics and Veterinary Dermatology at the University of Life Sciences in Lublin (referral clinic), Poland. The AH group was composed of 38.1% female and 61.9% male horses. The age range of the animals was 4 to 13 years (median age: 7.6 years). The inclusion criteria were year-round pruritus that intensifies in the summer season with various degrees of posttraumatic hypotrichosis, alopecia scaling, crusting or lichenification on the face, neck, thorax, abdomen, tail and legs. Other causes of pruritic skin diseases, such as parasitic, fungal and bacterial infections and adverse food reactions were excluded by using appropriate diagnostic tests and therapy (trichography, multiple skin scraping samples from various locations, cytological examinations, fungal and bacterial cultures, elimination diet, deworming). For all atopic horses, hypersensitivity to environmental allergens was demonstrated by intradermal tests (Agroskin RTU 20, Agrolabo Horse Panel, Scarmagio, Italy). No corticosteroid or antihistamine drugs were administered for at least 6 weeks prior to each scheduled testing. The HH group was composed of 47.6% female and 52.4% male horses. The age range of the animals was 2 to 14 years (median age: 5.6 years). Healthy horses had no history of skin or respiratory diseases before or during the performance of this study. All atopic and healthy horses lived in the same rural area and were kept loose in group housing stables during the winter and kept in pastures and stables during the summer.

Sample collection and IgE measurements were performed in summer seasons (between June and August) of 2008-2015. A volume of 5 ml of peripheral blood was collected by jugular vein venepuncture from each horse and centrifuged for 10 minutes at 4500g. Serum samples were kept cool in 4°C until an assay (5 to 12 hours after blood collection) without any freeze-thaw cykles. The serum allergen-specific IgE concentrations were determined using a monoclonal anti-IgE antibody (Polycheck Allergie NF Horse Panel, BioCheck GmbH, Munster, Germany) according to the manufacturer's instructions. The Northern and Central European allergen panel composed of 15 individual allergens and 5 allergen mixtures was used. In accordance with the guidelines provided by the manufacturer of the test, the reactions were interpreted as negative (<1 kU/l; NR), mild positive (1.0-2.0 kU/l; MPR), moderate positive (2.0-20 kU/l; MR) and strongly positive (> 20 kU/l; SR). The statistical analysis was performed using STATISTICA 10.0 for Windows (StatSoft, Tulsa, OK, USA). The analysis of significant differences between atopic and healthy horses was conducted using the Mann-Whitney U test, with p-values of $p<0.05$ considered to indi-

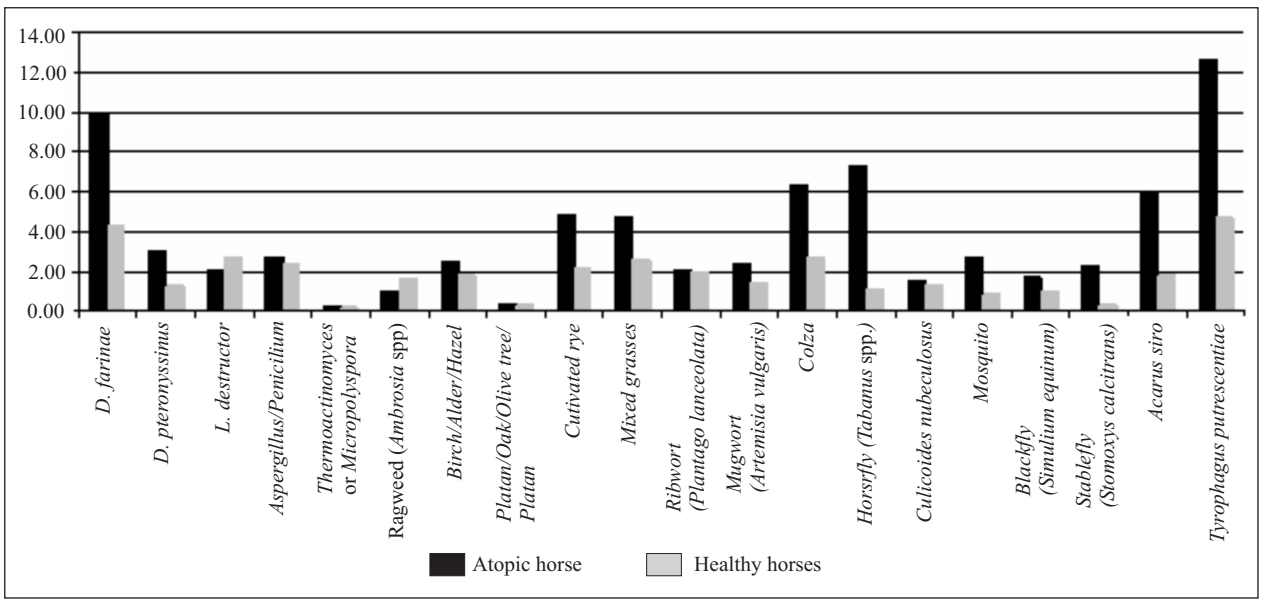

Fig. 1. Mean serum allergen-specific IgE concentrations (kU/l) in atopic and healthy horses.

cate a statistically significant difference. All investigations involving the use of animals were approved by the Local Ethics Committee.

Results

The mean allergen-specific IgE serum concentrations in atopic and healthy horses are shown in Fig. 1. The mean values and intensity percentiles for the IgE concentrations for all groups of allergens in the atopic and healthy horses are shown in Table 1. Significant statistical differences are indicated by * ($p<0.05$).

Specific IgE concentrations for all mite allergens (except *Lepidoglyphus destructor*) showed strong statistically significant differences between atopic and healthy horses. The most prevalent positive reactions and highest mean values were reported against the mites *Tyrophagus putrescentiae* (100% of atopic horses, range: 2.6-34 kU/l) and *Dermatophagoides farinae* (100%, 3.5-21 kU/l). Strong positive reactions against these two mite allergens were observed in this study. High antibody concentrations against *Acarus siro* (90.5%, 0.25-15 kU/l) and *Dermatophagoides pteronyssinus* (85.7%, 0.45-7.2 kU/l) were observed. The lowest intensity positive response was observed against *L. destructor* (76.2%, 0.53-5.2 kU/l). Among the healthy horses, mites were the most prevalent allergens, with positive reactions in 57.1% (*T. putrescentiae)*, 90.5% (*D. farinae*), 38.1% (*D. pteronyssinus*) and 42.9% (*L. destructor*) of horses. Among all allergens tests, the highest mean values were observed for *T. putrescentiae* (4.70 kU/l) and *D. farinae* (4.26 kU/l). Most reactions were moderate in both groups of horses.

The most prevalent positive reaction and the highest insect-specific IgE concentrations were observed among the atopic horses for the allergen of *Tabanus sp.* (100%, 1.5-16 kU/l), with a high percentage of moderate reactions. Lower mean insect-specific IgE concentrations were observed for mosquito (range: 0.23-9.4 kU/l), *Culicoides nubeculosus* (0.32-3.1 kU/l) and *Stomoxys calcitrans* (0.33-6.7 kU/l). Positive reactions were observed in approximately 70% of atopic horses. In the healthy group, very low mean IgE concentrations and prevalences of positive reactions were observed against *Tabanus*, *Culicoides*, mosquito (approximately 30%) and *Stomoxys* (9.5%). Significant differences in the IgE concentrations against all insect allergens were observed between the atopic and healthy groups.

High prevalences of positive reactions were detected for plant pollens, with the highest prevalence observed for the cultivated plant colza (90.5%, 0.54-14 kU/l) and cultivated rye (85.7%, 0.34-11 kU/l) in horses with atopic dermatitis. Approximately 80% of positive reactions were of moderate intensity. We also observed positive reactions for colza (66.7%) and cultivated rye (57.1%) in healthy horses, and these prevalences were significantly different from those among atopic horses. Similar results with statistically significant differences between atopic and healthy horses were observed for the grass allergen mixture (81%, 0.43-14 kU/l for atopic horses and 57.1%, 0.21-6.6 kU/l for healthy horses). Moderate reactions were detected in 71.5% and 52.4% of atopic and healthy horses, respectively.

For the mould allergen mixture of *Aspergillus* and *Penicillium*, the frequencies of positive reactions were

Table 1. Mean serum IgE concentrations (kU/l) and percentage of positive reactions for allergens in atopic and healthy horses. Significant statistical differences were indicated by * ($p<0.05$).

Allergen	Mean value (kU/l)		Positive reactions ≥1.0 kU/l (%)	
	atopic	healthy	atopic	healthy
Tyrophagus putrescentiae	12.58	4.70* p=0.001	100	57.1
Dermatophagoides farinae	9.93	4.26* p=0.001	100	90.5
Horsefly (*Tabanus* spp)	7.25	1.14* p=0.001	100	38.1
Colza (pollen)	6.32	2.74* p=0.021	90.5	66.7
Acarus siro	6.05	1.85* p=0.002	90.5	42.9
Cutivated rye (pollen)	4.82	2.17* p=0.046	81	57.1
Mixed grasses (pollens)	4.67	2.6* p=0.024	81	57.1
Dermatophagoides pteronyssinus	3.07	1.28* p=0.001	85.7	38.1
Aspergillus/Penicilium	2.73	2.35* p=0.011	71.5	47.6
Mosquito	2.71	0.91* p=0.009	61.9	23.8
Birch/Alder/Hazel (pollen)	2.49	1.83	85.7	52.4
Mugwort (*Artemisia vulgaris*) (pollen)	2.39	1.39* p=0.095	81	42.9
Stablefly (*Stomoxys calcitrans*)	2.8	0.35* p=0.001	76.2	9.5
Ribwort (*Plantago lanceolata*)	2.07	2	76.2	42.9
Lepidoglyphus destructor	2.04	2.68	76.2	42.9
Blackfly (*Simulium equinum*)	1.69	1.02* p=0.008	71.4	33.3
Culicoides nubeculosus	1.54	1.38* p=0.042	76.2	38.1
Ragweed (*Ambrosia* spp) (pollen)	1.01	1.64	14.3	47.6
Platan/Oak/Olive tree/Platan (pollen)	0.40	0.34	4.8	4.8
Thermoactynom/Micropolysp (bacteria)	0.21	0.17	0	0

71.5% (0.65-7.1 kU/l) and 47.6% (0,15-17 kU/l) for the atopic and healthy horses, respectively. Moderate reactions predominated in the atopic group, and low-value reactions predominated in the healthy group. Significant differences were observed between the two groups.

For the weed allergens, positive reactions were most common for *Artemisia vulgaris* (81%, 0.72-9.5 kU/l), followed by *Plantago lanceolata* (76.2%, 0.75-5.2 kU/l) and *Ambrosia* spp. (14.3%, 0.15-6.1 kU/l). Positive reactions against weed allergens were 50% more frequent among horses with atopic dermatitis than among healthy horses. Except for *Artemisia vulgaris*, the mean IgE values for the weed allergens were not significantly different between the atopic and healthy horses.

Among the tree pollens, a high prevalence of positive reactions was observed for the birch, alder and hazel mixture (85.7%; 0.54-6.4 kU/l), and a low prevalence and very low intensity reactions were observed for the platan, oak and olive tree mixture (4.8%, 0.15-1.4 kU/l). The frequency of detection of allergen-specific IgE for tree allergens was not significantly different between atopic and healthy horses.

For both groups of horses, no tests for allergen-specific IgE against *Thermoactinomyces* or *Micropolyspora* were positive.

In contrast, positive reactions were observed for *L. destructor* and *Ambrosia* spp., with higher mean IgE values in healthy horses than atopic horses. A higher prevalence of positive reactions in healthy horses was only observed for *Ambrosia spp.* allergens.

Discussion

To the best of our knowledge, this is the first study to compare allergen-specific IgE concentrations between healthy horses and horses with atopic disease for such a broad range of allergens. The comparable frequency of positive IgE reactivity across groups indicates that the presence of specific IgE against environmental allergens is widespread among both atopic and healthy horses (Kalina et al. 2003, Morgan et al. 2007, Wagner et al. 2009). However, our study showed statistically significant differences in the IgE concentrations for many groups of allergens between atopic and healthy horses.

Among atopic horses, the highest sensitization rates for environmental allergens were observed for the storage mite *T. putrescentiae*, the house dust mite *D. farinae*, the insect *Tabanus* spp. (horsefly) and the plant pollens of grasses and cultivated plants (colza and rye). Therefore, we considered these allergens to be important. Strong positive reactions were only observed for *T. putrescentiae* and *D. farinae*, and those allergens also had the highest percentage of moderate intensity reactions. In previous investigations conducted in horses suffering from atopic dermatitis and urticaria, three groups of allergens, mites (*D. farinae* and *D. pteronyssinus*), insects (*Chrysops* spp.) and grasses (Bermuda grass), were found to be the most common responsible for hypersensitivity reactions (Wagner 2009, Stepnik et al. 2012). Kalina et al. (2003) showed positive reactivity to grain mill dust, grasses, horsefly and mosquito, mites, moulds and western trees. The results of other studies showed high concentrations of specific serum IgE against *T. putrescentiae* in horses with RAO (Niedzwiedz et al. 2015). This indicates that mites, particularly *T. putrescentiae*, are significant factors causing hypersensitivity reactions in horses with skin and respiratory diseases.

Pollen producing trees typical of Central Europe, where this study was performed, include birch, alder and hazel. In our study, positive reactions were observed in 85% of atopic horses for the tree pollen allergen mixture of birch, alder and hazel, and in only 4.8% for the mixture of oak, olive tree and platan. Stepnik et al. (2012) observed positive reactions against tree pollens (olive, orange, red cedar and white alder tree) among 40% of horses. The geographical distribution of tree species critically impacts allergen-specific IgE concentrations against plant pollen allergens.

Our investigation showed that mould allergens play a role in the development of atopic dermatitis in horses. Positive reactions against *Aspergillus* and *Penicillium* were observed in more than 70% of horses with atopic dermatitis. Only a few anecdotal reports support the significance of mould allergens in equine atopic dermatitis. Stepnik et al. (2012) reported that 24 of 44 horses had a positive reaction to *Penicillium notatum*. Most previous investigations of mould allergy were performed in horses with recurrent airway obstruction (RAO). Some studies have detected IgE antibodies against crude extracts of *Aspergillus fumigatus* and *Alternaria alternata*, and the recombinant allergens Asp f 7, 8, 9 and Alt a 1 in bronchoalveolar lavage and serum of horses suffering from chronic bronchitis (Eder et al. 2001, Künzle et al. 2007, Wagner 2009, Niedzwiedz et al. 2015). In our study, elevated serum IgE concentrations against moulds in healthy horses were commonly found (49%). In studies of other authors in healthy horses and horses with RAO was revealed no positive reaction to these allergens with the exception of *Aspergillus fumigatus*. Only 14% (1 of 7 of healthy horses) had positive reactions for *A. fumigatus* (Niedzwiedz et al. 2015).

In studies of IgE concentrations performed in other species, no significant differences in the percentages of positive reactions to any allergens were observed between atopic and nonatopic dogs or cats. Similar to our study, other previous studies have been conducted in a selected breed predisposed to developing atopy. For most allergens, healthy control dogs and cats had significantly higher median IgE concentrations than atopic individuals (Roque et al. 2011, Lauber et al. 2012, Mueller et al. 2016). In contrast to these findings, our study demonstrated statistically significantly higher mean IgE concentrations in atopic horses than in healthy horses for most groups of allergens. However, a high incidence of positive reactions was also observed in healthy horses. This finding confirms that a positive allergen-specific IgE response is not pathognomonic for equine atopic dermatitis and cannot be considered as a diagnostic criterion for atopy in horses.

Our results showed that polysensitization (sensitization to ≥2 allergens) occurs frequently in atopic horses, and this phenomenon was observed in all allergic horses. The number of positive reactions in individual horses fluctuated between 10 and 17. Hypersensitivity to multiple allergens has also been shown in

humans, dogs and cats with atopic dermatitis (Diesel and DeBoer 2011, Roque et al. 2011, Kang et al 2014). Surprisingly, in contrast to other published data, IgE-negative horses in the atopic group were not found. Phenomenon such as dogs with a definite clinical diagnosis of atopic dermatitis which had negative specific-IgE test results is described as atopic-like dermatitis (Lian and Halliwell 1998, Diesel and DeBoer 2011). It is estimated that serological allergy tests are negative in approximately 30% of dogs with clinically diagnosed atopic dermatitis (Diesel and DeBoer 2011). In our study horses with atopic-like dermatitis were not found.

Conclusions

This study showed significantly higher concentrations of serum allergen-specific IgE in atopic horses than in healthy horses. Elevated serum concentrations of specific IgE cannot be used as a tool to distinguish between atopic and healthy horses due to the high incidence of positive reactions against various groups of allergens in healthy horses. Sensitization to multiple allergens (polysensitization) occurs very commonly in atopic individuals. The summer season is an appropriate time to measure allergen-specific IgE concentrations to determine causative allergens and desensitisation in atopic horses. Further investigations are needed to evaluate the role of allergen-specific IgE in the development of allergic diseases and to increase the effectiveness of specific immunotherapy in horses.

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