



Original Research

A Comparison of Multiple Allergen Simultaneous Tests Using Allergen-Specific IgE Concentration and Intradermal Skin Tests in Atopic Horses With Pollen Allergy

Piotr Wilkołek ^{a, *}, Marcin Szczepanik ^a, Beata Rodzik ^b, Wiesław Sitkowski ^a, Michał Pluta ^c, Iwona Taszkun ^a, Marcin Gołyński ^d

^a Sub-Department of Diagnostics and Veterinary Dermatology, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Głęboka 30, 20-612 Lublin, Poland

^b Institute of Mathematics, Maria Curie-Skłodowska University, Lublin, Poland

^c Equine Breeding and Management Unit, Department of Biology and Animal Breeding, University of Life Sciences in Lublin, Lublin, Poland

^d Nicolaus Copernicus University in Torun, Faculty of Biological and Veterinary Sciences, Torun, Poland

ARTICLE INFO

Article history:

Received 9 January 2020

Received in revised form

10 March 2020

Accepted 10 March 2020

Available online 17 March 2020

Keywords:

Pollen allergy

sIgE

Intradermal test

ROC curve

Horses

ABSTRACT

Intradermal tests (IDTs) and measurement of specific immunoglobulin E class (sIgE) levels in sera are the most common and reliable methods used in allergological clinical practice. The purpose of this study was to explore the sensitization of pollen allergy in atopic horses with pollinosis and to assess the diagnostic value of the multiple allergen simultaneous tests (MASTs) compared with that of the IDT. Twenty-one Malopolski horses with typical skin hypersensitivity symptoms during pollen seasons were enrolled. Intradermal tests were performed, and allergen-specific IgE concentrations in sera were measured using a monoclonal anti-IgE antibody to pollens of grass, weeds, trees, and cultivated plants. The highest sensitization rate was for *Secale cereale* (IDT, 76%; MAST, 66.7%), grass (IDT, 71%; MAST, 57%), and *Brassica napus* (IDT, 52.4%; MAST, 57%). Receiver operating characteristic (ROC) curve analysis and the area under the ROC curve (AUC) showed that *Betula* spp., *Alnus* spp., and *Corylus* spp. had the highest AUC at 0.854, followed by *Secale cereale* (AUC = 0.796), *Plantago lanceolata* (AUC = 0.726), *Brassica napus* (AUC = 0.704), and a grass allergen mixture (AUC = 0.695). The mean AUC for all allergens was 0.712 (0.604–0.867). The overall sensitivity of the sIgE plant horse panel was 78% (range, 68%–90%), the specificity was 86.3% (range, 64%–100%), and the accuracy was 79% (range, 64%–87%). Statistical kappa (κ) agreement between the MAST and IDT was reached for tree (κ = 0.767), rye (κ = 0.687), colza (κ = 0.671), and grass (κ = 0.664) pollen. The MAST showed a favorable agreement with the IDT and can be used for the detection of sIgE in atopic horses with pollinosis.

© 2020 Elsevier Inc. All rights reserved.

1. Introduction

Equine atopic dermatitis (EAD) is an inflammatory condition characterized by chronic relapsing pruritus leading to alopecia, excoriation, lichenification, recurrent urticaria or angioedema, and

eosinophilic granuloma. Affected individuals are genetically predisposed to develop IgE-mediated allergies to environmental allergens such as pollens of grasses, weeds, and trees but also to those of mites and mold [1–3]. Pollen allergies affect an estimated 40% of allergic individuals in the human population [4]. The flowering seasons of allergenic plants span the whole year, starting from early spring (trees), going over summer (grasses) and into late autumn (weeds). Specifically, the longest period of vegetation and exposure to allergens occurs in Europe in Mediterranean countries, whereas the allergen-exposure periods in northern European countries are shortened [5]. Pollen exposure and symptom development depend on the local plant species; thus, geographical differences enable distinct sensitizations [6,7].

Animal welfare/ethical statement: A comparison of multiple allergen simultaneous tests (MASTs) using allergen-specific IgE concentration and intradermal skin tests in atopic horses with pollen allergy.

Conflict of interest statement: The authors declare no conflicts of interest.

* Corresponding author at: Piotr Wilkołek, Sub-Department of Diagnostics and Veterinary Dermatology, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Głęboka 30, 20-612 Lublin, Poland.

E-mail address: pwilku@poczta.onet.pl (P. Wilkołek).

Only a few studies have investigated pollinosis in allergic horses and usually in the context of respiratory disorders [8–10]. The clinical skin signs of equine pollinosis are similar to those caused by insect hypersensitivity, and it is extremely common to have both diseases in the same horse [7]. Various tests have been evaluated for the *in vitro* etiological diagnosis of allergic disorders in horses, including serological allergy tests based on a specific IgE (sIgE) determination in sera, most commonly used in pollinosis, and functional tests, where histamine release or sulfidoleukotrienes are measured [11]. However, the intradermal test (IDT) and serological tests (STs) remain the most useful methods for detecting specific allergic triggers associated with EAD. Serological tests can be useful in determining the correct prescription of allergen-specific immunotherapy (ASIT). Favorable response to ASIT in horses associated with decreased concentrations of sIgE and steady to increased concentrations of allergen-specific IgG over time [12]. Multiple allergen simultaneous tests (MASTs) have become simple and low-cost serologic tests and screening methods used in clinical allergological practice for the quantitative determination of allergen-specific IgE in humans and animals [13–15]. Multiple allergen simultaneous tests simultaneously detect and identify common environmental allergens in one small value sample without necessity of horse transportation. The aim of this study was to assess the degree of agreement between the IDT and serological MAST to pollen allergens in horses with seasonal atopic dermatitis. Consequently, we determined whether the current diagnostic methods for sIgE determination against a whole extract of plant allergens are sufficient and whether they are associated with pollen allergy in horses.

2. Materials and Methods

2.1. Horses

Twenty-one privately owned Malopolski horses (16 females, 5 males) with atopic dermatitis were included in this study. The age range of the animals was 6–12 years (median age, 8 years). All horses showed clinical signs of atopic dermatitis with various degrees of post-traumatic hypotrichosis, alopecia scaling, crusting, or lichenification on the face, neck, thorax, abdomen, tail, and legs with a history of recurrent seasonal (spring and summer) pruritus. All equine patients were derived from the same geographic rural areas and kept outside in pasture and stables. Parasitic, fungal, and bacterial infections and adverse food reactions were excluded using diagnostic tests and therapy (multiple skin scraping tests, cytological examinations, fungal and bacterial cultures, elimination diets, and deworming). All horses had positive IDT results for plant allergens performed before serum sIgE concentrations were measured. No anti-inflammatory drugs, antibiotics, or antihistamines were administered for at least 4 weeks before each scheduled test.

2.2. Intradermal Tests

Intradermal tests were performed between September and November from 2010 to 2018. After the hair was clipped, the skin was cleaned with 70% alcohol. Injection sites in the middle region of the neck were marked approximately 5 cm apart. Injections of 0.1 mL using whole body allergen extract were placed intradermally using a 25 G needle; histamine was used as a positive control at a concentration of 1:1000 w/v, a phosphate buffer was used as a negative control, and extracts from cultivated plants (*Secale cereale*, rye; *Brassica napus*, colza), grasses (*Dactylis glomerata*, *Lolium perenne*, *Phleum pratense*), weeds (*Artemisia vulgaris*, mugwort; *Plantago lanceolata*, ribwort plantain; *Ambrosia* spp., ragweed), and

trees (*Betula* spp., birch; *Alnus* spp., alder; *Corylus* spp., hazel; *Quercus* spp., oak), mite, insect, and molds allergens were injected (Agroskin RTU 20, Agrolabo Horse Panel, Scarmagno, Italy). The test results were evaluated at 0.5 and 4 hours. Intradermal tests were scored semiquantitatively using a 0–5 point scale as proposed by Lebis based on the firmness and diameter of the wheals: 0 = no reaction, +/- = very flat reaction, + = reaction with slight palpable thickness, ++ = reaction with evident thickness and +++ = reaction identical to that for histamine [16].

2.3. Serological Tests

Serum samples were collected during the summer season between June and August. Peripheral blood was obtained by jugular venipuncture from each horse and centrifuged for 10 minutes at 4500 g. Serum samples were kept cool at 4°C until an assay was performed 2–10 hours after blood collection without any freeze-thaw cycles. The serum allergen-specific IgE concentrations were quantified using the immune-enzymatic panel method with whole-extract allergens and the monoclonal anti-IgE antibody (Polycheck Allergie NF Horse Panel, BioCheck GmbH, Münster, Germany). The European allergen panel was composed of the following plant allergens: rye, colza, grass mixture (*Dactylis glomerata*, *Lolium perenne*, *Phleum pratense*), weeds (*Artemisia vulgaris*, *Plantago lanceolata*, *Ambrosia* spp.), and 2 tree mixtures (tree I: *Betula* spp., *Alnus* spp., *Corylus* spp. and tree II: *Quercus* spp., *Platan* spp., *Olea* spp.) and mite, insect, and molds allergens were also used. The strips were sequentially incubated with horse serum, biotinylated monoclonal anti-equine IgE, anti-anti-equine IgE, avidin-alkaline phosphatase, and substrate. The resulting processed strips were dried and scanned on a scanner. Image analysis software (BioCheck GmbH) detected the densities of each band, along with the densities of a series of control bands of graded density, and expressed the results in arbitrary units/mL (U/mL). The results in accordance with the guidelines provided by the manufacturer of the test were given in 0–4 point allergy classes as follows: class 0, negative reaction at <1 kU/L; class 1, mild positive reaction at 1.0–2.0 kU/L; class 2, moderate positive reaction at 2.0–20 kU/L; class 3, strongly positive reaction > 20 kU/L.

2.4. Statistical Analysis

A receiver operating characteristic (ROC) curve analysis was performed to verify the performance of the IDT and MAST. The optimal cutoff points were determined by the Youden index (sensitivity + specificity–1) from the ROC curves assuming the equal costs for false-positive and false-negative errors. Cohen's kappa coefficient (κ) was used to evaluate multiple levels of agreement between the IDT and MAST using the qualitative positive-negative dichotomic parameter according to a range of κ values: less than 0, disagreement; 0–0.2, slight agreement; 0.2–0.4, fair agreement; 0.4–0.6, moderate agreement; 0.6–0.8, substantial agreement; and 0.8–1, almost perfect agreement. Data analyses were performed using STATISTICA version 13 (TIBCO Software Inc., College Station, Ireland).

3. Results

The most prevalent positive reactions and highest mean values in all performed tests were reported against *Secale cereale* (IDT, 76%; MAST, 76%), tree I: *Betula Alnus*, *Corylus* spp. (IDT, 71%; MAST, 67%), grass (IDT, 62%; MAST, 67%), and *Brassica napus* (IDT, 52%; MAST, 52%) allergens. The lowest intensity positive response was observed against the tree II allergens: *Quercus* spp., *Platanus* spp.,

Table 1Analysis of IDT results, IgE concentrations in sera, and agreement (κ) between both tests in atopic horses with pollen allergy.

Allergen	IDT (n = 21)			MAST IgE	Cohen's kappa coefficient	
	N (%) PRs			N (%) PRs		
	All	Early (0.5 hours)	Late (4 hours)	MV (R) kU/L	K	P-value
Rye; <i>Secale cereale</i>	16 (76)	12 (57)	7 (33)	16 (76) 7.3 (0.2–34)	0.475	.0148*
Colza; <i>Brassica napus</i>	11 (52)	9 (43)	5 (24)	11 (52) 3.2 (1.8–15)	0.618	.0023**
Grass; <i>Dactylis glomerata, Lolium perenne, Phleum pratense</i>	13 (62)	10 (48)	4 (19)	14 (67) 2.3 (1.2–7.3)	0.276	.1019
Mugwort; <i>Artemisia vulgaris</i>	5 (24)	4 (19)	2 (10)	7 (33) 1.5 (1.3–12)	0.077	.3586
Ribwort; <i>Plantago lanceolata</i>	5 (24)	5 (24)	1 (5)	9 (43) 2.6 (1.6–17)	0.382	.0273*
Ragweed; <i>Ambrosia</i>	9 (43)	6 (29)	4 (19)	10 (48) 5.2 (2.3–28)	0.329	.0651
Tree I <i>Betula, Alnus, Corylus</i>	15 (71)	13 (62)	4 (19)	14 (67) 9.0 (2.4–31)	0.667	.0011**
Tree II <i>Quercus</i>	3 (14)	3 (14)	0	1 (5) 0.09 (1.8)	−0.0769	.6621

Abbreviations: IDT, intradermal test; MAST, multiple allergen simultaneous test; N, number; PRs, positive reactions; MV mean values; R, ranges.

Significant values are indicated by * ($P < .05$), ** ($P < .01$), *** ($P < .001$).

and *Olea* spp. (IDT, 9.5%; MAST, 4.8%). Cohen's κ coefficients were varied depending on the type of allergen, with values ranging from 0.077 (*Artemisia* spp.) to 0.618 (*Secale cereale*). Agreement (κ) between the IDT and MAST was high when dichotomous outcomes were used, and fair to substantial agreement for 6 of 8 (0.276–0.667) allergens is shown (Table 1).

Using a mean IDT cutoff diameter of at least + and an IgE cutoff value ≥ 1.0 kU/L (manufacturer's standards), the overall sensitivity was 74% (range, 40%–87%) and the specificity was 78% (range, 64%–100%). The overall accuracy of the Polycheck Horse Pollen Panel and IDTs was 77% (range, 62%–90%). The AUC after ROC curve analysis (Fig. 1) for the MAST varied between 0.472 and 0.922 and showed

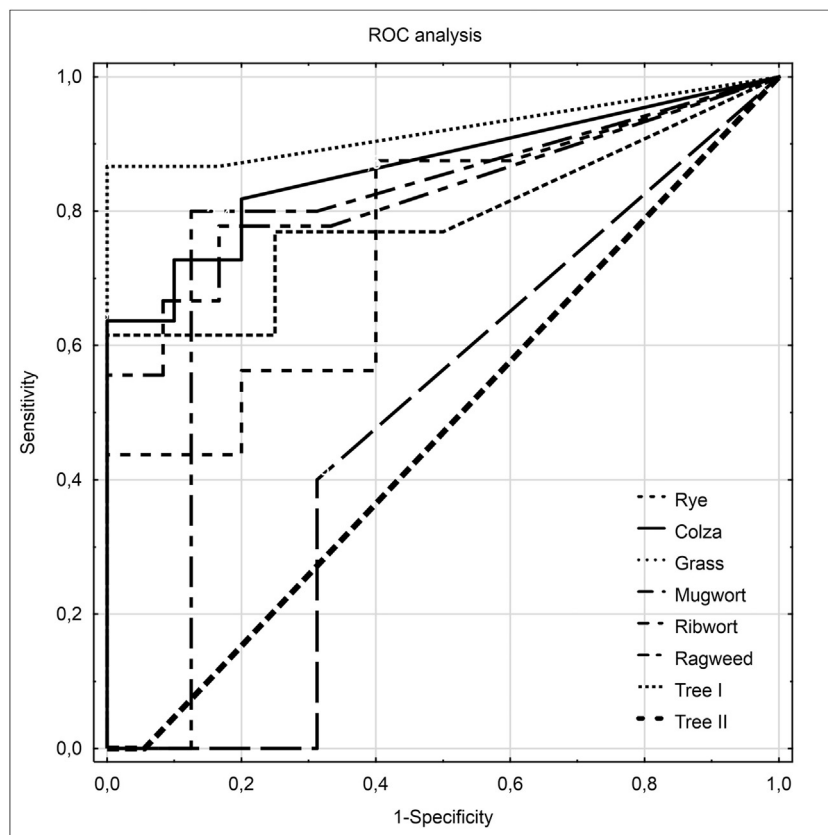


Fig. 1. Comparison of test performance based on receiver operating characteristic (ROC) area under the curve (AUC) for intradermal test and allergen-specific IgE to plant pollen allergens in horses with atopic dermatitis.

Table 2
Comparison of the diagnostic performance of IDT data and specific IgE measurements in response to pollen allergens in horses with atopic dermatitis calculated from the MAST.

Allergen	Cutoff value kU/L	Se (%)	Sp (%)	PPV (%)	NPV (%)	Acc (%)	Youden index	AUC 95% CI
Rye <i>Secale cereale</i>	Man ≥ 1 1.6	86	60	86	60	81	0.475	0.750** 0.515–0.985
	Opt 1.6	86	60	86	60	81	0.475	
Colza <i>Brassica napus</i>	Man ≥ 1 1.8	82	80	82	80	81	0.618	0.864*** 0.699–1.0
	Opt 3.8	64	100	100	71	81	0.636	
Grass <i>Dactylis glomerata</i> , <i>Lolium perenne</i> , <i>Phleum pratense</i>	Man ≥ 1 1.2	77	50	71	57	67	0.571	0.788*** 0.593–0.984
	Opt 2.7	62	100	100	62	76	0.857	
Mugwort <i>Artemisia vulgaris</i>	Man ≥ 1 1.3	40	69	29	79	62	0.088	0.481 0.210–0.753
	Opt 1.3	49	69	29	79	62	0.088	
Ribwort <i>Plantago lanceolata</i>	Man ≥ 1 1.6	80	88	44	92	71	0.488	0.769** 0.516–1.0
	Opt 2.6	80	88	67	93	86	0.675	
Ragweed <i>Ambrosia</i>	Man ≥ 1 2.3	78	67	64	80	71	0.444	0.824*** 0.623–1.0
	Opt 3.4	78	83	78	83	81	0.611	
Tree I <i>Betula</i> , <i>Alnus</i> , <i>Corylus</i>	Man ≥ 1 2.4	87	83	93	71	86	0.700	0.922*** 0.803–1.0
	Opt 3.6	87	100	100	75	90	0.867	
Tree II <i>Quercus</i> , <i>Platan</i> , <i>Olea</i>	Man ≥ 1 1.8	0	94	0	85	81	−0.056	0.472
	Opt 1.8	0	94	0	85	81	−0.056	0.125–0.820

Abbreviations: Opt, optimal; Se, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value; Acc, accuracy; AUC, area under the curve. Manufacturer (Man) and optimal (Opt) cutoff points were determined using the Youden index. Significant values are indicated by * ($P < .05$), ** ($P < .01$), *** ($P < .001$).

good predictive value with the sIgE to tree I pollen having the highest AUC value (AUC = 0.922), followed by that to colza (*Brassica napus*) (AUC = 0.864), ragweed (*Ambrosia* spp) (AUC = 0.824), grass allergen mixture (AUC = 0.788), ribwort (*Plantago lanceolata*) (AUC = 0.769), rye (*Secale cereale*) (AUC = 0.750). The statistically optimal cutoff value (based on Youden's index) defined from the ROC analysis overlapped with the manufacturer cutoff values in 3 of 8 allergens (37.5%), and the remaining 62.5% were higher than those recommended by the manufacturer (Table 2).

4. Discussion

In this study, we assessed the diagnostic value of a screening enzyme immunoassay for the quantitative determination of levels of allergen-specific IgE against widespread plants and common exposition in European countries in seasonal allergic dermatitis in horses [17]. To our knowledge, this is the first study investigating the analytical value of the MAST for an etiologic diagnosis of pollinosis in horses with seasonal atopic dermatitis. To date, little research has addressed the relevance of plant pollen in the development of atopic dermatitis in horses with seasonal allergy symptoms. Serological diagnosis in allergic dermatoses in humans and animals currently plays a key role in identifying allergenic factors [18,19] and displaces traditional tests *in vivo*. Simultaneous detection of sIgE to multiple analytes has replaced the determination of individual allergens, becoming the leading method in the practice of medicine and veterinary allergists. Such methods reduce the required amount of serum sample and the amount of time and labor needed, thereby decreasing the direct costs of the tests. In human medicine, tests such as the MAST have been studied using

approved *in vivo* skin prick tests and *in vitro* (Phadia, sIgE) methods [14,20].

Our results support a significant positive sensitization rate to cultivated plant, tree, and grass allergens in the studied horse population. The polysensitization pattern, which was more than 3/4 of the overall sensitization group, was apparently high. Previous studies have shown contradictory results, showing the highest sensitization rate for grass allergens. A possible reason for these discrepancies could be differences in animal selection. In our study, the main criteria for animal selection were cutaneous hypersensitivity and a positive IDT reaction to plant allergens. A comparison of ST results (sIgE in sera) and IDT was previously performed in horses mainly to study recurrent airway obstruction [1,21,22] and insect hypersensitivity [23–25] but very rarely to examine skin atopic hypersensitivity [11,12,26]. Tilley et al. showed a fair strength of agreement for molds and cultivated grass extracts, poor strength of agreement for wild grass extracts, and no agreement for tree and weed extracts. Morgan et al. showed a substantial agreement for only 2 (timothy and quack grasses) of the 61 allergens with κ values greater than 0.60. Using ELISA, Tahon et al. obtained positive sIgE reactions for yellow duck and timothy grass allergens. Thus, the sensitivity, specificity, and agreement of the different sIgE tests vary according to the group of allergens and the type of allergic disease. Veterinary laboratories use extracts prepared by different manufacturers and concentrations of major allergens as source material for their assays, thereby stymieing interlaboratory comparisons [26]. Recombinant allergen components generally reveal a higher correlation coefficient than the corresponding whole allergen [27]. In the future, the standardization of allergen recombinants may be useful for the standardization of the procedures in horses with allergies.

We also reevaluated MAST cutoff points to avoid false-positive results. On the basis of the Youden index, the optimal cutoff value defined by the Youden index overlapped with the manufacturer cutoff in 3 of 8 plant allergens. In the other cases (5 of 8), the optimal cutoff point shown here was higher than that given by the manufacturer. These results suggest that the specificity of the test should be increased at the expense of the sensitivity. Receiver operating characteristic analyses help determine a suitable cutoff point for each test, with the aim of approaching a 100% certainty of diagnosis [28]. Our results suggest that increasing the cutoff points could improve the accuracy and specificity of the MASTs.

False-negative reactions during asymptomatic periods of atopic dermatitis are one of the major restrictions of STs resulting from the periodic presence of sIgE in the blood. Scheduling testing during the symptomatic period, in which we can expect the presence of sIgE in sera, is crucial for STs, whereas IDTs are performed after drug withdrawal during remission in the fall and winter [15]. Recent studies have shown the potential impact of climate change on aeroallergens and, consequently, on the course of allergic diseases. Anemophilous pollen can be transported and deposited at great distances, impacting pollen amount, allergenicity, season, plant and pollen distribution, and other plant attributes [5,29]. In our studies, blood was collected during the symptomatology period and the point at which the pollen allergens were at their highest concentration in the environment. These periods correlate with the occurrence of the flowering period of the plants in the sampling area. The greatest severity of symptoms associated with allergies to pollens, where the studies were conducted, occurs from winter to spring (trees) and summer to autumn (grasses, weeds).

In conclusion, our study suggests that atopic horses, similar to humans and other species of animals, are sensitized to plant allergens. Cultivated plant, tree, and grass pollens had a higher sensitization rate, and polysensitization was the main type of sensitization observed. Simultaneous testing of allergen components may help distinguish among, for example, mite, pollen, and insect allergy but cannot be used to distinguish healthy from atopic horses because of the possibility of false-positive and -negative reactions. Further data collection and evaluation of the MAST in horses with atopic dermatitis from other populations and geographical regions and comparative studies with other sIgE measurement methods are needed.

References

- [1] Tahon L, Baselgia S, Gerber V, Doherr MG, Straub R, Robinson NE, Marti E. In vitro allergy tests compared to intradermal testing in horses with recurrent airway obstruction. *Vet Immunol Immunopathol* 2009;127:85–93.
- [2] Stepnik CT, Outerbridge CA, White SD, Kass PH. Equine atopic skin disease and response to allergen-specific immunotherapy: a retrospective study at the University of California-Davis (1991–2008). *Vet Dermatol* 2012;23:29–35.
- [3] Mueller RS, Jensen-Jarolim E, Roth-Walter F, Marti E, Janda J, Seida AA, DeBoer D. Allergen immunotherapy in people, dogs, cats and horses – differences, similarities and research needs. *Allergy* 2018;73:1989–99.
- [4] Asam C, Hofer H, Wolf M, Aglas L, Wallner M. Tree pollen allergens—an update from a molecular perspective. *Allergy* 2015;70:1201–11.
- [5] D'Amato G, Bonini S, Nunes C, Annesi-Maesano I, Behrent H, Liccardi G, Popov T, van Cauwenberge P. Allergenic pollen and pollen allergy in Europe. *Allergy* 2007;62:976–90.
- [6] Costa LRR, Johnson JR, Baur ME, Beadle RE. Temporal clinical exacerbation of summer pasture-associated recurrent airway obstruction and relationship with climate and aeroallergens in horses. *Am J Vet Res* 2006;67:1635–42.
- [7] Jensen-Jarolim E, Einhorn L, Herrman I, Thalhammer JG, Panakova L. Pollen allergies in humans and their dogs, cats and horses: differences and similarities. *Clin Transl Allergy* 2015;5:15.
- [8] Dixon PM, McGorum B. Pasture-associated seasonal respiratory disease in two horses. *Vet Rec* 1990;126:9–12.
- [9] Francqueville M, Sabbah A. [Allergic rhinitis in the horse: first case]. *Allerg Immunol (Paris)* 1990;22:56–60.
- [10] Ward MP, Couetil LL. Climatic and aeroallergen risk factors for chronic obstructive pulmonary disease in horses. *Am J Vet Res* 2005;66:818–24.
- [11] Lorch G, Hillier A, Kwochka KW, Saville WJ, Kohn CW, LeRoy BE. Comparison of immediate intradermal test reactivity with serum IgE quantitation by use of a radioallergosorbent test and two ELISA in horses with and without atopy. *J Am Vet Med Assoc* 2001;218:1314–22.
- [12] Radwanski NE, Morris DO, Boston RC, Cerundolo R, Lee KW. Longitudinal evaluation of immunological responses to allergen-specific immunotherapy in horses with IgE associated dermatological disease, a pilot study. *Vet Dermatol* 2019;30:255–e78.
- [13] Marti E, Gerber V, Wilson AD, Lavoie JP, Horohov D, Crameri R. Report of the 3rd havemeyer workshop on allergic diseases of the horse, Hólar, Iceland, June 2007. *Vet Immunol Immunopathol* 2008;126:351–61.
- [14] Wolthers OD, Staberg M. The usefulness of the multiple allergen simultaneous test-chemiluminescent as compared to the phadia immunocap IgE test panel system in children and adolescents. *Recent Pat Inflamm Allergy Drug Discov* 2013;7:96–9.
- [15] Wilkolek P, Pomorski ZJ, Szczepanik MP, Adamek Ł, Pluta M, Taszkun I, Gołyński M, Rozwód A, Sitkowski W. Assessment of serum levels of allergen-specific immunoglobulin E in different seasons and breeds in healthy horses. *Pol J Vet Sci* 2014;17:331–7.
- [16] Lebis C, Bourdeau P, Marzin-Keller F. Intradermal skin tests in equine dermatology: a study of 83 horses. *Equine Vet J* 2002;34:666–72.
- [17] Lane MJ, Pucheu-Haston CM, Kearney MT, Woodward M. Determination of irritant threshold concentrations of multiple tree, grass, weed and mould allergens for intradermal testing of horses residing in the southern USA. *Vet Dermatol* 2017;28:604–e147.
- [18] Lee KW, Blankenship K, McKinney B, Kern G, Bush J, Greenwood J, Brazis P, Drouot L, Tambone C, Faas R, Weaver G. Proficiency monitoring of monoclonal antibody cocktail-based enzyme-linked immunosorbent assay for detection of allergen-specific immunoglobulin E in dogs. *J Vet Diagn Invest* 2015;27:461–9.
- [19] Wagner B. Immunoglobulin E and allergy. *Equine Vet J* 2016;48:13–4.
- [20] Lee JH, Park KH, Kim HS, Kim KW, Sohn MH, Kim CH, Lee JS, Hong CS, Park JW. Specific IgE measurement using AdvanSure® system: comparison of detection performance with ImmunoCAP® system in Korean allergy patients. *Clin Chim Acta* 2012;413:914–9.
- [21] Tilley P, Sales JP, Ferreira MB. Comparison of skin prick tests with in vitro allergy tests in the characterization of horses with recurrent airway obstruction. *J Equine Vet Sci* 2012;32:719–27.
- [22] Niedzwiedz A, Jaworski Z, Kubiak K. Serum concentrations of allergen-specific IgE in horses with equine recurrent airway obstruction and healthy controls assessed by ELISA. *Vet Clin Pathol* 2015;44:391–6.
- [23] Ferroglio E, Pregel P, Accossato A, Taricco I, Bollo E, Rossi L, Trisciuglio A. Equine Culicoides hypersensitivity: evaluation of a skin test and of humoral response. *J Vet Med A Physiol Pathol Clin Med* 2006;53:30–3.
- [24] Langner KF, Darpel KE, Drolet BS, Fischer A, Hampel S, Heseler JE, Mellor PS, Mertens PP, Leibold W. Comparison of cellular and humoral immunoassays for the assessment of summer eczema in horses. *Vet Immunol Immunopathol* 2008;122:126–37.
- [25] Wilkolek P, Sitkowski W, Szczepanik M, Adamek Ł, Pluta M, Taszkun I, Gołyński M, Malinowska A. Comparison of serum concentrations of environmental allergen-specific IgE in atopic and healthy (nonatopic) horses. *Pol J Vet Sci* 2017;20:789–94.
- [26] Morgan EE, Miller Jr WH, Wagner B. A comparison of intradermal testing and detection of allergen-specific immunoglobulin E in serum by enzyme-linked immunosorbent assay in horses affected with skin hypersensitivity. *Vet Immunol Immunopathol* 2007;120:160–7.
- [27] Williams P, Önell A, Baldracchini F, Hui V, Jolles S, El-Shanawany T. Evaluation of a novel automated allergy microarray platform compared with three other allergy test methods. *Clin Exp Immunol* 2016;184:1–10.
- [28] Agarwal R, Aggarwal AN, Garg M, Saikia B, Chakrabarti A. Cut-off values of serum IgE (total and A. fumigatus -specific) and eosinophil count in differentiating allergic bronchopulmonary aspergillosis from asthma. *Mycoses* 2014;57:659–63.
- [29] Beggs PJ. Impacts of climate change on aeroallergens: past and future. *Clin Exp Allergy* 2004;34:1507–13.