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Comparison of Four Different Allergy Tests in Equine Asthma Affected Horses and Allergen Inhalation Provocation Test

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ABSTRACT

Potential triggers for equine asthma are allergens from hay and straw dusts, mold spores and storage mites. The contribution of these environmental trigger factors to equine asthma is still largely uncertain. The aim of this study was to compare results of four allergy tests from healthy and asthma-affected horses, and to evaluate the clinical relevance of allergens tested positive via specific inhalation provocation test. Fifteen horses were classified using a clinical scoring system as asthmatic (n = 9) or control (n = 6). Four different allergy tests (functional in vitro test, intradermal test, Fc-epsilon receptor test, and ELISA for allergen-specific IgE) were compared. A histamine inhalation provocation test as positive control was performed in all horses and the interpleural pressure was measured. In addition, two individual allergens were chosen for the allergen inhalation provocation test based on the results of the allergy tests and inhaled in increasing concentrations, until signs of dyspnea occurred. None of the four allergy tests could differentiate reliably between controls and asthma-affected horses. There was no agreement among the results of the four allergy tests. The interpleural pressure results showed a large individual variability. A clear positive reaction on the allergen inhalation provocation test was only detected in two asthma-affected horses 6 hours after allergen inhalation with *Aspergillus fumigatus* and *Cladosporium herbarum*. In most cases a purely type I immediate reaction is unlikely to be involved in causing the clinical signs of equine asthma. Because of a delayed reaction after allergen provocation in two horses, the involvement of cell-mediated type III or IV hypersensitivity may be possible. As all allergy tests used in this study can only detect IgE-mediated hypersensitivity, these tests are probably not suitable for an etiological diagnosis of equine asthma.

1. Introduction

Equine asthma is the most common non-infectious respiratory disease occurring in adult horses [1]. The disease has previously been referred to as recurrent airway obstruction (RAO), heaves, chronic obstructive bronchitis (COB), and chronic obstructive pulmonary disease (COPD), though the latter term is now considered obsolete, due to the difference in pathogenesis between equine asthma and human COPD. The presence of organic dust in bedding and feed, as well as the concentration of ammonia in the stable environment, play an important role in the manifestation of the disease. Numerous potentially pro-inflammatory particles have been identified in stable dust, including bacterial endotoxins, over 50

Abbreviations: BALF, bronchoalveolar lavage fluid; COPD, chronic obstructive pulmonary disease; RAO, recurrent airway obstruction; TBS, tracheobronchial secretion; IL, interleukin; IFN, interferon; Th, T-helper cell.

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Ethical Statement: The study was approved by the regional legal agency for animal experiments of the Government of Upper Bavaria, Germany (No. 55.2-1-54-2531-151-10). All of the horse owners signed an informed consent. The study was performed in accordance to the guidelines for animal studies (ARRIVE) and clinical trials (CONSORT).

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different mold species, peptidoglycans, proteases, microbial toxins, storage mites, plant particles, and inorganic dusts [2,3]. The correlation between the manifestation of equine asthma and the feeding of moldy hay has been confirmed in multiple studies. An association between the severity of the neutrophil response in equine asthma and the concentration of mold spores has been demonstrated, both in a study of the exposure to natural hay and straw [3], as well as the inhalation of a hay-dust suspension [4]. The inhalation of an extract of *Aspergillus fumigatus*, as well as *Faenia rectivirgula*, triggered an airway neutrophil response with pulmonary dysfunction in asthmatic horses in disease remission, but not in control horses [5,6]. In contrast to these results, *Faenia rectivirgula* led to an increase in airway neutrophilic granulocytes in both asthmatic horses and controls [7]. Similar results were achieved with the inhalation of a suspension containing mold spores, lipopolysaccharides and silica microspheres, which triggered a neutrophil response in both healthy horses and horses with asthma [8]. Mold spores seemed to be the allergen involved in the pathogenesis of equine asthma, whereby the severity was exacerbated by other organic dust particles, endotoxins, and factors such as cold, dry air, and harmful gases [9].

The exact immunological mechanisms causing the clinical signs of equine asthma are not yet fully explained, though many studies support the involvement of a hypersensitivity reaction [10]. This is characterized by a Th1/Th2 imbalance, in which the Th1 response, the predominant response in healthy horses, shifts to a Th2 response with increased production of Th2 cytokines [11]. Prolonged allergen exposure sustains mast cell activation, resulting in a chronic inflammatory response [12]. Multiple studies have identified excessive Th2 cytokine expression in the lungs of horses affected by equine asthma [13–15]. The chronic inhalation of dust particles causes the excessive stimulation of macrophages, which have a chemotactic effect on neutrophilic granulocytes by increased cytokine production, especially by interleukin-8 (IL-8) [16]. Neutrophilic granulocytes migrate to the airways within six to eight hours following allergen exposure [17].

Equine asthma seems unlikely to be a simple immediate hypersensitivity reaction, since the clinical signs generally only appear several hours after allergen exposure [11,18]. Allergen provocation resulted in neither an increased histamine concentration in the bronchoalveolar lavage fluid (BALF) nor an acute bronchospasm [5,19]. While some authors consider types I and III hypersensitivities the dominant mechanisms [20,21], other attribute equine asthma primarily to a delayed immune response [5,22]. They argue that this immune response causes an increase in neutrophilic granulocytes in the respiratory tract, and an elevated CD4+ T cell count can be measured in the BALF, but not in the serum. Whether this consists of T helper (Th) 1 cells, Th 2 cells, or a combination of both has yet to be determined. A combination of type I and type IV reactions has also been assumed [11]. As such, it has not yet been unequivocally determined whether equine asthma can definitely be attributed to one type of hypersensitivity or is a complex of various etiological and immunological manifestations.

Several serological tests are offered commercially to test for the possible allergic pathogenesis of equine asthma involving free IgE in serum despite conflicting data regarding their diagnostic value and many authors confirming their low ability to differentiate between asthma affected horses and controls [18,23–25]. Nevertheless, a type I hypersensitivity may still play a role in allergic pulmonary disease, as the hypersensitivity may not be triggered by free serum IgE antibodies with a very short half-life, but rather solely by degranulation of basophilic granulocytes and mast cells upon crosslinking of surface bound allergen-specific IgE by allergen [26]. The in vitro stimulation of peripheral basophilic granulocytes and mast cells, the effector cells of a Type I allergy, was reported to be an appropriate method for the identification of a sensitization to allergens, which play a role in the pathogenesis of equine asthma [27–29], but again, published data is controversial [25] and it could also be related to a mast cell asthma phenotype.

The intradermal test (IDT, also referred to as intracutaneous test, ICT) is considered the gold standard for allergen identification in atopic dermatitis by assessment of the functional sensitization of the mast cells asthma [30]. Diluted allergens are injected intradermally, physiological saline solution or allergen diluent is used as a negative control and histamine as a positive control. The local reaction to the injected allergen solution is then evaluated in relation to the negative and positive control [25]. The time-span from injection to positive reaction may hind to the type of allergy (30 min type I, 4 hours type III, 24–72 hours type IV [25]. Controversial results have been found in equine asthma [31–33].

To test for a local response of the bronchial epithelium, IgE levels were evaluated in lung tissue and bronchoalveolar lavage fluid (BALF). While some authors found increased levels [24,34], others did not [23,35]. Inhalation provocation tests might be a better approach and can be used to test for nonspecific or specific hyperreactivity: The histamine inhalation provocation test (HIPT), first described over 40 years ago for the horse, reflects the nonspecific reactivity of the airways [36]. Nonspecific hyperreactivity was shown in 25% of mildly and in 100% of severely affected asthmatic horses. Horses inhaled histamine solutions of increasing concentrations for two minutes each, until the first signs of dyspnea occurred. The inhaled histamine has a reflexive stimulatory effect on the vagal nerve, and a direct effect on the histamine receptors of the bronchial musculature, triggering a bronchospasm in hyperreactive horses [37].

A more specific approach is the allergen inhalation provocation test (AIPT), long and commonly used for the identification of specific allergens in the pathogenesis of human asthma [38]. It has also been used in horses for various allergens suspected to be involved in the pathogenesis of equine asthma [3,5,8,39,40]. The procedure is not standardized, most authors either use diluted allergen solutions, which are nebulized by an inhalation device, or expose the horses to moldy hay. Various instruments for the measurement of pulmonary pressure and hemodynamic monitoring are used to monitor and evaluate the reactions of the horses. Bronchial provocation tests with various allergens such as Micropolyspora faeni [5–7], Aspergillus fumigatus [5,6], and hay dust [4,6] led to an obstruction of the airways of predisposed horses within hours of exposure, while healthy control horses showed no reaction.

In this study, the results of different, commercially available allergy tests for equine asthma were compared with results of clinical scoring, tracheobronchial secretion (TBS) and bronchoalveolar lavage fluid (BALF) cytology as well inhalation challenge tests (histamine and specific allergens). The aim was examine the significance of individual allergens in the clinical manifestation and pathology of equine asthma and to evaluate the reliability of commercially available tests.

2. Materials and Methods

2.1. Study design and included subjects

In total, 15 horses participated in the study, including 6 horses without a history of respiratory disease ("control group") and 9 horses with a history of equine asthma of various degrees ("equine asthma group"). Participation in the study required that the horses were free of further diseases (specifically other allergic diseases such as insect-bite hypersensitivity), that the differential blood

Table 1
Clinical scoring modified from OHENSORGE et al. [30]: total score [RU] of the patients healthy (0–1 points), mild (2–3 points), moderate (4–6 points), or severe (>7 points) equine asthma.

Parameter		Score
Coughing	History, spontaneous or inducible coughing	1
Dyspnea at rest	Abdominal breathing	1
	Nostril flaring, heaves line	3
Auscultation	Rattling or wheezing	2
Percussion of the lung field	>1 hands enlarged	1
	> 2 hands enlarged	2
A-aDO2	7–14 mmHg	1
	>14 mmHg	2
Bifurcatio tracheae	Thickenend	1
Mucus scoring (amount, viscosity)	Moderately increased	1
	Severely increased	2
Neutrophilia in TBS/BALF cytology	prominent	1

count was within the normal range, and that the horses had not been treated with glucocorticoids for at least 6 weeks before the start of the study. All other medications, specifically bronchodilators, were discontinued at least 3 weeks before the start of the study.

The study was approved by the regional legal agency for animal experiments of the Government of Upper Bavaria, Germany (No. 55.2-1-54-2531-151-10). All of the horse owners signed an informed consent. The study was performed in accordance to the guidelines for animal studies (ARRIVE) and clinical trials (CONSORT).

For the identification of antigens, blood samples were taken 14 days ahead at the horses' accustomed environment for a functional in-vitro test as described below Clinical scoring and in-vivo provocation tests, Clinical examinations and provocation tests (intradermal, histamine and allergen inhalation provocation test) were performed over 5 days under an equine hospital setting.

2.2. Clinical scoring

The classification of the horses was achieved by a comprehensive pulmonary evaluation and use of a clinical scoring system for equine asthma, modified from Ohnesorge et al. [41] shown in Table 1. The variables examined included were breathing rate at rest, grade of dyspnea (relative units [RU] in the range of 0–3), nasal discharge (RU 0–3), coughing (RU 0–1), auscultation of lungs and trachea (RU 0–2), percussion of the lung field (RU 0–2), arterio-alveolar pressure gradient (RU 0–2), amount and viscosity of tracheal secretion (0–5) and tracheobronchial secretion (TBS) cytology. When horses were not in severe dyspnea, bronchoalveolar lavage and cytology was performed to confirm the results of TBS cytology using a balloon catheter (Bivona, Smith Medicals, St. Paul, USA) and 200 ml of sterile 0.9% saline.

2.3. Serological tests for identification of antigens (IgE ELISA)

Blood samples from the jugular vein were taken from all the horses in their accustomed environment for the implementation of the allergy tests 14 days before the provocation tests described below. Serum samples for the implementation of two IgE ELISA tests, the Fc-epsilon receptor test (ALLERCEPT, Heska Allergy Products) and the IBL test (Allergy-40 profile IgE horse ELISA, Allergovet GmbH), were frozen at −20°C and stored until assayed.

The Fc-epsilon receptor test measures specifically free IgE in ELISA absorbance (EA) units from 0 to 4000. Values above 150 are considered as positive. For a better comparison to the other allergy tests values were categorized from 0 to 5.

The IBL test is an enzyme immunoassay (EIA) for the quantitative determination of horse IgE. The resulting color was measured spectrometrically at 450 nm. The results were reported in units/ml using a validated rating scale. The spectrum ranges from 0 to >50 units/ml, where values of 0.7 and greater are considered positive.

2.4. Functional in-vitro test (FIT)

Whole blood samples (EDTA), also taken 14 days before the provocation tests, were sent immediately by overnight express service to the immunology working group at the University of Veterinary Medicine Hannover, Foundation, Germany for the implementation of the functional in vitro test (FIT) as described before [26]. For this test, peripheral blood cells are isolated and incubated with specific antigens for one hour. Subsequently, the cell-free supernatant is removed. With sufficient sensitized basophils present, the histamine released is detectable via an ELISA. Thus, the FIT exclusively determines the functional sensitization of the effector cells of a type I hypersensitivity. As sensitized basophils survive in vivo for months or even years, the FIT may also give positive results when clinical signs are absent.

2.5. Intradermal test (IDT)

The intradermal test (IDT) was performed on the left side of the neck after clipping of 20 × 30cm and setting of color markers 3 cm apart. The different mold, mite, pollen, insect and other allergen solutions **(**Artu Biologicals Europe B.V., Lelystad, Niederlande**)** were injected above the markers and scored 0.5–1 hours (early phase reaction) and 2–4 h (late phase reaction) as RU of 0–4 respectively. The intradermal tests were always read by the same examiner. Tested allergens are listed in Table 6.

2.6. Histamine inhalation provocation test (HIPT)

An esophageal catheter to measure interpleural pulmonary pressure (Venti-Graph, Boehringer Ingelheim, Germany) and an inhalation mask (SaHoMa-II mobile ultrasonic nebulizer, HM-200, NEBU-TEC Medical Products, Elsenfeld, Germany) were placed in the horses' accustomed environment. The nebulizer was powered by a battery pack, which was attached directly to the inhalation mask. The nebulizing capacity specified was 0.35–0.7 ml/min, with 67.86% of the nebulized particles <5µm according to the manufacturer.

Measurement of baseline value was followed by nebulization of a buffer solution (PBS, zero value) and then inhalation of histamine solutions of increasing concentrations starting with a concentration of 0.5 mg/ml in healthy and 0.125 mg/ml in asthma affected horses. Each concentration was inhaled for 2 minutes, followed by a 3-minute break before the next inhalation. Heart and respiratory rates as well as the subject's general condition were documented throughout the procedure. The provocation test was discontinued,

when the horse displayed defensive movements, pronounced dyspnea, increased coughing, a heart rate over 60 b/min, or an increase in interpleural pressure of more than 70% over the initial value.

2.7. Allergen inhalation provocation test (AIPT)

Following the HIPT, an AIPT was implemented on each of the next two days. Two allergen solutions (Artu Biologicals Europe B.V., Lelystad, Niederlande), selected based on the history and results of the allergy tests (shown in Table 7), were inhaled in 1:10, 1:100, and 1:1000 PBS dilutions of the original substance. When a horse reacted to the inhalation of the first allergen, no second allergen was inhaled on the following day due to safety reasons. Interpleural pressure measurement was performed as described for the HIPT.

In order to identify a possible delayed reaction, the subjects were monitored clinically over the following 24 hours. In addition, an esophageal catheter was inserted 6 hours after the completion of the AIPT and the interpleural pulmonary pressure was measured again.

2.8. Statistical Analysis

All statistical analyses were conducted using the Software IBM SPSS 23 Statistics for Windows and Microsoft Office Excel 2007. The mean value ± and standard deviation (SD) were calculated for all results. Furthermore, the data obtained was evaluated for normal distribution. Data was normally distributed except the interpleural pressure. Chi-Square test and Fisher's exact test were used to compare groups of control and asthma-affected horse. Statistical power was calculated with >80% with a postulated minimum difference of 40 percentage units. Agreement between individual tested allergens of four different allergy tests was calculated with Cohen's kappa for n rater. To evaluate the blood gas parameters, the simple t-test was used. For the statistical examination of the results of the inhalation challenge, especially the pleural pressure values, the t-test, the Mann-Whitney test and the Wilcoxon test were used.

3. Results

3.1. Included subjects

In total, 15 horses were included. The control group without a history or clinical signs of equine asthma (n = 6) had an average age of 17 years (±8.1), with an average size of 155 cm (±11), and average body weight of 497 kg (±76). The equine asthma group (n = 9) had an average age of 14.1 years (±4.6), an average size of 154 cm (±7.5), and an average body weight of 475.9 kg (±44.9). The horses were of various gender (one stallion, five mares, four geldings in the control group and two stallions, two mares, and four geldings in the asthma group) and breed (three trotters, two Haflinger, one warmblood in the control group and three warmbloods, two trotters, one Tinker, one Andalusian, one Pinto, and one Quarter in the asthma group).

3.2. Clinical scoring

Six horses without a history of respiratory disease were confirmed to be healthy (overall scoring 0–1 points), horses with a history of equine asthma were confirmed to suffer from moderate (n = 3, overall scoring 4–6 points) to severe (n = 6, overall scoring 7–12 points) equine asthma. Moderate (10%–25%) to severe (>25%) neutrophilia was confirmed in BALF cytology for three and four horses, respectively, in two horses, no BAL was performed due to severe dyspnea and hypersecretion. The results of the clinical scoring, modified from Ohnesorge et al. [41], are shown in Table 2.

Table 2
Clinical results [relative units, RU], arterial blood gas values [mmHg], Mucus quantity and quality in the trachea [RU], Cytology of the tracheobronchial mucus [RU] and total score [RU] of the patients modified from OHNESORGE et al. [41].

Horse no.	Breath per minute	Nasal discharge [RU]	Coughing [RU]	Dyspnea at rest [RU]	Auscultation [RU]	Percussion of lungfield	PaO_2 [mmHg]	$PaCO_2$ [mmHg]	A-aDO_2 [mmHg]	Amount of tracheal secretion [0-5 RU]	Viscosity of secretion [0-5 RU]	Cytology of TBS [RU]	Total score [RU]
1	10	0	0	0	0	0	105	37	0	1	3	0	0
2	10	0	0	0	0	0	89	44	1	0	0	0	0
3	12	0	0	0	0	0	89	43	2	0	0	1	1
4	14	0	0	0	0	0	93	43	0	2	1	0	0
5	10	0	1	0	0	0	95	44	0	1	2	0	1
6	10	0	0	0	0	0	96	42	0	1	3	0	0
7	18	1	1	1	0	0	90	37	7	3	2	1	6
8	16	1	1	0	0	0	87	47	4	3	3	1	4
9	24	1	1	3	2	1	73	41	25	2	4	1	12
10	12	1	1	0	2	0	95	42	2	3	3	1	5
11	24	0	1	0	2	0	93	39	10	3	3	1	7
12	22	1	1	3	2	0	82	49	11	5	4	1	11
13	20	1	1	3	2	0	73	48	19	4	4	1	12
14	18	1	1	3	2	0	87	49	7	4	3	1	11
15	28	1	1	3	2	1	86	48	5	5	4	1	11

Horses classified as controls are marked in gray.

Table 3
Results of Fc-epsilon-receptor test.

	Control group (n = 6)				Equine asthma group (n = 9)			
Allergens	mean	min	max	n (%) +	mean	min	max	n (%) +
Mould								
Alternaria alternata	28	6	71	**0**	2	0	19	**0**
Aspergillus fumigatus	20	4	51	**0**	8	0	52	**0**
Cladosporium herbarum	23	5	68	**0**	2	0	15	**0**
Penicillium sp.	19	1	59	**0**	1	0	5	**0**
Mites								
D. farinae	163	32	377	**2 (33%)**	219	33	708	**5 (56%)**
D. pteronyssinus	119	27	301	**2 (33%)**	170	52	591	**3 (33%)**
Tyrophagus p.	104	38	237	**1 (17%)**	238	40	703	**4 (44%)**
Acarus siro	78	2	145	**0**	86	12	376	**1 (11%)**
L. destructor	51	16	148	**0**	26	5	55	**0**
Pollen								
Gramineae mixtum	34	8	58	**0**	28	6	77	**0**
Agrostis alba	42	19	78	**0**	48	0	121	**0**
Cynodon dactylon	36	19	94	**0**	48	0	138	**0**
Sorghum halepensis	42	11	76	**0**	64	0	170	**1 (11%)**
Rumex crispus	56	13	212	**1 (17%)**	84	0	301	**3 (33%)**
Plantago lanceolata	158	20	414	**3 (50%)**	39	0	138	**0**
Artemisia vulgaris	20	10	32	**0**	16	0	41	**0**
Chenopodium album	58	25	114	**0**	55	17	117	**0**
Urtica dioica	50	11	147	**0**	5	0	27	**0**
Ambrosia mixtum	1	0	4	**0**	3	0	14	**0**
Parietaria officinalis	54	17	185	**1 (17%)**	24	8	45	**0**
Salsola kali	78	8	208	**2 (33%)**	123	2	377	**2 (22%)**
Betula sp.	108	6	214	**3 (50%)**	20	0	94	**0**
Alnus sp.	140	0	333	**2 (33%)**	81	5	289	**2 (22%)**
Quercus sp.	35	8	79	**0**	5	0	24	**0**
Cypressus sp.	10	0	29	**0**	19	0	111	**0**
Corylus avellana	79	6	184	**2 (33%)**	51	11	116	**0**
Ulmus campestris	27	6	88	**0**	42	0	172	**1 (11%)**
Fagus sylvatica	85	0	277	**1 (17%)**	36	0	133	**0**
Populus sp.	117	0	266	**2 (33%)**	46	5	177	**1 (11%)**
Acer pseudoplantus	46	1	110	**0**	23	0	77	**0**
Salix sp.	68	0	209	**1 (17%)**	8	0	30	**0**
Olea euopaea	201	8	646	**2 (33%)**	42	0	146	**0**
Cryptomeria sp.	22	1	52	**0**	45	0	111	**0**
Insects								
Ctenocephalides salivae	22	0	105	**0**	10	0	31	**0**
Blattela germanica	79	5	292	**1 (17%)**	14	0	109	**0**
others		0	0			0	0	
Felis catus epithelium	25	0	104	**0**	11	0	37	**0**
Total positive				**26 (12%)**				**23 (7%)**

Abbreviations: max = largest value measured; min = smallest value measured.
Allergen-specific serum IgE level, measured in the horses of the control group and the equine asthma group by the Fc-epsilon receptor tests (Allercept).
n (%) + = Number and percentage of horses with a positive result. mean = mean of all allergy units (AU) measured in the group; IgE-Level "cut off" of 150 allergy units.

3.3. Serological tests for identification of antigens (IgE ELISA)

Fc-ε-receptor test (Allercept): Half of the controls and 7/9 asthmatic horses tested positive for at least one allergen. Overall, control horses showed more positive reactions (26/216, 12%) than asthmatic horses (23/324, 7%; Fig. 2a), but this difference was tested insignificant. No horse had an IgE concentration over the manufacturer's cut off level of 150 allergy units (AU) for assumed clinical significance against any of the tested mold allergens (Fig. 2b). Results are shown in detail in Table 3.

IBL Test (Allergy-40 profile IgE horse ELISA Allergovet): Overall, control horses tested positive for 11/240 (5%) and asthmatic horses for 28/360 (8%) of allergens, the difference between groups was not significant including mold antigen (Fig. 2a). None of the controls tested positive for any mold antigen, asthmatic horses showed 10/54 (19%) reactions (no significant difference). Results are shown in detail in Table 4.

3.4. Functional in-vitro test (FIT)

All horses tested positive for at least one allergen preparation. Overall, there were 77/162 (48%) positive reactions in controls and 104/243 (43%) positive reactions in asthmatic horses. Again, there was no significant difference between groups. All asthmatic and 4/6 control horses tested positive for "mold-mix." Results are shown in detail in Table 5.

3.5. Intradermal test (IDT)

All horses of both groups showed more than one positive reaction. Overall, control horses had 85/252 (34%) positive reactions (40 early and 45 late-phase reactions) and asthmatic horses 111/378 (29%) positive reaction (Fig. 2a; 75 early and 36 late-phase reactions). Mold antigens led to positive reactions in only 1/18 possible reactions in controls and 3/27 in asthmatic horses (Fig. 2b),

Table 4
Results of the IBL test.

	Control group (n = 6)	Equine asthma group (n = 9)
Allergenes	**n (%) +**	**n (%) +**
Mould		
Alternarium alternata	**0**	**1 (11%)**
Aspergillus fumigatus	**0**	**3 (33%)**
Epicoccum nigrum	**0**	**0**
Cladosporium herbarum	**0**	**2 (22%)**
Penicillium notatum	**0**	**4 (44%)**
Ustilago tritici	**0**	**0**
Mites		
Acarus	**0**	**0**
D. farinae	**0**	**0**
D. pteronyssinus	**0**	**0**
Tyrophagus	**0**	**1 (11%)**
Glycyphagus domesticus	**0**	**0**
Lepidoglyphus destructor	**0**	**0**
Pollen		
Birch (betula)	**0**	**0**
Beech (fagus sylvatica)	**0**	**0**
Alder (alnus)	**1 (17%)**	**1 (11%)**
Hazel (corylus avellana)	**0**	**0**
Willow (salix)	**0**	**2 (22%)**
Gräsermix	**1 (17%)**	**1 (11%)**
Rye (secale cereale)	**1 (17%)**	**2 (22%)**
Raps	**0**	**1 (11%)**
Ragweed	**0**	**1 (11%)**
Common Sorrel (rumex acetosa)	**1 (17%)**	**2 (22%)**
Plantain (plantago)	**1 (17%)**	**1 (11%)**
Lamb's quarters (chenopodium album)	**0**	**2 (22%)**
Dandelion (taraxacum)	**0**	**1 (11%)**
Stinging nettle(urtica)	**0**	**0**
Mugwort (artemisia vulgaris)	**0**	**0**
Wheat (triticum)	**1 (17%)**	**0**
Barley (hordeum vulgare)	**0**	**1 (11%)**
Oat (avena)	**0**	**0**
Corn (zea mays)	**1 (17%)**	**0**
Treacle	**0**	**0**
Soya	**0**	**0**
Yeast	**0**	**0**
Alfalfa (medicago sativa)	**0**	**0**
Insects		
Flea	**2 (33%)**	**1 (11%)**
Horse fly (Tabanus spp.)	**0**	**0**
Biting midge (Culicoides spp.)	**0**	**0**
Stable fly (Stomoxis spp.)	**2 (33%)**	**1 (11%)**
Blackfly (Simulium spp.)	**0**	**0**
Total positiv	**11 (5%)**	**28 (8%)**

Number of positive results from the IBL (Allergovet) of both groups in comparison.
n (%) + = number and percentage of horses with a positive result. Test results with a value ≥ 2 (classification according to laboratory report) were considered positive.

while all controls and 8/9 asthmatic horses tested positive for mosquito allergens. The results are shown in detail in Table 6.

3.6. Histamine inhalation provocation test (HIPT)

The HIPT was performed in all control and in 8/9 asthmatic horses, one horse (No. 15) showed severe dyspnea and coughing, so it was impossible to place the esophageal catheter. Control horses showed clinical signs of coughing, severe dyspnea or adverse reactions at a mean histamine concentration of 26 mg/ml (4–64 mg/ml), while asthmatic horses reacted to 1.95 mg/ml (0.125–4 mg/ml). This difference was tested significant ($P = .003$). Results are shown in detail in Fig. 1.

Interpleural pressure (IPP) measurements were found to be very unreliable, some horses were still irritated by the esophageal catheter after 30 min and baseline values differed significantly from day to day and different times of the day. The interpleural pressure values measured showed large individual variability and were also significantly increased in some of the horses in the control group. At no time was there a significant increase of the heart or respiratory rate.

3.7. Allergen inhalation provocation test (AIPT)

The AIPT was performed in 13 horses with the allergen of highest relevance in the allergy tests, 10 horses received a further inhalation with a second allergen based on the allergy tests. Two horses (Nos. 9 and 15) were not subjected to the AIPT due to poor general respiratory condition and the unforeseeable consequences of a positive reaction. For the AIPT, the same allergen preparations from the same company were used as for the IDT (ARTU, ALK Netherlands).Three horses (Nos. 4, 13, and 14) required treatment with an inhalative bronchodilator (salbutamol 2 µg/kg, Salbutamol ratiopharm) and an inhalative glucocorticoid (beclomethasone 3 µg/kg, Sanasthmax) to control the acute dyspnea after the inhalation with allergen 1. For this reason, no second allergen was administered.

Table 5
Results of functional in vitro test.

	Control group (n = 6)		Equine asthma group (n = 9)	
Allergens	mean	n (%) +	mean	n (%) +
Mould				
Mould mix	2	**4 (67%)**	3	**9 (100%)**
Skin fungus mix	1	**1 (17%)**	0	**1 (11%)**
Mites				
D. pteronyssinus	2	**4 (67%)**	2	**5 (56%)**
L. destructor	1	**2 (33%)**	1	**4 (44%)**
Acarus siro	2	**4 (67%)**	2	**6 (67%)**
Pollen				
Herbage mix (pollen)	0	**0**	0	**1 (11%)**
Tree mix (early)	1	**1 (17%)**	1	**3 (33%)**
Tree mix (late)	1	**2 (33%)**	1	**3 (33%)**
Pollen late mix	1	**1 (17%)**	1	**2 (22%)**
Corn (zea mays)	1	**2 (17%)**	1	**2 (22%)**
Rye (secale cereale)	1	**3 (50%)**	1	**2 (22%)**
Wheat (triticum)	0	**1 (17%)**	1	**2 (22%)**
Oat (avena)	1	**1 (17%)**	1	**4 (44%)**
Barley (hordeum vulgare)	1	**2 (33%)**	1	**2 (22%)**
Hazel (corylus avellana)	0	**0**	0	**1 (11%)**
Rape (brassica napus)	3	**5 (83%)**	2	**6 (67%)**
Birch	1	**1 (17%)**	1	**2 (22%)**
Herbage-mix (grain)	1	**3 (50%)**	1	**3 (33%)**
Insects				
Culicoides nubeculosus	2	**5 (83%)**	1	**4 (44%)**
Stable fly	2	**4 (67%)**	1	**3 (33%)**
Blackfly (Simuliidae)	2	**4 (67%)**	1	**4 (44%)**
Mayfly (ephemeroptera)	3	**5 (83%)**	2	**6 (67%)**
Moth	3	**5 (83%)**	2	**8 (89%)**
Mosquito (culicidae)	3	**5 (83%)**	2	**9 (100%)**
Horsefly	3	**5 (83%)**	1	**4 (44%)**
Housefly (musca domestica)	2	**3 (50%)**	1	**3 (33%)**
Fire ant (solenopsis invicta)	2	**4 (67%)**	2	**5 (56%)**
Total positive		**77 (48%)**		**104 (43%)**

Comparison of the results of the functional in vitro tests (FIT) for each group.
mean = mean; n (%) + = number and percentage of horses with a positive result. Test results were considered positive with a value ≥ 2 (internal classification), which corresponds to the laboratory's value 1+, which according to the laboratory is a positive result.

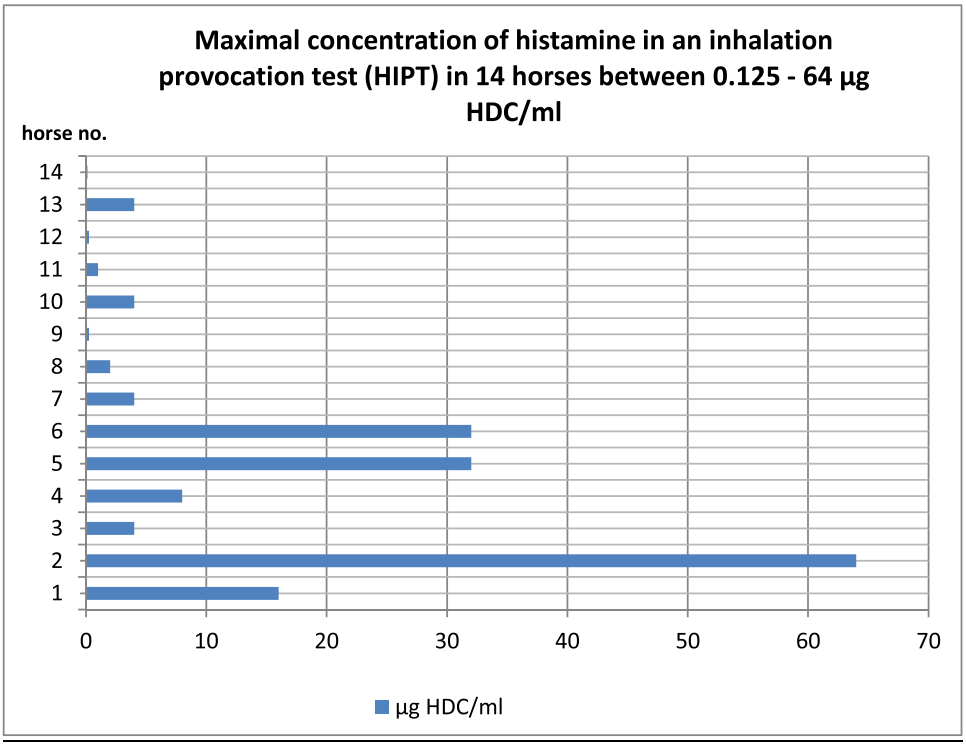

Fig. 1. Maximal concentration of histamine in an inhalation provocation test (HIPT) in 14 horses. HDC = histamine dihydrochloride; horses Nos. 1 to 6 = control group, horses Nos. 7 to 14 = equine asthma group.

Table 6
Results of intradermal test (IDT).

Allergens	Control group (n = 6)			Equine asthma group (n = 9)		
	early (0.5–1 h)	Late (2–4 h)	total	early (0.5–1 h)	late (2–4 h)	total
Mould						
Alternaria alternata	1	0	**1**	0	1	**1**
Aspergillus fumigatus	0	0	**0**	0	1	**1**
Cladosporium herbarum	0	0	**0**	0	1	**1**
Mites						
D. farinae	4	1	**5**	5	4	**9**
D. pteronyssinus	3	2	**5**	4	5	**9**
T. putrescentiae	1	2	**3**	5	0	**5**
Acarus siro	3	1	**4**	3	0	**3**
Lepidoglyphus destructor	1	0	**1**	7	1	**8**
Pollen						
Beech (fagus sylvatica)	0	0	**0**	0	0	**0**
Acer	1	0	**1**	3	1	**4**
Hazel (corylus avellana)	1	0	**1**	2	0	**2**
White poplar (populus alba)	1	0	**1**	1	0	**1**
Couch grass (elymus/agropyron repens)	1	0	**1**	3	2	**5**
Rye-grass (lolium perenne)	1	3	**4**	4	5	**9**
Lamb's quarter (chenopodium album)	3	1	**4**	2	1	**3**
Mugwort (artemisia vulgaris)	2	3	**5**	2	0	**2**
Goldenrod (solidago)	1	3	**4**	1	3	**4**
Cocksfoot 1:100 (dactylis glomerata)	0	0	**0**	0	0	**0**
Cocksfoot 1:5 (dactylis glomerata)	1	0	**1**	1	0	**1**
Black bent (agrostis gigantea)	1	0	**1**	0	0	**0**
Bluegrass (poa pratensis)	0	0	**0**	2	0	**2**
Meadow foxtail (alopecurus pratensis)	0	0	**0**	3	0	**3**
Bermudagrass (cynodon dactylon)	0	0	**0**	1	0	**1**
Ragweed (ambrosia)	1	0	**1**	3	0	**3**
Field sorrel (rumex acetosella)	1	2	**3**	1	1	**2**
Velvet grass (holcus lanatus)	1	2	**3**	1	0	**1**
Ribwort 1:100 (plantago lanceolata)	0	0	**0**	0	0	**0**
Ribwort 1:5 (plantago lanceolata)	0	0	**0**	0	0	**0**
pellitory 1:100 (parietaria diffusa)	0	0	**0**	0	0	**0**
pellitory1:5 (parietaria diffusa)	1	4	**5**	3	2	**5**
Rape 1:100 (brassica napus)	1	0	**1**	0	0	**0**
Rape 1:5 (brassica napus)	0	6	**6**	4	3	**7**
Tree pollen 1	1	4	**5**	1	0	**1**
Tree pollen 2	0	5	**5**	0	0	**0**
Herbage pollen mixture	0	3	**3**	2	0	**2**
Allergens	Control group (n = 6)			Equine Asthma group (n = 9)		
	Early (0.5–1 h)	Late (2–4 h)	Total	Early (0.5–1 h)	Late (2–4 h)	Total
Insects						
cockroach	2	1	**3**	3	0	**3**
housefly	0	0	**0**	0	0	**0**
mosquito	4	2	**6**	4	4	**8**
flea	1	0	**1**	3	1	**4**
other						
sheep's wool	1	0	**1**	0	0	**0**
epithelia mixture	0	0	**0**	0	0	**0**
cats epithelia	0	0	**0**	1	0	**1**
Total positive	**40**	**45**	**85 (34%)**	**75**	**36**	**111 (29%)**

Number of positive reactions (Score ≥2) in the intracutaneous test at the given times of the horses in the control group and the equine asthma group.
0 = no reaction (cf. negative control NaCl), 4 = strongly positive reaction (cf. positive control histamine), 1–4 degrees of manifestation of wheal in comparison to the negative and positive controls (degree 5: stronger reaction than to histamine).

None of the control or asthmatic horses showed a significant increase in heart or respiratory rate. Again, IPP measurements were not feasible in all horses. For further analysis of the data obtained during the first AIPT, the relative percent increase was calculated for all values, in comparison to the inhalation with PBS (=100%). Asthmatic horses showed a significant increase in IPP in comparison to the PBS inhalation six hours after the inhalation with allergen 1 ($P = .016$). This was based on the prominent rises in IPD of horses No. 13 (allergen: Cladosporium herbarum) and No. 14 (allergen: Aspergillus fumigatus). These showed an increased IPP of 336.8% and 367.5%, respectively, 6 hours after the allergen challenge, although no increase in IPP had been determined during the inhalation itself. Both horses displayed increased coughing during the allergen inhalation, so horse No. 14 only received the lowest allergen concentration (1:1000).

Inhalation of the second antigen did not lead to any clinical reaction or increase in IPP in any of the horses. Results of the AIPT compared to the results of other allergy tests are shown in detail in Table 7.

3.8. Comparison of allergy tests

Agreement between individual tested allergens of four different allergy tests was calculated with Cohen's kappa for n rater. Agreement was very low between 0.064 and 0.118. The overall number of positive results of the four allergy tests (Fc-ε-receptor, IBL, FIT, and IDT test) did not differ significantly between control and asthmatic horses (Fig. 2a). There was also no agreement between the results of the different tests. Positive results against mold allergens differed strongly between the tests, while there was no positive re-

Table 7
Results of the antigen inhalation provocation test (AIPT).

Pferd	Clnical symptoms according to the season	Allergen 1	IPP (PBS)	IPP (1:1000)	IPP (1:100)	IPP (1:10)	IPP (4-6h)	FIT	IDT	Heska	IBL	Allergen 2	IPP (PBS)	IPP (1:1000)	IPP (1:100)	IPP (1:10)	IPP (4-6h)	FIT	IDT	Heska	IBL
1	none	Brassica napus	15.1	21.8	22.7	22.1	–	3	2	n.t.	0	Dermatophagoides farinae	14.8	17.1	17.7	23.7	–	n.t.	3	2	0
2	none	Lolium perenne	5.9	6.5	6.9	6.2	6.4	0	4	0	0	Dermatophagoides farinae	7.5	6.5	7.9	7.7	7.1	n.t.	4	0	1
3	none	Brassica napus	16.5	14.2	15.0	13.7	15.0	2	3	n.t.	0	Tyrophagus putrescentiae	15.2	15.7	17.5	22.8	13.8	n.t.	3	0	0
4	none	Dactylis glomerata	23.4	30.5	35.5	–	–	0	3	0	0	No 2nd antigen tested positive in FIT									
5	none	Brassica napus	27.0	31.9	32.3	30.1	2.9	3	2	n.t.	0	Acarus siro	29.5	32.7	28.4	26.4	–	3	4	0	0
6	none	Brassica napus	6.4	5.7	4.5	4.4	4.8	4	2	n.t.	0	Tyrophagus putrescentiae	6.2	6.6	7.1	7.6	5.1	n.t.	2	0	0
7	whole year	Aspergillus fumigatus	6.9	7.9	8.3	8.4	7.7	3	0	0	0	Lolium perenne	8.2	8.1	8.5	10.5	11.0	4	2	0	0.
8	Only during winter time (pollen can be excluded)	Dermatophagoides pteronyssinus	2.9	3.4	3.8	2.7	3.1	3	4	3	1	Lepidoglyphus destructor	3.1	3.2	3.3	3.1	3.3	2	3	0	0
9	whole year		AIPT impossible due to severe dyspnea										AIPT impossible due to severe dyspnea								
10	whole year (winter worse)	Aspergillus fumigatus	9.7	11.4	15.1	15.4	12.6	3	1	0	1	Dermatophagoides pteronyssinus	14.8	19.2	16.9	16.7	14.4	3	3	3	0
11	Only during spring and summer time	Aspergillus fumigatus	6.1	5.5	5.6	6.9	13.1	3	0	0	2	Acarus siro	13.2	15.2	10.0	11.4	6.1	2	2	0	0
12	Only winter time (pollen can be excluded)	Aspergillus fumigatus	5.9	4.5	6.7	4.5	7.2	4	1	0	0	Brassica napus	5.0	5.9	6.2	6.0	5.9	3	2	n.t.	0
13	whole year	Cladrosporium herbarum	6.8	8.3	7.8	6.6	22.9	3	3	0	1	No second allergen was tested because of strong reaction with necessary medical treatment	–	–	–	–	–				
14	Only during winter time (pollen can be excluded)	Aspergillus fumigatus	8.0	12.8	–	–	29.4	3	2	0	2	No second allergen was tested because of strong reaction with necessary medical treatment	–	–	–	–	–	2			
15	whole year		AIPT impossible due to severe dyspnea										AIPT impossible due to severe dyspnea								

Allergens 1 and 2 identified based on functional-in-vitro test (FIT) results, mean delta interpleural pressure (IPP) at baseline (PBS), under antigen-dilutions 1:1000, 1:100, 1:10, and 4–6 hours post inhalation, compared to the results of FIT, intradermal test (IDT), Fc-epsilon-receptor test (HESKA) and IBL-test.
Controls marked in grey, n.t. = not tested (not included in the panel).

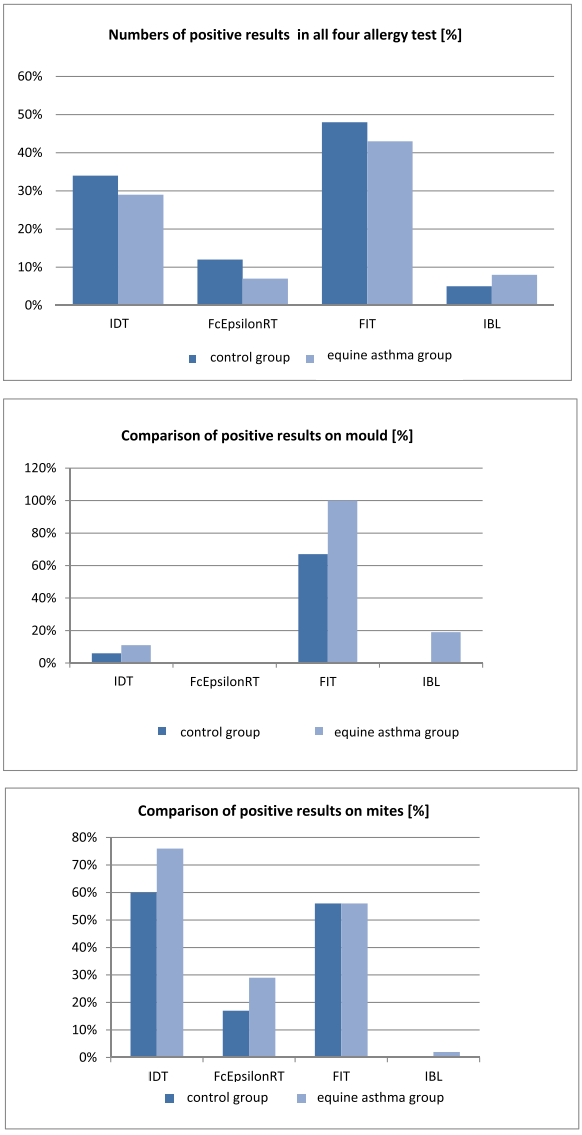

Fig. 2. Comparison of allergy test results. Number of all positive results in the four allergy tests in [%] in the comparison between the control group (dark blue) and the equine asthma group (light blue). Comparison of the positive results from mold spores in [%]. Comparison of the positive results from dust mites in [%].(For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

sult in the Fc-ε-receptor test against any of the four mold antigens, all asthmatic horses and 4/6 control horses tested positive in the FIT. The IBL separated best between control and asthmatic horses concerning six mold allergens, there was no positive result in the control group, but 10/54 possible reactions (19%) in the asthmatic group (Fig. 2b).

Large variation between the allergy tests was also evident for mite antigens. While 56%–76% of control and asthmatic horses tested positive using the IDT and FIT test, only 2%–29% tested positive using the serum IgE ELISAs.

4. Discussion

In this study, no agreement was found between the results of four different, commercially available allergy tests for equine asthma. None of the four allergy tests could differentiate reliably between healthy and asthma-affected horses. According to the results of the present study, a purely type I immediate immune reaction seems unlikely to be the major cause of equine asthma. Since two horses showed a reaction to the allergen inhalation after 6 hours, a type I late-phase reaction or other allergy mechanisms, such as a delayed immune response, must be suspected for some horses affected by equine asthma. Since all of the allergy tests used here only evaluate type I hypersensitivity, these tests must be considered unsuitable for the etiological diagnosis of equine asthma. In a small part of equine asthma horses IgE-mediated reactions to mold allergens may also be important according to the positive results of the IBL test on molds.

Numerous studies have reported that serological tests do not give reliable results [42,43]. No significant differences in serum IgE titers against mold extracts and storage mite allergens were found between healthy and asthma-affected horses (significant differences were found with recombinant Aspergillus fumigatus allergens) [23,25,44]. Although both tests (Fc-epsilon and IBL test) measured free allergen-specific IgE serum antibodies, there were no good agreement between the results of both tests to the same allergens or allergen groups, although the total number of positive results in both tests was similar. Since both tests measure free IgE antibodies, these varying results must be attributed to the different binding capacities of the allergen solutions to the Fc-epsilon receptors. It is crucial to note though that both tests utilize allergen solutions that are prepared in different ways, so that they cannot be compared. Furthermore, the evidence of free antibodies in the serum against a certain antigen says nothing about a sufficient sensitization of the type I effector cells of the patient and their response capacity to the given antigen [45]. Equine asthma may be a local event without effects on the concentration of serum antibodies in the serum. In unison with prior studies, the diagnostic value of serum IgE titers was low in our study.

The FIT showed most positive results of all tests, but there was no significant difference between the asthmatic and the control group. In contrast to our study, other authors identified less than 10% positive reactions in asthmatic, and none in control horses [46]. Although a history of IBH (insect bite hypersensitivity) was an exclusion criterion for present study, the majority of control and asthmatic horses tested positive with insect allergens. This sensitization of the basophilic granulocytes to insect allergens was obviously not clinically relevant at the time of testing. It may have been better to use the cellular antigen stimulation test (CAST), which is an ELISA measuring sulfide-leukotriene concentrations instead of histamine. These leukotrienes are produced after antigen stimulation by basophils, eosinophils, neutrophils and monocytes. The CAST was found to have a very high specificity for IBH and sulfide-leukotriene and histamine release correlate well [47,48].

In addition, the mast cells of the lung may not possess the same activating Fc-receptors, as the basophilic granulocytes in the blood. As a consequence stimulation of pulmonary mast cells might be more appropriate for equine asthma than using blood basophils [28]. Thus, a sensitization of the blood cells may be without great significance for allergic pulmonary diseases, and would therefore be unsuitable for the etiological diagnosis of equine asthma. Since the present study determined similar numbers of positive sensitizations in both horse groups for all allergen groups, especially mold and mites, the practical benefit of this test's results for equine asthma is very limited.

The IDT showed no significant differences between the control and the equine asthma group, similar to the findings of other studies. Other authors reported a comparable range of 40% positive reactions, but also no differences between horses with asthmatic and healthy horses [31]. Our study revealed more positive reactions in the control group than in the equine asthma group. In contrast, up to 55% positive reactions were observed in another study and the average number of positive reactions after 30 minutes, 4 hours, and 6 hours was significantly greater in asthmatic horses [32]. A

reaction after 6 hours could also be due to a late phase reaction of type I hypersensitivity. Therefore, it cannot be excluded that in some patients type I plays a role, but types III and IV might also play a role. In our study, the vast majority of reactions was noticed within one hour of injection. Three other studies [25,49,50] also observed more positive reactions in asthmatic horses in the IDT. Tahon et al. [25] could only identify a significantly greater number of positive reactions in the equine asthma group after 4 hours, but not at the other reading times before and up to 48 hours. Halliwell et al. [50] identified significantly more positive reactions after 30 minutes and after 4 hours compared to healthy horses. The allergen selection was very different from the allergen selection in the present study, so that the results are difficult to compare. However, based on these contrasting results, positive reactions on the IDT may not be related to equine asthma, but rather reflect an indication of the responsiveness of the skin mast cells, independent of a pulmonary disease. The notably high percentage of positive test results for mite antigens in the present study and mosquitoes correspond to the results of other studies [25]. Comparable percentages of positive results were evident with the allergen solutions from rapeseed and ryegrass, for which no published values from other studies exist, to the best of the authors' knowledge. In contrast, the present study identified only few positive reactions to the molds tested, which is similar to the results of comparable studies [25,32]. Only Mcphearson et al. [6] could identify numerous positive reactions to the molds *Aspergillus fumigatus* and *Micropolyspora faeni*. Wong et al. [51] required a higher concentration of the mold preparation, especially with *Aspergillus fumigatus*. False positive results are common in IDTs. As in our study, no significant differences were found between groups [5] and only three studies report more positive reactions in asthmatic horses compared to controls [50,51,52]. Therefore, the diagnostic value of mold and hay dust allergens in the IDT been questioned by some authors [5,17,48]. Positive skin test results in non allergic individuals are also identified in human medicine [53]. These problems with the IDT are still relevant after more than 40 years, and applicable to the IDT used for horses. A positive skin test result only indicates that the mast cells of the skin are sensitized to that allergen, not necessarily, that this allergen causes clinical signs. This may explain the multiple positive reactions in various studies including ours without the clinical phenotype of asthma. Furthermore, the sensitization of cutaneous effector cells may differ significantly from the sensitization of the immune cells in other tissues [33]. During HIPT and AIPT, some control horses had significantly higher IPP values during the entire duration of the measurement including baseline, although all other examination parameters, including the final histamine concentration reached, gave no indication of pulmonary disease. It can only be presumed that the elasticity and compliance of the lungs of these horses had strongly declined, due to age and predominant stabling with rare exercise. In human medicine, it is known that the lack of physical activity leads to a stiffening of the bony thorax and therefore, in addition to the age-related fibrosis of the connective tissue, leads to an increase in pulmonary pressure with increased age [54,55]. In contrast to this, some older horses in the equine asthma group, which were in clinical remission at the time of the examination, displayed physiological normal values. Therefore, the increases in pressure in percentage were taken as the starting point for the statistical calculations. Interestingly, the Pplmax occasionally decreased in some horses with a drastic increase in the respiratory rate, a phenomenon also observed by other authors [36]. One reason for the difficulty in evaluating the results was the occurrence of improvements in the pulmonary pressure values in various test phases. These short-term improvements in pulmonary function could be due to an adrenaline rush from excitement, explaining the results in the current study. According to Klein and Deegen [36], this improvement lasts for 5–10 minutes, which agrees with our own observation. The evaluation of the HIPT based solely on the changes in pulmonary pressure measured by an esophageal catheter is possibly not sensitive enough and too prone to faults for an objective assessment. Dynamic compliance is the best option for the evaluation of the bronchopulmonary response to inhaled histamine solutions [36]. Future studies should aim at using additional measurements via pneumotachography or plethysmography, which were not available for the current study.

The pulmonary pressure in the AIPT increased significantly in the equine asthma group 6 hours after the inhalation with allergen 1, but not during the inhalation. This was due to the significant increase in pulmonary pressure of over 300% in two horses after the inhalation with *Aspergillus fumigatus* and *Cladosporium herbarum*, respectively. These results mirror those of MCGORUM et al. [5], who also measured an increase in airway resistance after 5 hours, but not after 1.5 hours. The nebulization of the allergens seems to be necessary to provoke a bronchoconstriction, as transendoscopic endobronchial provocation with *Micropolyspora faeni* and an extract of moldy hay did not cause any reaction [56]. Nevertheless, positive reactions in an AIPT of asthmatic animals may be the result of an unspecific reaction to the inhaled substances [5]. As in our study, results of the IDT did not correlate with the results of AIPT [6].

Although the AIPT results of this study could only show a delayed reaction in two out of nine asthma-affected horses, this could be interpreted as evidence for a type IV hypersensitivity. This is in accordance with the hypothesis of multiple authors that equine asthma is not a pure type I immediate-type allergy [2,5,19,25,33,56–58]. Other allergy mechanisms must be involved in at least some horses affected. A cytokine-induced late response form of the type I hypersensitivity or also a type III or type IV hypersensitivity could be responsible. It is also possible that further, less obvious positive reactions to the allergen inhalation were not recognized, due to the substantial individual variability of the initial values of all parameters.

As all of the allergy tests utilized in the present study (except for the late reactions in the IDT) can only identify the allergy potential for a type I allergy, these tests may not be helpful for the diagnosis of the asthma etiology in horses. Currently, targeted allergen inhalation seems to be a better option to identify an allergic cause for pulmonary disease of the horse. However, since its implementation, especially for the evaluation of multiple allergens, is both time and material-consuming, it remains reserved for individual cases and research for the moment. Further studies with larger numbers of subjects involved and possibly more sensitive measuring devices for example, via plethysmography with an elastic girth over multiple hours or days are necessary to gain new insights into the complex, but very significant disease incidence of equine asthma.

Owners' Agreement

All horse owners signed an informed consent.

Off-label Antimicrobial Declaration

Authors declare no off-label use of antimicrobials.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Financial disclosure

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