

Allergen-specific IgE levels against crude mould and storage mite extracts and recombinant mould allergens in sera from horses affected with chronic bronchitis

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Abstract

Immunoglobulin E antibody (IgE) levels against four recombinant (r) mould allergens (r-*Aspergillus fumigatus* [rAsp f] 7, 8 and 9; r-*Alternaria alternata* 1 [rAlt a1]) and crude mould (*Aspergillus fumigatus*, *Alternaria alternata*, *Penicillium notatum*) and storage mite extracts were determined by ELISA in sera from 24 pulmonary sound control horses and 26 horses suffering from chronic bronchitis/bronchiolitis (CB), also called chronic obstructive pulmonary disease (COPD). Serum IgG and IgA titres were also determined against *Aspergillus fumigatus* extract and rAsp f 8.

IgE against the crude extracts could be measured in all sera, but there was no significant difference between CB-affected and control horses. In contrast, only 8–30% of the horses, depending on the r-allergen tested, had detectable IgE levels in serum against the r-allergens. Horses with CB had significantly more often detectable IgE levels than controls against rAlt a 1 (10/26 and 3/24, respectively, $p=0.054$), rAsp f 7 (13/26 and 2/24, respectively, $p<0.01$) and rAsp f 8 (11/26 and 1/24, respectively, $p<0.01$). Only four horses (three CB-affected and one healthy, $p>0.05$) had detectable IgE levels against rAsp f 9. Furthermore, CB-affected horses were often sensitised against two or more r-allergens (13/26 of the CB-affected horses) while only one of the 24 healthy horses had positive IgE levels against more than one r-allergens. Similarly to IgE levels, no significant differences between CB-affected and healthy horses were found for IgG titres against the *Aspergillus fumigatus* extract. However, horses with CB had significantly higher serum IgG titres against rAsp f 8 than healthy controls (median=28 versus 10 relative ELISA units [REU], $p<0.01$). Additionally, horses with detectable IgE titres against rAsp f 8 had significantly higher IgG

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titres against this r-allergen than horses with undetectable IgE titres (median IgG titres=46 and 13 REU, respectively; $p<0.01$). For serum IgA titres, neither differences between healthy and CB-affected animals nor correlations between IgA and IgG or IgE titres could be found.

These results show that horses suffering from CB are more often sensitised to some *Aspergillus fumigatus* and *Alternaria alternata* allergens than control horses and that they are partly sensitised to the same fungal proteins as mould-allergic human patients. Furthermore, this study shows that r-allergens allow a much more sensitive determination of specific serum antibody levels by ELISA than crude mould extracts. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Horse; Immunoglobulin E; Crude allergen extracts; Recombinant allergens; Chronic bronchitis

Abbreviations: Ab, antibody; Alt a, *Alternaria alternata*; AP, alkaline phosphatase; Asp. f, *Aspergillus fumigatus*; BAL, bronchoalveolar lavage; BALF, bronchoalveolar lavage fluid; CB, chronic bronchitis/bronchiolitis; COPD, chronic obstructive pulmonary disease; ELISA, enzyme linked immunosorbent assay; ESA, equine serum albumin; IgA, Immunoglobulin A; IgE, Immunoglobulin E; IgG, Immunoglobulin G; ns, not significant ($p>0.05$); PSG, porcine skin gelatine; r, recombinant; REU, relative ELISA units; T, Tween-20; TBS, tris buffered saline

1. Introduction

Chronic bronchitis/bronchiolitis (CB), also called chronic obstructive pulmonary disease (COPD), is a condition affecting 12–50% (Robinson et al., 1996) of stabled horses and is characterised by cough, nasal discharge, expiratory dyspnoea and reduced performance. Removal of hay and straw often leads to remission of the disease. Although many aspects of the pathogenesis of CB are still unknown, different studies have shown that CB is a hypersensitivity reaction to moulds in hay and straw (see Robinson et al., 1996 for a review). In particular, *Aspergillus fumigatus*, *Faenia rectivirgula* (Lawson et al., 1979; McPherson et al., 1979; Derksen et al., 1988), and *Alternaria alternata* (L. Viel, Ontario Veterinary College, University of Guelph, Canada, pers. commun.) have been shown to be involved in the pathogenesis of CB. Due to the lack of commercially available reagents specific for equine IgE, the presence of allergen-specific IgE antibodies has only been investigated by Halliwell et al. (1993). Horses affected with COPD had higher IgE-levels against *Aspergillus fumigatus* and *Micropolyspora faeni* in bronchoalveolar lavage fluid (BALF) than healthy controls. No differences between COPD-affected and healthy horses were found in serum. Similar results were obtained with recombinant (r)-*Aspergillus fumigatus* I/a allergen (Schmallenbach et al., 1998).

Mould extracts are complex mixtures of a large number of proteins and glycoproteins and the allergenic proteins represent only a small percentage of the total protein content. Furthermore, the composition of the extracts varies between preparations, hampering a reproducible diagnosis as well as the study of the pathogenesis of mould-associated disease. Over the last 10 years much effort has been devoted to identify, clone and produce biotechnologically allergens of medical importance (Kraft et al., 1998). In particular, Cramer and colleagues have identified and produced with molecular techniques 13 different allergens from *Aspergillus fumigatus* (Asp f) relevant to aspergillosis, of which three, rAsp f 4, 6 and 8, allow to discriminate with serological

methods between IgE-mediated asthma and life-threatening allergic bronchopulmonary aspergillosis (Crameri, 1998).

The aim of the presented study was to investigate by enzyme linked immunosorbent assay (ELISA) whether horses with CB have higher serum IgE levels against different mould extracts (*Alternaria alternata*, *Aspergillus fumigatus*, *Penicillium notatum*) and storage mites (*Acarus siro*, *Glycophagus domesticus* and *Tyrophagus*) than pulmonary sound control horses.

Furthermore, we wanted to analyse if horses show serum IgE-binding to the same specific mould antigens as human patients and if these recombinant antigens can be used for the serological diagnosis of CB. For that purpose, three rAsp f allergens were chosen: rAsp f 7, a 112 amino acid long protein with yet unknown function (Crameri, 1999); rAsp f 8, which is the 60S Acidic Ribosomal Protein P2 of *Aspergillus fumigatus* (Mayer et al., 1999) and rAsp f 9, a 302 amino acid protein with unknown function (Crameri, 1999). A recombinant allergen from *Alternaria alternata*, rAlt a 1 was also included in this study.

Finally, with the *Aspergillus fumigatus* extract and with rAsp f 8, we have investigated if allergen-specific serum IgG and IgA levels were altered in CB-affected compared to healthy horses, and if a correlation between allergen-specific serum IgE, IgG and IgA levels exists.

2. Material and methods

2.1. Criteria used for the diagnosis of CB

All horses diagnosed as suffering from CB had a history of chronic respiratory disease. Except for few horses that were not removed from hay or that were slaughtered, it was known that withdrawal of hay and straw led to remission of the disease. CB-affected horses showed some or all of the typical clinical signs for CB: cough, nasal discharge, increased expiratory depth and/or increased respiratory rate and increased and/or abnormal lung sounds. Bronchoscopy showed increased mucotracheal secretions and swelling of the carina. Horses were afebrile and based on blood count there was no evidence of infection. Clinical examinations were carried out before and after exercise. Control horses had neither a history nor clinical signs of CB or of any other respiratory disease, and had less than 5% neutrophils in their bronchoalveolar lavage fluid (BALF).

2.2. Horses

2.2.1. Control horses

The 24 healthy horses were of mixed breeds and included two stallions, five mares and 17 geldings. The age ranged from 4 to 31 years. Fifteen controls were from Stable A and three from Stud B; the remaining six horses belonged to different private owners and were all living at different places. All horses were stabled and fed hay. They had no history of respiratory disease over the preceding year(s). A clinical examination, including endoscopy and cytology of BALF, was carried out in all but five of the privately owned

horses. These five horses had been known by one investigator for many years and had never shown signs of respiratory diseases during that time.

2.2.2. CB-affected horses

The 26 CB-affected horses were also of mixed breeds and included two stallions, eight mares and 16 geldings. Their age ranged from 4 to 26 years. Four of them were from Stable A, three from Stud B and three were privately owned and known by the investigator for a long time as suffering from CB. The remaining 16 horses were CB-affected patients from our equine clinic. All horses had a history of CB and had been subjected to a thorough clinical examination including endoscopy and cytology of tracheobronchial secretion (TBS). Furthermore, in 11 of them, cytology of BALF had been performed. Cytology of TBS or/and BALF revealed elevated numbers of neutrophils. All affected horses showed moderate to severe symptoms of CB and had been kept stabled and fed hay before blood sampling.

2.3. Serum samples

Blood samples were taken from all horses and serum was frozen in aliquots at the latest 12 hours after blood sampling and then stored at -70°C until assayed. From one CB-affected horse there was only enough frozen serum for IgE, but not for IgG and IgA determinations.

2.4. Allergens

Alternaria alternata tenuis, *Aspergillus fumigatus* and *Penicillium notatum* extracts were purchased from Allergopharma, Hamburg, Germany. Storage mites, consisting of a mixture of *Acarus siro*, *Glycophagus domesticus* and *Tyrophagus* were provided by Trimedal, Brüttisellen, Switzerland. r*Alternaria alternata* 1 (rAlt a 1) was purchased from Biomay, Linz, Austria. rAsp f 7, rAsp f 8 and rAsp f 9 were produced as described (Cramer, 1998).

2.5. Allergen-specific IgE ELISA

The ELISA for the detection of allergen-specific serum IgE was carried out as described by Marti et al. (1997) with the following modifications: Immulon² polystyrene microtitre plates (Dynatech Lab., Chantilly, VA, USA) were coated overnight at 4°C with 10 (storage mites), 5 (rAlt a 1) or 1 (all rAsp f) $\mu\text{g/ml}$ allergen in 0.2 M sodium carbonate-bicarbonate buffer, pH 9.4 (Pierce, Rockford, IL, USA). The *Aspergillus*, *Alternaria* and *Penicillium* extracts were used at a dilution of 1:100. Plates were blocked with TBS containing 0.2% equine serum albumin (ESA). After washing, sera were diluted 1:3 (rAlt a 1, rAsp f 7), 1:5 (rAsp f 9; *Alternaria*, *Aspergillus*, *Penicillium* and storage mite extracts) or 1:6 (rAsp f 8) in TBS containing 0.1% ESA and 4% Tween-20 (T). Starting from these dilutions, three 1.5-fold serial dilutions were made in the plate, followed by an incubation for 90 min at 37°C . Subsequently, an affinity purified chicken antibody (Ab), specific for equine IgE (Marti et al., 1997), was added for 2 h at room

temperature on a shaker, diluted to a concentration of 1 µg/ml in TBS 0.1% ESA–1% T. Because the chicken Ab reacted slightly with the rAsp f allergens it had to be preincubated for 30 min on a shaker at RT with rAsp f 7 (900 ng/ml), rAsp f 8 (650 ng/ml) or rAsp f 9 (360 ng/ml) before use. After washing the plate, an AP-labelled goat anti-chicken IgG (H+L) Ab (Kirkegaard and Perry Laboratories, Gaithersburg, MA, USA) was added. ELISA plates were developed with 1 mg/ml p-nitrophenyl phosphate (Sigma Chemical, St.-Louis, MO, USA) in 10% diethanolamine buffer (Fluka Chemie AG, Buchs, Switzerland) with 0.5 mM MgCl₂ at pH 9.8 for 45 min. Absorbance readings were measured at 405 nm with a Molecular Devices Reader (Sunnyvale, CA, USA) and converted to relative ELISA units (REU). For that purpose, an equine serum positive for the tested allergen was used as reference serum and assigned a value of 100 REU. Serial dilutions of the reference serum were used to generate a standard curve and the test sample results were calculated from the curve using an ELISA software program (SOFTmax[®] 2.31 for WindowsTM, Molecular Devices, Sunnyvale, CA, USA). Serum from one horse was used to monitor intra- and inter-assay variations.

2.6. *Aspergillus fumigatus* extract - and rAsp f 8 - specific IgG ELISA

The ELISA for IgG detection was carried out as above but the wells of the ELISA plate were blocked with TBS containing 2% porcine skin gelatine (PSG; Fluka Chemie AG). Sera were two fold serially diluted starting at 1:200 for the *Aspergillus* extract and at 1:50 for rAsp f 8, in TBS containing 1% PSG and 2% T. For detection an AP-conjugated goat anti-horse IgG Ab, Fc fragment-specific (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) was used at a dilution of 1:2000 in TBS 1% PSG–1% T.

2.7. *Aspergillus fumigatus* extract - and rAsp f - 8 specific IgA ELISA

The same blocking and Ab dilution buffers as for IgG detection were used, but horse sera were diluted 1:5 for *Aspergillus* extract and rAsp f 8 specific serum IgA Ab measurements. Two-fold serial dilutions followed. A monoclonal, equine IgA specific Ab (Serotec, Oxford, UK) diluted 1:100 was then added. An AP-labelled goat anti-mouse IgG (H+L) Ab (Jackson ImmunoResearch Laboratories) was used 1:2000 as detection Ab.

2.8. Statistical analyses

Statistical analyses were carried out with the statistical software package SPSS SYSTAT for Windows, version 7.0. The nonparametric Mann–Whitney U-Test was used to compare the age of the healthy and CB-affected horse groups. For IgE and IgG titres against the crude extracts and for rAsp f 8 specific IgG titres, comparison of the responses obtained for the two groups of horses was also done with the Mann-Whitney U-Test. IgE levels to r-allergens and IgA levels to *Aspergillus* extract and rAsp f 8 were subdivided into positive and negative results and evaluated with the Fisher's exact two tailed test. Positive and negative IgE and IgA levels in CB-affected horses were also analysed with the Fisher's exact test.

Correlations between IgE levels against the different allergen extracts were calculated with the Pearson correlation coefficient (r). This correlation coefficient was also used to evaluate the relationships between IgE and IgG levels against the *Aspergillus* extract. The rAsp f 8 specific IgG levels of horses with negative rAsp f 8 IgE levels were compared to those of horses with positive rAsp f 8 specific IgE levels with the Mann–Whitney U test. p -values ≤ 0.05 were considered statistically significant.

3. Results

3.1. Age distribution of CB-affected and healthy horses

Comparison of the age between the two horse groups showed that CB-affected horses were slightly older (mean age=11 years, SD=5.3 years) than the control animals (mean age=9.8 years, SD=6.7 years) but this difference was statistically not significant ($p>0.1$).

3.2. Assay precision

The reproducibility of the different ELISA was high. The intraplate coefficients of variation were below 4% and the interplate coefficients of variation were below 9% for the IgE and IgA ELISA and below 14% for the IgG ELISA.

3.3. Allergen-specific IgE against the crude extracts

IgE against the crude *Aspergillus*, *Alternaria* and *Penicillium* extracts, as well as against the mixture of storage mites could be detected in all horse sera but there were no statistically significant differences ($p>0.26$) between CB-affected and healthy horses as shown in Table 1.

3.4. Correlations between the IgE levels against the different allergen extracts

There were significant positive correlations between the IgE levels against the different allergen extracts, i.e. a horse with a high IgE titre against *Aspergillus* usually also had

Table 1

Serum IgE antibody levels in relative ELISA Units (REU) to different allergen extracts in sera from 24 controls and 26 chronic bronchitis (CB)-affected horses

Allergen extract	Horses				Mann–Whitney U-Test
	Controls (<i>N</i> =24)		CB (<i>N</i> =26)		
	Median	Range	Median	Range	<i>p</i> -value
<i>Alternaria alternata</i>	94	62–147	94	44–144	ns ^a
<i>Aspergillus fumigatus</i>	102	83–144	119	37–216	ns
<i>Penicillium notatum</i>	97	63–203	96	30–151	ns
Storage mites	95	61–179	92	25–195	ns

^a ns — not significant.

Table 2

Pearson correlation coefficient (r) between serum IgE levels against different allergens in all 50 horses

	<i>Alternaria alternata</i>	<i>Aspergillus fumigatus</i>	<i>Penicillium notatum</i>
<i>Aspergillus fumigatus</i>	0.777*** ^a		
<i>Penicillium notatum</i>	0.832***	0.755***	
Storage mites	0.740***	0.447**	0.731***

^a **0.01 > *p* > 0.001; ****p* < 0.001.

high IgE titres against most of the other allergen extracts (Table 2). Interestingly, all calculated correlation coefficients, but one, were stronger in the CB-affected than in the healthy horses (data not shown, Δr between healthy and CB ≥ 0.25).

3.5. Specific IgE levels against the recombinant allergens

With the r-allergens, IgE could only be detected in 8–30% of the sera, depending on the r-allergen used. The other sera contained specific IgE levels below the detection limit of the assay. More CB-affected than control horses (Fig. 1) showed IgE binding to rAlt a 1 (10/26 and 3/24, respectively, *p* = 0.054), rAsp f 7 (13/26 and 2/24, *p* < 0.01) and rAsp f 8 (11/26 and 1/24, *p* < 0.01). Only four of the 50 horses (three with CB and one healthy) had detectable IgE levels against rAsp f 9; for this r-allergen the difference between CB-affected and healthy horses was not significant. These results are summarised in Table 3. Interestingly, 50% of the CB-affected horses were sensitised to two or more different r-allergens, while only one of the six positive healthy animals reacted with more than one r-allergen (Table 4).

When all the four r-allergens were evaluated together (Table 3), 18 of the 26 CB-affected horses (69%) had IgE against at least one of the r-allergens while only six of the 24 healthy horses (25%) had detectable IgE levels against one of the r-allergens (*p* < 0.01).

3.6. *Aspergillus fumigatus* extract and rAsp f 8 specific IgG titres

Similar results as for the IgE levels were obtained when IgG titres (Fig. 2) were measured: with the *Aspergillus* extract no differences between healthy controls and CB-affected horses were found (median IgG titre for CB-affected horse = 63 REU versus 61

Table 3

Comparison of serum IgE binding to recombinant (r)-*Alternaria alternata* 1 (rAlt a 1), r-*Aspergillus fumigatus* 7 (rAsp f 7), rAsp f 8 and rAsp f 9 in 24 control and 26 chronic bronchitis (CB)-affected horses

Horses	Number (%) horses with IgE binding to recombinant allergens				
	rAlt a 1	rAsp f 7	rAsp f 8	rAsp f 9	Any of them
Controls (N=24)	3 (13%)	2 (8%)	1 (4%)	1 (4%)	6 (25%)
CB (N=26)	10 (38%)	13 (50%)	11 (42%)	3 (12%)	18 (69%)
Fisher's exact test <i>p</i> -value	=0.054	<0.01	<0.01	ns ^a	<0.01

^a ns — not significant.

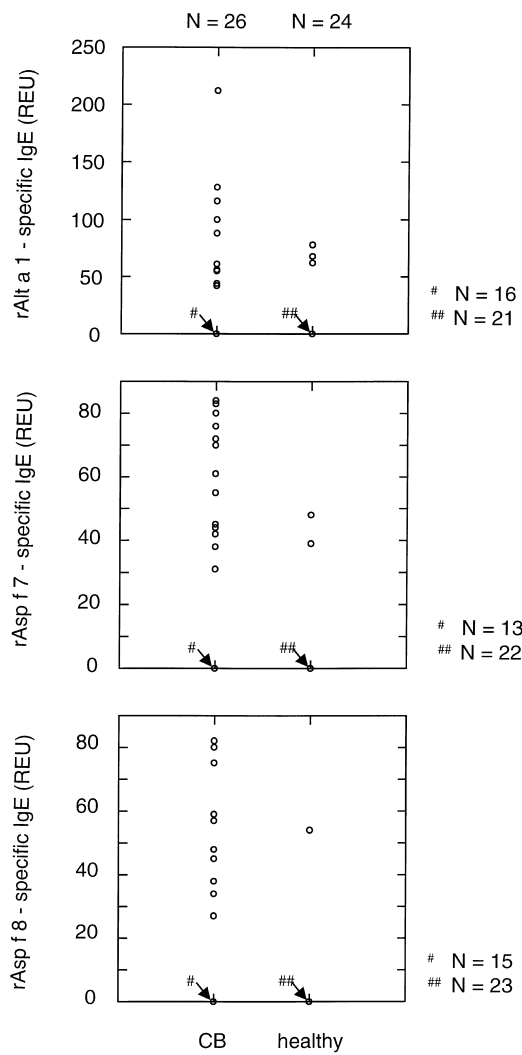


Fig. 1. IgE antibody titres in relative ELISA units (REU) against recombinant (r) *Alternaria alternata* 1 (rAlt a 1), r *Apergillus fumigatus* 7 (rAsp f 7) and r *Aspergillus fumigatus* 8 (rAsp f 8) in sera from 26 chronic bronchitis (CB)-affected and 24 healthy control horses. The symbols indicate the number of horses with undetectable IgE levels within each horse group (# for CB-affected and ## for healthy horses).

Table 4
Number (%) of horses with IgE binding to different numbers of recombinant allergens

Horses	Number of recombinant allergens				
	None	One	Two	Three	Four
Healthy (N=24)	18 (75%)	5 (21%)	1 (4%)	0 (0%)	0 (0%)
CB (N=26)	8 (31%)	5 (19%)	9 (34%)	2 (8%)	2 (8%)

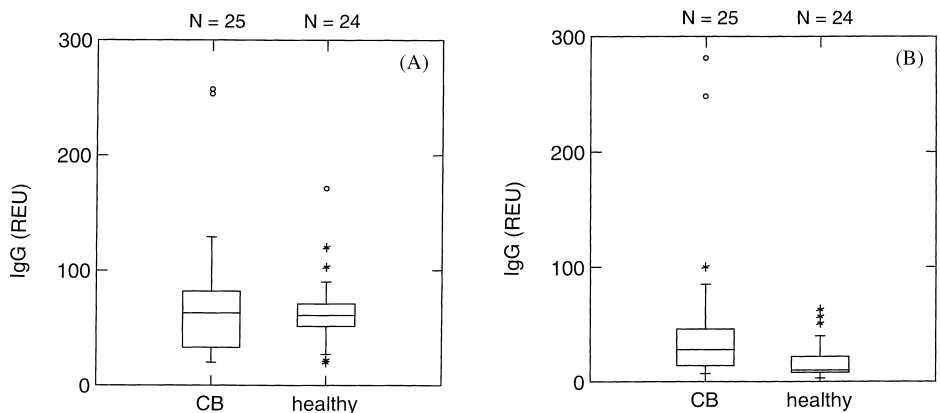


Fig. 2. IgG antibody titres in relative ELISA units (REU) against (A) *Aspergillus fumigatus* extract and (B) recombinant *Aspergillus fumigatus* 8 (rAsp f 8) in sera from 25 chronic bronchitis (CB)-affected and 24 healthy control horses. CB-affected and healthy horses had not significantly different IgG titres against *Aspergillus fumigatus* extract, but CB-affected horses had significantly ($p<0.01$) higher rAsp f 8 specific IgG titres than the healthy animals.

REU for healthy animals). However, CB-affected horses had significantly ($p<0.01$) higher IgG titres (median=28 REU) than controls (median=10 REU) against the recombinant allergen rAsp f 8.

3.7. *Aspergillus fumigatus* extract and rAsp f 8 specific IgA titres

Although the starting serum dilution for IgA determination was only 1:5, Ab levels were below the detection limit of the assay in 41% of the horses for *Aspergillus* extract specific and in 71% of the horses for rAsp f 8-specific IgA measurements. 16 of the 24 (67%) controls and 13 of the 25 (52%) CB-affected horses had detectable IgA titres against the *Aspergillus* extract (healthy versus CB =ns). Only six out of the 24 (25%) healthy and eight of the 25 (32%) CB-affected horses had positive IgA titres against rAsp f 8 (healthy versus CB=ns; Table 5).

Interestingly, only one of the eight CB-affected horses with detectable IgA titres against rAsp f 8 had also a measurable IgE titre against this r-allergen while, from the 17

Table 5
Comparison of serum IgA binding to *Aspergillus fumigatus* and recombinant (r)-*Aspergillus fumigatus* eight in 24 healthy and 25 chronic bronchitis (CB)-affected horses

Horses	Number (%) horses with IgA binding to	
	<i>Aspergillus fumigatus</i> extract	r- <i>Aspergillus fumigatus</i> 8
Healthy (N=24)	16 (67%)	6 (25%)
CB (N=25)	13 (52%)	8 (32%)
Fisher's exact test p-value	ns ^a	ns

^a ns — not significant.

CB-affected horses with undetectable IgA levels against rAsp f 8, 10 had a positive IgE titre against this r-allergen ($p=0.042$). No similar results were found with the *Aspergillus* extract.

3.8. Correlations between allergen-specific IgE and IgG levels

There was a significant ($p<0.01$) positive, although not very strong correlation ($r=0.4$) between *Aspergillus* extract-specific IgE and IgG titres.

The Pearson correlation coefficient was not calculated for rAsp f 8 specific IgE and IgG titres because of the high number of horses with undetectable IgE levels for this antigen. However, horses with positive IgE titres ($N=12$) against rAsp f 8 had significantly ($p<0.01$) higher IgG levels than horses with undetectable IgE titres ($N=37$) (median IgG levels in REU=46 versus 13, respectively).

4. Discussion

This study shows that IgE Ab against different mould or storage mite allergens can be measured by ELISA in horse sera. While IgE against the crude extracts could be measured in all sera, independently of the health status of the horse, IgE against the recombinant allergens could only be detected in some of them. In contrast to the results obtained with the crude extracts, significantly more CB-affected than healthy control horses had IgE Ab against the r-allergens. These results suggest that CB-affected horses are often sensitised to the same mould allergens as human mould allergic patients. The CB-affected animals that did not react with any of the four tested r-allergens may be sensitised to other fungal proteins. We are aware that a number of other r-*Aspergillus fumigatus* (Cramer, 1999) and r-*Alternaria* antigens are available and could be tested. This was not possible because the equine IgE-specific chicken Ab displayed a high reactivity with some of the rAsp f allergens, as for example with rAsp f 4. A preliminary screening indicated that rAsp f 11 is probably not important for the horse, as no horse sera with IgE-binding to this r-allergen were found. This is also true for rAsp f 9: only four of the 50 tested horse sera reacted with this r-allergen.

The sensitisation of horses without clinical signs of CB against the recombinant mould allergens is rather high (4–12.5%, depending on the r-allergen) compared to the human healthy controls in the study of Cramer (1998). This may be due to the fact that horses spend most of their time in an environment full of fungal spores and are thus exposed to a much higher degree to these allergens than humans. Furthermore, it is well known in humans that the presence of allergen-specific IgE Ab does not always coincide with manifestation of clinical allergic diseases but merely indicates sensitisation to given allergens (Pastorello et al., 1995). This may also apply for the horse.

With the crude allergen extracts our findings are concordant with Halliwell et al. (1993): They did not find differences between CB-affected and control horses when mould allergen specific IgE levels were determined in serum, but CB-affected horses had higher IgE and IgG titres in BALF than controls. Unfortunately, we were not able to determine IgE levels by ELISA in BALF with the chicken anti-horse IgE Ab because

BALF is too diluted. Concentrating BALF immunoglobulins by ammonium sulphate precipitation or with other procedures results in denaturation of the very labile epitope(s) recognised by this IgE-specific Ab (Marti et al., 1997).

Our investigation shows that for the horse the ELISA is probably not a suitable method for serum IgE detection with complex protein mixtures, like crude mould or mite extracts. Although our results with the r-allergens show that CB-affected horses are more often sensitised against *Aspergillus* and *Alternaria* allergens than healthy animals, no significant differences between CB-affected and healthy horses were observed with these extracts. The concentration of the relevant allergens is probably too low in these crude mixtures or they are 'hidden' by other proteins. Interestingly, this phenomenon does not seem to be due to competition for binding to the relevant allergenic proteins by the IgG Ab, present in a much higher concentration in serum than IgE, as similar results were obtained for serum IgG levels: When IgG titres against the crude *Aspergillus* extract were determined, no differences between CB-affected and healthy horses were found. However, CB-affected horses had significantly higher IgG levels against rAsp f 8 than controls. As known in humans (Ali et al., 1983; Berrens and Homedes, 1991; Boluda et al., 1997; Shibasaki et al., 1997) and as shown by the positive correlation between specific IgE and IgG titre in this study, the specific IgE response is accompanied most of the time by an IgG response. IgG4 is the subclass mainly correlating with IgE levels in man (Olsen et al., 1995). It remains to be determined in the horse if some IgG subclasses show a stronger correlation with the IgE response than others.

The role of IgA Ab in the pathogenesis of hypersensitivity reactions against inhaled allergens is still controversial in humans (Björkstén and Gerrard, 1990): Some authors suggest a protective effect of secretory IgA by blocking foreign antigens and thus reducing the possibility of a sensitisation, while others find higher IgA levels in atopic than in non-atopic subjects. In the horse, total (Mair et al., 1988) and mould-specific (Halliwell et al., 1993) IgA levels were increased in BALF but not in serum of COPD-affected horses compared to healthy controls. In our study there were no significant differences between serum IgA levels in CB-affected and control horses. Interestingly, most of the horses with detectable rAsp f 8 – specific IgA levels had undetectable IgE Ab against this r-allergen. We do not know if serum IgA titres reflect secretory IgA levels in the horse.

Our data suggest that high or low IgE responders may exist in the horse like in other species (Marsh and Blumenthal, 1990). The positive correlation between IgE levels against the different allergen extracts and the fact that these correlation coefficients were stronger in the CB-affected horses than in the controls indicate a general disposition for high IgE responses in some individuals. Similar results were found with the r-allergens: CB-affected horses often had IgE Ab against more than one r-allergen. A reliable determination of total serum IgE levels, but keeping in mind that factors such as degree of infestation with endoparasites will influence the result, may allow to define high and low IgE responders in the horse.

Previous investigations have shown that the frequency of CB increases with age (Marti et al., 1991) and allergen-specific IgE levels may also increase with age due to a longer exposure to moulds. The effects of age on allergen-specific antibody levels could unfortunately not be evaluated in this study because the number of tested horses was too

small. In order to get age balanced groups, six privately owned horses were included in the controls. We were unfortunately not allowed to perform a bronchoscopy in five of these six horses, but they had been known by the investigators for many years, were fed hay and had not shown clinical signs of respiratory diseases over the last 2 years. Furthermore, a thorough clinical examination revealed no signs of CB. In our opinion these facts allow to add these horse to the control group also without performing a bronchoscopy and cytology of BALF.

In conclusion, this study shows that horses suffering from CB are more often sensitised to some *Aspergillus fumigatus* and *Alternaria alternata* proteins than control horses, as shown by determination of specific serum IgE with recombinant mould allergens but not with crude allergen extracts. Furthermore, horses are partly sensitised to the same fungal proteins as mould-allergic human patients. Additional studies will have to be undertaken to evaluate the effects of environment, age and sex on allergen-specific IgE levels and to further investigate the role of IgE Ab in the pathogenesis of CB. It is only then that a serological in vitro test for CB could be taken into consideration as a diagnostic tool.

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