



Allergen-specific IgE in Icelandic horses with insect bite hypersensitivity and healthy controls, assessed by FcεR1α-based serology

Rebecka Frey^{a,*}, Kerstin Bergvall^b, Agneta Egenvall^c

^a *Norsholms Djursjukhus, Biskop Henriks v. 6, S-610 21 Norsholm, Sweden*

^b *Department of Clinical Sciences, Division of Small animal Clinical Sciences, Swedish University of Agriculture, Box 7054, SE-750 07 Uppsala, Sweden*

^c *Department of Clinical Sciences, Division of Ruminant Medicine and Epidemiology, Swedish University of Agriculture, Box 7054, SE-750 07 Uppsala, Sweden*

Received 1 November 2007; received in revised form 15 June 2008; accepted 25 June 2008

Abstract

Insect bite hypersensitivity (IBH) and atopy can both be causes of pruritus in horses and are associated with allergen-specific IgE to biting insects and environmental allergens respectively. Information with respect to differences in IgE levels in diseased and healthy animals is crucial in enabling an understanding of the clinical relevance of results of allergen-specific IgE tests.

The aim of this study was (i) to evaluate and compare levels of allergen-specific IgE, using an ELISA method, in Icelandic horses, with and without IBH, from Iceland and Sweden respectively; (ii) to investigate patterns of allergen-specific IgE to insects, pollens, moulds and mites in those groups of horses; and (iii) to investigate the clinical significance of employing two different cut-off levels for the ELISA.

The study compromised a total number of 99 horses from Iceland and Sweden, with and without IBH, divided in 5 groups. Sera from the horses were analysed blindly with the use of AllerceptTM, a non-competitive, solid-phase ELISA-test, designed to detect the presence of allergen-specific IgE in sera using the recombinant alpha chain of the high-affinity IgE receptor (FcεR1α). The distribution of the ELISA values was shown for each insect, mould, mite and pollen allergen, in the different groups using 10th, 50th and 90th percentiles. The use of two cut-off levels, 150 EA and 300 EA, did not eliminate the false positives. Horses with IBH had a higher number of positive reactions, counting all the 29 allergens, than healthy controls and this was borderline significant ($P = 0.053$).

In this study it was shown that serological testing with an ELISA that uses the high-affinity IgE receptor (FcεR1α) is presently not suitable as a tool for establishing a diagnosis of IBH or equine atopy. The importance of establishing a correct cut-off level for the ELISA for the different allergens is emphasised.

© 2008 Elsevier B.V. All rights reserved.

Keywords: Horse; ELISA; Insect bite hypersensitivity; Atopy; Allergy

1. Introduction

Insect bite hypersensitivity (IBH), also known as summer eczema or sweet itch, is a widely distributed

common pruritic skin disease of horses, associated with IgE-mediated reactions to biting insect allergens (Wilson et al., 2001; van der Haegen et al., 2001; Wagner et al., 2006). Horses with IBH present with a characteristic distribution of pruritus and varying degrees of skin lesions, typically involving the mane and tail, but also other parts of the body (Halldórsdóttir and Larsen, 1991).

* Corresponding author. Tel.: +46 733666782; fax: +46 11 105746.
E-mail address: rebecka.frey@telia.com (R. Frey).

The seasonality of the condition from spring until autumn reflects the active period of the biting midges, *Culicoides* spp., one of the biting insects implicated in the pathogenesis of the disease (Broström et al., 1987; Halldórsdóttir and Larsen, 1991; Baselgia et al., 2006; Bjornsdóttir et al., 2006). The incidence of IBH has been shown to be high in the Icelandic breed (Broström et al., 1987; Halldórsdóttir and Larsen, 1991; Bjornsdóttir et al., 2006). *Culicoides* spp. do not exist in Iceland and the horses in Iceland do not develop IBH, whereas horses exported to Europe are at much higher risk of developing the disease than those born on the continent which get exposed to the midges early in life (Broström et al., 1987; Halldórsdóttir and Larsen, 1991; Bjornsdóttir et al., 2006).

The term atopic dermatitis has been used for pruritic horses with allergen-specific IgE to environmental allergens other than biting insects (Scott and Miller, 2003). Equine atopy is not as clearly characterised as is canine atopy (Willemse, 1986). Horses diagnosed as having atopic dermatitis commonly show pruritus during non-insect season or year round, although equine atopy and IBH can present with overlapping clinical presentations. Furthermore horses with IBH, initially showing a typical seasonality, can progress to a year round dermatitis (Scott and Miller, 2003). The demonstration of allergen-specific IgE by intradermal testing or by serological testing (Lorch et al., 2001; Ferroglia et al., 2006) has been used to determine offending allergens and discriminate between atopy and IBH. Information with respect to differences in IgE levels in diseased and healthy animals is crucial in enabling an understanding of the clinical relevance of results of allergen-specific IgE tests. The use of serology as compared to skin tests in the horse has many advantages (Scott and Miller, 2003). It is much easier to take a blood sample than to perform an intradermal skin test. Furthermore, the skin test in the horse is more difficult to interpret than it is in the dog and is usually undertaken only by dermatologists.

The aim of this study was (i) to evaluate and compare levels of allergen-specific IgE, using an ELISA method, in Icelandic horses, with and without IBH, from Iceland and Sweden respectively; (ii) to investigate patterns of allergen-specific IgE to insects, pollens, moulds and mites in those groups of horses; and (iii) to investigate the clinical significance of employing two different cut-off levels for the ELISA.

2. Materials and methods

2.1. Study population

Icelandic horses, from Sweden and Iceland, with and without clinical signs compatible with IBH were identified in Sweden and in Iceland during September and October 2006. The study comprised 99 horses that were divided into five groups, namely (a) healthy controls: born and living in Iceland (group 1, healthy Ice), born in Iceland and exported to Sweden (group 2, healthy exp), born in Sweden or Denmark (group 3, healthy Swe-born), and (b) horses with IBH: born in Iceland and exported to Sweden (group 4, IBH exp) and born in Sweden (group 5, IBH Swe-born). The Swedish horses came from the middle part of Sweden and the Icelandic horses from the Institute of Experimental Pathology at Keldur, Iceland.

The number of horses in each group, mean age, gender, severity of eczema and exposure to *Culicoides*, in groups 1–5, are listed in Table 1. From Sweden 77 horses were included and from Iceland 22 horses.

Horses were considered healthy if they had no previous history of skin problems, were not using insect blankets and were kept outdoors during the insect period, had not received any medical treatment except deworming and regular vaccination and showed no signs of IBH upon clinical examination. Only horses of 3 years of age or more were included in the healthy

Table 1

The median age, gender, severity of eczema and extent of exposure to *Culicoides* (number of summers spent in Sweden) of horses included in the study

Group	No. of horses	Median age ^a	Ratio males:mares	Number with mild/moderate/severe eczema	Mean no. of seasons in Sweden
1, Healthy Ice	22	11.5	20:2	NA ^b	NA
2, Healthy exp	14	18 (13)	9:5	NA	11.5
3, Healthy Swe	18	7	7:11	NA	7.9
4, IBH exp	30	18.5	12:18	4/15/11	7.3
5, IBH Swe	15	11	1:14	2/10/3	4

^a Based on number if different from *n*.

^b NA: not applicable.

groups of horses. Horses classified as having IBH all used insect protecting blankets or were stabled during insect season. The presence of IBH was based on history and clinical signs at the examination and included the following classifications: healthy, mild, moderate and severe eczema. Mild signs of eczema were defined as pruritus affecting mane and tail with self-induced hairloss but no visible skin lesions, moderate signs of eczema was defined as pruritus and dermatitis affecting mane and tail area and severe eczema was defined as pruritus and dermatitis of mane and tail and also on other parts of the body. The feeding management was not controlled in any of the groups.

Blood was collected by venipuncture of vena jugularis from all horses. The blood was allowed to clot and following centrifugation the serum was harvested and stored at -20°C .

2.2. Laboratory methods

Sera from the horses were analysed blindly with the use of AllerceptTM, a non-competitive, solid-phase ELISA-test, designed to detect the presence of allergen-specific IgE in sera using the recombinant alpha chain of the high-affinity IgE receptor (FcεR1α). The test uses the cloned human alpha chain which has shown reactivity with canine, feline and equine IgE. A standard AllerceptTM protocol was used: 100 μl of the diluted sample (1:6) was added to coated micro wells and incubated overnight (16–18 h) at 4–8 °C. The wells were subsequently washed once with Tris buffered saline containing 0.05% Tween-20 (TBST) and 100 μl of appropriately diluted biotinylated Fc receptor (FcεR1α) was added. After 2 h incubation at 22 °C, the wells were again washed once with TBST, and 100 μl of appropriately diluted streptavidin–alkaline phosphatase (SA–AP) was added. After 60 min incubation at 22 °C a final washing (four times with TBST) was performed and 100 μl of p-NPP substrate was added. The enzymatic color reaction was stopped after 60 min by the addition of 50 μl of 20 mM cysteine. The optical density (OD) was determined at 405 nm.

The results were calculated as follows: OD minus blank times Normalisation Factor (value obtained from assay's calibrators) times 1000. The final value was reported in arbitrary EA units (ELISA Absorption units). The cut-off value has been set at 150 EA units in the commercial assay. All samples were tested blindly against 5 insects, 9 molds, 12 pollens and 3 mites (Table 3).

2.3. Statistical analysis

The distribution of the ELISA values was shown for each insect, mould, mite and pollen allergen, in groups 1–5, using 10th, 50th and 90th percentiles. Two cut-off levels, 150 and 300 EA, were used in an attempt to demonstrate clinically relevant cut-off values. The number and proportions of horses, positive for each insect, mould, mite and pollen allergen, were shown by group, together with 95% exact binomial confidence intervals (CI), the latter indicating statistical significance between groups when non-overlapping. In addition, in situations when confidence levels overlapped by <15%, group 2 (healthy exp) and group 4 (IBH exp) and group 3 (healthy Swe-born) and group 5 (healthy Swe-born) were compared. The Wilcoxon test was used for continuous data and Fisher's exact test for categorical data.

At the 150 cut-off, the number of positive reactions to all 29 allergens was compared between the healthy groups, namely groups 2 (healthy exp) and 3 (healthy Swe-born) and the IBH groups, namely 4 (IBH exp) and 5 (IBH Swe-born), using the Wilcoxon method. The same calculation was made for IBH horses in relation to the eczema scoring. *P*-values <0.05 were considered statistically significant.

3. Results

3.1. Anti-insect IgE

3.1.1. Healthy horses born and living in Iceland (group 1)

The 10th percentiles for all insect allergens were lowest in horses born and living in Iceland (Table 2). Only one horse had levels >150 EA, to *Simulium* and *Tabanus* (Table 3). All other insect allergens tested were negative. No horse had levels >300 EA (Table 4).

3.1.2. Horses in groups 2–5

Levels >150 EA were most commonly registered for *Tabanus* followed by *Culicoides* (Table 3). In group 2 (healthy exp), 43% of the horses had levels >150 EA to *Tabanus* compared with group 4 (IBH exp) where 83% had levels >150 EA. The same trend was noted for *Culicoides*. In group 2 (healthy exp) 29% had levels >150 EA compared with 63% in group 4 (IBH exp). For both *Tabanus* and *Culicoides* 95% CIs overlapped.

Table 2

Percentiles (10, 50 and 90) of the ELISA values for insects, moulds, mites and pollens in the 5 groups of horses

		Percentiles (10%, 50%, 90%)														
		Healthy Ice			Healthy exp			Healthy Swe			IBH exp			IBH Swe		
		1 (n = 22)			2 (n = 14)			3 (n = 18)			4 (n = 30)			5 (n = 15)		
Insects	Simulium	0	0	24	39	145	263	33	111	281	66	176	284	0	104	332
	Culex	0	0	6	13	110	51	15	76	207	38	127	291	0	78	221
	Tabanus	0	0	13	47	118	445	56	196	355	139	251	768	18	186	467
	Stomoxys	0	0	0	3	47	445	1	23	100	13	35	88	0	21	115
	Culicoides	0	0	0	8	109	223	11	131	508	40	227	535	0	171	855
Moulds	Alternaria	0	0	16	18	51	99	11	32	62	19	34	66	17	32	64
	Aspergillus	0	2	21	10	29	52	8	23	42	7	23	54	9	17	35
	Cladosporium	0	0	6	3	16	34	1	8	25	0	13	26	3	11	20
	Epicoccum	0	0	11	11	28	46	10	21	35	9	21	46	8	16	50
	Helminthosp	0	2	9	11	23	59	5	18	31	5	17	42	9	15	36
	Penicillium	2	8	20	7	30	65	5	16	43	4	18	43	10	18	53
	Fusarium	0	0	14	0	11	53	0	14	33	0	14	52	4	14	40
	Ustilago	0	0	0	1	22	32	2	23	40	1	16	68	5	22	68
	Rhizopus	0	0	0	0	0	0	0	0	25	0	0	11	0	0	16
Mites	Acarus	0	27	137	37	133	237	75	117	316	41	160	361	63	124	311
	Dpt + Dfarinae	56	108	348	76	142	253	84	148	377	56	168	476	87	183	337
	Tyrophagus	0	0	12	0	29	188	24	177	453	0	114	259	12	182	317
Pollens	Orchard grass	6	13	33	30	48	98	17	50	143	25	54	149	23	87	138
	Timothy	0	4	19	31	41	114	23	37	66	24	53	109	26	82	152
	Rye culture	1	16	34	15	41	112	20	32	89	18	64	150	18	53	119
	Mugwort	0	0	16	4	50	86	14	36	64	24	41	73	14	39	75
	Lamb's Quarter	0	0	0	7	38	75	8	36	83	15	37	80	10	31	95
	Ribwort	7	22	63	54	76	129	31	65	242	46	96	243	46	112	510
	Nettle	3	14	21	26	46	74	23	34	58	26	42	73	39	71	96
	Yellow Dock	0	0	15	22	66	168	14	79	596	44	98	260	57	88	609
	Birch	10	25	55	44	96	215	22	48	271	38	72	205	36	88	549
	Salow	1	15	68	33	53	69	26	50	117	30	68	159	42	87	297
	Hazel	4	12	27	43	53	75	20	42	101	29	48	138	40	76	334
	Beech eur	33	69	410	86	158	551	25	102	705	71	202	858	64	147	1987

At the 300 cut-off level for *Tabanus*, 14% of the horses in group 2 (healthy exp) had levels >300 EA compared to 43% in group 4 (IBH exp). In group 3 (healthy Swe-born), 28% had levels >300 EA compared to 40% in group 5 (IBH Swe-born) though 95% CIs overlapped for both comparisons. At the 300 EA cut-off level for *Culicoides*, 7% of the horses in group 2 (healthy exp) had levels >300 EA compared to 33% in group 4 (IBH exp) and in group 3 (healthy Swe-born) 17% had levels >300 EA compared to 40% in group 5 (IBH Swe-born). However 95% CIs overlapped.

Overlap less than 15% was found only for *Tabanus* (6%) and *Culicoides* (14%) at the 150 EA level comparing group 2 (healthy exp) and 4 (IBH exp). The number of positive reactions was significantly greater for *Tabanus* ($P = 0.012$) but not significant for *Culicoides* ($P = 0.052$). Comparing the original continuous values (Wilcoxon), the P -values were 0.008 and 0.026 respectively.

3.2. Anti-mould IgE

3.2.1. Horses in groups 1–5

All horses were negative towards *Alternaria*, *Cladosporium*, *Epicoccum*, *Helminthosporium*, *Penicillium* and *Fusarium*. Few positive reactions ($n = 6$) distributed in all groups were seen towards *Aspergillus*, *Ustilago* and *Rhizopus*, and no difference in pattern was seen using the 300 EA cut-off (Tables 3 and 4).

3.3. Anti-pollen IgE

3.3.1. Horses in groups 1–5

All sera tested were negative towards Mugwort. In all groups levels >150 EA were most commonly registered towards Beech and the levels ranged between 27% and 60% as seen in Table 3. At the 300 EA cut-off levels ranged between 9% and 40%, with the highest percentage in group 5 (IBH Swe-born) as seen in Table 4. The highest 90th percentile

Table 3
Number and percent positive results using ELISA values with a cut-off of 150 units

		Healthy Ice 1 (n = 22)			Healthy exp 2 (n = 14)			Healthy Swe 3 (n = 18)			IBH exp 4 (n = 30)			IBH Swe 5 (n = 15)		
		n	%	95%CI	n	%	95%CI	n	%	95%CI	n	%	95%CI	n	%	95%CI
Insects	Simulium	1	5	0.1–23	7	50	23–77	5	28	10–53	18	60	41–77	6	40	16–68
	Culex	0	0	0–15 ^a	6	43	18–71	6	33	13–59	11	37	20–56	4	27	8–55
	Tabanus	1	5	0.1–23	6	43	18–71	10	56	31–78	25	83	65–94	9	60	32–84
	Stomoxys	0	0	0–15 ^a	2	14	2–43	1	6	1–27	0	0	0–12 ^a	1	7	0.2–32
	Culicoides	0	0	0–15 ^a	4	29	8–58	9	50	26–74	19	63	44–80	8	53	27–79
Moulds	Alternaria	0	0	0–15 ^a	0	0	0–23 ^a	0	0	0–19 ^a	0	0	0–12 ^a	0	0	0–22 ^a
	Aspergillus	1	5	0.1–23	0	0	0–23 ^a	0	0	0–19 ^a	0	0	0–12 ^a	0	0	0–22 ^a
	Cladosporium	0	0	0–15 ^a	0	0	0–23 ^a	0	0	0–19 ^a	0	0	0–12 ^a	0	0	0–22 ^a
	Epicoecum	0	0	0–15 ^a	0	0	0–23 ^a	0	0	0–19 ^a	0	0	0–12 ^a	0	0	0–22 ^a
	Helminthosp	0	0	0–15 ^a	0	0	0–23 ^a	0	0	0–19 ^a	0	0	0–12 ^a	0	0	0–22 ^a
	Penicillium	0	0	0–15 ^a	0	0	0–23 ^a	0	0	0–19 ^a	0	0	0–12 ^a	0	0	0–22 ^a
	Fusarium	0	0	0–15 ^a	0	0	0–23 ^a	0	0	0–19 ^a	0	0	0–12 ^a	0	0	0–22 ^a
	Ustilago	1	5	0.1–23	1	7	0.2–34	0	0	0–19 ^a	1	3	0.1–17	0	0	0–22 ^a
	Rhizopus	0	0	0–15 ^a	0	0	0–23 ^a	1	6	1–27	1	3	0.1–17	0	0	0–22 ^a
Mites	Acarus	2	9	1–29	6	43	18–71	7	39	17–64	17	57	37–75	5	33	12–62
	Dpt + Dfar	7	32	14–55	7	50	23–77	8	44	22–69	20	67	47–83	9	60	32–84
	Tyrophagus	0	0	0–15 ^a	3	21	5–51	11	61	36–83	13	43	25–63	10	67	38–88
Pollens	Orchard grass	0	0	0–15 ^a	0	0	0–23 ^a	2	11	1–35	3	10	2–27	2	13	2–40
	Timothy	0	0	0–15 ^a	1	7	0.2–34	0	0	0–19 ^a	2	7	0.8–22	2	13	2–40
	Rye culture	0	0	0–15 ^a	1	7	0.2–34	1	6	1–27	3	10	2–27	1	7	0.2–32
	Mugwort	0	0	0–15 ^a	0	0	0–23 ^a	0	0	0–19 ^a	0	0	0–12 ^a	0	0	0–22 ^a
	Lamb's Quar	0	0	0–15 ^a	0	0	0–23 ^a	1	6	1–27	1	3	0.1–17	0	0	0–22 ^a
	Ribwort	0	0	0–15 ^a	1	7	0.2–34	6	33	13–59	6	20	8–39	6	40	16–68
	Nettle	0	0	0–15 ^a	1	7	0.2–34	0	0	0–19 ^a	0	0	0–12 ^a	0	0	0–22
	Yellow Dock	0	0	0–15 ^a	2	14	2–43	4	22	6–48	10	33	17–53	5	33	12–62
	Birch	0	0	0–15 ^a	4	29	8–58	3	17	4–41	5	17	6–35	4	27	8–55
	Salow	0	0	0–15 ^a	1	7	0.2–34	1	6	1–27	5	17	6–35	3	20	4–48
	Hazel	0	0	0–15 ^a	0	0	0–23 ^a	1	6	1–27	3	10	2–27	2	13	2–40
	Beech eur	6	27	11–50	8	57	29–82	6	33	13–59	18	60	41–77	7	47	21–73

^a One-sided 97.5% confidence interval.

for 10 out of 12 pollens was in group 5 as seen in Table 2.

3.4. Anti-mite IgE

3.4.1. Horses in groups 1–5

In group 1 (healthy Ice) all horses were negative towards Tyrophagus. In all groups levels >150 EA were most commonly registered towards *D. farinae*/*D. pteronyssinus* and the levels ranged between 32% up to 67%. At the 300 EA cut-off levels ranged between 9% and 40% (Tables 3 and 4).

3.5. Number of positive reactions in relation to groups and to eczema scoring

The mean value of positive reactions, at the cut-off >150 EA, from all 29 allergens was in group 2 (healthy

exp) 4.3, in group 3 (healthy Swe-born) 4.6, in group 4 (IBH exp) 6.0 and in group 5 (IBH Swe-born) 5.6. The difference in mean number of positive reactions between groups 2 and 3, versus 4 and 5 was borderline significant ($P = 0.053$).

The number of positive reactions for the ELISA to all 29 allergens in relation to severity of clinical signs in IBH horses did not differ statistically.

4. Discussion

To our knowledge, this is the first study when a serological test for allergen-specific IgE towards insects is used within a control group of native horses where IBH and *Culicoides* spp., which is the major source of allergens, does not exist. The high incidence of the disease in exported Icelandic horses (Broström et al., 1987; Halldórsdóttir and Larsen, 1991; Björnsdóttir

Table 4

Number and percent positive results using ELISA values and a cut-off of 300 units

		Healthy Ice 1 (n = 22)			Healthy exp 2 (n = 14)			Healthy Swe 3 (n = 18)			Healthy exp 4 (n = 30)			Healthy Swe 5 (n = 15)		
		n	%	95% CI	n	%	95% CI	n	%	95% CI	n	%	95% CI	n	%	95% CI
Insects	Simulium	0	0	0–15 ^a	1	7	0.2–34	2	11	1–35	2	7	0.8–22	2	13	2–40
	Culex	0	0	0–15 ^a	1	7	0.2–34	1	6	1–27	3	10	2–27	1	7	0.2–32
	Tabanus	0	0	0–15 ^a	2	14	2–43	5	28	10–53	13	43	25–63	3	20	4–48
	Stomoxys	0	0	0–15 ^a	0	0	0–23	0	0	0–19	0	0	0–12	1	7	0.2–32
	Culicoides	0	0	0–15 ^a	1	7	0.2–34	3	17	4–41	10	33	17–53	6	40	16–68
Moulds	Alternaria	0	0	0–15 ^a	0	0	0–23 ^a	0	0	0–19 ^a	0	0	0–12 ^a	0	0	0–22 ^a
	Aspergillus	0	0	0–15 ^a	0	0	0–23 ^a	0	0	0–19 ^a	0	0	0–12 ^a	0	0	0–22 ^a
	Cladospo	0	0	0–15 ^a	0	0	0–23 ^a	0	0	0–19 ^a	0	0	0–12 ^a	0	0	0–22 ^a
	Epicoccum	0	0	0–15 ^a	0	0	0–23 ^a	0	0	0–19 ^a	0	0	0–12 ^a	0	0	0–22 ^a
	Helmintosp	0	0	0–15 ^a	0	0	0–23 ^a	0	0	0–19 ^a	0	0	0–12 ^a	0	0	0–22 ^a
	Penicillium	0	0	0–15 ^a	0	0	0–23 ^a	0	0	0–19 ^a	0	0	0–12 ^a	0	0	0–22 ^a
	Fusarium	0	0	0–15 ^a	0	0	0–23 ^a	0	0	0–19 ^a	0	0	0–12 ^a	0	0	0–22 ^a
	Ustilago	0	0	0–15 ^a	1	7	0.2–34	0	0	0–19 ^a	0	0	0–12 ^a	0	0	0–22 ^a
	Rhizopus	0	0	0–15 ^a	0	0	0–23	1	6	1–27	0	0	0–12 ^a	0	0	0–22 ^a
Mites	Acarus	0	0	0–15 ^a	1	7	0.2–34	3	17	4–41	7	23	10–42	2	13	2–40
	Dpt + Dfarinae	3	14	5–51	1	7	0.2–34	3	17	4–41	7	23	10–42	2	13	2–40
	Tyrophagus	0	0	0–15 ^a	1	7	0.2–34	4	22	6–48	4	13	4–31	2	13	2–40
Pollens	Orchard gr	0	0	0–15 ^a	0	0	0–23 ^a	0	0	0–19 ^a	2	7	0.8–22	0	0	0–22 ^a
	Timothy	0	0	0–15 ^a	0	0	0–23 ^a	0	0	0–19 ^a	0	0	0–12 ^a	0	0	0–22 ^a
	Rye culture	0	0	0–15 ^a	0	0	0–23 ^a	0	0	0–19 ^a	0	0	0–12 ^a	0	0	0–22 ^a
	Mugwort	0	0	0–15 ^a	0	0	0–23 ^a	0	0	0–19 ^a	0	0	0–12 ^a	0	0	0–22 ^a
	Lamb's Quar	0	0	0–15 ^a	0	0	0–23 ^a	0	0	0–19 ^a	0	0	0–12 ^a	0	0	0–22 ^a
	Ribwort	0	0	0–15 ^a	1	7	0.2–34	1	6	1–27	3	10	2–27	2	13	2–40
	Nettle	0	0	0–15 ^a	0	0	0–23 ^a	0	0	0–19 ^a	0	0	0–12 ^a	0	0	0–22 ^a
	Yellow Dock	0	0	0–15 ^a	0	0	0–23 ^a	4	22	6–48	3	10	2–27	3	20	4–48
	Birch	0	0	0–15 ^a	1	7	0.2–34	2	11	1–35	3	10	2–27	2	13	2–40
	Salow	0	0	0–15 ^a	0	0	0–23 ^a	0	0	0–19 ^a	0	0	0–12 ^a	2	13	2–40
	Hazel	0	0	0–15 ^a	0	0	0–23 ^a	0	0	0–19 ^a	1	3	0.1–17	2	13	2–40
	Beech eur	2	9	1–29	3	21	5–51	4	22	6–48	8	27	12–46	6	40	16–68

^a One-sided 97.5% confidence interval.

et al., 2006) makes this breed suitable for studies of IBH and the presence of allergen-specific IgE. The conclusions from this study are that the AllerceptTM test often yields positive reactions in clinically healthy individuals and determination of offending allergens by serology testing in IBH and atopic horses can have a low specificity when testing for specific allergens. Different studies in dogs and horses have compared intradermal testing with serology testing with poor sensitivity and specificity results and most studies have used the intradermal test as a golden standard (Bond et al., 1994; Lorch et al., 2001; Foster et al., 2003; Saevik et al., 2003; Morgan et al., 2007). There are a number of different tests that employ IgE serology.

Radioallergsorbent test (RAST) and different enzyme-linked immunosorbent assays (ELISA) using mono- or polyclonal anti-IgE-antibodies can detect relative levels of allergen-specific IgE. Cross-reactivity between IgE

and IgG, causing false positive reactions, can occur and isotype-specific reactivity must be carefully assessed (DeBoer and Hillier, 2001; Morgan et al., 2007). In this study the sera were analysed for allergen-specific IgE by a non-competitive, solid-phase ELISA-test based on the FcεR1α receptor of IgE, a molecule that binds specifically and exclusively to IgE (Stedman et al., 2001). Nevertheless, positive results according to the ELISA were commonly recorded in the healthy control groups in Sweden but to a lesser extent in the healthy groups in Iceland. The frequent finding of high levels of allergen-specific IgE in the clinically healthy horses might be due to the animals suffering from sub-clinical disease and who could be at risk of developing the disease. Another possibility might be that the animals were indeed exposed previously and hence reactive, but not currently exposed to an extent sufficient to induce disease. Another explanation could be that allergen-specific IgE is not

the only immunoglobulin responsible for hypersensitivity reactions in horses or that IgE is only a part of the pathogenesis. Hellberg et al. (2006) showed that IgG subclasses can play a role and Wagner et al. (2006) found that IgGT can bind to skin mast cells and might contribute to allergy. Hellberg et al. (2006) also showed that every horse had its own characteristic IgE-binding pattern, which changed from year to year, which further explains the complexity of the disease. Interestingly, horses living in Iceland showed binding of IgGa and IgGT to salivary gland extracts from *Culicoides*, a biting midge that is not found in Iceland. The explanation for this could be that different insect species share proteins in the saliva and exposure to *Simulium vittatum*, an insect native to Iceland could have been responsible. In this study only one horse in the healthy group in Iceland had slightly elevated levels towards *Simulium* and *Tabanus*. None of those horses had positive reactions against *Culicoides*. The low levels of allergen-specific IgE to *Simulium* could be explained by the minor yearly exposure time as the insect season in Iceland is short.

There is thus a possibility that horses susceptible to the disease can be sensitised to insects already in Iceland. Baselgia et al. (2006) demonstrated that IBH-affected horses are sensitised to both *Culicoides* and *Simulium* extracts. Hellberg et al. (2006) also found that IgE antibodies bind to protein bands of the same molecular weight in immunoblots from both insect species.

The insect extracts used in the test were whole body extracts and one explanation for the low specificity could be lack of purification of the offending allergen or that the major allergen is missing. More than 80 species of *Culicoides* exist in Sweden and there is no information as to which are most commonly implicated in equine IBH. In this study antigen from *C. nubeculosus* was used and antigenic cross-reactivity among *Culicoides* species has been described (Andersson et al., 1993; Hellberg et al., 2006; Baselgia et al., 2006). In flea bite hypersensitivity, a common allergic skin disease in dogs, allergen-specific IgE to purified flea salivary proteins correlated with clinical disease (McCall, 1997). Studies have demonstrated at least 15 different antigens in flea saliva and when using whole body extracts only <0.5% of the antigens were present (Cook, 1998). Hellberg et al. (2006) demonstrated that the biting midges, *Culicoides* spp., have many IgE binding sites. In the salivary glands of the insect, more than 10 proteins with the ability to bind IgE have been found. Whether several proteins are relevant, or there is one major allergen responsible for the immunopathogenesis remains to be elucidated.

Many of the healthy control horses in Iceland had elevated levels of allergen-specific IgE to house dust

mites (32%) and pollens (27%, to beech) according to the ELISA. House dust mites are amongst the major allergens responsible for atopic dermatitis in dogs (Sture et al., 1995; Hill and DeBoer, 2001), but there have been no extensive studies in horses. If this is an indication of horses with sub-clinical disease, or individuals classified as high IgE responders, it could be of great importance. These horses might be at a greater risk of developing severe IBH than those lacking such reactivity if exported to the continent where the horses are massively exposed to *Culicoides* spp., the major allergen in IBH.

All horses in all groups showed high levels of IgE towards beech. In Iceland beech is very uncommon (Margret Hallsdóttir, personal comm.) and only rarely seen in gardens. Birch is however common in Iceland and cross-reactivity between beech and birch has been described (Weber, 2003). However it is noteworthy that all horses in Iceland tested negative towards birch, the main pollen in Iceland. Small numbers of horses in all groups showed IgE reactivity against moulds. It seems that mould is not an important allergen in these horses, although mould reactivity may well be more important in horses housed in an environment with high levels of mould. The low levels may be reflective of the fact that these horses were kept under good conditions outdoors.

Positive allergen-specific reactions were recorded employing the use of two different cut-off levels in this study. With a cut-off at 300 EA fewer apparently false positive reactions were seen compared with the 150 EA cut-off, although healthy horses still showed positive reactions. Ideally, the most relevant cut-off level should be determined for each allergen.

In allergic dogs, it is assumed that clinical signs develop if a pruritic threshold is exceeded (Marsella and Candace, 2001). In the present study horses in the IBH groups had positive reactions towards a higher number of allergens than did the healthy horses. There was no significant difference in the number of positive reactions between groups with different severity of eczema. Group 5 (IBH, Swe-born) had the highest percentiles for 10 out of 12 pollen allergens and this could indicate that these horses have a more atopic pattern (Table 2).

Further research on atopic dermatitis and the relationship between atopy and IBH in the horse may identify differences in susceptibility, severity of signs, clinical picture, heritability and gene expression.

Admittedly, the population used in the present study was not randomly selected. However the authors judge that the horses were representative of the Icelandic horse population, with and without IBH, in the sampled

locations, even though group 1 (healthy Ice) was sampled from one location and that this group of horses therefore may have been affected by similar environmental factors. Further, larger groups could have yielded stronger statistical significance.

5. Conclusions

The results of this study show that serological testing with an ELISA that uses the high-affinity IgE receptor (FcεR1α) is presently not suitable as a tool for establishing a diagnosis of IBH or atopy. It is merely a guide to determine which allergens are involved in the disease. The use of two cut-off levels, 150 EA and 300 EA, did not eliminate the apparent false positives. The correct cut-off level has to be better established for the different allergens.

Acknowledgements

The authors would like to thank Heska AG, Fribourg, Switzerland for analysing the sera, especially Cornelia Boschung who did the laboratory work.

We also would like to thank Professor Richard Halliwell for giving helpful advice on the manuscript.

References

- Andersson, G.S., Belton, P., Kleider, N., 1993. Hypersensitivity of horses in British Columbia to extracts of native and exotic species of *Culicoides* (Diptera: Ceratopogonidae). *J. Med. Entomol.* 30, 657–663.
- Baselgia, S., Doherr, M.G., Mellor, P., Torsteinsdottir, S., Jermann, T., Zurbriggen, A., Jungi, T., Marti, E., 2006. Evaluation of an in vitro sulphidoleukotriene release test for diagnosis of insect bite hypersensitivity in horses. *Equine Vet. J.* 38, 40–46.
- Bjornsdottir, S., Sigvaldadottir, J., Brostrom, H., Langvad, B., Sigurdsson, A., 2006. Summer eczema in exported horses: influence of environmental and genetic factors. *Acta Vet. Scand.* 48 (1), 3–26.
- Bond, R., Thorogood, S.C., Lloyd, D.H., 1994. Evaluation of two enzyme linked immunosorbent assays for the diagnosis of canine atopy. *Vet. Rec.* 135 (6), 130–133.
- Brostrom, H., Larsson, A., Troedsson, M., 1987. Allergic dermatitis (sweet itch) of Icelandic horses in Sweden: an epidemiological study. *Equine Vet. J.* 19 (3), 229–236.
- Cook, C.A., 1998. The in vitro diagnosis of flea bite hypersensitivity: flea saliva versus whole-flea extracts. In: Kwochka, K.W., et al. (Eds.), *Advances in Veterinary Dermatology III*. Butterworth Heinemann, Boston, p. 494.
- DeBoer, D.J., Hillier, A., 2001. The ACVD task force on canine atopic dermatitis (XVI): laboratory evaluation of dogs with atopic dermatitis with serum-based “allergy” tests. *Vet. Immunol. Immunopathol.* 81, 277–287.
- Ferroglio, E., Pregel, P., Accossato, A., Taricco, I., Bollo, E., Rossi, L., Trisciuglio, A., 2006. Equine *Culicoides* hypersensitivity: evaluation of a skin test and of humoral response. *J. Vet. Med. A.* 53, 30–33.
- Foster, A.P., Littlewood, J.D., Webb, P., Wood, J.L., Rogers, K., Shaw, S.E., 2003. Comparison of intradermal and serum testing for allergen specific IgE using a Fcεpsilon R1alpha-based assay in atopic dogs in the UK. *Vet. Immunol. Immunopathol.* 93 (1–2), 51–60.
- Halldorsdottir, S., Larsen, H.J., 1991. An epidemiologic study of summereczema in Icelandic horses in Norway. *Equine Vet. J.* 23 (4), 296–299.
- Hellberg, W., Wilson, A.D., Mellor, P., Doherr, M.G., Torsteinsdottir, S., Zurbriggen, A., Jungi, T., Marti, E., 2006. Equine insect bite hypersensitivity: immunoblot analysis of IgE and IgG subclass responses to *Culicoides nubeculosus* salivary gland extract. *Vet. Immunol. Immunopathol.* 113 (1–2), 99–112.
- Hill, P.B., DeBoer, D.J., 2001. The ACVD task force on canine atopic dermatitis (IV): environmental allergens. *Vet. Immunol. Immunopathol.* 81, 169–186.
- Lorch, G., Hillier, A., Kwochka, K.W., Saville, W.J., Kohn, C.W., LeRoy, B.E., 2001. Comparison of immediate intradermal test reactivity with serum IgE quantitation by use of radioallergen sorbent test and two ELISA in horses with and without atopy. *J. Am. Vet. Med. Assoc.* 218 (8), 1314–1322.
- Marsella, R., Candace, A.S., 2001. The ACVD task force on canine atopic dermatitis (XIII): threshold phenomenon and summation of effects. *Vet. Immunol. Immunopathol.* 81, 251–253.
- Morgan, E.E., Miller W.H.Jr., Wagner, B.A., 2007. 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.* 19.
- McCall, C.A., 1997. FcεR1α-based measurement of anti-flea saliva IgE in dogs. *Comp. Cont. Educ. Pract. Vet.* 19 (Suppl.), 24.
- Saevik, B.K., Ulstein, T.L., Larsen, H.J., 2003. Evaluation of commercially available enzyme-linked immunosorbent assay for the detection of allergen-specific IgE antibodies in dogs. *Res. Vet. Sci.* 74 (1), 37–45.
- Scott, D.W., Miller, W.H., 2003. Skin immune system and allergic skin disease. In: Scott, D.W., Miller, W.H. (Eds.), *Equine Dermatology*. Elsevier Science, USA, pp. 427–462.
- Stedman, K., Lee, K., Hunter, S., Rivoire, B., McCall, C., Wassom, D., 2001. Measurement of canine IgE using the alpha chain of the human high affinity IgE receptor. *Vet. Immunol. Immunopathol.* 78 (3–4), 349–355.
- Sture, G.H., Halliwell, R.E.W., Thoday, K.L., van den Broek, A.H.M., Henfrey, J.I., Lloyd, D.H., Mason, I.S., Ferguson, E., 1995. Canine atopic disease: the prevalence of positive intradermal skin tests at two sites in the north and south of Great Britain. *Vet. Immunol. Immunopathol.* 44, 293–308.
- van der Haegen, A., Griot-Wenk, M., Welle, M., Busato, A., van Tscarner, C., Zurbriggen, A., Marti, E., 2001. Immunoglobulin-E-bearing cells in skin biopsies of horses with insect bite hypersensitivity. *Equine Vet. J.* 33 (7), 699–706.
- Wagner, B., Miller, W.H., Morgan, E.E., Hillegas, J.M., Erb, H.N., Leibold, W., Antczak, D.F., 2006. IgE and IgG antibodies in skin allergy of the horse. *Vet. Res.* 37, 813–825.
- Weber, R.W., 2003. Patterns of pollen cross-allergenicity. *J. Allergy Clin. Immunol.* 112 (2), 229–239 quiz 240.
- Willemse, A., 1986. Atopic skin disease: a review and a reconsideration of diagnostic criteria. *J. Small Anim. Pract.* 27, 771.
- Wilson, A.D., Harwood, L.J., Bjornsdottir, S., Marti, E., Day, M.J., 2001. Detection of IgG and IgE serum antibodies to *Culicoides* salivary gland antigens in horses with insect dermal hypersensitivity (sweet itch). *Equine Vet. J.* 33, 707–713.