



Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm



Conference

Report of the 3rd Havemeyer workshop on allergic diseases of the Horse, Hólar, Iceland, June 2007

ARTICLE INFO

Article history:
Received 18 July 2008

ABSTRACT

Allergic diseases occur in most mammals, although some species such as humans, dogs and horses seem to be more prone to develop allergies than others. In horses, insect bite hypersensitivity (IBH), an allergic dermatitis caused by bites of midges, and recurrent airway obstruction (RAO), a hyperreactivity to stable born dust and allergens, are the two most prevalent allergic diseases.

Allergic diseases involve the interaction of three major factors: (i) genetic constitution, (ii) exposure to allergens, and (iii) a dysregulation of the immune response determined by (i) and (ii). However, other environmental factors such as infectious diseases, contact with endotoxin and degree of infestation with endoparasites have been shown to influence the prevalence of allergic diseases in humans. How these factors may impact upon allergic disease in the horse is unknown at this time. The 3rd workshop on Allergic Diseases of the Horse, with major sponsorship from the Havemeyer Foundation, was held in Hólar, Iceland, in June 2007 and focussed on immunological and genetic aspects of IBH and RAO.

This particular venue was chosen because of the prevalence of IBH in exported Icelandic horses. The incidence of IBH is significantly different between Icelandic horses born in Europe or North America and those born in Iceland and exported as adults. Although the genetic factors and allergens are the same, exported adult horses show a greater incidence of IBH. This suggests that environmental or epigenetic factors may contribute to this response. This report summarizes the present state of knowledge and summarizes important issues discussed at the workshop.

1. Introduction

The 3rd Havemeyer workshop focussed on immunological and genetic aspects of insect bite hypersensitivity (IBH) and recurrent airway obstruction (RAO).

IBH, or sweet itch, is a recurrent pruritic allergic dermatitis of the horse occurring in summer and caused by IgE-mediated reactions to bites of midges. The allergens causing IBH are likely salivary gland proteins from these insects, but the individual allergens have not yet been identified. IBH occurs in horses of various breeds worldwide, except for regions, like Iceland, where *Culicoides* midges are absent. However, IBH has an especially high prevalence in horses of the Icelandic breed exported from Iceland to geographic areas where *Culicoides* spp. are present. IBH causes considerable discomfort to the affected

horses, which scratch excessively especially in the mane and tail regions, causing secondary skin lesions. Presently, no long term effective treatment exists.

RAO or heaves, is a severe, potentially debilitating, inflammatory airway disease typically affecting middle-aged horses and resulting in coughing, increased respiratory effort and exercise intolerance due to bronchospasm and mucus accumulation in the airways. RAO, caused in part by inherited factors and influenced by environmental conditions, is common in stabled horses fed hay. It is initiated by the inhalation of organic dust and, once sensitized, a horse becomes hypersensitive to airborne particles and requires careful management. However, the relative importance of IgE-mediated reactions, Th1- and Th2-type profiles, innate immunity, and individual cytokines is still unclear.

0165-2427/\$ – see front matter
doi:[10.1016/j.vetimm.2008.07.008](https://doi.org/10.1016/j.vetimm.2008.07.008)

Please cite this article in press as: version="1.0" encoding="utf-8">Report of the 3rd Havemeyer workshop on allergic diseases of the Horse, Hólar, Iceland, June 2007. Vet. Immunol. Immunopathol. (2008), doi:[10.1016/j.vetimm.2008.07.008](https://doi.org/10.1016/j.vetimm.2008.07.008)

This summary report presents the results of a workshop held at Hólar, Iceland in June 2007. Advances in immunology, such as the detection of new pathways by microarray technology, as well as progress in the identification, cloning and delivery of recombinant allergens were presented. In genetics, the completion of the horse genome sequence and the development of new genetic tools for the horse, open new avenues for the study of both diseases. Extensive sampling of RAO and IBH-affected horses is on-going. Presentations and state-of-the-art lectures also addressed new reagents and technologies for the study of these diseases and new therapeutic concepts. Summary statements presented by working groups formed at the meeting and plenary discussions identified important questions to be addressed, potential investigative strategies, and research areas for collaborative efforts.

2. Immunopathology of RAO and IBH and immune response of foals

2.1. RAO (heaves)

In conventional indoor stable environments with hay feeding and straw bedding, the air in the breathing zone of horses contains very high levels of organic dust with allergens and non-specific irritants like endotoxin (Woods et al., 1993). Since the use of various terms referring to environment-induced lower airway disease has led to confusion, consistent working definitions were proposed based on the state-of-the-art of our knowledge (Robinson, 2001): RAO, according to these definitions, is a severe, debilitating disease characterized by coughing and increased breathing effort due to cholinergic bronchospasm and airway hyperreactivity as well as neutrophil and mucus accumulation in the airways believed to result from hypersensitivity reactions to various inhaled allergens. Removal of hay and straw often leads to remission of the disease. While its phenotype is defined, the immunopathophysiology of heaves remains ill understood.

In the last decade, a number of studies have addressed the role of T cells in equine diseases. There are now examples of predominant Th1 (e.g. Herpesvirus; Slater et al., 2006) and Th2 (e.g. parasite defense) type responses in the horse, supportive for the basic concept of the Th1 vs. Th2 paradigm in horses (Horohov, KY, USA).

To date, there are reports of a predominant Th2-type response in RAO and Summer pasture associated (SPA)-RAO with increased numbers of interleukin (IL)-4 and IL-5 secreting (*in situ* hybridization) cells and increased mRNA levels of IL-4 and IL-13 in BAL of RAO and SPA-RAO horses (Lavoie et al., 2001; Beadle et al., 2002; Cordeau et al., 2004). However, there are also reports of either a Th1-type profile (Ainsworth et al., 2003), or studies suggesting no involvement of either type responses in heaves (Kleiber et al., 2005). Th1-type cytokines, such as interferon (IFN)- γ , can also be increased in crisis and may persist longer during remission. Furthermore, non-specific responses to endotoxin, alone and through potentiation of reactions to mould allergens, play a role in RAO (Pirie et al., 2003) and endotoxin increases Toll-like receptor (TLR)-4 and TLR2 mRNA in horse lungs (Singh Suri et al., 2006). It was

proposed that some contradictory results could in part be due to investigations of different groups of RAO-affected horses at different time-points in the course of the disease (Horohov, KY, USA). Information presented at the workshop supports the involvement of a Th2-type response in RAO, and also showed the potential of microarray expression technology for the identification of new candidate genes.

IL-8, a potent chemokine for neutrophils, was shown to be increased in BALF (Franchini et al., 2000) and the epithelium (Ainsworth et al., 2006) of RAO-affected horses. Based on reports that IL-17 is increased in stabled RAO-affected horses (Debrue et al., 2005) and on findings in other species, Ainsworth/Wagner (NY, USA) hypothesized that mononuclear cell derived IL-17 and IL-23 may drive epithelial chemokine production in RAO. They found, however, that neutrophil-depleted BAL cells of horses with heaves and controls showed a similar inherent expression of IL-17, IL-23, IL-8 and IL-1 β as well as a similar increase of these cytokines after treatment with LPS, β -glucan and hay dust extracts (Ainsworth et al., 2007). These results suggest that production of these cytokines by mononuclear cells plays a minimal role in the propagation of airway neutrophilia in RAO. Evidence presented by Lavoie (Montreal, Canada) showed that IL-4, a major Th2-type cytokine, has the potential to contribute to neutrophil activation: recombinant equine IL-4 causes morphological changes in blood neutrophils, increases IL-8 mRNA levels and potentiates effects of LPS and TNF- α on IL-8 expression by pulmonary artery endothelial cells. Receptors for IL-4 (IL-4R), however, were not increased on neutrophils of RAO-affected compared to healthy horses. This does, however, not exclude an involvement of the IL-4R in the pathogenesis of RAO, as Gerber (Bern, Switzerland) presented evidence for a genetic linkage of polymorphisms in IL-4R- α with RAO and increased expression of this receptor in one high-prevalence family of horses but not in another one (see below).

A model of ovalbumin (OVA) sensitized horses, which develop airway obstruction and neutrophilia and a Th2-type profile (Bowles et al., 2002) was presented (Horohov, KY, USA). These results are consistent with the development of Th2-type response in horses exhibiting some of the main characteristics of RAO. As in other species, the effectiveness of parasite defence in the horse relies on a Th2-type response, specifically the production of IL-4, IL-5 and IL-13 cytokines. In this model, acute parasitism (infection with *Strongylus vulgaris*) in parasite-free horses exacerbated the allergic response to OVA inhalation. A similar effect of parasitism on allergy development has been proposed in humans (Carvalho et al., 2006).

Gene microarrays, developed for human gene expression studies, have helped identify new candidate genes involved in both innate and adaptive immunity. These new candidate genes may also play a role in RAO (Ramery et al., 2008; Ramery, Liège, Belgium). Differential expression of the genes PTX3, CYBB, MARCKS was confirmed with real time RT-PCR. There is evidence from other species that PTX3 overexpression can contribute to lung injury and the down regulation of CYBB to the recruitment of neutrophils. The importance of these mediators in the initiation and expression of RAO remains to be determined.

The following consensus statement on RAO immunology for future research was made (Lavoie, Canada; Horohov, USA; Ramery, Belgium; Wilson, UK; Gerber, Switzerland):

1. While the RAO phenotype has been clinically well-defined, no accepted recommendations exist for standardized exposure protocols and sampling times in experimental studies of RAO. This, and the as yet ill-defined overlying variation from chronicity and age-related changes, make comparisons between studies difficult.
2. A further methodological issue is to define which tissue is best to study: bronchoalveolar lavage (BAL) fluid; BAL separated cells; bronchial brushings or lung tissue biopsies.
3. The role of IgE and individual allergens in RAO is still unclear. An experimental protocol for the definitive investigation of this question would need to:
 - a. Identify potential major recombinant allergens in RAO-affected individuals by serological screening and confirm the relevance of putative recombinant allergens by functional *in vitro* tests for each affected individual.
 - b. Perform regional bronchial challenge with the identified recombinant allergens and measure reaction by BAL transfer containing allergen-specific IgE to a naïve unaffected individual.
 - c. Perform regional bronchial challenge and compare reaction before and after transfer.
4. Exposure experiments in neutrophil-depleted RAO-affected horses are needed to determine the relevance and pathophysiological contribution of neutrophils in RAO.
5. Longitudinal, epidemiological studies are necessary to identify predisposing factors in the environment of foals and young horses that may influence the development to RAO.

2.2. Insect bite hypersensitivity (IBH)

Compared to RAO, the pathogenesis of IBH is better characterized. Various studies have suggested that IBH is an IgE-mediated reaction to bites of insects, mainly *Culicoides* species but other insects have been implicated in some cases. Affected horses most often show immediate type reactions after intradermal injections of *Culicoides* extract and sometimes also react to extracts from other insects. In a subset of horses, delayed type reactions have been observed following intradermal tests with insect extracts. Peripheral blood leukocytes of IBH-affected horses release significantly more sulfidoleukotriene or histamine after stimulation with *Culicoides* and to some extent also with *Simulium* extract than those of control horses (Baselgia et al., 2006). Sera of IBH-affected horses also have significantly more often IgE and IgG abs binding to *Culicoides* salivary proteins (Wilson et al., 2001; Hellberg et al., 2006).

A modified Prausnitz-Küstner experiment has provided the first direct evidence that IgE mediates classical Type-I allergy in horses and plays a major role in the pathogenesis of IBH (Wagner, NY, USA): IgE antibodies were purified with anti-IgE affinity columns from serum of IBH-affected

horses. These purified IgE abs were injected intradermally in four clinically healthy horses. The following day, *Culicoides* allergen extract was injected at the same sites. Skin reactions were not observed in response to allergen alone, and in two horses after stimulation at any previous IgE injection site. However, the other two healthy horses showed an immediate skin reaction at the previous injection sites of IgE obtained from allergic horses (Wagner et al., 2006a,b). These findings support an early study showing that reagin-like antibodies can be transferred in the horse (Matthews et al., 1983).

In the skin, IBH lesions are characterized by oedema, infiltration with eosinophils, increased numbers of IgE-bearing cells, mast cells (van der Haegen et al., 2001), and increased expression of Th2 cytokines (Marti, Berne, Switzerland). Chronic lesions are characterized by hyperkeratosis and T cell infiltrates. There is presently no evidence of increased expression of Th1 cytokines (IFN- γ) in chronic IBH-lesions as known in human atopic eczema. However, further studies are necessary to confirm these findings as only a small number of chronic lesions were tested so far (Marti, Berne, Switzerland).

In contrast to human atopic eczema, clinically normal skin of horses affected with IBH is not characterized by increased levels of IL-1 β mRNA. Intradermal injection of *Culicoides nubeculosus* extract stimulates local IL-1 β transcription in all horses independent from disease status, but the extent of upregulation from basal levels only reaches statistical significance in IBH-affected horses during the active stages of the disease. In humans and mice, IL-1 RA down-regulates antigen specific IL-4 and IgE responses with concomitant enhancement of IFN- γ production *in vitro* and *in vivo*. There is no indication so far that cutaneous IL-1RA has a modulatory effect in equine IBH (Kolm et al., 2006).

In summary, an IgE-mediated mast cell degranulation, i.e. a type I hypersensitivity reaction is sufficient to explain the acute phases of IBH. This, however, does not exclude that other immunological mechanisms are also involved in chronic stages of the disease.

A special focus was then given to the specific aspects of IBH in Icelandic horses. IBH is a serious problem in exported Icelandic horses but also occurs in Icelandic horses born abroad. The disease is not seen in the native horse population because the causative agents, *Culicoides* spp., are not present in Iceland. As introduced Björnsdóttir (Hólar, Iceland) summarised a recently published study on the prevalence of IBH in Icelandic horses (Björnsdóttir et al., 2006). Overall, IBH was diagnosed in 34.5% of 330 horses exported from Iceland to Denmark, Sweden or Germany. The number of affected horses increased with duration of exposure to midges. At two years post export, the prevalence was 49.5%, and even reached 54% in horses that had lived in areas heavily infested by *Culicoides* spp. A slightly lower prevalence of IBH of 47% was found in another group of 191 horses exported to Norway or Sweden that had been living in *Culicoides* spp. rich environments for at least two years. Age at export, gender, sire and country of examination were not associated with the prevalence of IBH in exported Icelandic horses. Information about the time of onset of the disease

was available for 49 horses and ranged from 1 to 8 years after export. Most commonly the first signs of IBH were detected 2–3 years after export (mean 2.4 years). By comparison, only 4.6% of 1192 Icelandic horses born in Germany in *Culicoides* infested areas were affected with IBH. It was concluded that Icelandic horses are predisposed to IBH most likely as a result of a lack of exposure to *Culicoides* bites in early life and possibly because of the great environmental changes following export. The Icelandic horse does not seem to be inherently more susceptible to IBH than other breeds if the individuals are born in an environment where midges are present.

Hamza (Berne, Switzerland) compared the Th1/Th2 polarity in healthy and IBH-affected horses of the Icelandic breed imported from Iceland to Europe (1st generation horses) or born in Europe (2nd generation horses). During summer, after *in vitro* stimulation, PBMC from IBH-affected horses produced significantly more IL-4 and less IFN- γ than those of healthy horses (Hamza et al., 2007). Most interestingly, upon polyclonal or antigen-specific stimulation, lymphocytes of 1st generation Icelandic horses expressed significantly higher levels of Th2-cytokines than those of 2nd generation Icelandic horses matched with regard to disease state. This may indicate that Th2-type cytokine expression is suppressed in horses born in Switzerland. These results raise the question whether the IL-4 response in 2nd generation horses is suppressed by regulatory cytokines. To test this hypothesis, supernatant from PBMC cultures of healthy 2nd generation horses were added to cultures of IBH-affected 1st generation horses. Addition of these supernatants led to a significant decrease of IL-4 production by PBMC of IBH 1st generation horses. This effect could be mimicked by addition of IL-10 and TGF β 1. On the other hand, neutralisation of IL-10 and TGF β 1 in PBMC cultures of healthy 2nd generation horses led to a significant increase of IL-4 production (Hamza et al., 2008). These findings indicate that in 1st generation horses the immune response is Th2 biased and that this could be due to decreased regulatory cytokine expression (Hamza, Berne, Switzerland). Further studies are necessary to investigate the presence of regulatory T cells in the horse and their potential role in allergic disease.

The discussion then focussed on factors which may lead to this Th2 biased immune response which results in a high prevalence of IBH in imported Icelandic horses. The timing of exposure to *Culicoides* bites was considered to be a major factor: horses born in Europe are first exposed to *Culicoides* allergens at the age of 2–3 months when their own IgE production is low and maternal IgE levels are present although decreasing (Wilson, Bristol, UK). There is presently no evidence supporting priming by maternal IgE (Wilson, Bristol, UK; Wagner, NY, USA) and whether maternal factors such as passive IgG antibody transfer have a protective effect remains unknown. Horses imported from Iceland are usually imported as adults i.e. the first contact with the *Culicoides* allergens happens with a mature immune system. However, besides age at first exposure to *Culicoides* allergens other environmental factors may also contribute to the high prevalence of IBH in imported horses. A recent study had shown that

horses living in Iceland have high total IgE levels, which decreased some time after import to Switzerland and remained relatively low in horses remaining unaffected by IBH, while IgE levels increased again in horses developing IBH (Wilson et al., 2006). As high total IgE levels are induced by helminth infections, it was thus hypothesized that horses living in Iceland (where anthelmintic use is infrequent) may have a higher endoparasitic burden than horses living in Europe. It has been previously shown that horses imported from Iceland to Norway have a lower incidence of IBH if they arrive during the summer compared to the winter (Halldorsdóttir and Larsen, 1991). Taking this observation together with the results reported here we propose that horses exposed to *Culicoides* immediately after import would have a high level of total IgE (presumably to helminths) which may block the Fc ϵ receptors on mast cell, thus competitively preventing a new IgE response to *Culicoides*. In contrast, total IgE levels of horses imported in winter will have dropped until the following spring and sensitization of mast cell by exposure to *Culicoides* at this time may be facilitated.

Infestation with helminths also influences the immune response at the Th-level, leading to a strong Th2 response. A pilot study, however, showed that there is no evidence for high IL-4 production following polyclonal activation or stimulation of PBMCs with helminth extracts in horses living in Iceland (Hamza, Berne, Switzerland). Studies in humans, however, have shown that chronic infections with endoparasites lead a strong regulatory network (Carvalho et al., 2006). Regulatory cytokines need now to be determined in these horses. Further environmental factors specific for the Iceland environment are also the absence of some viral infections such as equine influenza and EHV-1 and -4 (Svansson, Reykjavik, Iceland). Viral diseases are known to induce a Th1-type immune response, inhibiting a Th2 response. This inhibition loop may thus be absent in horses living in Iceland favoring a higher Th2 response in exported horses exposed to insect bites in the new environment.

Post export, Icelandic horses are usually immediately de-wormed and vaccinated, and depending on the season of export, exposed to *Culicoides* bites. It was agreed that studies are needed to investigate whether *Culicoides* bite exposure alone or other environmental factors are involved in the high prevalence of IBH in horses exported from Iceland to Europe.

In summary, while the pathogenesis of IBH is relatively well-defined and clearly related to Th2-type, IgE-mediated reactions, the pathogenesis of RAO is less clear. Different Th-type immune responses are probably involved in the pathogenesis of RAO depending on time point, exposure to antigens and airway irritants and also depending on the genetic background of the horse.

2.3. Foals

As the differences in the immune response between foals and adult horses may be one of the factors contributing to the differences in IBH prevalence in horses exposed for the first time to bites of *Culicoides* as adults (i.e. horses imported from Iceland to Europe) as compared to foals born in an environment where IBH allergens are

present, data on immune responses in foals were also presented and discussed at this workshop.

While transfer of IgG occurs in all vertebrates, IgE is not transferred in all mammal species. Wagner (NY, USA) and Wilson (Bristol, UK) showed that in the horse there is maternal transfer of IgE with the colostrums (Wagner et al., 2006b): IgE antibodies detected in foals in the first 3–4 months of life are of maternal origin and levels are not correlated with those later in life. Furthermore, there is evidence for IgE binding to mast cells directly after birth. IgE positive intestinal mast cells are found in the lamina propria of neonatal foals but disappear after a few weeks and re-appear later (Wilson, Bristol, UK). They can be frequently found in lymph nodes (Wagner, NY, USA). IgE then becomes undetectable for a variable period of time until the foal's endogenous production begins at 6–12 months of age. The levels of early endogenous IgE production at six months of age are correlated with levels later in life at least until three years of age.

How maternal IgE present in young foals may influence and modulate their immune response and development is currently unknown. The relatively late onset of endogenous production and the lack of a correlation between maternal and endogenous levels may explain, however, why in horses IgE-mediated allergic diseases are usually not observed before puberty.

Further characterisation of foals' immune development has come from the study on cytokine profiles over time (Horohov, Lexington, KY, USA): IL-13 may be initially elevated in newborn foals and then declines over time. Further, diminished INF- γ , IL-4, IL-5 and IL-10 production characterizes neonatal foals compared to adult horses. Thus, there is currently no evidence supporting Th2 biases in foals, and the role of these cytokine profiles and fluctuations in the onset of allergy is unclear. Low INF- γ both at the mRNA and protein level, however, may predispose foals to intracellular pathogens like *Rhodococcus equi* and rotavirus (Breathnach et al., 2006).

Cunningham (London, UK) then gave an overview of new potential therapeutic targets in equine allergic diseases, such as inhibition of cytokines and chemokines or inhibition of transcription factors which should result in decreased synthesis of multiple inflammatory proteins which are described in more details by Cunningham and Dunkel (2008).

3. Serology, other *in vitro* allergy tests and intradermal testing in RAO and IBH

There is older evidence suggesting IgE-mediated type I hyperreactivity (Halliwell et al., 1993; Hare et al., 1999) in RAO. Newer studies showed that within the same environment, unrelated RAO-affected horses have increased serum IgE levels against *Aspergillus fumigatus* and *Alternaria alternata* allergens (Künzle et al., 2007). However, environmental as well as genetic factors influence mould allergen-specific IgE levels in the horse (Eder et al., 2001; Curik et al., 2003). In horses living under various environmental conditions there was no significant difference in serum IgE levels against *A. alternata* and *A. fumigatus* extracts between RAO-affected and control

horses (Eder et al., 2000). Increased levels in RAO horses were only found for specific IgE against the recombinant allergens rAsp f 7 and 8 and rAlt a 1. Evidence presented at this workshop indicates that total IgE is not associated with the RAO phenotype (Gerber, Berne, Switzerland). Contrarily, total IgE is influenced by environmental factors (predominantly parasite loads) and decreases with age (Marti, Berne, Switzerland). In contrast, allergen-specific IgE (rAsp7) and, more strongly, the IgG subclass IgG₄ (IgG1) are associated with the RAO-phenotype in two Warmblood halfsibling high-prevalence families. Both IgG₄ and allergen-specific IgE were also higher in offspring of one compared to offspring of another RAO-affected sire.

Overall, commercial allergy tests have not been shown to be useful for the diagnosis of RAO so far. Comparing a serological IgE ELISA (the FcRI α -based Test; ALLERCEPTTM, Heska), an *in vitro* sulfidoleukotriene release assay (Cellular Antigen Stimulation Test (CAST[®]), DPC Bühlmann, Switzerland) and intradermal testing (IDT) Marti (Berne, Switzerland) found some agreement between the tests: the majority of positive reactions in all tests were found with mite extracts, but none of the tests showed a significant difference between RAO-affected and control horses. In contrast, another commercial serological IgE ELISA test, showed high sensitivity (92%) and acceptable specificity (76%) in different groups of RAO-affected and control horses. However, the nature of the reagent in this ELISA was not disclosed, and therefore the specificity of the IgE detection remains unclear.

Various tests have been evaluated for *in vitro* diagnosis of IBH recently. They include serological (FcRI α -based Test (ALLERCEPTTM from Heska) and Polychex Allergie NF Horse Panel, BioCheck GmbH, Münster, Germany) as well as functional *in vitro* tests such as the Functional *In vitro* Test (FIT, Hannover), where histamine release is measured, or the Cellular Antigen Stimulation Test (CAST[®], DPC Bühlmann, Switzerland), where released sulfidoleukotrienes are measured after incubation of peripheral blood leukocytes with presumptive allergen extracts.

In the first study presented (Kolm-Stark, Vienna, Austria) the FcRI α -based serological test, the FIT and the CAST[®] were compared to intradermal testing, using the same five insect extracts (*Tabanus*, *Simulium*, *Culex*, *Stomoxys* and *C. nubeculosus*). Sixteen horses with and 29 horses without IBH were included in the study. In summary, there was fair to moderate agreement (kappa statistics) between the three *in vitro* tests and clinical diagnosis of IBH.

In the second study (Frey, Norrköping and Uppsala, Sweden) sera from 99 Icelandic horses were tested in the FcRI α -based Test (ALLERCEPTTM, Heska). IgE levels against five insect species were determined (*Culicoides*, *Simulium*, *Tabanus*, *Stomoxys* and *Culex*). All horses living in Iceland, with one exception, had IgE levels below the cut off of the test. Within the group of horses born in Iceland and exported to Sweden, 45% of the IBH-affected and 35% of the healthy horses gave a positive reaction with at least one insect extract. Within the horses born in Sweden, the proportion of horses showing a positive reaction with at least one insect extract was lower both in the IBH-affected group (17%) as well as in the healthy animals (10%). The

results of this study will be presented in more details in Frey et al. (in press).

A test for determination of allergen-specific IgE in horse sera using a monoclonal anti-IgE antibody, which is presently being developed (Polycheck Allergie NF Horse Panel, BioCheck GmbH, Münster, Germany), was evaluated (Marti, Berne, Switzerland) for diagnosis of IBH. For this purpose, sera of 48 IBH-affected and 49 healthy control horses, usually living in the same environment as the IBH-affected horses, were used. A receiver operator curve (ROC) analysis was first performed to determine the best cut off of the assay. Using this cut off, the test with *C. nubeculosus* extract had a sensitivity of 71% and a specificity of 92%. Sensitivity and specificity of the test were lower with a *Simulium vittatum* extract (67% and 69%, respectively) confirming a previous study with the CAST[®] (Baselgia et al., 2006). These findings provide further evidence, together with the fact that *Culicoides* but not *Simulium* are absent in Iceland, that the main allergens for IBH are derived from *Culicoides* spp.

In conclusion, there was a fair to moderate agreement between the *in vitro* tests presented here and the clinical diagnosis of IBH. The performance of these tests needs to be improved and this can be achieved by the use of high affinity IgE reagents (monoclonal antibodies) and of pure recombinant allergens instead of the whole body extracts which are presently being used. For RAO the situation is more complex as it is still controversial if and to what extent IgE-mediated reactions are involved in the pathogenesis of this disease. Further studies are also needed to identify the specific antigens that are involved in RAO.

4. Allergens

While no recent data were presented on the allergens that might be responsible for RAO, a number of studies are now underway to identify the specific allergens causing IBH. Previous studies had shown that *Culicoides* whole body extracts contain a wide range of IgE-binding antigens and that all allergic horses show positive reactions with these antigens (Wilson et al., 2001). In a recent study using *C. nubeculosus* salivary gland extract (Hellberg et al., 2006), ten different IgE-binding protein bands were identified, with molecular weights ranging between 75 and 12 kDa. It was shown that serum IgE of most of the allergic horses bind to at least one salivary gland protein but with variable affinity, and that each horse shows an individual IgE-binding pattern to the different allergens present in the salivary gland extract (Marti, Berne, Switzerland).

A proteomics approach was used to identify candidate allergens for IBH which is described in more details in Wilson et al. (2008). In brief, this study showed that *Culicoides* salivary gland extract contains a limited number of highly expressed proteins corresponding to abundant mRNA species and that a group of proteins with basic pI and molecular weights of about 40 kDa are the most frequent IgE-binding proteins in 2D blots (Wilson, Bristol, UK).

A “candidate allergen” approach was used by Björnsdóttir (Reykjavík, Iceland): Hyaluronidase and antigen 5 are known allergen in bee and/or vespid venom and EST

sequences from *C. sonorensis* with homology to antigen 5 and hyaluronidase are available. Based on these sequences, antigen 5 and hyaluronidase have been isolated from a *C. nubeculosus* salivary gland library. These are now in the process of being expressed as recombinant proteins.

In summary two different cDNA libraries have been made from *C. nubeculosus* salivary glands and cDNA clones encoding putative allergens are available including D7 proteins, antigen 5, hyaluronidase, trypsin as well as various clones with homology to novel *C. sonorensis* salivary gland sequences with unknown function (Wilson, Bristol, UK). In particular, one additional candidate allergen, a glycoprotein with an observed molecular weight of about 45–50 kDa and a calculated pI of 9.1 which was similar to the *C. sonorensis* salivary protein AY603640 was identified.

Using a different approach, clones expressing IgE-binding proteins from *S. vittatum* have been isolated using phage surface display technology. Seven putative *S. vittatum* salivary gland allergens have been cloned and expressed in *E. coli*, including a protein with homology to antigen 5. This protein appears to be an interesting candidate allergen as it binds significantly more often IgE antibodies from sera of IBH-affected horses than from healthy control horses (Marti, Berne, Switzerland). Interestingly, antibodies produced against the r-antigen 5 protein from *S. vittatum* also bound to a protein band from *C. nubeculosus* SGE of a similar molecular weight, suggesting that the antigen 5 protein from *S. vittatum* and *C. nubeculosus* are closely related.

The question to which extent the various *Culicoides* species contain conserved allergens was raised at the workshop. Studies have shown that horses with IBH usually showed positive reactions in intradermal testing with different species of *Culicoides* and even with *Culicoides* species which are not native to the areas where the horses are living (Anderson et al., 1993; Langner et al., 2008). The fact that most IBH-affected horses show IgE-binding to at least one salivary gland protein from *C. nubeculosus*, a species which is only rarely found in Switzerland seems to confirm these findings (Hellberg et al., 2006). On the other hand, comparison of cDNA sequences of *C. sonorensis* EST sequences with sequences generated from *C. nubeculosus* sequences suggests that for some sequences, although they share 85% amino acid homology, this was seldom in stretches long enough to provide an immune epitope (Wilson, Bristol, UK; Wilson et al., 2008).

In summary, the search of the specific allergens responsible for IBH is progressing. It was agreed that there is a need to find and express putative recombinant allergens for IBH both in *E. coli* and insect cells. These putative recombinant allergens then need to be tested *in vitro* and *in vivo* for their relevance as allergens for IBH. It was also stated that it would be very useful to establish an international bank of recombinant *Culicoides* salivary proteins.

5. New treatment of allergy

As IBH is often due to IgE-mediated reactions and the specific IBH allergens are in the process of being identified, there is good chance to implement a successful allergen-

specific immunotherapy (SIT) for this condition in the future. On the other hand, SIT for treatment of RAO cannot be presently envisaged due to the lack of knowledge about the molecular structures involved in the immunopathogenesis of RAO which still need to be identified.

There are no recent studies published on the efficacy of immunotherapy for IBH. However, it is generally recognised that the success of specific immunotherapy for IBH is currently limited, probably because of the use of *Culicoides* whole body extracts instead of pure salivary gland proteins. In humans, an effective immunotherapy leads to a change in the Th1/Th2/Treg ratios and the induction of regulatory T cells. A high antigenicity leads to a Th1 response, which results in a counter regulatory induction of IL-10 and T_{regs} (Larché et al., 2006). However, the immunological changes leading to a successful SIT in humans require a long time to establish, which strongly compromises the patient's compliance and, as a consequence thereof, the number of patients which are choosing SIT as a therapeutic option for allergy (Cramer, 2007).

Novel approaches for SIT against IBH were discussed at this workshop, including DNA vaccination, the use of viral vectors or protein vaccination with allergens coupled to modular antigen translocating (MAT) molecules, which enhance the antigen presentation through intracellular targeting of the MHC class II presentation pathway (Cramer, Davos, Switzerland; Cramer et al., 2007).

Different pilot studies have been performed with the aim to improve immunotherapy for equine allergy. As the specific allergens for IBH have not been identified yet, studies aimed at shifting the balance of the immune response from a Th2 to a Th1 phenotype were performed using human serum albumin (HSA) as a model allergen. DNA vaccination of horses with HSA resulted in a very weak immune response which did not abrogate the IgE response after boosting with HSA protein in aluminium hydroxide (Svansson, Reykjavik, Iceland). The aim of the next pilot study was then to improve HSA expression from the vectors with a Kozak sequence and the cytomegalovirus (CMV) intron A. Insertion of CMV intron A diminished the expression of HSA from the pcDNA vectors whereas insertion of a Kozak sequence upstream of the gene increased the *in vitro* expression both in COS7 cells and primary horse cells derived from skin, lung, duodenum and kidney (Ólafsdóttir, Reykjavik, Iceland; Ólafsdóttir et al., submitted for publication). The use of adjuvants inducing a Th1 shift is another option for improvement of specific immunotherapy. Monophosphoryl-lipid (MLP) was tested as an adjuvant and compared to aluminium hydroxide (alum) in a further pilot study. Horse immunised with HSA/MLP had a slightly higher IFN- γ /IL-4 ratio than horses immunised with HSA/aluminium hydroxide. However, both groups responded with similar IgG subclass profiles. The HSA-specific IgE response was boosted to high levels in the HSA/MLP horses but was more transient than in the HSA/alum horses that showed a strong and persistent IgE response (Torsteinsdóttir, Reykjavik, Iceland). It was concluded that unlike in laboratory mice, MLP does probably not induce a sufficiently strong Th1 immune response to induce T_{regs} and inhibit a Th2 response in the horse.

The following issues regarding specific immunotherapy (SIT) were discussed (Lunn, CO, USA; Cramer, Davos, Switzerland; Svansson, Reykjavik, Iceland): If the specific allergens are used as proteins for SIT their immunogenicity could be improved through coupling to modular antigen translocating molecules (Cramer, Davos, Switzerland). Alternatively, DNA vaccination could be used. However, although DNA vaccination induces a Th1 response in the horse it seems to be quite inefficient. This could be improved by physically targeting the lymph nodes, testing various CpG motifs as adjuvants, and by the addition of cytokines. Another option could be the use of viral vectors. The immune response in viral infections is generally Th1-mediated and could thus induce T_{regs}. Several groups have been working with human and animal herpes viruses as a model for potential vectors for gene delivery. It was shown that the immunological response to EHV-2 is strongly Th1 biased (Svansson, Reykjavik, Iceland). An EHV-2 vector could thus be engineered as a vector for vaccination against IBH. The approach to vaccination using EHV-1 as vaccine vector has recently been described in the horse (Rosas et al., 2007). Alternatively, a commercial non-persistent canary pox vector could be used (Lunn, CO, USA). This vector is presently used for vaccination against various viral diseases in the horse with good results (Poulet et al., 2007; Siger et al., 2006).

Finally, it was also discussed that for the Icelandic horses, a preventive immunisation with the relevant IBH allergens before export to *Culicoides* infested areas could be a possibility to reduce the high incidence of IBH in these horses.

6. Genetics of RAO and IBH

Antczak (NY, USA) presented recent advances in the understanding and information gained on the horse genome. Sequencing of the whole equine genome has been completed; this and other advances offer new tools to identify candidate genes for disease susceptibility. A concerted international effort has led to the development of tools such as a synteny map, a genetic linkage map, a cytogenetic map, a radiation hybrid (RH) map, and the ongoing effort to produce a high-resolution BAC-based physical map of the horse genome. To date the 'Newmarket Horse Genetic Linkage Map' consists of 950 microsatellite markers covering all autosomes and the X chromosome (Swinburne et al., 2000; Swinburne et al., 2006). Furthermore, The National Human Genome Research Institute of the USA has funded and completed a 7 $\times$ sequence of the horse genome, with SNP discovery. These tools are now becoming available to the equine research community.

A genetic predisposition for RAO has been previously shown in different full- and half-sibling groups (Gerber, 1989; Marti et al., 1991). Gerber (Berne, Switzerland) presented the validation of a new scoring system for larger epidemiological and genetic studies. A standardized questionnaire was used to gather information on the horses' history of chronic coughing, respiratory distress, increased breathing effort after exercise, and nasal discharge, all graded into categories of frequency and severity of symptoms. This information was used sepa-

rately for analyses and compounded into a Horse Owner Assessed Respiratory Signs Index (HOARSI): healthy (no symptoms observed = HOARSI 1), mildly affected (some nasal discharge and/or occasional cough = HOARSI 2), moderately affected (frequent coughing = HOARSI 3), severely affected (moderate to severe coughing and poor performance = HOARSI 4). Using the HOARSI, a strong heritability for RAO was confirmed in two large Warmblood half-sibling families of offspring from RAO-affected sires (Ramseyer et al., 2007). Coughing, compared to increased nasal discharge, poor performance and abnormal breathing pattern most markedly distinguished genetically predisposed RAO-affected offspring. Compared to maternal half-siblings, the risk of offspring from affected stallions was 4–5.5 fold increased. From preliminary segregation analyses, the investigators conclude that a single major gene cannot explain the observed phenotypes. The high heritabilities suggest the presence of additional major genes.

Association and segregation analysis of markers for epithelial signaling factors and other microsatellites located near atopy candidate genes as well as gene expression studies indicate IL-4RA as a candidate gene with disease association in one family but not in the other, indicating possible genotypic locus heterogeneity for this disease (Jost et al., 2007). Results from a whole genome scan showed association with other chromosomal regions in addition to the one around IL-4RA. Furthermore, preliminary results indicate that offspring from one high-prevalence family have lower parasitic burden (decreased fecal egg counts) than control pasture mates (Gerber, Berne, Switzerland).

A genetic basis of IBH has been demonstrated in Swiss Warmbloods and other breeds (Marti et al., 1992; Lazary et al., 1994). Björnsdóttir (Hólar, Iceland) found an overall prevalence of 4.6% in close to 1200 Icelandic horses born in Germany. When both parents were affected by IBH the prevalence was significantly higher (12.2%) compared to offspring with one of the parents affected (6.5%) or offspring with two unaffected parents (2.9%). Interestingly, disease status of the stallions was significant only when affected stallions were born in Germany, but not when they had been born in Iceland. Thus, the high prevalence of IBH in exported Icelandic horses appears to reflect environmental rather than genetic factors. Frey (Norrköping and Uppsala, Sweden) presented an on-going project in 27 Swedish-born Icelandic horse half-sib families consisting of 1211 individuals classified by questionnaire data into healthy, mild, moderate and severe IBH. The main goal of the project is to use samples from all affected individuals (8.3%) and healthy controls from each family to perform a whole genome association analysis by genotyping with a SNP chip.

Horin (Brno, Czech Republic) reported that out of 56 breeding mares of the Old Kladruber breed, which is genetically isolated and inbred, 17 were classified as IBH susceptible (showing clinical signs of IBH in at least one of nine seasons) and 39 as resistant (no clinical signs of IBH over nine years). A genome scan using 50 microsatellites and 9 single nucleotide polymorphisms (SNP) in 5 candidate genes identified significant associations between the microsatellite *AHT04* as well as SNPs in the

FcεR1 alpha and the *IFNγ* genes. Furthermore, a significant difference of total IgE levels was found between *IFNγ* intron 1 SNP heterozygotes and homozygotes.

The following issues were discussed and summarised (Antczak, NY, UK; Horin, Brno, Czech Republic; Gerber, Berne, Switzerland; Björnsdóttir, Hólar, Iceland; Frey, Norrköping and Uppsala, Sweden):

Both RAO and IBH have a large environmental component and both are probably complex traits, with more than 2 genes with major effects involved. Overall, there is no breed specificity for RAO or IBH. However, there is a high incidence of IBH in exported Icelandic horses, which is probably due to environmental factors (first exposure to allergen as adults). The fact that only 50% of these imported Icelandic horses become IBH-affected still suggests that genetic factors may be involved and the question was raised whether it would be possible to breed out IBH susceptible horses. For RAO, the identification of susceptible animals prior to developing the condition could allow prevention of the clinical manifestation of the disease through management such as dust/allergen poor environment and help preventing high risk matings. Furthermore, identification of genes responsible for RAO susceptibility could contribute to a better understanding of the pathogenesis of this disease.

For the future, it was recommended to collect samples (DNA bank) from phenotypically well characterized cases and controls. Furthermore, once candidate genes have been identified it will be needed to demonstrate that they translate into immunological anomalies. For functional genomics different samples, i.e. for RNA isolation, will be needed (genotyping versus expression studies). Samples should also be collected from different breeds. However, there should be enough samples from each breed to perform an analysis within breed. Samples can be from unrelated individuals or families. As both RAO and IBH are complex traits with many genes involved, a larger number of cases and controls will be needed (>100 per group).

New genomic resources (e.g. SNP chips) and bioinformatic approaches will likely become available within the next three years. They are currently expensive but costs will decrease. As cheaper alternative, small, targeted SNP arrays may be useful for some genetic studies.

7. Conclusion

In conclusion, this workshop has shown that while it is still controversial whether RAO is an allergic reaction and if so, which are the causative allergens for RAO, there is an improved understanding of the pathophysiology leading to RAO. The presence of a genetic predisposition for this condition has been thoroughly documented and the availability of DNA samples from families of RAO-affected sires may allow, in the future, to identify the genes involved in RAO susceptibility. The genetics approach may also be a way to better understand the pathogenesis of this disease as shown with the example of the IL-4Rα gene.

The immunopathogenesis of IBH is better characterized. However, the next task will be to understand which immunological mechanisms lead to the high prevalence of IBH in horses imported from Iceland to Europe. This may

allow identifying a way to prevent IBH in exported Icelandic horses and contribute to the understanding of the increase of allergy prevalence in humans. The first priority for research on IBH is now to identify the allergens at the molecular level. This will open the way to a causative treatment of the disease and to a better understanding of the immunopathogenesis underlying this allergic condition. Genetic studies are presently also being performed for IBH. First insights into the immune response of foals contribute to understand susceptibility of foals to some infectious diseases, such as *Rhodococcus* infections. Additionally, the low levels of serum IgE early in life may explain why clinical signs of allergy are usually not occurring in horses before puberty.

Acknowledgements

The authors are grateful to the Dorothy Russell Havemeyer Foundation for financial support and to Dr. Sigríður Björnsdóttir, Holar, Iceland, for the excellent local organization of the workshop.

References

- Ainsworth, D.M., Grunig, G., Matychak, M.B., Young, J., Wagner, B., Erb, H.N., Antczak, D.F., 2003. Recurrent airway obstruction (RAO) in horses is characterized by IFN-gamma and IL-8 production in bronchoalveolar lavage cells. *Vet. Immunol. Immunopathol.* 96, 83–91.
- Ainsworth, D.M., Wagner, B., Franchini, M., Grünig, G., Erb, H.N., Tan, J.Y., 2006. Time-dependent alterations in gene expression of interleukin-8 in the bronchial epithelium of horses with recurrent airway obstruction. *Am. J. Vet. Res.* 67, 669–677.
- Ainsworth, D.M., Wagner, B., Erb, H.N., Young, J.C., Retallick, D.E., 2007. Effects of in vitro exposure to hay dust on expression of interleukin-17, -23, -8, and -1beta and chemokine (C-X-C motif) ligand 2 by pulmonary mononuclear cells isolated from horses chronically affected with recurrent airway disease. *Am. J. Vet. Res.* 68, 1361–1369.
- Anderson, 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., Torsteinsdóttir, 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.
- Beadle, R.E., Horohov, D.W., Gaunt, S.D., 2002. Interleukin-4 and interferon-gamma gene expression in summer pasture-associated obstructive pulmonary disease affected horses. *Equine Vet. J.* 34, 389–394.
- Björnsdóttir, S., Sigvaldadóttir, J., Broström, H., Langvad, B., Sigurdsson, A., 2006. Summer eczema in exported Icelandic horses: influence of environmental and genetic factors. *Acta Vet. Scand.* 48, 3.
- Bowles, K.S., Beadle, R.E., Mouch, S., Pourciau, S.S., Littlefield-Chabaud, M.A., Le Blanc, C., Mistic, L., Fermaglich, D., Horohov, D.W., 2002. A novel model for equine recurrent airway obstruction. *Vet. Immunol. Immunopathol.* 87, 385–389.
- Breathnach, C.C., Sturgill-Wright, T., Stiltner, J.L., Adams, A.A., Lunn, D.P., Horohov, D.W., 2006. Foals are interferon gamma-deficient at birth. *Vet. Immunol. Immunopathol.* 112, 199–209.
- Carvalho, E.M., Bastos, L.S., Araujo, M.I., 2006. Worms and allergy. *Parasite immunol.* 28, 525–534.
- Cordeau, M.E., Joubert, P., Dewachi, O., Hamid, Q., Lavoie, J.P., 2004. IL-4, IL-5 and IFN-gamma mRNA expression in pulmonary lymphocytes in equine heaves. *Vet. Immunol. Immunopathol.* 97, 87–96.
- Cramer, R., 2007. Allergy vaccines: dreams and reality. *Exp. Rev. Vaccines* 6, 991–999.
- Cramer, R., Flückiger, S., Daigle, I., Kündig, T., Rhyner, C., 2007. Design, engineering and in vitro evaluation of MHC class-II targeting allergy vaccines. *Allergy* 62, 197–206.
- Cunningham, F.M., Dunkel, B., 2008. Equine recurrent airway obstruction and insect bite hypersensitivity: understanding the diseases and uncovering possible new therapeutic approaches. *Vet. J.* 177, 334–344.
- Curik, I., Fraser, D., Eder, C., Achmann, R., Swinburne, J., Binns, M., Cramer, R., Brem, G., Sölkner, J., Marti, E., 2003. Association between the MHC gene region and variation of serum IgE levels against specific mould allergens in the horse. *Genet. Selection Evol.* 35, 117–190.
- Debrue, M., Hamilton, E., Joubert, P., Lajoie-Kadoch, S., Lavoie, J.P., 2005. Chronic exacerbation of equine heaves is associated with an increased expression of interleukin-17 mRNA in bronchoalveolar lavage cells. *Vet. Immunol. Immunopathol.* 105, 25–31.
- Eder, C., Cramer, R., Mayer, C., Eicher, R., Straub, R., Gerber, H., Lazary, S., Marti, E., 2000. Allergen-specific IgE levels against crude mould and storage mite extracts and recombinant mould allergens in sera from horses affected with chronic bronchitis. *Vet. Immunol. Immunopathol.* 73, 241–253.
- Eder, C., Curik, I., Brem, G., Cramer, R., Bodo, I., Habe, F., Lazary, S., Sölkner, J., Marti, E., 2001. Influence of environmental and genetic factors on allergen-specific immunoglobulin E levels in sera from Lipizzan horses. *Equine Vet. J.* 33, 714–720.
- Franchini, M., Gill, U., von Fellenberg, R., Bracher, V.D., 2000. Interleukin-8 concentration and neutrophil chemotactic activity in bronchoalveolar lavage fluid of horses with chronic obstructive pulmonary disease following exposure to hay. *Am. J. Vet. Res.* 61, 1369–1374.
- Frey, R., Bergvall, K., Egenvall, A., 2008. Allergen specific IgE in Icelandic horses with insect bite hypersensitivity and healthy controls, assessed by FcεR1-based serology. *Vet. Immunol. Immunopathol.*, in press, Jul 4. [Epub ahead of print].
- Gerber, H., 1989. Sir Frederick Hobday memorial lecture. The genetic basis of some equine diseases. *Equine Vet. J.* 21, 244–248.
- Halldorsdóttir, S., Larsen, H.J., 1991. An epidemiological study of summer eczema in Icelandic horses in Norway. *Equine Vet. J.* 23, 296–299.
- Halliwell, R.E.W., McGorum, P.C., Irving, P., Dixon, P.M., 1993. Local and systemic antibody production in horses affected with chronic pulmonary disease. *Vet. Immunol. Immunopathol.* 38, 201–215.
- Hamza, E., Doherr, M.G., Bertoni, G., Jungi, T.W., Marti, E., 2007. Modulation of allergy incidence in Icelandic horses is associated with a change in IL-4-producing T cells. *Int. Arch. Allergy Immunol.* 144, 325–337.
- Hamza, E., Wagner, B., Jungi, T.W., Mirkovitch, J., Marti, E., 2008. Reduced incidence of insect-bite hypersensitivity in Icelandic horses is associated with a down-regulation of interleukin-4 by interleukin-10 and transforming growth factor-beta1. *Vet. Immunol. Immunopathol.* 122, 65–75.
- Hare, J.E., Viel, L., Conlon, P.D., Marshall, J.S., 1999. In vitro allergen-induced degranulation of pulmonary mast cells from horses with recurrent airway obstruction (heaves). *Am. J. Vet. Res.* 60, 841–846.
- Hellberg, W., Wilson, A.D., Mellor, P., Doherr, M.G., Torsteinsdóttir, 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, 99–112.
- Jost, U., Klukowska-Rötzler, J., Dolf, G., Swinburne, J.E., Ramseyer, A., Bugno, M., Burger, D., Blott, S., Gerber, V., 2007. A region on equine chromosome 13 is linked to recurrent airway obstruction in horses. *Equine Vet. J.* 39, 236–241.
- Kleiber, C., McGorum, B.C., Horohov, D.W., Pirie, R.S., Zurbriggen, A., Straub, R., 2005. Cytokine profiles of peripheral blood and airway CD4 and CD8 T lymphocytes in horses with recurrent airway obstruction. *Vet. Immunol. Immunopathol.* 104, 91–97.
- Kolm, G., Knapp, E., Wagner, R., Klein, D., 2006. Increased interleukin-1beta mRNA expression in skin biopsies of horses with *Culicoides* hypersensitivity following challenge with *Culicoides nubeculosus* extract. *Vet. Immunol. Immunopathol.* 113, 90–98.
- Künzle, F., Gerber, V., Van der Haegen, A., Wampfler, B., Straub, R., Marti, E., 2007. IgE-bearing cells in bronchoalveolar lavage fluid and allergen-specific IgE levels in sera from RAO-affected horses. *J. Vet. Med. A: Physiol. Pathol. Clin. Med.* 54, 40–47.
- Langner, K.F., Darpel, K.E., Drolet, B.S., Fischer, A., Hampel, S., Heselhaus, J.E., Mellor, P.S., Mertens, P.P., Leibold, W., 2008. Comparison of cellular and humoral immunoassays for the assessment of summer eczema in horses. *Vet. Immunol. Immunopathol.* 122, 126–137.
- Larché, M., Akdis, C.A., Valenta, R., 2006. Immunological mechanisms of allergen-specific immunotherapy. *Nat. Rev. Immunol.* 6, 761–771.
- Lavoie, J.P., Maghni, K., Desnoyers, M., Taha, R., Martin, J.G., Hamid, Q.A., 2001. Neutrophilic airway inflammation in horses with heaves is characterized by a Th2-type cytokine profile. *Am. J. Respir. Crit. Care Med.* 164, 1410–1413.
- Lazary, S., Marti, E., Szalai, G., Gaillard, C., Gerber, H., 1994. Studies on the frequency and associations of equine leucocyte antigens in sarcoid and summer dermatitis. *Anim. Genet. (Suppl. 1)*, 75–80.

- Marti, E., Gerber, H., Essich, G., Ouhlela, J., Lazary, S., 1991. On the genetic basis of equine allergic diseases. I. Chronic hypersensitivity bronchitis. *Equine Vet. J.* 23, 457–460.
- Marti, E., Gerber, H., Lazary, S., 1992. On the genetic basis of equine allergic diseases: II Insect bite dermal hypersensitivity. *Equine Vet. J.* 24, 113–117.
- Matthews, A.G., Imlah, P., McPherson, E.A., 1983. A reagin-like antibody in horse serum: 1. Occurrence and some biological properties. *Vet. Res. Commun.* 6, 13–23.
- Ólafsdóttir, G., Svansson, V., Ingvarsson, S., Marti, E., Torsteinsdóttir, S., 2008. In vitro analysis of expression vectors for DNA vaccination of horses. The effect of a Kozak sequence, submitted for publication.
- Pirie, R.S., Dixon, P.M., McGorum, B.C., 2003. Endotoxin contamination contributes to the pulmonary inflammatory and functional response to *Aspergillus fumigatus* extract inhalation in heaves horses. *Clin. Exp. Allergy* 33, 1289–1296.
- Poulet, H., Minke, J., Pardo, M.C., Juillard, V., Nordgren, B., Audonnet, J.C., 2007. Development and registration of recombinant veterinary vaccines. The example of the canarypox vector platform. *Vaccine* 25, 5606–5612.
- Ramery, E., Closset, R., Bureau, F., Art, T., Lekeux, P., 2008. Relevance of using a human microarray to study gene expression in heaves-affected horses. *Vet. J.* 177, 216–221.
- Ramseyer, A., Gaillard, C., Burger, D., Straub, R., Jost, U., Boog, C., Marti, E., Gerber, V., 2007. Effects of genetic and environmental factors on chronic lower airway disease in horses. *J. Vet. Int. Med.* 21, 149–156.
- Robinson, N.E., 2001. Chairperson's introduction: International Workshop on Equine Chronic Airway Disease, Michigan State University, 16–18 June 2000. *Equine Vet. J.* 33, 5–19.
- Rosas, C.T., Tischer, B.K., Perkins, G.A., Wagner, B., Goodman, L.B., Osterrieder, N., 2007. Live-attenuated recombinant equine herpesvirus type 1 (EHV-1) induces a neutralizing antibody response against West Nile virus (WNV). *Virus Res.* 125, 69–78.
- Siger, L., Bowen, R., Karaca, K., Murray, M., Jagannatha, S., Echols, B., Nordgren, R., Minke, J.M., 2006. Evaluation of the efficacy provided by a Recombinant Canarypox-Vectored Equine West Nile Virus vaccine against an experimental West Nile Virus intrathecal challenge in horses. *Vet. Ther.* 7, 249–256.
- Singh, S., Janardhan, K.S., Parbhakar, O., Caldwell, S., Appleyard, G., Singh, B., 2006. Expression of Toll-like receptor 4 and 2 in horse lungs. *Vet. Res.* 37, 541–551.
- Slater, J.D., Lunn, D.P., Horohov, D.W., Antczak, D.F., Babiuk, L., Breathnach, C., Chang, Y.W., Davis-Poynter, N., Edington, N., Ellis, S., Foote, C., Goehring, L., Kohn, C.W., Kydd, J., Matsumura, T., Minke, J., Morley, P., Mumford, J., Neubauer, T., O'Callaghan, D., Osterrieder, K., Reed, S., Smith, K., Townsend, H., van der Meulen, K., Whalley, M., Wilson, W.D., 2006. Report of the equine herpesvirus-1 Havermeier Workshop, San Gimignano, Tuscany, June 2004. *Vet. Immunol. Immunopathol.* 111 3–13.
- Swinburne, J., Gerstenberg, C., Breen, M., Aldridge, V., Lockhart, L., Marti, E., Antczak, D., Eggleston-Stott, M., Bailey, E., Mickelson, J., Roed, K., Lindgren, G., von Haeringen, W., Guérin, G., Bjarnason, J., Allen, T., Binns, M., 2000. First comprehensive low-density horse linkage map based on two 3-generation, full-sibling, cross-bred horse reference families. *Genomics* 66, 123–134.
- Swinburne, J.E., Boursnell, M., Hill, G., Pettitt, L., Allen, T., Chowdhary, B., Hasegawa, T., Kurosawa, M., Leeb, T., Mashima, S., Mickelson, J.R., Raudsepp, T., Tozaki, T., Binns, M., 2006. Single linkage group per chromosome genetic linkage map for the horse, based on two three-generation, full-sibling, crossbred horse reference families. *Genomics* 87, 1–29.
- Wagner, B., Miller, W.H., Morgan, E.E., Hillegas, J.M., Erb, H.N., Leibold, W., Antczak, D.F., 2006a. IgE and IgG antibodies in skin allergy of the horse. *Vet. Res.* 37, 813–825.
- Wagner, B., Flaminio, J.B.F., Hillegas, J., Leibold, W., Erb, H.N., Antczak, D.F., 2006b. Occurrence of IgE in foals: evidence for transfer of maternal IgE by the colostrum and late onset of endogenous IgE production in the horse. *Vet. Immunol. Immunopathol.* 110, 269–278.
- Wilson, A.D., Harwood, L.J., Björnsdóttir, 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.
- Wilson, A.D., Harwood, L., Torsteinsdóttir, S., Marti, E., 2006. Production of monoclonal antibodies specific for native equine IgE and their application to monitor total serum IgE responses in Icelandic and non-Icelandic horses with insect bite dermal hypersensitivity. *Vet. Immunol. Immunopathol.* 112, 156–170.
- Wilson, A.D., Heesom, K.J., Mawby, W.J., Mellor, P.S., Russell, C.L., 2008. Identification of abundant proteins and potential allergens in *Culicoides nubeculosus* salivary glands. *Vet. Immunol. Immunopathol.* 122, 94–103.
- Woods, P.S., Robinson, N.E., Swanson, M.C., Reed, C.E., Broadstone, R.V., Derksen, F.J., 1993. Airborne dust and aeroallergen concentration in a horse stable under two different management systems. *Equine Vet. J.* 25, 208–213.

E. Marti*

V. Gerber

Department of Clinical Veterinary Medicine,
Vetsuisse Faculty, University of Berne,
3001 Berne, Switzerland

A.D. Wilson

Division of Pathology Infection and Immunity,
University of Bristol School of clinical Veterinary Science,
Langford House, Langford BS40 5DU, UK

J.P. Lavoie

Department of Clinical Sciences,
Faculty of Veterinary Medicine,
Université de Montréal, Quebec, Canada

D. Horohov

Department of Veterinary Science,
Gluck Equine Research Center,
University of Kentucky, Lexington, KY 40546, USA

R. Cramer

Swiss Institute of Allergy and Asthma Research (SIAF),
Obere Strasse 22, CH-7270 Davos, Switzerland

D.P. Lunn

Department of Clinical Sciences,
James L. Voss Veterinary Teaching Hospital,
College of Veterinary Medicine and Biomedical Sciences,
Colorado State University, 300 West Drake Road,
Fort Collins 80523-1620, Colorado

D. Antczak

Baker Institute for Animal Health,
College of Veterinary Medicine,
Cornell University, Ithaca, NY 14853, USA

S. Björnsdóttir

Icelandic Food and Veterinary Authority,
dep. Hólar, Hólar, IS-551 Saudárkrókur, Iceland

T.S. Björnsdóttir

Institute for Experimental Pathology,
University of Iceland,
Keldur, v/Vesturlandsveg, Reykjavík, Iceland

F. Cunningham

Department of Veterinary Basic Sciences,
The Royal Veterinary College,
Hawkshead Campus, Hertfordshire AL9 7TA, UK

M. Dérer
HESKA AG, Grandes-places 16,
1700 Fribourg, Switzerland

R. Frey
Norsholms Djursjukhus,
Biskop Henriksv.6, 610 21 Norsholm, Sweden

E. Hamza
Department of Clinical Veterinary Medicine,
Vetsuisse Faculty, University of Berne,
3001 Berne, Switzerland

P. Horin
Institute of Animal Genetics,
Faculty of Veterinary Medicine,
University of Veterinary and Pharmaceutical Sciences,
612 42 Brno, Czech Republic

M. Heimann
Institute for Experimental Pathology,
University of Iceland, Keldur,
v/Vesturlandsveg, Reykjavík, Iceland

G. Kolm-Stark
Department of Natural Science,
University of Veterinary Medicine Vienna,
1210 Vienna, Austria

G. Ólafsdóttir
Institute for Experimental Pathology,
University of Iceland, Keldur,
v/Vesturlandsveg, Reykjavík, Iceland

E. Ramery
Department of Functional Sciences,
Faculty of Veterinary Medicine,
University of Liege, Belgium

C. Russell
Division of Pathology Infection and Immunity,
University of Bristol School of clinical Veterinary Science,
Langford House, Langford BS40 5DU, UK

A. Schaffartzik
Department of Clinical Veterinary Medicine,
Vetsuisse Faculty, University of Berne,
3001 Berne, Switzerland

Swiss Institute of Allergy and Asthma Research (SIAF),
Obere Strasse 22, CH-7270 Davos, Switzerland

V. Svansson
Institute for Experimental Pathology, University of Iceland,
Keldur, v/Vesturlandsveg, Reykjavík, Iceland

S. Torsteinsdóttir
Institute for Experimental Pathology,
University of Iceland, Keldur, v/Vesturlandsveg,
Reykjavík, Iceland

B. Wagner
Department of Population Medicine & Diagnostic Sciences,
College of Veterinary Medicine,
Cornell University, Ithaca, NY 14853, USA

*Corresponding author at:
Department of Clinical Research VPH,
Vetsuisse Faculty, University of Berne,
Länggass-str. 124, PO Box 3001,
Berne, Switzerland.
Tel.: +41 31 631 23 30;
fax: +41 31 631 26 30
E-mail address: eliane.marti@itz.unibe.ch
(E. Marti)

18 July 2008