From the

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Insect bite hypersensitivity in Icelandic horses:

The latest advances in pathomechanisms, differential diagnosis and treatment (a literature review), and the comorbidity with equine asthma (a clinical data analysis)

Diploma thesis

University of Veterinary Medicine, Vienna

presented by

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Dedication and acknowledgments

To Julia Strauß, who helped with English language and grammar.

Declaration of authorship

I declare that this Diploma Thesis has been written by myself. I have not used any other than the listed sources, nor have I received any unauthorized help.
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Abbreviations

AIT	allergen immunotherapy
Alum	aluminium
APC	antigen presenting cells
ASIT	allergen-specific immunotherapy
BALF	bronchoalveolar lavage fluid
B-cells	bone marrow cells
BDT	basophile degranulation test
CAST	cellular antigen stimulation test
CCL	chemokines (C-C-motif)-ligand
CCR3	cysteine chemokine receptor 3
CD4+ T cells	cluster of differentiation 4 positive T cells
CD8+ T cells	cluster of differentiation 8 positive T cells
CpG-ODN	CpG-rich oligodesoxynucleotides
Cul	*Culicoides*
Cul o 2	*Culicoides* antigen 2
Cul o 3	*Culicoides* antigen 3
$CuMV_{TT}$	cucumber mosaic virus containing a tetanus toxoid
DC	dendritic cell
DTH	delayed type hypersensitivity
EA	equine asthma
ECA	equine chromosome A
eIL	equine interleukin
ELA	equine leucoyte antigen
ELISA	enzyme-linked immunosorbent assay
FIT	functional *in-vitro* test

H	histamine
HRT	histamine release test
IBH	insect bite hypersensitivity
IAD	inflammatory airway disease
ID	Insol®-Dermatophyton
IDT	intradermal testing
IFNγ	interferon gamma
Ig	immunoglobulin
IL	interleukin
ILC	innate lymphoid cells
JAK	Janus kinase
JAK-STAT	Janus kinase signal transducer and transcription
LT	leukotriene
MAP	mitogen- activated protein
MAPK	mitogen-activated protein kinase
MCP	monocyte chemotactic protein
mEA	mild equine asthma
MHC	major histocompatibility complex
MHS	multiple hypersensitivity
MPLA	monophosphoryl lipid-A
mRNA	messenger ribonucleic acid
NKT cells	natural killer T cells
OVA	ovalbumin
PAF	platelet-activating factor
PAMP	pathogen-associated molecular patterns
PBMC	peripheral blood mononuclear cells

PC	phosphatidylcholine
PE	phosphatidylethanolamine
PI	phosphatidylinositol
PI3K	phosphatidylinositol 3 kinase
RAO	recurrent airway disease
RIA	radio-immuno assay
SAA	serum amyloid A
sEA	severe equine asthma
SLIT	sublingual immunotherapy
SM	sphingomyelin
SNPs	single nucleotide polymorphisms
T cells	thymus cells
Th2 cells	thymus helper 2 cells
TLR	toll like receptor
TNFα	tumour necrosis factor alpha
Treg	regulatory T cells
TSLP	thymic stromal lymphopoietin
VLP	virus-like particle
WBE	whole body extract

1. Introduction

Insect bite hypersensitivity is an allergic skin disease in adult horses and is known by several different names, like summer eczema, *Culicoides* hypersensitivity, sweet itch, and summer seasonal recurrent dermatitis.[2-11] Insect bite hypersensitivity (IBH) is a rather frequent problem of horses imported from foreign countries, for example from Iceland, to continental Europe, especially within the breed of Icelandic horses. IBH is a hypersensitivity reaction caused by the saliva of *Culicoides* to which horses develop an allergic dermatitis. The signs are present from April until November in most European countries.

It has also been suggested that IBH is a notable problem for stakeholders concerning welfare and economics. However, there is a tendency to involve veterinary practitioners only in more severe cases.[1]

Recently, there was a lot of research on the immunological response of horses to *Culicoides* bites and treatment options modulating the immune response[2,]. It was also found that horses with IBH develop equine asthma more easily and *vice versa*, so co-morbidity between IBH and equine asthma was suggested[3].

In the present thesis, recent knowledge on pathomechanisms as well as advances in treatment options are reviewed. In addition, clinical data bases of two veterinary hospitals were screend for (i) the prevalence of horses affected by IBH and EA, and (ii) potential co-morbidities between EA and IBH. The hypothesis was that horses suffering of EA are affected more often with IBH than horses not suffering of EA.

2. Aims

The aim of the first part of this thesis was to summarise the latest advances concerning IBH, with a focus on current knowledge regarding the pathomechanisms and the latest therapeutical options. Furthermore, it should give a short overview of equine asthma and a potential comorbidity.

The second part consists of a clinical data analysis at the University of Veterinary Medicine Vienna and the Veterinary Faculty of the Ludwig-Maximilians-Universtiy Munich, investigating (i) the prevalence of Icelandic horses affected by IBH and EA, and (ii) potential co-morbidities between EA and IBH. The hypothesis was that horses suffering of EA are affected more often with IBH than horses not suffering of EA.

3. Literature review of the latest advances in insect bite hypersensitivity and the comorbidity with equine asthma in horses

The literature review lasted from September 2021 until July 2022 and was conducted with help of the search engines PubMed, Google scholar and Scopus.

The following search terms were used: "Sommerekzem", "sweet itch", "sweet itch symptoms", "sweet itch histology", "insect bite hypersensitivity", "equine asthma", and "RAO". Papers with a publication date of no earlier than 2016 have been included.

3.1. Epidemiology of insect bite hypersensitivity

Insect bite hypersensitivity can affect all horse breeds worldwide in geographic areas where biting midges like *Culicoides* are present, so Iceland, New Zealand and Antarctica are excluded. It is the most common equine allergic skin reaction. The reaction is mainly due to the bites of *Culicoides spp*, but other biting insects can be involved as well.[2-11] The prevalence of IBH in the different horse breeds varies between 4% and 70%.[2-11] The reasons for this variation might be due to multiple factors influencing the disease like the horse's environment, genetic predisposition, and age.[2-11] In particular, Icelandic horses born in Iceland and exported to continental Europe as adults have a high risk of developing this disease with a high prevalence of more than 50%.[4] This is because of the non-existence of *Culicoides* in Iceland, so the horses have a lack of immunological tolerance development in early life. As a result, they develop an allergy more easily once exposed to the foreign *Culicoides* antigen.[4] Clinical signs usually commence in the second summer of exposure. In contrast, Icelandic horses born outside of Iceland show a similarly low prevalence of developing IBH as other horse breeds (between 6.7% and 8%).[2-11] Additionally, an interesting fact is that horses which are exported from Iceland to continental Europe and which are exposed to *Culicoides* in this new country before seven months of age have the same prevalence of developing the disease as locally born horses.[2-11] Thus, it seems that early exposure to the allergens is really important for developing an immune tolerance.[2-11]

The heritability ranges from 0.16 to 0.30 in different breeds and is at 0.27 in Icelandic horses.[5,6] It has also been shown that, in general, offspring of mares affected by IBH have a higher risk of developing the disease than offspring of non-affected mares. The prevalence in this case is between 0% and 30%.[5,6,7]

The inheritance of IBH is polygenetic. Recently, a study tried to find single nucleotide polymorphisms (SNPs) associated with IBH.[8] If such gene regions were found, the

prevalence of IBH could be reduced by selecting and breeding horses less prone to developing the condition.[8] Due to the low heritability, reducing the prevalence through traditional breeding would take many generations, so genomic information could help to overcome these limitations.[8] A few candidate genes have recently been published, for example the equine leucocyte antigen (ELA) class II region and some non-ELA genes, which regulate immunity and allergy.[8] It has also been shown that Icelandic horses have a greater genetic variation than other breeds, which makes it even more difficult to find the right genomic regions.[8] Besides the breed, the environment might play an important role as well when it comes to genetic variations. Consequently, the polygenetic nature of the multifactorial condition of equine IBH was shown as well as the large number of genomic regions, each explaining only a small fraction of the genetic variance.[8]

Genomic regions associated with IgE levels against *Culicoides* allergens were found in Icelandic horses on ECA1:62-64 Mb, ECA1:107-111Mb, ECA2:52–63 Mb, ECA3:78 Mb and ECA15:14–21 Mb.[4]

For horse breeds born in areas with exposure to *Culicoides*, genetic predisposition for insect bite hypersensitivity is well documented, and gene regions connected with IBH have been reported.[9] Such findings are still missing for Icelandic horses born in Iceland and exported to Europe. There is no genetic basis for IBH established in these horses.[9]

By comparing the IgE concentration in the sera from IBH-affected Icelandic horses with those of other horse breeds, it was shown that Icelandic horses more often have a positive reaction in the IgE serologic test compared to other breeds.[10]

Until now, there are also no findings about whether gender, age of the adult horses at import, or season of import affect the clinical outcome.[9]

Furthermore, climate changes have to be considered, especially considering that due to global warming the spread of *Culicoides* changes over time and result in different patterns of midge occurrence and IBH prevalence and severity.[11] This is supported by the first reports of the occurrence of *Culicoides* in the south of Iceland. But due to the strong winds present in Iceland in combination with the limited ability of *Culicoides* to fly, spreading across the country might be difficult.[11]

Environmental factors may also influence the prevalence of IBH. There is a significant difference in the severity of IBH in connection with stabling during the night or whether there are wetlands close by. Horses which are in closed stables during the night have a lower level

of IBH compared to horses in open boxes or free stall barns. Stables located close to running waters are associated with an increased risk of the horses developing IBH compared to stables with a higher distance to open water sources.[12]

Further, biodiversity might affect IBH prevalence as well. The biodiversity hypothesis describes the interplay of improved hygiene, altered nutrition and changes in microbiome affecting viral, bacterial or parasitic diversity, all potentially contributiong to the increase in allergic diseases.[11]

3.2. Clinical signs

Insect bite hypersensitivity is caused by an IgE mediated type-I hypersensitivity against proteins in saliva of the biting midges *Culicoides spp.*[7,13] Affected horses develop a highly pruritic, seasonal recurrent allergic dermatitis.[7,13] The first signs of IBH appear in spring when the presence of *Culicoides* starts, then the clinical signs worsen during the summer months and last until November, followed by an asymptomatic phase over the winter.[7,13] The initial clinical signs are severe pruritus, which is the main sign of IBH, leading to self-excoriations with alopecia, irritation of the skin, and open wounds.[7,13] Because of this, hair loss can occur and the skin can become inflamed, crusty and sore, leading to a higher risk of infection (Figure 1).[7,13] It has been shown that the existence and severity of the clinical signs of IBH correlate with the activity of the *Culicoides*.[7,13]

(A) (B) (C)

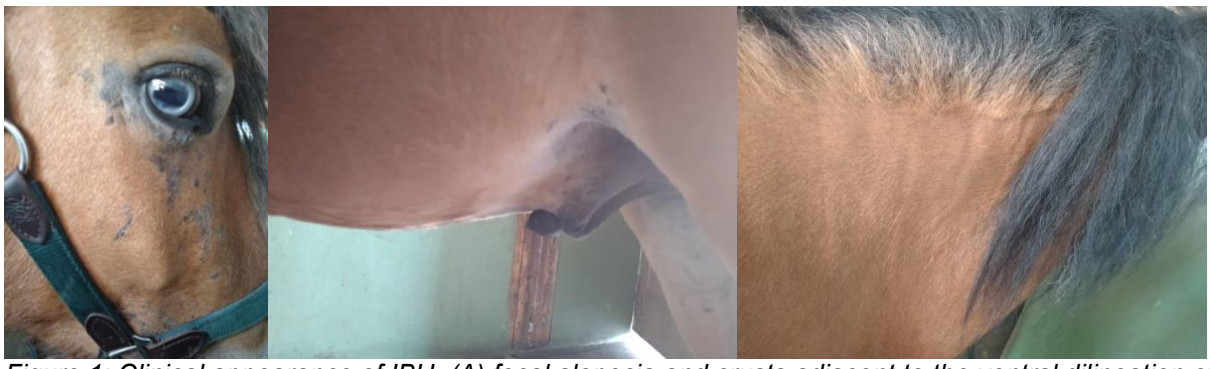

Figure 1: Clinical appearance of IBH, (A) focal alopecia and crusts adjacent to the ventral dilineation of the right eye and facial crest, (B) focal alopecia and crusts adjacent to the prepuce, (C) mane with broken hair and patches of alopecia. ©Lindlbauer Kathrin/VetMedUni Vienna

The ventral midline, head, chest, withers, neck, back, the base of the mane and tail are typical locations for skin irritation and wounds and sometimes the face, ears and legs can be affected as well in severe cases.[3-6,8,10-12,14,16-23]

Three different distribution patterns have been described:[7,13]

1. Dorsal distribution over the mane, croup, tail and later also the ears, neck, shoulder and the dorsal parts of the thorax.[7,13]

2. Ventral distribution over the ventral part of the thorax and abdomen as well as the axilla and the groin, and later also the legs.[7,13]

3. A hybrid form of the dorsal and the ventral distribution patterns.[7,13]

Most likely the distribution patterns are mirrored by the preferred landing site of the *Culicoides species*.[7,13]

Because of the long duration over the whole summer until the end of autumn, the initially acute dermatitis turns into a chronic state.[3-6,8,11,14,16-23] Often there result secondary changes to the skin like crusted papules, hyperkeratosis and thickening of the skin, as well as diffuse or complete alopecia. Sometimes there can be a secondary bacterial infection as well.[3-6,8,11,14,16-23]

Apart from the dermatological disease, the discomfort of the horses caused by the presence of the insects plays an important role as well. [5,6,18] The horses can show restlessness, impatience, bad mood and lower food uptake. This leads to losing weight, less well-being of the horse and lower performance. [5,6,18] Self-harming with tail swishing, headshaking and rubbing, excessive grooming and rolling are a problem, too.[5,6,18] When insects are around, horses might also become anxious. The signs normally become less severe in fall and disappear during winter due to absence of the biting insects.[5,6,18]

3.3. Pathohistology, pathogenesis, and immunological response

Pathohistology

Insect bite hypersensitivity is histologically characterised by mixed perivascular to diffuse cellular infiltrates of mononuclear cells, lymphocytes and eosinophils in the dermis in inflamed regions.[15,16] Mast cells and MHC class II positive cells can be found in increased numbers. In the epidermis, strong acanthosis and hyperkeratosis are frequently present.[15,16]

In the stratum corneum there is an epidermal proliferation and parakeratosis, in the upper regions oedema can be found.[7,11,12] Blood and lymph vessels in this region are dilated and modified and the endothelial cells are swollen.[7,11,12] Hence, there is a release of serum and leucocytes, mainly eosinophil granulocytes, which leads to a sub-epithelial oedema typical of IBH and a perivascular infiltration with mostly eosinophilic granulocytes.[7,11,12] Furthermore, there can also be collagen lysis.[7,11,12] This leads to hyperplasia and fibrosis of the dermis, and to epidermal hyperkeratosis in chronically affected horses.[7,11,12]

Pathogenesis of hypersensitivity reactions

In IBH, the acute and the late-phase hypersensitivity type I reactions play an important role, as well as the type IV hypersensitivity in the chronic phase of the disease.[17] The hypersensitivity type I is characterised by a reaction against specific antigens with the production of IgE-antibodies and degranulation and mediator release of mast cells. After antigen crosslinking of the IgE bound onto the mast cell, proinflammatory substances like histamine are released and production of prostaglandins, leukotrienes and PAF is stimulated (Figure 2).[17]

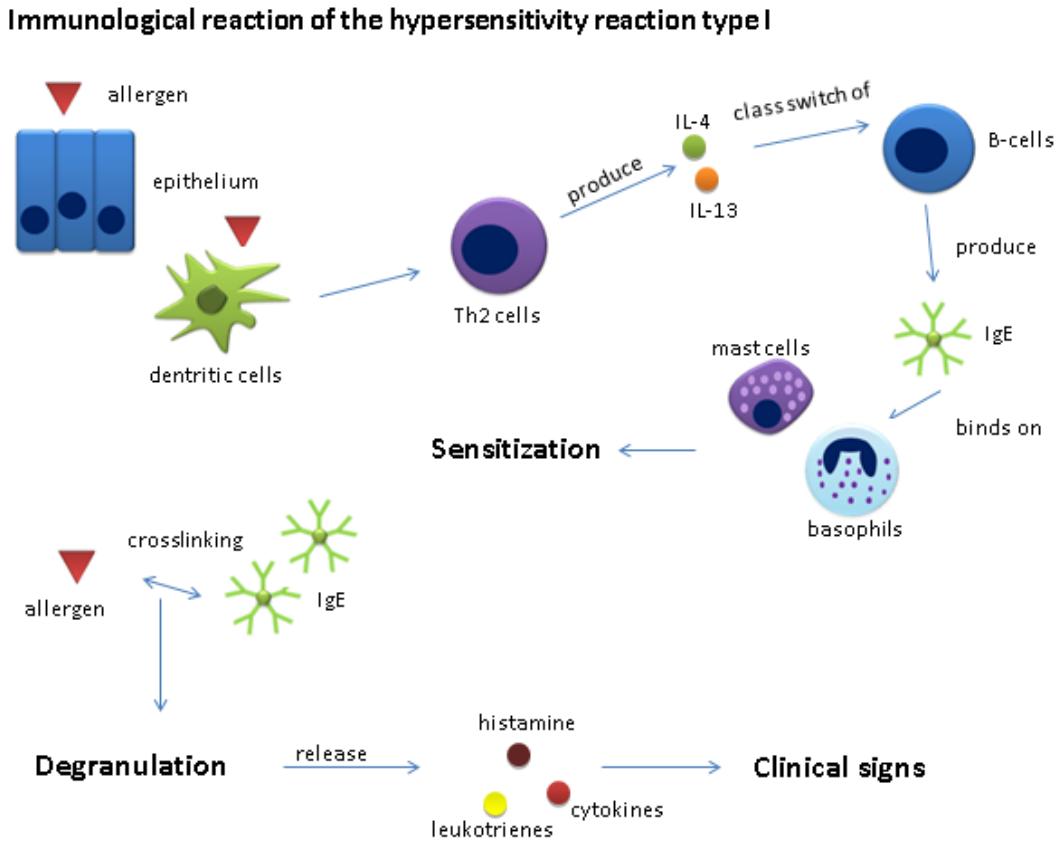

Figure 2: Immunological reaction of the hypersensitivity reaction type I. At first contact of the allergens with the epithelium dentritic cells send information to Th2 cells then producing IL-4 and IL-13, which leads to a class switch of B.-cells. These B-cells then produce IgE binding on mast cells and basophils, which leads to sensitization. After antigen crosslinking of the IgE bound onto the mast cell, proinflammatory substances like histamine are released and production of prostaglandins, leukotrienes and PAF is stimulated, which leads to clinical signs. Created with PowerPoint by Kathrin Lindlbauer

The acute hypersensitivity type I reaction appears within a few minutes after coming in contact with the antigen and the late-phase reaction appears within four to eight hours.[17] Eosinophilic granulocytes and neutrophils infiltrate the tissue followed by Th2 cells.[17]

Furthermore, it has been suggested that there is a transient shift from an early IgE-dominated stage to an eosinophil-dominated later stage, which is mediated by T cell plasticity. This is due to the phenomenon that T cells can re-differentiate into another T cell subset and hence modify their mediator expression characteristics.[11] In the early stages of allergy, Th2 cells mainly secrete IL-4 and IL-13 and thereby promote IgE production. In the chronic stage, the Th2 cells shift towards IL-5 secreting T cells which lead to eosinophilia.[11]

A possible involvement of delayed-type hypersensitivity type IV in the pathogenesis of IBH has been suggested as well.[17] During the hypersensitivity type IV reaction, the antigen is presented to the naive T cells by antigen-presenting cells like Langerhans cells in the dermis [5,6,7,18] The T cells are then differentiated into the different types of T-helper cells and the Th2 cells are the major part in type IV allergy.[5,6,7,18] They release cytokines such as IL-4, IL-5 and IL-13. Interleukin 4 induces the class change from IgG to IgE, IL-5 stimulates the proliferation, the activation and the differentiation of eosinophils and IL-13 induces the production of IgE.[5,6,7,18] This hypersensitivity type IV reaction appears 24-48 hours after the antigen exposure.[5,6,7,18] It can be divided into different subtypes based on the predominant cell types. Type IVb is the relevant subtype for IBH and is associated with IL-5 producing Th2 cells and eosinophilia.[17] Eosinophils are present in both the late-phase reaction of type I hypersensitivity and the type IV hypersensitivity.[17] They play a major role in IBH, as the severity of IBH is associated with eosinophilia and the allergic lesions are often characterized by infiltration with eosinophils.[17]

It is clinically manifested as an inflammation of the skin with oedema and eosinophil accumulation in perivascular clusters in the dermis. IL-5 displays multiple effects on eosinophils and affects their differentiation, migration activation and survival.[17,24] The eosinophils activated by IL-5 release granule enzymes and effector molecules like leukotrienes and major basic protein. These mediator substance in turn trigger degranulation of mast cells and basophils, which forms a vicious circle of allergic inflammation(Figure 3).[17,24]

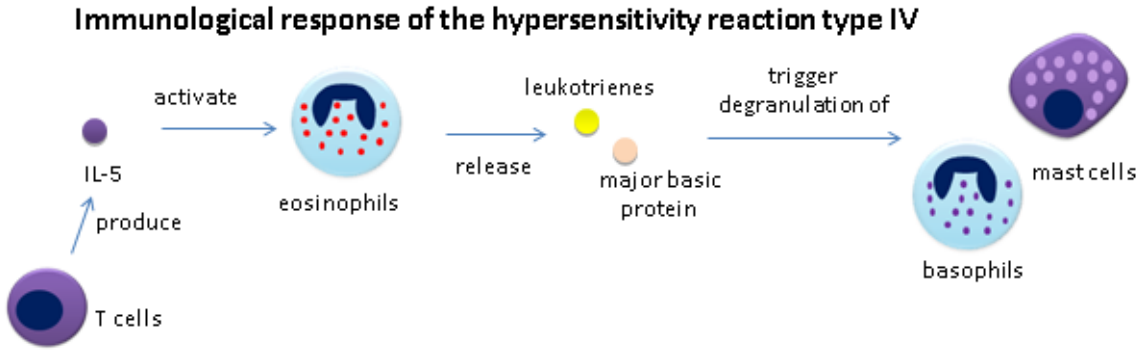

Figure 3: Immunological response of the hypersensitivity reaction type IV. T cells produce IL-5 activating eosinophils, which then release leukotrienes and major basic proteins. These trigger the degranulation of basophils and mast cells and that leads then to the reaction. Created with PowerPoint by Kathrin Lindlbauer

Increased levels of mRNA which encodes the chemokines CCL11 (Eotaxin-1) and CCL2 (monocyte chemotactic protein 1, MCP1) have also been found. Eotaxin-1 binds to the receptor CCR3 and is a potent eosinophil chemoattractant, also involved in eosinopoiesis.[25] Eotaxin-1 is synergistic with IL-5 and together they induce blood eosinophilia and stimulate eosinophils to migrate from blood to tissue and to produce a large number of pro-inflammatory and toxic mediators.[25] Consequently, it was shown that eosinophils are not only upregulated locally in the lesions but also systemically in the blood. [25] These concentrations of blood eosinophils strongly correlate with the severity of the disease.[25]

Most Icelandic horses produce *Culicoides*-specific IgE, meaning they undergo a silent sensitisation, but only a few of them develop a clinical allergic response due to subsequent allergen exposure. Both allergic and non-allergic Icelandic horses develop an inflammatory response during the exposure to *Culicoides* allergens during the summer months, but in a different way. It is assumed that non-IBH-affected horses develop a T helper type 1 skewed immune response against the allergens and thus remain healthy, i.e. without clinical symptoms. Allergic horses, on the contrary, develop a T helper type 2 response and clinical signs. [26] In affected horses, the T cells produce elevated IL-4 mRNA transcripts and this leads to a decreased proportion of allergen-specific T regulatory cells compared to non-affected horses.[26] Another aspect is that the immune cells in the skin of allergic horses have an increased frequency of CD4 + T-cells, a lower ratio of T regulatory cells to CD4+T cells, and an increased expression of IL-10 mRNA and IL-13 mRNA.[26]

Another point that has been shown is an imbalance between a Th2 and T regulatory (Treg)/ Th1 immune response in IBH. An increased number of CD4+ and not of CD8+ T cells have been shown with a decreased mRNA expression of the Treg marker FoxP3 and an increased mRNA expression of IL-13 in the skin of IBH-affected horses compared to non-affected horses. Therefore, it has been suggested that IL-13 is the key Th2 cytokine driving inflammation in the periphery, while IL-4 has a more central effect.[15,16,17]

Additionally, it has been shown that Icelandic horses affected by IBH often have a high IgE serum concentration against *Culicoides* antigens.[14] The IgE serum concentration increases considerably between before-exposure and first-exposure, but is nearly the same between first and second exposure.[2] Horses born in Iceland and subsequently exposed to *Culicoides* allergens showed a higher IgE concentration and reacted to more different *Culicoides* allergens than locally born horses.[2] Interestingly, there was no significant difference of the IgE concentration between non-affected horses born in continental Europe compared to non-

affected horses born in Iceland and exported to continental Europe.[2] This indicates that the difference occurs in the immune response between European-born and Icelandic-born Icelandic horses, and that Icelandic-born Icelandic horses respond more strongly. It has also been shown that IBH-affected horses imported from Iceland as adults have a strong Th2-biased immune response.[27] This results in the release of histamine, other inflammatory mediators and cytokines such as IL-4, IL-9, IL-13 and TNF- α.[13] Importantly, it has previously been shown that horses exposed to *Culicoides* who do not develop IBH are not immunologically ignorant to the antigens but develop a more balanced antigen-specific Th1/Treg immune response.[2]

These cellular mediators are really important in the pathogenesis of the allergic disease. Besides them there might also be important IgE-binding monocytes, such as IgE+, CD14, MHCII, CD16-, CD163 and CD23 cells, which represent about 6% of the total peripheral monocytes.[21] Moreover, additional factors like environmental exposure, parasite burden and individual immune response variations, which also influence IgE receptor crosslinking dynamics, need to be considered.[21]

Another type of T cells are the regulatory T cells (Treg), which inhibit responses of Th2 cells and release the anti-inflammatory interleukin 10.[5,6,7,18] Hence, T cells play an important role in the pathogenesis of IBH.[5,6,7,18] In the lesional skin, there are more CD4+ receptors compared to non-lesional skin of affected horses or non-lesional skin of non-affected horses.[5,6,7,18] There is also more interleukin 13, but not more interleukin 4, so it is suggested that IL-4 is only secreted initially and that the process is kept up by IL-13.[5,6,7,18] Affected horses have fewer Treg cells in their skin and blood compared to non-affected horses, and the concentration of IL-10 in the skin and in the blood is lower than in non-affected horses as well. Also, a leak of the barrier of the skin is described in IBH affected horses, as a result of which the antigens can pass through the skin more easily, further driving the ongoing allergic response.[5,6,7,18]

Thus, for an earlier diagnosis and treatment, the differences in immune landscape between allergic and non-allergic Icelandic horses in the early sensitisation phase, even before clinical signs become visible, might be valuable.[26] Mast cells and basophils are the main inflammatory cells responding quickly to allergen exposure.[17,26] Mast cells can be found in the skin, the submucosa of the respiratory tract and the lungs of IBH affected horses and are long-lived tissue resident myeloid cells.[17,26] Contrary to mast cells, basophils are short-lived circulating cells.[17,26] The production of leukotrienes and cytokines is induced by IgE receptor

crosslinking. IL-4 as important cytokines in the development of allergy is also produced by mast cells and basophils.[17,26]

The role of Interleukin-4

Interleukin 4 supports immunoglobulin class switching from IgG to IgE and thereby the development of allergy.[13] It has been considered that IL-4 is also important during the development of an allergen-specific Th2 response and that it has a crucial effect on immunoregulatory functions on immune cells like B-cells, T-cells, monocytes and dendritic cells, and on non-immune cells such as endothelial cells and fibroblasts. It is being debated whether the initial IL-4 signal, which leads to the differentiation of naïve T-cells into Th2 cells, either comes from T-cells themselves or from basophils or NKT-cells.[13] Once Th2 cells have developed during an allergic reaction, every additional allergen exposure further promotes the production of IL-4 and so provides a positive feed-back loop which maintains allergen-specific Th2 cells.[13] Additionally, IL-4 can be produced by innate immune cells like macrophages, mast cells, eosinophils, monocytes and basophils.[13] It was also shown that the IL-4 responses vary throughout the year. This might be due to the fact that immune cells quickly become exhausted at the local sites of inflammation in the skin of allergic horses due to continuous allergen stimulation.[13] A similar effect of exhaustion caused by continuous allergen-specific stimulation might also lead to a lack of differences in IL-4 production in peripheral blood cells from allergic and non-allergic horses during environmental *Cul* exposure.[13] Additionally, the percentage of IL-4+ cells after *Cul* stimulation was increased in allergic horses compared to non-allergic ones, but they had a similar percentage of IL-4+ cells following IgE crosslinking.[13] Furthermore, peripheral blood basophils produce a high amount of IL-4 in IBH-affected horses after exposure to *Cul* allergens and the percentage of basophils remains higher in allergic horses throughout the year than in non-allergic horses.[13] So, basophil-derived IL-4 could be an early signal for immune induction, which modulates the immune responses towards Th2 immunity and IgE production.[13] It has been shown that the majority of basophils produce IL-4 after *Cul* stimulation, whereas only a small percentage of IL-4+/CD4+ T-cells were found in response to the stimulation, and the IL-4 production was not induced in CD8+T-cells, or IgM or IgG producing B-cells.[13] When the midges disappear during winter, the cells can recover and are not triggered anymore by the constant environmental allergen challenge, and the difference in IL-4 production capacities between allergic and non-allergic horses becomes detectable again.[13] An interesting fact is that few days-old foals show a population of peripheral IL-4+ basophils stimulated by colostrum-

acquired IgE and this might be important for developing an immune landscape against future allergy development.[26]

The role of interleukin-31

Another important factor is the inflammatory interleukin 31 (IL-31). It is associated with chronic inflammatory diseases and with cellular immunity against pathogens.[28] Type 2 helper T cells are the main source of IL-31, but mature dendritic cells are described as a source as well.[28] The signal pathway starts with the binding of interleukin 31 to its receptor complex. Then, the intracellular signalling activates the Janus kinase-signal transducer and transcription (JAK-STAT), the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) pathways.[28] The IL-31 receptor complex is expressed by several cell types such as epithelial cells, monocytes, keratinocytes, macrophages and eosinophils.[28] The immune and the nervous system are linked by this cytokine by binding of IL-31 to the IL-31 receptor on dorsal root ganglia cells, which evokes pruritus independent of histamine.[11] (Figure 4) IL-31 is only expressed in lesional skin of IBH-affected horses but not in non-lesional skin or in skin of non-affected horses.[11,29]

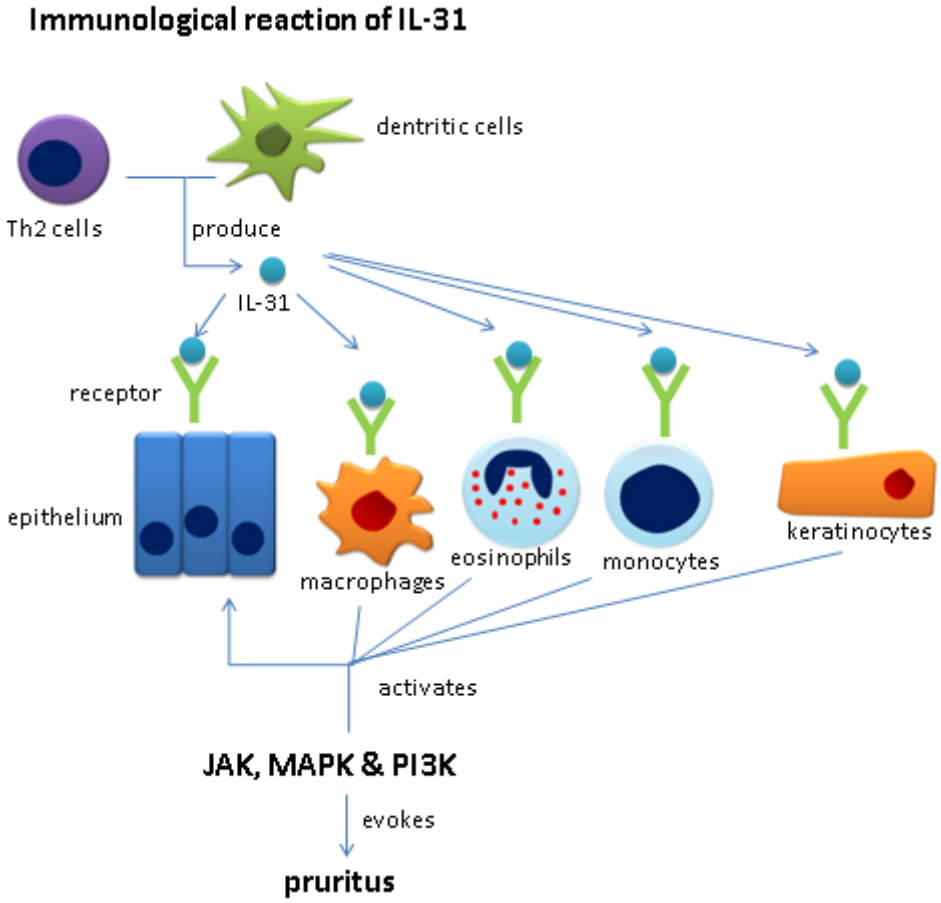

Figure 4: Immunological reaction of IL-31. Dentritic cells and Th2 cells produce IL-31, which binds on receptors on the epithelium, macrophages, eosinophils, monocytes and kerationocytes. These then activates JAK, MAPK and PI3K, which evoke pruritus. Created with PowerPoint by Kathrin Lindlbauer

The role of Interleukin-8

Another cytokine associated with an IgE-mediated allergy is IL-8, which recruits neutrophils and basophils to promote inflammation and is produced by monocytes in response to LPS stimulation.[20] IL-8 production is also induced by IgE cross-linking by both peripheral blood basophils and IgE-binding monocytes.[20] The percentage of IL-8 releasing IgE binding monocytes after IgE cross-linking was significantly higher in allergic horses compared to non-allergic horses, whereas the percentages of IL-8 basophils after IgE cross-linking were similar in all horses.[20] Hence, it can be concluded that IgE-binding monocytes from allergic horses have an increased capacity for IL-8 production and likely contribute to the recruitment of innate immune cells during IgE-mediated allergy and promotion of inflammation during

repeated allergen contact (Figure 5).[20] Interleukin 8 production was also found in response to IgE cross-linking very rapidly. So, it has been suggested that IL-8 is involved in the early IgE-mediated recruitment of inflammatory cells and that it contributes to inappropriate or excessive inflammation in allergic diseases.[20]

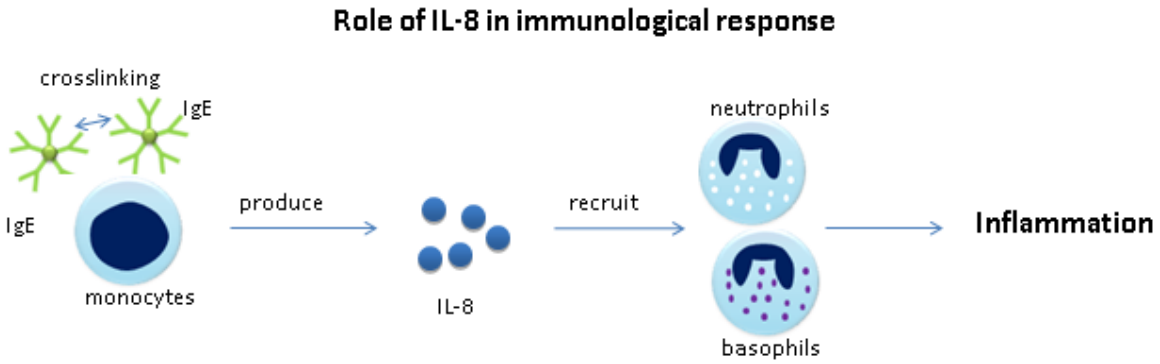

Figure 5: Role of IL-8 in immunological response. Crosslinking between IgE and monocytes leads to a production of IL-8 recruiting neutrophils and basophils, which lead to Inflammation. Created with PowerPoint by Kathrin Lindlbauer

In addition, IgE-binding monocytes, which express a variant of high-affinity IgE receptors and promote allergic inflammation through IL-8, and CD23+ B-cells, which also bind IgE on their surface and promote the allergic response through facilitated antigen presentation, circulate in peripheral blood of allergic horses.[26] Monocytes have highly plastic functions and they are responsible for the rapid migration towards sites of inflammation, phagocytosis of invading pathogens or allergens and the presentation of foreign epitopes to T cells in the regional lymph nodes.[21] Furthermore, monocytes secrete chemokines and cytokines during these processes. These cytokines and chemokines then help the immune response to an appropriate response to invading allergens or pathogens.[21] This regulatory response fails to induce adequate tolerance to allergens when it comes to *Culicoides* allergens in IBH affected horses.[21]

Thymic stromal lymphopoietin

Thymic stromal lymphopoietin (TSLP) is released together with IL-25 and IL-33 during sensitisation from injured or inflamed epithelial cells. This release then induces type 2 innate

lymphoid cells to produce IL-5 and IL-31.[15] Furthermore, TSLP leads to scratching behaviour by acting on sensory neurons. [29]

Previous studies have shown that thymic stromal lymphopoietin is also involved in the pathogenesis of IBH, besides the already known Th2 cytokines IL-4, IL-5, IL-13 and IL-31.[15] In addition to TSLP, IL-25 and IL-33 are also released from inflamed and injured epithelial cells during sensitisation, and they induce type 2 innate lymphoid cells (ILC2) to produce IL-5 and IL-13.[15,11,28] Under the influence of these cytokines, the immune response is directed by antigen presenting cells (APCs) towards Th2 cells with production of IL-4 and IL-13.[15,11,28] It has been demonstrated that TSLP is the linking factor between the environmental stimuli and the type of immune response, so it is hypothesised that the expression of TSLP is upregulated in primary equine keratinocytes by Toll-like receptor (TLR) ligands.[30] Toll-like receptors recognise pathogen-associated molecular patterns (PAMPs) on various microbes and play an important role in innate immunity.[30] The activation of several signalling pathways, such as mitogen-activated protein- (MAP-) kinases inducing expression of various genes involved in host defence like IL-6 and IL-1, chemokines, major histocompatibility complex (MHC) and co-stimulatory molecules, is caused by the binding of ligands to TLRs.[30] It has been shown that equine TSLP is upregulated in lesional skin of IBH-affected horses compared to non-lesional skin, indicating a potential role of this cytokine in the pathogenesis of IBH.[30]

Another point which has been hypothesised is that stimulation of TLR on keratinocytes by bacterial or viral PAMPs from the environment might lead to an increased release of TSLP in horses affected by an IgE mediated skin allergy during the allergy. This might be due to the allergic cytokine microenvironment in the skin of these horses.[30] So a crucial role for TSLP in the pathogenesis of insect bite hypersensitivity is suggested because of the increased mRNA expression of it in the skin of IBH-affected horses as well as by the strong ability of equine keratinocytes to produce TSLP in response to TLR ligands, which are present in the skin microenvironment and possibly in the saliva of the causative insects as well.[30]

Epithelial barrier

It is quite well known now that the epidermal barrier plays an important role in the process of allergic sensitisation.[15,16] Keratinocyte activation, following mechanical damage of the skin caused by the bites of *Culicoides* and the cocktail of salivary proteins injected while feeding, might induce this increased TSLP expression. The production of TSLP in the skin might be

caused by the allergic cytokine milieu present in the lesions as well and possibly also by secondary bacterial infections.[15,16] Important changes in the barrier epithelium, such as genes affecting processes of epithelial cell differentiation, desmosome organisation as well as regulation of epithelial morphogenesis enrichment have been found when comparing the transcriptome of lesion IBH skin to non-lesional skin, while the expression of most of the genes involved in terminal differentiation of keratinocytes, like filaggrin, involucrin and loricrin, was not changed in IBH affected horses.[15,16] Furthermore, small proline rich proteins, another component of the cornified layer, were upregulated in IBH lesions, the expression of many keratins was altered and the expression of genes involved in formation of adherents and tight junctions were downregulated as well as the fibroblast growth factors and fibroblast growth factor receptors.[15,16] Conversely, matrix metalloproteinases as well as many different serine proteases were significantly upregulated in lesional skin.[15,16]

Insect bite hypersensitivity is characterised by a downregulation of genes involved in tight junction formation, alterations in keratins and substantial immune signature of both Th1 and Th2 types with particular upregulation of IL-13, as well as involvement of the hypoxic pathway.[16] A different expression of the genes involved in metabolism of epidermal lipids, pruritus development, as well as IL-25 was found in the epidermis of IBH affected horses compared to non-affected ones.[16] Thus, an impairment of the epithelial barrier in IBH-affected horses that may act as a predisposing factor for IBH development has been suggested.[16]

Immunglonulin G antibodies

Another tested factor was the *Culicoides*-specific IgG subclass antibody concentration of imported horses. IgG1, IgG1/3, IgG4/7 and IgG5 were the tested subclasses.[14] There were only a few but quite interesting significant differences between the non-affected horses and the IBH affected ones. In the second summer, the levels of IgG1 and IgG5 were significantly higher in the affected horses than in the control group.[14] Also, there was a significant increase in the concentration between the second summer and before-exposure in the IBH affected horses.[14] The IgG1/3 titres in affected horses were higher than in the non-affected horses at each point in time. The IgG5 concentration was higher too in the IBH affected horses than in the control group, and this was already the case before the first exposure.[14] The appearance of the higher antibody concentration of IgG1 and IgG1/3 in the affected group compared to the non-affected group was always there but the time point of time when it became significant varied.[14] The study shows that IgG5 is strongly associated with allergy

in horses.[14] The horses which developed IBH after the import to continental Europe already had significantly higher IgG5 concentration against *Culicoides* antigens before exposure than non-affected horses. A previous study[31] demonstrated that there might be a cross-reactivity between the antigen 5-like allergen from *Simulium vittatum*, which is present in Iceland, and the *Culicoides* antigens. This heightened IgG5 level, due to the cross-reactivity between *Simulium* and *Culicoides* antigens, in Icelandic horses before exposure to *Culicoides* might have a predictive value for IBH.[14]

IgG3 and IgG5 are the isotypes associated with Th2 responses and it has recently been shown that they are already present in horses before they develop clinical signs, and that they are higher in affected horses than in non-affected ones before, during and after clinical signs of allergy.[26]

Phospholipids

It was also found that the concentration of useable phospholipid in the sera of affected horses is much lower than in non-affected horses.[32,33] The hypothesis is that affected horses overproduce lipids and that these lipids then form abnormal aggregate complexes, which cannot interact with their receptors anymore.[32,33] Sphingomyelins, for example, are involved in many biochemical reactions, especially in skin and nervous tissue.[32,33] Besides the function as a structural component of the skin, they also function as bioactive regulators and messengers in cutaneous homeostasis and immune mediated responses, particularly in mast cell regulation.[32,33]

Eosinophils

Furthermore, it has been shown that younger horses with clinical signs of IBH in their first or second year of the disease have less elevated and more transient blood eosinophil counts. A systemic marker for eosinophilia is represented by IL-5, and the local tissue counterpart for IL-5 is eotaxin-1, a chemokine which is involved in the migration and chemotaxis of eosinophils into resident organs.[11]

Pruritus: Histamine and Interleukine-31

A cardinal sign of insect bite hypersensitivity is pruritus, which also causes many of the other clinical signs due to subsequent self-trauma induced by rubbing.[15] A well-known mediator of this pruritus is histamine, which acts by direct stimulation of H1 and H4 receptors expressed in sensory nerve endings, resulting in an immediate itch response.[15] Histamine is produced by mast cells and it is present in the saliva of blood feeding insects, where its presumed

function is to cause vasodilation through its action on H1 receptors and further inflammatory reaction via H4 receptors on leucocytes and mast cells. A major impact in pruritus development is mediated by IL-31.[15]

The pathway of pruritus is still not completely understood and very complex.[29] In the epidermis and dermal-epidermal junction, pruritus originates and is transmitted into the periphery by two types of itch-selective C nerve fibres.[29] Some of them are sensitive to histamine, but the majority of them are not.[29] A complex interplay between histamine-independent C fibres and T cells, neutrophils, eosinophils, mast cells and keratinocytes, combined with the release of cytokines, neuropeptides and proteases lead to exacerbation of pruritus. The inflammatory cytokine IL-31 is also involved in this histamine-independent pruritus.[29]

3.4. Diagnosis

To reach a confirmed diagnosis of IBH, a thorough and structured diagnostic work-up is essential. A detailed history including the duration, the time of year of onset, lesion progression, behaviour of the horse, treatment response and environmental conditions, thorough clinical exam, and potentially further advanced diagnostic tests such as intradermal skin tests, measurement of the IgE levels, the histamine-release-test (HRT) or the functional in vitro test (FIT), and the cellular antigen stimulation tests (CAST) or basophile degranulation test (BDT) can be done.[6,7,11,18,22,34,35] Not all of these tests are currently available to the equine practitioner, however.[34] Another test that is sometimes used is provocative exposure. Here, the horse is in a kind of isolation area until it is free of clinical signs and then previous environmental materials are re-introduced step by step.[6,18] The benefit of *in vitro* testing is that there is no involuntary sensitisation of the horses.[34]

Clinical assessment

The first step in the diagnosis is taking of a thorough history, followed by the clinical assessment with the typical clinical appearance of IBH. Differential diagnoses that may cause similar clinical signs have to be excluded (Table 1).[6,7,11,18,22,35]

Table 1: differential diagnoseis of IBH

Differential diagnosis	Clinical appearance	Difference to IBH	Ref.
atopic dermatitis	Pruritic disease normally in the non-insect season, appearance quite similar to IBH	Seasonal or not seasonal	[36]
psoroptic mange	Ear disease or truncal dermatitis focused around the ears, mane and tail, variable pruritus in pruritic horses: non-follicular papule, crusts, excoriation and alopecia	Also ear disease possible No age, breed, or sex predilections Skin scrapings for diagnosis	[37]
sarcoptic mange	Pruritus, beginning on the head, ears and neck and spreads caudally, crusts, excoriations, non-follicular papules, alopecia and licheninfection	No age, breed, or sex predilections Diagnosis with skin scrapings and skin biopsy Treatment response	[37]
chorioptic mange	Pruritus intense or absent Tail rubbing, pastern dermatitis	No age or sex predilections Especially during winter Skin scrapings for diagnosis	[37]
demodectic mange	Asymptomatic alopecia, scaling, maybe papules and pustules Affected areas are face, neck, shoulders and forelimbs	No age, breed, or sex predilections Skin scrapings and skin biopsy for diagnosis	[37]
trombiculidiasis	Seasonal in the late summer and fall Papules at the muzzle, nares, false nostril, face, ears, neck, distal limbs and ventral thorax and abdomen, variable pruritus, cutaneous oedema, ulceration, exudation, crusts possibly sneezing or headshaking	No age, breed, or sex predilections Visible larvae Skin scrapings for diagnosis	[37]

dermatophytosis	Circular patches of alopecia with variable scaling and crusting, minimal pruritus, secondary bacterial infection, urticaria, dilvery scaling Face, neck, dorsolateral thorax, girth are mostly affected; legs, mane and tail are rarely affected Quite common	Only minimal or non pruritus Mane and tail only rarely affected Fungal tests (specimen collection, direct examination, fungal culture) for diagnosis	[37]
lice infestation	Different degrees of scaling, alopecia and pruritus Areas affected: dorsolateral trunk, mane, tail and fetlocks	No age, breed, or sex predilections Clinical signs most obvious in winter Demonstration of lice or nits for diagnosis	[37]

Intradermal skin testing

In addition to clinical signs, intradermal skin testing using extracts from environmental substances can help to confirm the diagnosis. The intradermal skin test is an *in vivo* provocation test to see whether the horse reacts to an allergen.[4,7,38] For this, a small amount of allergen extract (0.05ml – 0.1 ml) is injected intradermally, and after thirty minutes, four and six hours the skin's reaction is checked for redness and swelling. There is also a positive control with histamine and a negative control with NaCl.[4,7,38] The outcome of this test varies considerably, as there may be crossreactivity due to a faulty allergen preparation. Additionally, there is also a substantial number of false positive and false negative results.[34] It has been shown that non-allergic horses react less frequently to intradermal injection of allergen extracts than allergic horses do.[4,7,38] But there are also some difficulties with intradermal testing. First, interpretation of test results may be difficult, as there are no real threshold values for when the outcome is positive and when not, and so the interpretation is quite subjective. Second, there is variation in the skin's reaction for the used allergen extracts, so there can also be false negative results, and third, the adjuvant extracts are kind of irritating themselves. [4,7,38]

Normally the test contains allergens from *Camponotus*, *Solenopsis, Periplaneta, Blattella, Culicodes, Chrysops, Ctenocephalides, Tabanus, Musca, Culicidae* and *Lepidoptera.* Furthermore, the allergen extracts can be chosen dependent on the region. [39]

Hence, intradermal testing is not the best of diagnostic tools and is also rather inconsistent, as it leads to quite a number of false positive results.[4,7,38]

In vitro tests

One example of *in vitro* testing is the immune-enzymatic multiple allergen simultaneous test. For this test, blood sample collection and IgE measurements are done during the summer months. Peripheral blood is collected and centrifuged. Allergen-specific IgE concentrations of the serum are determined by using whole body extract (WBE) and monoclonal anti-IgE antibody. Individual allergen, including *Tabanus spp.*, *Culicoides spp*., *Simulium spp*. and *Stomoxys spp*., and five allergen mixtures are tested. Based on guidelines the results can then be interpreted. An important aspect is that no corticosteroids should be administered for at least six weeks before testing.[35] This test has been used for quantitative determination of IgE in allergic horses.[35] Due to low costs and simple technology, *in vitro* tests have become a good tool for identifying allergy factors.[35] A limiting factor, however, is the possibility of numerous false positive reactions and a small risk of false negative reactions.[35] Therefore, multiple allergen simultaneous tests should only be used in connection with clinical signs and the clinical history, and cannot be used to distinguish IBH affected horses from non-IBH affected horses. Hence, they should not be used as a screening test.[35]

Released mediator substances

Further possibilities for *in vitro* testing include measurement of sulphidoleukotriene or histamine, which are released form basophils and mast cells by IgE binding.[7] But the significance of serologic tests is not particularly high, and there is also no real cut-off value, so there are many wrong positive outcomes.[7] For testing the level of sulphidoleukotrienes, blood leukocytes are incubated with a *Culicoides* antigen and then the level is measured with an enzyme-linked immunosorbent assay (ELISA). This test has quite good sensitivity and high specificity. An ELISA is also used for the measurement of histamines that are released after stimulation with *Cul* allergens. This test also provides a lot of wrong positive outcomes.[7] The release of sulphidoleukotrienes has shown to be a good *in vitro* diagnosis with a sensitivity level of 80% and a specificity level of 97%.[9] In vivo the test is only positive in horses which have already developed clinical signs, so it is not possible to make predictions based on the value of the release of sulphidoleukotrienes.[9]

Functional in vitro test

The functional in vitro test (FIT) is a histamine release test. The principle of the test is that the basophils are brought in contact with the assumed allergen in different concentrations. When enough sensitising antibodies are on the surface of the cells, the allergen-specific antibodies get cross-linked and provoke the release of pre-formed histamine. The histamine is then quantified with a RIA, compared to the concentration of histamines released due to physical reasons, and by doing so it can be determined whether the horse is sensitised or not.[34] For this, the histamine concentration from the peripheral blood is measured, which is released in whole blood provoked with allergens.[34] Histamine is the only mediator that is secreted by basophils during the first hour of incubation with antigens. So, the FIT can only detect the functional sensitisation of the blood effector cells of the type I allergy without measuring the unspecific mediators, which can be released due to manipulation during the test.[34] There are a number of false positive results with this test as well. It has been shown that the specific sensitisation of basophils is also there in winter, so the potential for IBH can also be predicted, which might be of interest when for example buying a horse.[34] The FIT has only been available in the institute for immunology of the Veterinarian University of Hannover and nowadays is not available anymore.[34] The FIT was used for hypersensitivity against *Culicoides*, *Stomoxis*, *Simulium*, *Ephemeridae*, *Culex*, *Tabanidae* and *Musca Domestica*. Unfortunately, there is no correlation between the severity of the clinical signs and the height of the FIT value.[34]

Enzyme-linked immunosorbent assay

Another option of diagnosing IBH is an indirect ELISA test that measures the allergen-specific IgE concentration in the serum. Affected horses show a higher titre of allergen-specific IgE compared to non-affected horses.[4] These ELISA tests provide the opportunity to have an objective, independent and quantitative test besides the subjective clinical scoring.[4] However, it is still unclear whether measurement of IgE concentration is a good diagnostic tool. This is because of a high number of false negative results as well as the missing correlation between the severity of the clinical signs and the height of the IgE level as well as the correlation between the IgE level and the intensity of allergen exposure. That is also the reason why the test is not being used for diagnostic.[34] Different options are employed, such as recombinant antigens that are derived from different *Culicoides spp* or whole body extracts.[4] Another problem of IgE-quantifications tests are allergen-specific IgGs. The IgGs

compete for the binding sites and so interfere with the sensitivity. Therefore, IgGs should probably be depleted prior to IgE testing.[11]

Cellular antigen stimulation tests

Another option of *in vitro* testing are the Cellular antigen stimulation tests (CAST). Similar to the FIT, the blood is incubated with allergens.[34] In the CAST, the level of inflammatory mediators, such as sulphidoleukotrienes, is measured with an ELISA.[34] The CAST is an alternative to the FIT and is available in a few laboratories in Germany, Austria and Switzerland.[34] Benefits of this test include better detection of inflammatory cells and production of leukotrienes only by intact cells, so there are no false positive results due to cytotoxic activity.[34] The sensitivity of the CAST is at 78% and the specificity at 97%, thus the test is quite a good diagnostic tool.[34] As there is only a threshold value and no severity level, there is no guarantee that the value correlates with clinical signs.[34]

Specificity of Culicoides allergens

An important point of diagnosis is to identify the causative allergen. The specificity of the test is hampered by different geographical regions dominated by different *Culicoides species* and IgE cross-reactivity between the different species and interspecies cross-reactivity.[11] Whole body extract (WBE) of *Culicoides* is used in commercially available serological tests, which varies in quality and thus reduces sensitivity and specificity of the diagnostic results.[11] Furthermore, WBE does not contain purification of allergens and therefore contains a large proportion of undefined components including those that may lead to degradation of the relevant allergens.[11] Another problem is that the relevant allergens are only present in a very low concentration, and that the WBE is usually made from laboratory-bred *Culicoides*, which are not in the surroundings of horses. [2,15] Recently, IgE serology results have been shown to be better when WBE is produced from *Culicoides* collected from the horse's environment. Still, the commercially available serological tests have low sensitivity and specificity and remain unsatisfactory.[15]

Nowadays, 30 *Culicoides* allergens have been characterised at the molecular level and produced as recombinant (r) proteins. Pooling the different r-allergens in one ELISA test might be a time- and cost-saving *in vitro* test to confirm clinical diagnosis of IBH. But this would not allow identifying the specific r-allergen to which the individual is sensitised.[2,15] A recent study showed that a combination of different specific *Culicoides* allergens, particularly *Cul o 8, Cul o 1P, Cul o 2P, Cul o 10, Cul o 9, Cul o 11* and *Cul o 7*, leads to a great ELISA

test performance with a sensitivity of 90% and a specificity of 96%. These 7 allergens are the major allergens in IBH, no matter which origin or breed. In Icelandic horses, *Cul o 7, Cul o 5* and *Cul o 3* play an important role as well.[10,15]

In summary, a mixture of recombinant *Culicoides* allergens instead of WBEs for the common use seems to have the potential to increase the test's sensitivity and specificity.[11] A limiting factor of the development of improved ELISA using recombinant allergens is the identification of major allergens to which the majority of horses react.[11] Different horses show different allergic patterns.[11]

Table 2: Pros and cons of the different diagnostic methods.

Methods	Pros	Cons	Ref.
Clinical examination	Low costs Easy to perform No additional sensitisation of the horse Always available	No definite diagnosis No definite outcome	6 18 35 22 7 11
Intradermal skin test	Relatively low costs Good availability	Variable outcomes, Crossreactivities Sensitisation of the horse Numerous false positive and false negative results Results difficult to interpret Time consuming	34 38 7 4
Provocative exposure	reliable outcome small risk of false results no sensitisation of the horse	Difficult to perform Limited availability	6 18
Functional in-vitro test	Easy to perform No sensitisation of the horse Diagnosis without clinical signs possible	relatively high costs Numerous false positive results Not available anymore No correlation between the severity of the clinical signs and the height of the FIT value	34
Cellular antigen	relatively low costs	Only available in a few	34

stimulation test	Easy to perform No sensitisation of the horse Low risk of false positive results due to cytotoxic activity reliable outcome	laboratories in Germany, Austria and Switzerland Only a threshold value but no severity level	
Serum allergy test	Relatively low costs Easy to perform No sensitisation of the horse Low risk of false positive results Good availability	No real cut-off value	7 9

3.5. Treatment, prevention and management

For treatment of IBH, there are three different methods. On the one hand, there is preventive therapy, which includes arrangements in management, on the other hand there is supportive complementary treatment, which can be used systemically or topically, and on the other hand there is the attempt to revoke the allergy. The aim of the supportive treatment is to reduce the horses' pruritus and secondary lesions.[7]

Topical treatment

The easiest way of topical treatment is washing the horse with cold water. Cold water has a cooling effect, and leads to vasoconstriction and, therefore, a reduced absorption of allergens.[7] Additionally, shampoo, for example with local anaesthetics, can be used. Corticosteroids can be used topically as well in the form of shampoo or sprays for reducing pruritus.[7]

To ease the irritation and inflammation of the skin, lotions can be used, but they will not deter another midge attack.[5,18] A cream containing omega-3-fatty acids, humectants and emollients, for example, was able to improve clinical lesions, but not the pruritus.[17]

Also antiseptic products should be mentioned to reduce the secondary infections. [5,18]

Corticosteroids

The first pharmaceutical remedy, which is currently used to control pruritus and discomfort in allergic horses, are corticosteroids, also called glucocorticoids. They are an effective choice in controlling the extreme itch of IBH affected horses, but may also have a dose-dependent unwanted side effects like immunosuppression and disruption in the hypothalamic-pituitary axis.[23] For systemic treatment, mostly glucocorticoids such as prednisolone are used.[7]

Corticosteroids are the most-effective anti-inflammatory and immunosuppressive drugs that are available for treating chronic inflammatory and immune diseases.[11,40] Most of the anti-inflammatory and immunosuppressive effects are exerted by glucocorticoids diffusing across the cell membrane and binding the glucocorticoid receptors in the cytoplasm. Then they release receptor chaperon proteins and translocate into the cell nucleus, where they alter gene expression.[11,40] The anti-inflammatory effects are mostly due to gene transrepression, which leads to repression of transcription factors and downregulation of inflammatory chemokines and cytokines as well as adhesion molecules.[11,40] Glucocorticoids result in gene transactivation following direct DNA binding as well, which leads to an increased release of anti-inflammatory mediators, but also to undesirable metabolic effects.[11,40] An increase in peripheral blood neutrophils and a decrease in lymphocytes are induced by corticosteroids in horses.[11,40]

Worth mentioning are the side effects of glucocorticoids, which can occur in several different organs. One of the side effects, which can occur after systemic administration of corticosteroids, is the hypothalamic-pituitary-adrenal axis suppression with a resultant decrease in endogenous cortisol production and possibly an adrenocortical dysfunction. Other side effects include hepatopathy, muscle wasting, altered bone metabolism, polyuria, polydipsia, hyperglycaemia and an increased susceptibility to infections.[11,40] Laminitis has also been described as a side effect, but appearance is very low.[11,40] In consequence, an optimal glucocorticoid should have a low mineralocorticoid-to-glucocorticoid receptor affinity ratio, high tissue specificity, a low transactivation-to-transrepression ratio and increased nongenomic effects.[11,40] To lower the risk of side effects, some additional strategies could be employed, such as the use of short-acting systemic corticosteroids when local treatment is not possible or not sufficient, the use of the minimum dosage and duration necessary, the use of combinations whenever possible to reduce the dose and the duration, and morning administration of corticosteroids to minimize disruption of normal circadian cycles.[11,40]

Antihistamines

The effects of histamine are exerted via the interaction with H1-receptors, which are present in most tissues. Antagonists to these receptors, such as tripelennamine, promethazine and chlorpheniramine, are used in IBH affected horses, but the effect of this treatment has not been proven so far, and there is often a lack of success for antihistamines. Therefore, antihistamines are not the best of treatment options. [11,36]

Second-generation H1-antagonists, like cetirizine, a metabolite of hydroxyzine and a non-sedative histamine H1-antagonist, are another possibility and might bring some relief.[5,11,18,36] But the effects are very variable and a quite high dose might be needed as well.[5,11,18] They are not efficient enough for severe pruritus, but can potentially reduce the use of glucocorticoids.[7] The reaction to the different antihistamines varies between each individual. Antihistamines need to be used carefully in horses with glaucoma, heart arrhythmia, intestinal atony and urinary retention because of their anticholinergic characteristics.[7]

The lack of success for antihistamines so far raises the question whether IBH is a pure type I hypersensitivity, and whether the role of histamine is more complex and not fully understood yet. An important role for IgE as well as for IgG antibodies could be confirmed. The finding that IgGs might trigger IBH lesions similar to IgE might point towards an involvement of additional hypersensitivity reactions and might explain the lack of efficacy of antihistamines.[11,36] Several other mediators, such as H4 receptors, might play an important role in the pruritic responses, as well, and for this reason H1-antogonists, like cetirizine, might be less effective.[36]

Cetirizine can be given orally at 0.4 mg/kg twice a day for 3 weeks.[36] However, no difference in the reduction of dermatitis between the placebo groups and the treated groups after three weeks of treatment with this dose was found.[36]

Another point is that histamine plays a more important role in the initial phase of the pruritic diseases, and so antihistamines might be more effective at this time, if at all.[36]

Important to mention is also the fact that antihistamines are prohibited drugs in competions in many countries, so they should be used carefully.[41]

In summary it can be said that there are only a few studies concerning the use of antihistamines, but that their use is quite often prescribed in clinical practice to bring some relief of clinical signs.[41] Due to the fact, that IBH might not just be a histamine-mediated disease the relief of clinical signs is limited.[41]

Immunotherapy

Another option which can be tried is immunotherapy, also known as desensitisation, to try to reduce or modify the inappropriate immune response to certain allergens. However, success of these therapies is variable.[5,18]

Allergen-specific immunotherapy (ASIT)

The only causative treatment for type I hypersensitivities is the allergen-specific immunotherapy (ASIT), which should lead to a shift from a Th2 immune response to a regulatory immune response. This re-directed immune response produces IgG antibodies which block allergens that would otherwise bind to allergen specific IgE antibodies.[2,11] Until now, the efficacy of this treatment is still questionable, as no significant difference between the placebo control group and the ASIT treated group could be found.[2,17] This might be due to the fact that whole body extracts were used instead of pure allergens.[2,17] Lately, thirty *Culicoides* allergens as recombinant proteins have been produced to improve ASIT. If the primary sensitising allergens could be found, there might also be the possibility of developing a preventive ASIT against IBH.[2,17] The ASIT can also stop the “allergic march”, which is sensitisation to further allergens and development of new allergic signs like asthma.[15,42] A shift of the immune response from Th2 towards a regulatory and/or Th1 response and the induction of IgG antibodies, which are able to block the binding of allergen-specific IgE antibodies to allergens and prevent mast cell degranulation, are the main immunological mechanisms of allergen immunotherapy.[15,42]

When high-quality antigens are used, allergen specific immunotherapy has proven to be relatively inexpensive, highly effective and long-lasting. But the standardisation of allergens can be difficult, they can vary greatly in potency, the amount may be insufficient and immunogenicity of individual allergens and major allergens can be lacking.[17]

Another treatment option, which has been tested in a study[43], is an immunisation with recombinant allergens using the adjuvant aluminium hydroxide (alum) alone or combined with monophosphoryl lipid A (MPLA) to generate a preventive immunisation against IBH. The vaccine was used intra-lymphatically three times. The effect of the vaccination was induced by allergen-specific antibodies that block the binding of the allergen specific IgE so that no IgE-mediated reactions could happen. Both combinations showed good clinical effects.[43]

Another efficient method to increase antigen immunogenicity is dendritic cell (DC)-targeting. It has been shown that the IL-10 response in horses is enhanced by immunomodulators, like

the Toll-like receptor 4 (TLR-4) agonist monophosphoryl lipid-A (MPLA), and that Th1 or regulatory responses in horses with equine asthma are induced by CpG-rich oligonucleotides (CpG-ODN), acting as TLR-9 agonists.[44] Antigen-uptake by equine DC was significantly enhanced by the fusion of DC-binding peptides to ovalbumin (OVA). A significantly higher T-cell proliferation compared to the corresponding control antigens was induced by DC primed with DC-binding peptides coupled to OVA or *Cul o 3*.[44] A significant increase in the proinflammatory cytokines IFNγ, IL-4, as well as the anti-inflammatory IL-10 was elicited by PBMC stimulation with DC binding peptides coupled to *Cul o 3*.[44] The addition of adjuvant further increased the effect of the DC-binding peptides by increasing the production of IL-4, IL-10 and IFNγ by CpG-ODN and IL-10 by MPLA, while suppressing IFNγ, IL-4 and IL-17 production by MPLA.[44] In summary, targeting the equine DC with allergens fused to DC-binding peptides might be useful in increasing the equine immune response against recombinant antigens due to enhancing antigen-uptake and T-cell activation.[44] In view of this, the combination with MPLA might be a promising option to improve the efficacy of AIT in horses.[44]

Autoserum therapy

Recently, a new therapy method has been introduced for horses.[7,32,33] It is an autoserum therapy, which is based on serum phospholipids and it is prepared from the horse's own serum and administrated orally.[7,32,33] The principle of the therapy is to increase the level of useable phospholipids, such as phosphatidylcholine (PC), sphingomyelin (SM), phosphatidylinositol (PI) and phosphatidylethanolamine (PE), which usually are too low in IBH affected horses.[7,32,33] The underlying hypothesis is that IBH-affected horses overproduce lipids and that these lipids then form abnormal aggregate complexes, which cannot interact with their receptors on cells anymore and so cell signalling is not working. These complexes are resolved during the preparation process, so they can be used for biological reactions again. In addition, changes in the concentration of these phospholipids are closely connected with the clinical signs of IBH.[7,32,33]

In order to evaluate the results of the treatment, long-term information about this treatment was collected from the owners.[45] According to the owners, 70% of the horses benefited from the autoserum therapy and 16% did not. 14% of the owners did not give a clear opinion, because they had not been able to tell whether the IBH signs had improved due to the treatment or due to other conditions.[45] The clinical signs of IBH were significantly milder in horses treated with autoserum after a 5 year follow-up, and there had also been no harmful

side effects.[45] The first positive finding during the first four weeks of treatment was that skin lesions had healed more quickly and that no new damage had developed. This might be due to less pruritus and subsequently fewer self-inflicted injuries.[45] It needs to be mentioned that this was a placebo controlled study and that there is always the problem with the bias when it comes to studies with the opinion of the horse's owner. [45]

Equine interleukine-31 vaccine

A new treatment option, the eIL-31- cucumber mosaic virus containing a tetanus toxoid ($CuMV_{TT}$°) vaccine, is currently being tested.[28] IL-31, a mainly Th2 cell-derived cytokine, plays a crucial role in the allergic pruritus by directly interacting with the nervous system via the IL-31 receptor expressed by dorsal root ganglia cells in the skin. Also, IBH affected horses have a significantly higher level of IL-31 in skin lesions compared to non-affected horses.[17] The vaccine should be used two times before the IBH season starts, which should delay the onset of clinical signs.[15,11,28] For further reduction of the IBH lesions, a booster vaccination should be administered in the middle of IBH season.[15,11,28] The vaccine is aimed at the allergic itching, which is caused by allergic immune response in the skin. This way, the IL-31-mediated pruritus should be lowered and so the self-inflicted trauma and scratching should be reduced. However, the anti-IL-31 therapy is no causal treatment and therefore will not help against the allergic immune reaction itself.[15,11,28]

Moreover, it has been shown that the pruritic behaviour was reduced by the IL-31 vaccine in more than 76% of the treated horses according to reports from their owners.[29] After two vaccinations, the lesions at the neck and the tail root disappeared.[29] As a result, the skin lesions healed and the hair coat grew back due to the reduction of the pruritus through the vaccination.[29] Generally, some safety aspects have to be fulfilled when it comes to vaccination against self-targets, in this case the equine IL-31 molecule. It must be assured that (i) the vaccine-induced anti-self antibody responses are reversible; (ii) anti-self antibodies are not induced by endogenous self-proteins, and (iii) self-reactive T cells are not induced by the vaccine, which has been the case with this vaccine.[29]

Equine interleukine-5 vaccine

In the pathogenesis of IBH, eosinophils play a significant role, so they are an important therapeutic target as well.[10,11,14,17,24-27,41,42] Eosinophils were found in IBH skin lesions as well as in peripheral blood which correlated to IBH severity. Recently, an active immunisation against the eosinophilic master-molecule IL-5 has been found, targeting equine IL-5 (eIL-5),[25]

which is a classic Th2 cytokine involved in eosinophil mediated inflammation. [10,11,14,17,24-27,41,42] A virus-like particle (VLP) based IL-5 vaccine, which can overcome B cell unresponsiveness, has been developed in order to induce IL-5 specific neutralising antibodies.[10,11,14,17,24-27,41,42] Vaccinated horses show a high level of IL-5 specific antibodies and a reduction of clinical signs already in the first season of treatment and even more so in the second treatment season.[10,11,14,17,24-27,41,42] The basic vaccination consists of three injections, two initial and one mid-season booster, and long-term management could consist of a single booster at the beginning of the season. One vaccine dose contains 300µg of eIL-5 $CuMV_{TT}$. Recombinant *Culicoides* allergens are injected intradermally or intralymphatically, or are administered orally.[10,11,14,17,24-27,41,42] Horses develop antibodies against IL-5 and $CuMV_{TT}$ and, in consequence, a reduced systemic eosinophil level. The antibody levels negatively correlate with the course of eosinophil levels, which indicates that anti-IL-5 antibodies cause the reduction of eosinophil levels.[10,11,14,17,24-27,41,42] Horses tolerated the vaccination well and there was no difference in helminth presence, indicating that eosinophil effector function against parasites is not dramatically impaired.[10,11,14,17,24-27,41,42] Also, no other side effects were noticed, and neither the liver nor the spleen parameters differed, nor was the Serum Amyloid A higher.[10,11,14,17,24-27,41,42] Overall, it has been shown that vaccination with a cytokine linked to a VLP is a safe way to induce auto-antibodies with the induction of reversible, neutralising, long-lived and not-endogen-induced IgG antibody responses, and without any induction of auto-reactive T cells.[10,11,14,17,24-27,41,42] By induction of auto-antibodies, the general health status of the horses is not affected, and it also leads to reduction of the clinical signs and thus to an improved quality of life for the horses.[10,11,14,17,24-27,41,42]

Interleukine-5 vaccination combined with Interleukine-31 vaccination

In order to limit allergy-induced eosinophilia and simultaneously reduce pruritus-associated self-inflicted trauma, a combined treatment with both vaccines, IL-5 and IL-31, could be desirable.[11] This way, the vicious circle of tissue damage and itching could be broken simultaneously in long-term treatment, while at the same time aiming for a stepwise and sustainable reduction of IBH clinical signs.[11] For this, however, further studies are needed. It has also been suggested that the reduction of IL-5 levels is not only limited to eosinophil depletion but possibly also interferes with other allergic pathways.[11] In addition, a substantial reduction of basophils in targeted eosinophil therapy might contribute to the clinical effects.[11]

Insol®-Dermatophyton

A vaccine against dermatophytosis, which contains different inactivated colonies of *Trichophyton*, *Microsporum* and *Nannizia*, is another treatment option that is sometimes suggested.[17]

Insol®-Dermatophyton has an immunomodulatory effect as well. So the concentration of IL-10, TNFα and IFN γ increased significantly after the ID treatment, which means a shift from the Th2 (allergy) immune response to a Th1 (tolerance) response. [42] It has been shown that horses treated with Insol®-Dermatophyton (ID), a vaccine against fungi, showed somewhat less severe clinical signs of IBH in summer.[42] The vaccine was used intramuscularly with a dose of 0.6 ml for horses of less than 400kg of weight and 1.0 ml for horses of over 400kg of weight three times in the interval of 2 weeks.[42] It was shown that ID treated horses, which had been suffering from IBH for less than two years, had less severe clinical signs compared to ID treated horses that had been having IBH for more than two years. [42] In conclusion, it has been shown that treatment with ID can be successful for single individuals, but it was not sufficient for all horses.[42]

Janus kinase (JAK) inhibitors

The JAK inhibitor oclacitinib, which is being used for the treatment of atopic dermatitis in dogs, but not registered for treatment of horses, was suggested to be effective as symptomatic treatment of IBH.[17]

Cytokines involved in allergic dermatitis are inhibited by oclacitinib maleate, which is a Janus kinase inhibitor, most potent in the JAK1 pathway.[23] This pathway targets pro-inflammatory cytokines like IL-2, IL-4, IL-6 and IL-13 as well as the pruritus-inducing cytokine IL-31. These cytokines are well documented in pathogenesis of atopic dermatitis across species.[23] Oclacitinib maleate is currently only licensed for treatment of atopic dermatitis and pruritus in dogs. The dosage in dogs is between 0.4 and 0.6 mg/kg orally twice a day for 2 weeks and then once a day for maintenance therapy.[23] For horses, a dosage of 0.25 mg/kg intravenously and 0.2 mg/kg orally was described lately and a treatment duration of at least 30 days to show improvements in skin lesions and pruritus was suggested.[23] In order to better understand dosage and efficacy, further studies are needed.[23]

Secondary prevention

A major part of allergy treatment is the management of avoiding allergen contact.[7,15,18,19] For this reason, stabling is recommended in the mornings and in the evenings, both of which are the main time of activity of *Culicoides*.[7,15,18,19] Putting on blankets (Figure 6) and insect repellents, like pyrethroids, can also minimise exposure to insects.[7,15,18,19] Another possibility would be to move the horses away from wet land near waters and forests, and preferably keep the horses on windy and dry pastures.[15,18,19]

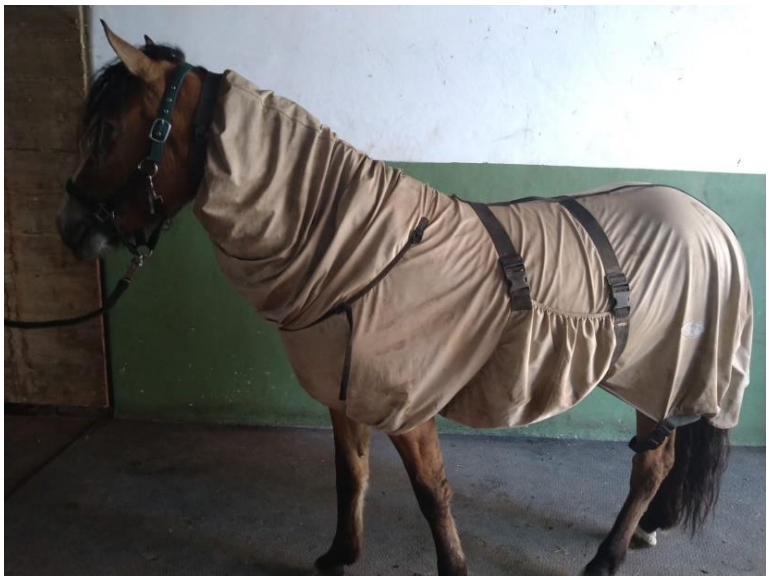

Figure 6: Use of a veterinary blanket. Icelandic horse wearing a veterinary blanket. ©Lindlbauer Kathrin/VetMedUni Vienna

Preventive immunotherapy

The use of preventive immunotherapy is another approach to control allergies.[15,17] Crucial factors in successful immunotherapy are changing the balance towards an allergen specific Th1 response and generating regulatory T cells. IgG antibodies, which have the capacity to inhibit IgE binding to the specific allergen, are another important immune parameter of induced clinical tolerance. [48] This can be applied in high-risk individuals before sensitisation occurs or in individuals with increased allergen-specific IgE before developing clinical signs. In ideal circumstances, preventive immunotherapy should be used before the first exposure to the allergens.[15,17] Not only the source of allergens and the type of adjuvants have to be considered for the development of prophylactic vaccines, but also the injection route may play an important role.[15,17] For the injection route, the intralymphatic application has shown to be a good option, because it is more efficient than the subcutaneous route and fewer

injections are required.[15,17] The goal of a prophylactic vaccination is to induce a Th1/Treg immune response with production of IgG-blocking antibodies.[15,17] One possibility would be the adjuvant IC31®, a TLR agonist, which induces a strong antibody response. It is used three times at 4-week intervals, delivering 10 µg of allergen/injection either intradermally or intralymphatically.[15,17] Another possibility would be Alum/MPLA mixed with *Culicoides* r-allergens, which results in a Th1/Treg immune response with induction of high specific IgG antibody levels after intralymphatic injections.[15,17] Until now, there are only a few studies on this topic, so further research is necessary until it will be available. An alternative to that could be sublingual immunotherapy (SLIT), where porridge of transgenic barley expressing *Culicoides* allergens is brought in contact with the oral mucosa. A weak IgG antibody response could be shown after using this transgenic barley six times over a period of 20 weeks.[15,17]

Research on plant-based protein expression has been greatly enhanced by the rising demand for therapeutic proteins because of their eukaryotic protein machinery, which improves safety and lowers costs.[48] Barley grain, for example, is an excellent source of proteins and ideal for stable storage.[48] The use of non-injection routes of allergen specific immunotherapy is increasing. An immune privileged site is the mucosa of the oral cavity, which is constantly bathed in various antigens derived from food and microbes. As such, it has an effective immunological network, which is able to enforce tolerogenic mechanisms.[48] An IgG1 response, which was significantly higher than in the control group, was seen after barley consumption (in total 500g in seven feedings, in a treatment period of 20 weeks), while there was no IgG5 response.[48] It was also shown that there was an IgE blocking capacity of the sera after treatment.[48] A further finding was that the IgG1 response could be effectively boosted with one barley dose eight months after the last treatment, and that this response was higher after the boost than after the initial treatment.[48] The IgG1 response was also observed in the saliva of treated horses.[48] The IgG5 response was very low or absent in both serum and saliva.[48] The sera from the horses treated with the transgenic barley were able to partly block the binding of IgE, indicating that a beneficial immune response had been induced.[48]

Table 3: (Dis-) Advantages of options for treatment, secondary prevention and immunotherapy for IBH in horses

Treatment and secondary prevention	**Advantages**	**Disadvantages**	**Ref.**
Local treatment			
Cold water	Easy application (topical) No side effects or long term effects Easy availability Cooling effect Vasoconstriction	Will not deter another midge attack Needs to be used every day over the whole summer will not improve clinical signs and pruritus	5 7 18
Shampoo with local anaesthetics	Easy application (topical) No side effects or long term effects Easy availability Reducing pruritus	Will not deter another midge attack Needs to be used every day over the whole summer Will not improve clinical signs	5 7 18
Cream containing omega 3 fatty acids	Easy application (topical) No side effects or long term effects Easy availability Improve clinical signs	Will not deter another midge attack Needs to be used every day over the whole summer Will not improve pruritus	5 7 18
Corticosteroids			
Prednisolone	Quite easy application (oral) Good for controlling extreme itch	Needs to be used every day as long as necessary Numerous side effects	7 11 23 40
Antihistamines			
Cetirizine	Quite easy application (oral) The use of Corticosteroids can be reduced	Needs to be used every day over the whole summer Variable effects, no effective reduction of clinical signs Side effects like gastrointestinal problems and tachycardia Not in pregnant horses	5 7 11 18
Immunotherapy			
Allergen-specific	Relatively inexpensive,	Application not that easy	2 15 11 17

immunotherapy	long-lasting	(intralymphatically) Efficacy is questionable Side effects and long term effects are questionable	43 42
Autoserum therapy	Individual therapy	Application not that easy (intravenously) A duration of 4 weeks	7 32 33
eIL-31-$CuMV_{TT}$°vaccine	No side effects Good efficacy in the clinical tests	Application not that easy (intravenously or intralymphatically) Vaccination scheme needs to be followed	7 15 11 17 25-28 46 47
eIL-5-$CuMV_{TT}$°vaccine	No side effects Good efficacy in the clinical tests	Application not that easy (intravenously or intralymphatically) Vaccination scheme needs to be followed	15 11 17 28
Insol®-Dermatophyton	Less severe clinical signs	Application not that easy (intamuscullary) Quite high number of injections are needed Not significant, efficacy questionable	17 42
JAK kinase inhibitors (oclacitinib)	Quite easy application possible (oral) Good efficacy in some clinical tests	Application once a day for 30 days needed Side effects like swelling of the skin, gastrointestinal problems, lethargy Not registered for treatment of horses	17 23
Secondary prevention			
Insect repellents	Easy to manage, Easy application (topical) No real side effects or long term effects Easy availability	Needs to be used every day over the whole summer	7,15,18,19
Management (blankets, stabling)	No side effects or long term effects	possibly difficult to manage Needs to be done every day over the whole summer	7,15,18,19

3.6. Equine asthma

Equine asthma (EA) is a chronic respiratory disease in horses. On the one hand, there is severe equine asthma (sEA), also called recurrent airway obstruction (RAO). On the other hand, there is mild to moderate equine asthma (mEA), also called inflammatory airway disease (IAD).[49,50] The sEA is a chronic respiratory disease which is associated with lower airway inflammation and obstruction as well as with frequent coughing, nasal discharge, higher respiratory effort at rest and performance limitation, and normally affects horses over seven years of age. Up to 20% of adult horses in the Northern hemisphere are affected. [49,50] The clinical signs of mEA include poor performance and casually coughing with normal breathing at rest. It can affect horses at any age. [49,51] Furthermore, massive accumulation of mucus in the airway is a characteristic of both forms. [49,51]

Clinical signs

Equine asthma is a respiratory disease limiting the performance of the animal, and can resemble an allergic phenotype. However, in contrast to the type I hypersensitivity phenotype of common human asthma, horses do not display a large eosinophil response despite a reported Th2 response in equine asthma.[50] Typical signs of severe equine asthma upon exposure to causative antigens are dyspnoea, tachypnoea, coughing, nasal discharge, flared nostrils, and subsequent exercise intolerance. In more severe cases, weight loss and anorexia might appear. [3,18] At clinical examination, a prolonged expiratory phase, wheezing and tracheal rattle due to mucus production and over-expanded lung fields can be found. These signs are mostly caused by bronchoconstriction and compromised gas exchange. [3,18]

Pathogenesis of mild equine asthma

The pathogenesis of mEA is still not completely clear, and the role of age and exposure to particulates is also still unclear. [49,51] By definition is an inflammatory airway disease described by a dysregulation of the homeostasis of inflammatory cells in the lumen of the airway, which leads to clinical signs of different severity. [49,51] Several etiological agens might be involved and their contribution to develop mEA might vary due to the environmental conditions they are exposed to.[49,51] In the development of mild equine asthma, non-infectious agens might play an important role. Therefore, horses, which are mostly in the stable, have a higher risk of developing mEA due to the high dust concentration and gases in their environment. [49,51] Furthermore, an involvement of aeroallergens is suggested because of the presence of high eosinophil, neutrophil and mast cell counts in BALF as well as Th2

cytokines like IL-4 and IL-5. [49,51] However, further studies will be needed in order to evaluate the involvement of eosinophil in the pathogenesis and its effect on performance.[49,51]

Pathogenesis of severe equine asthma

The pathogenesis of sEA is still not completely clear either. The disease develops when genetically susceptible animals are exposed to an environment in which the airborne respirable particles are highly concentrated and when these particles then are able to induce airway inflammation.[50] Many different allergens have been included in the etiology of sEA, such as for example fungal spores, bacterial endotoxins, forage and storage mites, microbial toxins, pollen, and plant debris.[45-47,50]

Severe equine asthma is normally characterised by a neutrophilic response, and so there is no typical presentation of a type I hypersensitivity.[50] Since the clinical signs of EA only appear several hours after exposure to allergens, it seems unlikely that equine asthma is a simple immediate type I hypersensitivity reaction, even though a Th2 cytokine profile has been found in sEA-affected individuals. [45-47,50] A late phase response has been described. This response leads to a neutrophilic bronchiolitis, which is associated with an increased level of CD4+ T cells in the BALF.[50] Hence, it is suggested that a type III hypersensitivity response might be involved in the pathogenesis. This hypersensitivity response results in antibody-antigen complexes and activates the complement cascade.[50] However, many of the features described in a type III hypersensitivity are not present in sEA, so it is still unclear whether this type of response accounts for the main immunological features of this disease.[50] Furthermore, the expression of IL-4 and IL-5 was seen in the BALF of affected horses.[50] However, airway neutrophilia but not eosinophilia accompanied the Th2 cytokine profile of these horses.[50] An increased expression of IL-1, IL-8, IL-17, IFN and TNF in the BALF and thus a Th1 mediated response has also been suggested.[50] In addition, a mixed Th1/Th2 cytokine profile which involves mediators like IL-4 and IFN has been proposed.[50] This might show the heterogeneity of the cytokine profile in sEA affected horses and might suggest the existence of different endotypes of the disease.[50] This heterogeneity could also be considered as the existence of different phenotypes, for example allergic asthma, non-allergic asthma and late-onset asthma.[50] A Th2 mediated response would thereby characterise the allergic asthma phenotype and so would be associated with other allergic diseases like IBH or atopy.[50] Nevertheless, more studies are needed in order to fully understand the pathogeneses of sEA.[50] The presence of neutrophils in the airways in normally associated with the non-allergic phenotype, which also reflects the involvement of a

Th1 response.[50] The last phenotype, the late-onset asthma, is age-associated. This phenotype occurs in sEA as well and affects mature adults.[50]

Underlining this allergen provocation and lack of eosinophils neither results in an acute bronchospasm nor in an increased histamine concentration in the BAL.[45-47] Until now, it has not been possible to determine whether equine asthma can definitely be attributed to one type of hypersensitivity or if it is a complex of various etiological and immunological manifestations.[3,52] It seems that the type I hypersensitivity reaction plays a major role in the pathogenesis of severe EA, whereas the type IV hypersensitivity reaction is a major part in milder forms.[53]

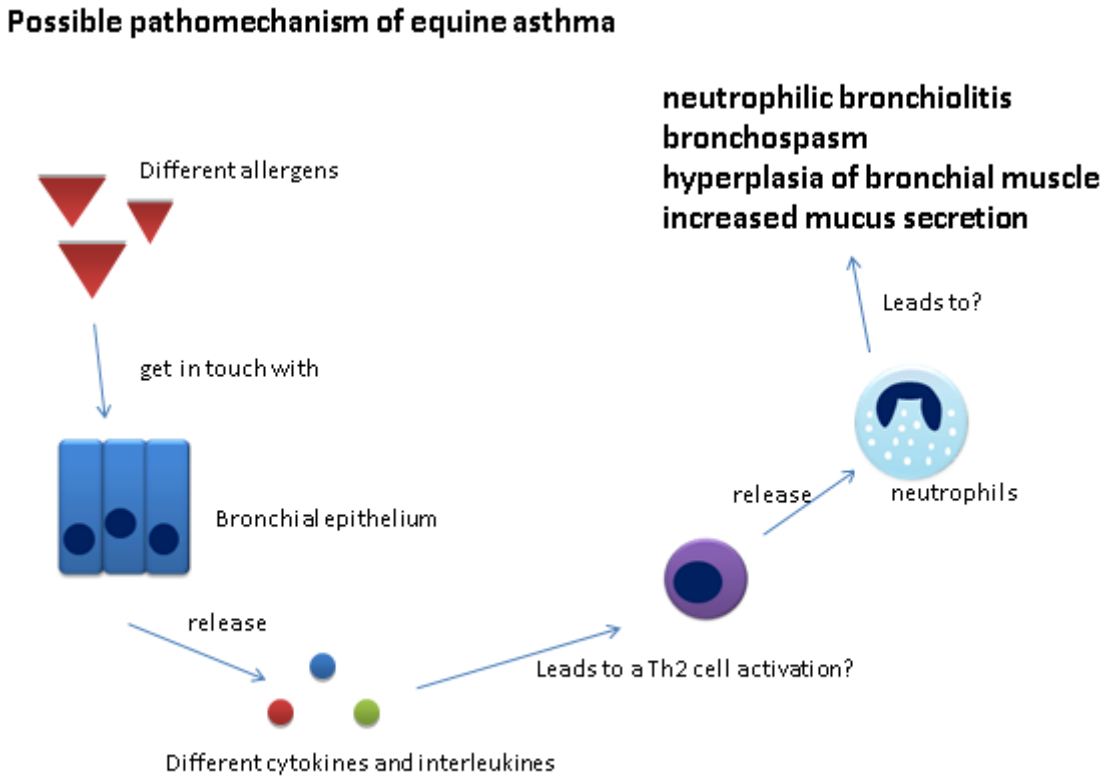

Figure 7: A possible pathomechanism of EA. Different allergens get in touch with the bronchial epithel releasing different cytokines and interleukines. These may lead to a Th2 cell inflammation and a release of neutrophils, which then may lead to neutrophilic bronchiolitis, bronchospasm, hyperplasia of bronchial muscle and increased mucus secretion. Created by PowerPoint by Kathrin Lindlbauer

Diagnosis

The diagnosis of mEA is based on different parameters. Firstly, there is the presence of clinical signs of lower airway disease such as poor performance and chronic coughing.[49,51] Secondly, there is the documentation of lower airway inflammation. This can be detected due to excess mucus production on endoscopy, BALF, cytology or abnormal lung function. In practice, the BALF cytology is recommended to confirm the diagnosis of mEA and in the end the importance of the results of this cytology should be seen in the light of the history, the clinical signs and the endoscopical findings.[49,51] Thirdly, there is the exclusion of infectious and other respiratory diseases.[49,51]

Generally, equine asthma diagnosis is a combination of the history, clinical signs of reversible airway obstruction after exposure to trigger factors, and of endoscopic examination with mucus score and BAL cytology.[50,54,55,49] For further diagnosis of EA, the local response of the bronchial epithelium to what can be tested and the IgE concentration in lung tissue and BAL can be evaluated. Another approach is the inhalation provocation test, which can be used to test for non-specific or specific hyperreactivity, but this test is not being used.[3,52]

One option for the diagnosis of allergy-mediated EA is the use of allergen-specific IgE tests, which can be measured in both serum and BAL.[54] This test is adapted from use in humans, where it is known that allergic asthma is common, and that eosinophils and IgE are what causes the clinical signs.[54] An IgE ELISA test with the screening panel for house mites, storage mites, mould, and pollen can be used to measure the allergen-specific IgE in BAL and serum.[54] No correlation between serum and BALF was found in the results of the allergen panel testing, wheras a significant correlation was identified between mould-specific and pollen-specific IgE in the BALF and IL-5 and CD14 gene expression.[54] In conclusion, further studies are needed to determine whether this test actually works in horses.[54]

Intradermal testing (IDT) is another test to identify hypersensitivities to specific allergens in horses.[53] In the test, different allergens, including moulds, mites, insects, plants, animals and feedstuffs, are used and reactions are evaluated at 30 minutes (immediated reaction), 4h (late-phase reaction), 24h and 48h (delayed reaction).[53] After the inoculation of the allergen, binding of allergen-specific IgE to high-affinity receptors on the surface of mast cells is taking place and a signal transduction cascade is activated. This cascade induces mast cell degranulation and, as a consequence, various preformed inflammatory mediators are released and a wheal-and-flare reaction can develop.[53] The evaluation of the reactions is

based on visual inspection and digital palpation on the basis of wheal diameter, thickness, turgidity, warmth, and itch.[53] One reason for not acknowledging the IDT as an additional standard diagnostic tool in EA affected horses is the fact that there are contrasting results, which might be due to non-standardised allergens used in the IDT.[53]

Lung function tests are further diagnostic tools.[56] There are different tests to evaluate the lung mechanics, for example the rebreathing method or the measurment of the airflow immediately after strenuous exercise.[49,51] Furthermore, there are more sensitive lung function tests, such as forced expiration and impulse oscillometry.[49,51] An effective way to demonstrate the increased maximal pleural pressure and the higher pulmonary resistance in severe EA is the esophageal balloon/pneumotachometer method, but it is not sufficient to demonstrate the abnormal function in mild EA.[56] Another test, which can be performed in the field and which shows quite good outcome, is the airway hyperresponsiveness, which is a common clinical sign.[49,51]

A primary method of diagnosis of EA is the examination of airway secretions.[56] A limitation of the examination of airway secretions, however, is that the relationship between BAL cytology and clinical outcome is still unclear.[56] Overall, the importance of BAL cytology should be seen together with the history, the clinical signs and the endoscopic findings.[49]

Endoscopic visualisation of mucus is another diagnostic tool, which is used in many clinics.[56] With this, the tracheal mucus correlates quite well with the performance of the horse.[56]

Endobronchial biopsies are a method one can use to view the histopathology of the larger airways, although deeper layers cannot be accessed. With this method, EA can be distinguished from mild EA or EA in remission.[56]

Another diagnostic tool is radiography. It can be helpful to support the diagnosis of EA by excluding differential diagnoses, but it is insufficient for individual diagnosis because the sensitivity is insufficient.[49,51] Only endobronchial ultrasound can show thickening of airway smooth muscle in severe EA.[56] In addition, there was also no association between the radiographic changes and the BALF cytology or the pulmonary function tests.[49,51]

In the future there might be a development of new portable and sensitive devices to measure the lung function of horses, such as forced oscillation or flow interruption techniques. Additionally, blood biomarkers for EA might possibly be discovered, which would facilitate the diagnosis of EA in clinical practice.[56]

Treatment and prevention

As with IBH, management of horses affected by equine asthma is the most important aspect of treatment and prevention. It is crucial to avoid the allergen and provide supportive treatment, such as bronchodilators.[18] Most of the time, medical treatment is focused on decreasing the inflammation of the lung and is together with the management of environment quality.[49,51]

Usage of glucocorticoids for the management of equine asthma is the most common use of glucocorticoids in equine medicine.[40] An improvement of lung function could be observed after the intravenous, intramuscular and oral administration of glucocorticoids as well as after inhaled glucocorticoids.[40,49,51] Dexamethasone and prednisolone are normally used for systemic medication, with the advantage of improving clinical sings and lung function in EA-affected horses rapidly and effectively.[49,51] For treatment with inhaled glucocorticoids beclomethasone, ciclosonid and fluticasone are used.[49,51,57] Whenever possible, inhaled corticosteroids should be used because of the advantage of reaching the lungs in high concentrations while minimising systemic side effects due to low bioavailability. Additionally, use of bronchodilators and antigen avoidance should be considered.[40]

Bronchodilator therapy is still an important part of equine asthma management, although bronchoconstriction is mostly due to the inflammatory events that take place in the airways.[58] Bronchodilators commonly used target beta2 or muscarinic receptors on airway smooth muscle, although beta2 agonists additionally have some anti-inflammatory properties.[58] The only beta2 agonist authorised for the use in horses is clenbuterol, which can be used as long term treatment.[58] Another option is the muscarinic receptor antagonist atropine, which is an effective bronchodilator, but it is not selective and so systemic use may lead to reduced gastrointestinal tract motility and other unwanted side effects, and thus should be used only for emergencies.[58] These side effects may be limited by the use of the inhaled muscarinic antagonist ipratropium bromide.[58]

N-butyl-scopolammonium bromide, a nonspecific antagonist at the muscarinic receptor and a potent bronchodilator, is another treatment option for emergencies.[59] It reaches its maximum effect ten minutes after intravenous administration and lasts for about one hour.[59] The side effects of N-butyl-scopolammonium bromide are minimal and include transient tachycardia, decreased borborygmi and transient pupillary dilatation.[59] The used dosage is 0.3 mg/kg i.v..[59] The standard lung mechanics improved, the pulmonary resistance became less, and

breathing frequency decreased.[59] It was possible to show that the administration of N-butylscopolammonium bromide is capable of reversing signs of airway obstruction during an acute EA crisis in horses.[59]

Furthermore, there is the treatment option to use mucolytic and mucokinetic agents like acetylcysteine, bromhexine, ammonium chloride and potassium iodide infusions or hyperinfusion treatment. They have been used in practice for a long time, although their efficacy is still unclear.[49,51]

Another important point are management strategies like reducing the exposure to airborne dust.[49,51] Therefore, changing bedding from straw to low-dust material such as wood shavings, and feeding from hay to haylage for example can be very helpful.[49,51]

3.7. Insect bite hypersensitivity and its potential association with equine asthma

Besides IBH, multiple hypersensitivities can manifest in horses.[3] Prevalence of equine asthma is particularly increased in IBH affected horses and *vice versa*.[3] The hypothesis is that a dysfunction of the skin barrier leads to an allergen sensitisation and a systemic T-helper-2 immunity. This is then a predisposing factor for developing a respiratory allergy.[3]

As in IBH, IgE and histamine have been implicated in the pathogenesis of equine asthma as well, although the delayed response to intradermal allergen is suggestive of a type IV in addition to a type I reaction.[58] Inflammatory cell infiltration of the skin with early eosinophil accumulation followed by T cell recruitment is induced by the presence of antigens in the skin of affected horses. An increased IL-4, IL-5 and IL-13 mRNA expression in acute lesions and increased chemokine mRNA expression in established lesions in the skin has been described.[58] Also, an increased expression of IL-1β mRNA, which might be due to mechanical trauma incurred when the affected horses rub to relieve the pruritus, has been detected. Such self-excoriations may in part be responsible for the structural changes like lichenification that are evident in chronically affected horses.[58]

Both in equine asthma and IBH, antigen-induced local inflammatory responses develop, although clearly different conditions affect disparate organ systems.[58] The local inflammatory response is thought to be responsible for the subsequent short and long term structural and functional changes in the lungs or skin, which involve recruitment and activation of circulating granulocytes and where lymphocytes seem to play a key role.[58] Therefore, allergen

avoidance or administration of drugs with a broad profile of anti-inflammatory actions like glucocorticoids are effective approaches in the treatment of both IBH and equine asthma when management strategies are insufficient.[58] Key mediators in orchestrating the immune response are cytokines and chemokines and, as such, represent a therapeutic target in allergic horses.[58] Increased mRNA expression for a range of cytokines and chemokines in the bronchoalveolar lavage fluid, epithelium or lymphocytes of asthmatic horses and in the lesional skin of IBH affected horses has been described[58]. It has also been shown that IL-4 and IL-5 are increased in both the lesional skin of IBH affected horses and in the BAL cells of symptomatic asthmatic horses.[58] TNFα is increased in BAL cells of asthmatic horses as well, although no change in the level of expressed protein was detected.[58] Another interleukin playing a key role in the pathogenesis of both diseases is IL-13, which was shown to be increased in BAL fluid of asthmatic horses and in acute IBH lesions.[58] Increased IL-8 concentration have also been described in epithelial cells and BAL fluid of asthmatic horses, and this neutrophil activation chemokine has been implicated in causing neutrophilia in the airways.[58]

Due to recent genetic and epidemiologic evidence, it has been suggested that different manifestations of hypersensitivity can occur together in horses.[53,60,61] A common immunogenetic background, route of sensitisation, or both, leading to these multiple hypersensitivities (MHS), were suggested.[53,60,61] Interleukin 4 is described to have a high prevalence in EA, but has recently not been associated with MHS in horses.[53,60,61] A mutation in a profilaggrin/filaggrin gene and a genetic variant in IL-13, a gene playing a related role in the Th2-type response as IL-4, are associated with both airway hypersensitivities and skin allergies.[53,60,61] It has also been shown that there is a link between IL-4 and IL-13 polymorphisms in equine asthma.[53,60,61] Even though this finding of IL-13 has triggered these investigations in MHS in horses, one still needs to consider that IBH and EA exhibit important immunopathologic differences in context with genes like IL-13 and IL-4.[53,60,61] Hence, IBH is known as a type I hypersensitivity reaction with IgE- and Th2-type driven immunopathogenesis and lesional eosinophil and mast cell involvement, whereas the exact immunopathology of EA is still unclear.[53,60,61] Furthermore, the integrity of epithelial barrier function has been found to be of great importance in the development of allergies. It has been suggested that the skin has an important role as a route of sensitisation against asthma-inducing allergens. Thus, it seems like skin and mucosal barrier dysfunction

increases the risk of sensitisation to allergens by facilitating crossing of the epidermal barrier by allergens and inducing sensitisation.[53,60,61]

4. Retrospective investigation of data on icelandic horses at two university hospitals concerning the comorbidity between EA and IBH

4.1. Introduction

One of the most common causes of allergic dermatitis of horses is insect bite hypersensitivity. It is due to an allergic reaction to the bites of *Culicoides* and is characterised by intense pruritus, which leads to hair loss, appearance of papules and lesions on the mane, neck and tail area. All horse breeds worldwide are affected, with the exception of a few areas where *Culicoides* are not present, such as Iceland for example. Therefore, Icelandic horses are especially prone to develop IBH, because they miss allergen contact at a young age and thus no tolerance development against *Culicoides* has taken place.[27,43]

Another common disease occurring in adult horses is equine asthma. It is one of the most common non-infectious respiratory diseases. An important role in the development of equine asthma is played by the presence of dust in feed and bedding, as well as the ammonia concentration in the stable. A lot of different pro-inflammatory particles have been found in the stable environment, such as bacterial endotoxins, different mould species, microbial toxins, plant particles and more. A hypersensitivity to inhaled organic dust causes the clinical signs like cough, exercise intolerance, and severe respiratory distress.[52,58]

Consequently, both IBH and equine asthma are quite common allergic conditions in horses and are both caused by a type I hypersensitivity. [58]

Recent findings suggest that different manifestations of hypersensitivity might occur together in horses. Despite the different pathomechanims, where insecte bite hypersensitivity is an allergic dermatitis caused by *Culicoides* bites and equine asthma is caused by a hypersensitivity to inhaled allergens, horses with IBH show a higher risk of developing equine asthma and *vice versa.*[60]

Therefore, the aim of this part of the thesis was to investigate if there is a potential comorbidity between EA and IBH. So the hypothesis is that horses which suffer from equine asthma are more often affected by IBH than non-asthmatic horses.

4.2. Methods

Data retrieval

The data collection for this investigation took place at the Universities of Veterinary Medicine of Vienna and the Veterinary Faculty of the Ludwig-Maximilians-University Munich.

All digitally stored patient records of all Icelandic horses presented at the clinics between the period of 1 January 2014 until 31 May 2022 were included. The records were then screened for the horses' age, their sex, the reason they were presented at the hospital and whether they had IBH and/or EA. The data screening was performed during May and June 2022.

Statistics

Descriptive statistics and descriptive graphics have been conducted with the software Microsoft-"EXCEL". Furthermore, the Chi Square test was calculated with a value of p=0.05 with the software Microsoft "EXCEL". To show the outcomes the percentage of the horses for each categorie was calculated and presented. Additionally, the mean age as well as the minimum and maximum age was determined. For categorical data, frequencies of occurrence are presented.

4.3. Results

The findings are presented here as (i) the frequency of horses presented with IBH or EA, and (ii) the comorbidity between these two. Unfortunately, the data were not always complete, so the horses' origins were not mentioned most of the time, their age was sometimes missing, and documentation of the allergic diseases varied a lot between the files.

Horses

In total, 826 files of Icelandic horses are included in this evaluation. These 826 horses were assigned to two groups. One group includes the Icelandic horses suffering from equine asthma and the other group includes the Icelandic horses not suffering from equine asthma. In the EA group are 181 horses from the investigated horses and in the non-EA group are 645 horses from the study population.

There are 332 mares in total, 81 suffering of EA and 251 non-affected mares. Furthermore, there are 414 geldings in total, 92 affected by EA and 322 non-affected ones and 80 stallions in total, eight suffering of EA and 72 non-affected horses. (Figure 8)

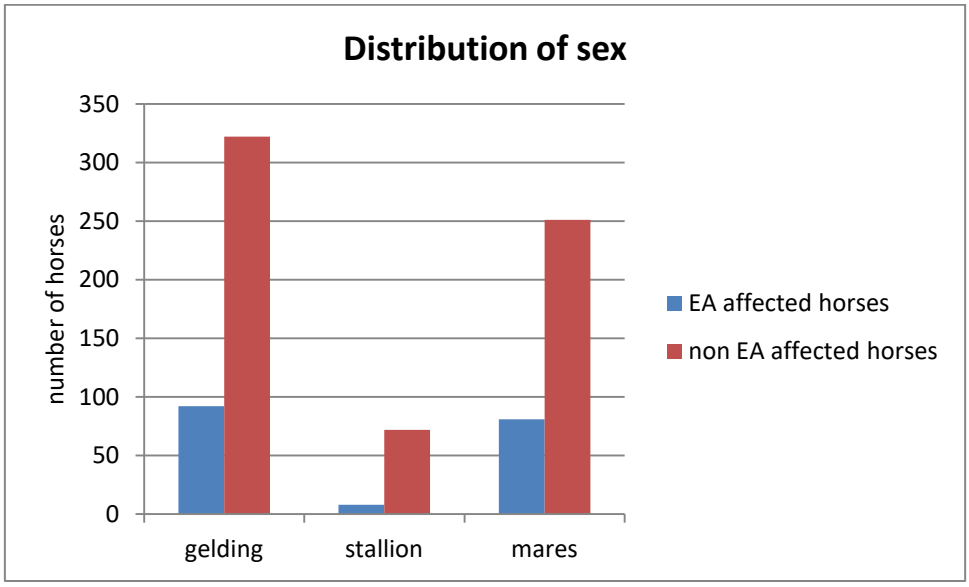

Figure 8: Distribution of sex in the investigated Icelandic horse population of the two groups. The distribution of the sex in the EA affected horses and in the non-EA affected horses. Created with Excel by Kathrin Lindlbauer

The mean age of all investigated Icelandic horses was 17.71 years, with the youngest horse only being a few days old and the oldest being 41 years. The median of the age of EA affected horses is at 19 years and of non-affected horses at 16 years. The youngest horse suffering from EA is 4 years old and the oldest 39 years old. In the group of non-affected horses the youngest one is only a few days old and the oldest 41 years.

Most common reasons for presentation at the hospital

The reasons for presenting an Icelandic horse at one of either hospitals were numerous, and were categorised into the following different groups: gastrointestinal problems, orthopaedic problems, respiratory problems, dental problems, ophtalmological problems, reproductive problems, tumours, neurological problems, renal problems, liver problems, dermatological problems, cardiological problems, more than one problem, other problems, or as company animal for other patient horses.

For all 680 horses presented at the hospital in Vienna, the most common reasons were gastrointestinal problems with 22.5% (n=153) and orthopaedic problems with 22.1% (n=150), followed by horses with more than one problem with 12.2% (n=83) and horses with dental problems with 10.6% (n=72). Less common reasons were respiratory problems with 8% (n=55), ophtalmological problems with 6% (n=41), reproductive problems with 4.7% (n=32) and tumours with 2.1% (n=14). At the hospital in Vienna 5.6% (n=38) of the horses were

because of a different problem and 2.4% (n=16) were only there as company. The least common reasons were renal problems with 1.5% (n=10), liver problems with 1.3% (n=9), neurological problems with 0.6% (n=4) and dermatological problems with 0.4% (n=3). Two out of the three horses with dermatological problems were presented because of IBH, but none of these two had EA as well, and one was presented because of mite infestation. The reasons for presenting the horses with respiratory problems were various: 33 of the 55 horses were presented with EA, of which only six had IBH as well, twelve horses were presented because of strangles, four had a problem with the diverticulum tubae auditivae, three had a bronchopneumonia, one was presented because of a pulmonary oedema, one because of dyspnoea, and one because of left sided recurrent laryngeal neuropathy.

For the 146 horses presented at the hospital in Munich, the most common reasons were ophtalmological problems with 23.3% (n=34) and respiratory problems with 20.5% (n=30), followed by reproductive problems with 14.4% (n=21) and gastrointestinal problems with 12.3% (n=18). Less common reasons for presenting were neurological problems with 6.2% (n=9), dermatolgical problems with 2.7% (n=4) and liver problems with 2.1% (n=3). The least common reasons were renal problems with 1.4% (n=2), dental problems with 0.7% (n=1) and tumours with 0.7% (n=1) as well. No horse was presented at the hospital in Munich with an orthopaedic problem. 10.9% (n=16) were at the hospital for a different reason, 0.7% (n=1) were at the hospital with more than one problem and 4.1% (n=6) were there as company. All horses with dermatological problems were presented because of IBH, but none of them had EA as well. For presenting horses with respiratory problems there were different reasons: 22 of them were presented with EA and two of these also had IBH, further reasons were dyspnoe, which affected four horses, dorsal displacement of the soft palate which affected three horses, and one horse suffered from bronchiolitis.

When the results of both hospitals are analysed together, the most common reason was gastrointestinal problems, such as colic, with 20.7% of the horses, closely followed by orthopaedic problems with 18.2%. Other quite common reasons for being presented at the hospitals include ophtalmological problems (9.1%), respiratory problems (10.3%), dental problems (8.8%), or with more than one problem (10.2%). Less common reasons were problems with the liver or the kidneys (1.5% each), neurological problems (1.5%) or dermatological problems (0.8%).

Frequency of horses with IBH

For this study, all Icelandic horses with a history or a diagnosis of IBH were defined as IBH affected.

When counted together 64 Icelandic horses, which are 7.7% of the study population in both hospitals, were affected by IBH. Only six of these 64 horses were diagnosed with IBH at the hospitals, for the rest the problem was known before. (Figure 9)

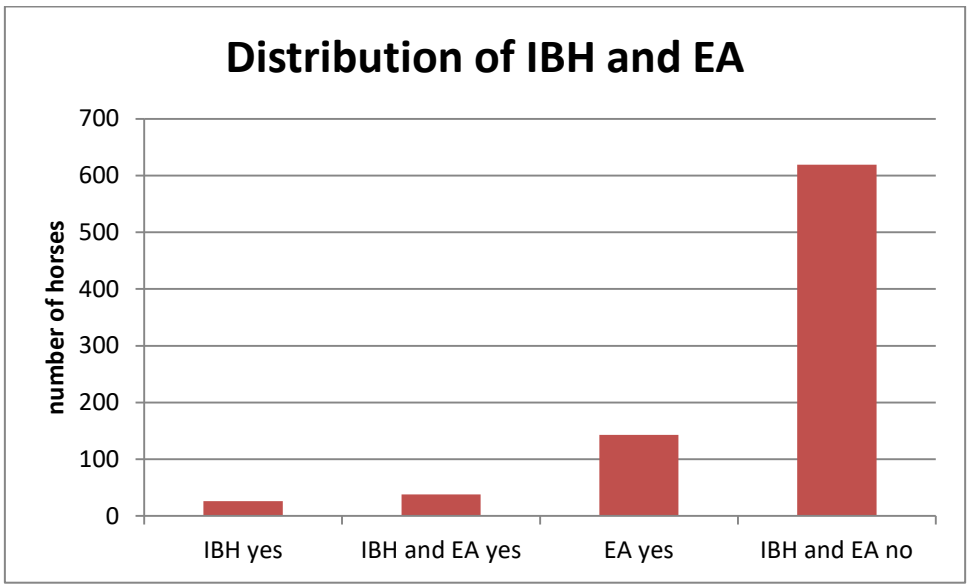

Figure 9: Distribution of allergic diseases among investigated Icelandic horses. Each horse is just in one category. Created with Excel by Kathrin Lindlbauer

Frequency pf horses with EA

Similar to IBH, all horses with either a history of EA or with EA diagnosed at the hospital were counted as EA affected in this study.

When counted together, 181 horses were affected by EA, which are 21.9% of all here investigated horses in both hospitals. Among these 181 horses, 55 were diagnosed with EA during their hospitalvisit, while the remaining cases had a pre-existing diagnosis.

Comorbidity between EA and IBH

A comorbidity of IBH and EA was present in this study if the horses either had a reported history of both IBH and EA, or if they had a history of one of the illnesses and received a diagnosis of the other one at the hospital, or if they were diagnosed with both IBH and EA when presenting at the hospital.

In the EA-affected group 26.57% (n=38) was also affected by IBH, whereas in the non-EA-affected group only 4.2% (n=26) was also affected by IBH.

With a value of 56.6 the Chi square test showed a significant connection between EA-affected horses and IBH-affected horses ($p<0.05$).

4.4. Discussion of data evaluation

In this study 826 files of Icelandic horses in the hospitals of Vienna and Munich were collected and examined.

In total, 7.7% (n=64) of the 826 horses were affected by IBH and 21.9% (n=181) were affected by EA.

The prevalence of IBH of 7.7% that was identified in this study is within the range of the published prevalence in Icelandic horses born in continental Europe of 6.7 to 8%.[1-11]

The prevalence of EA was found to be at 21.9% in this project, which corresponds with other studies were prevalence of EA has been determined to be at up to 20% of adult horses in the Northern hemisphere. [49-50]

The comorbidities between IBH and EA have been calculated in the investigated animals. 26.57 % of the EA-affected horses were also affected by IBH, whereas only 4.2% of the non-EA-affected horses were affected by IBH: 38 of the 64 IBH-affected horses also had EA and 38 of the 181 EA-affected horses also had IBH. The Chi square test in this study turned out to be significant with a value of 56.6. In other studies, it is only mentioned that horses affected by IBH can suffer from EA more easily and *vice versa*, but there is no concrete data on comorbidity.[3,58]

To see if the comorbidity between the two diseases is real further studies with more horses will be needed and maybe only horses with allergic diseases should be included to become a more specific result. In the further studies the origin of the horse and the stable environment

should be included. Also, horses in outpatient's clinic should be included because the number of IBH there might be higher than in the clinic because IBH is often diagnosed in outpatient's clinic and do not show up in the clinic anymore.

Incomplete documentation at the hospitals could be a potential problem, because in some cases the allergies were not mentioned in detail. Additionally, during winter season there might be some remission of IBH and it might not always be detected during the first examination. This issue was not investigated in this study, as the season when the horse was presented to the hospital was not mentioned in all cases. Therefore, there could have been a number of horses that have not been diagnosed with an allergic disease, even though they may have had one. Various studies also mention that the clinical signs of IBH become less in winter and worsen again in summer. [7,13]

In addition, it could be that Icelandic horses with IBH are treated in an outpatient's clinic and, therefore, may not present at the hospital and thus would not be included in this study. So the numbers of patients included in this study may not correlate with the real numbers in the whole Icelandic horse population.

Furthermore, it would be interesting to conduct a study concerned with EA in Iceland particularly because none such study could be found in the research for this thesis. This would be interesting when it comes to the comorbidity of EA and IBH because of the non-appearance of insect bite hypersensitivity in Iceland.

5. Summary

The aim of this thesis was to summarise the latest advances in IBH with a focus on current knowledge regarding the pathomechanisms and the latest therapeutical options.

The second part was about a clinical data analysis at the Universities of Veterinary Medicine of Vienna and Munich in order to investigate (i) the prevalence of horses affected by IBH and EA, and (ii) potential co-morbidities.

The literature review for the first part was conducted with help of the search engines PubMed, Google scholar and Scopus and with the following search terms: “Sommerekzem”, “sweet itch”, “sweet itch symptoms“, “sweet itch histology“, “insect bite hypersensitivity”, “equine asthma”, and “RAO”.

The data collection for the second part took place at the Universities of Veterinary Medicine of Vienna and the Veterinary Faculty of the Ludwig-Maximilians-University Munich. All digitally stored patient records of Icelandic horses presented at the clinics between the period of 1 January 2014 until 31 May 2022 were included. The records were then screened for the horses’ age, their sex, the reason why they were presented at the hospital and whether they had IBH and/or EA. Descriptive statistics and descriptive graphics were conducted with the software Microsoft-“EXCEL”. Furthermore, the Chi square test was calculated with the software Microsoft “EXCEL”. To show the outcomes the percentage of the horses for each categorie was calculated and presented. Additionally, the mean age as well as the minimum and maximum age was determined.

Insect bite hypersensitivity (IBH) is one of the most common allergic problems in horses, and it appears nearly everywhere in the world. Because of the non-appearance of the allergy causing *Culicoides* in Iceland, Icelandic horses transported to other countries are more often affected by this disease.

The first signs of IBH show up in spring when the *Culicoides* start to appear. Pruritus is the main sign of IBH and causes self-harming, irritation of the skin, wounds and alopecia. Besides these problems, there can also be restlessness that is caused by the insects.

Insect bite hypersensitivity is a type I hypersensitivity reaction against the proteins in the saliva of *Culicoides*. It is also being discussed if a type IV hypersensitivity reaction may be part of IBH.

There are different possibilities for diagnosis. The easiest way to diagnose IBH is the anamnesis and clinic evaluation. Furthermore, there are also intradermal tests and serological tests.

Even though IBH is one of the most common problems in horses, there is still no optimal treatment. On the one hand, there is the possibility to cover the horses in blankets and use insect repellents on them in order to protect them from the insects. On the other hand, the allergy can be treated with corticosteroids. But success of these options is rather variable. Recently, there have been a number of studies on vaccinations against IL-5 or IL-31, but these vaccinations still need to be tested in large long-term studies and approved.

Lately, the question of whether there is a connection between IBH and equine asthma has also been brought up. Studies have shown that horses with IBH have a higher prevalence of suffering from equine asthma as well and *vice versa*.

The clinical data analysis in the present work showed a similar prevalence (7.7%) of IBH as other studies before (6.7% to 8%). The prevalence of EA in this study (21.9%) also correlated with the prevalence in other studies before (over 20%). Furthermore there was a significant connection between the two diseases.

It turned out that, even though considerable research has been conducted over the last few years, the prevalence of IBH in Icelandic horses and the pathomechanisms of IBH and EA are still not fully understood. Moreover, therapeutical options have not been completely successful yet. In light of this, more research needs to be done when it comes to IBH.

6. Kurzzusammenfassung

Das Ziel dieser Studie war es im ersten Teil die letzten Erkenntnisse zum Thema Sommerekzem vor allem im Hinblick auf die Pathomechanismen und die neuesten therapeutischen Möglichkeiten darzustellen.

Im zweiten Teil der Arbeit ging es darum klinische Daten der Universitätsklinik von Wien und München auszuwerten. Dabei wurden (i) die Prävalenz der Pferde, die an Sommerekzem und an equinem Asthma litten ermittelt und (ii) nach einer möglichen Comorbidität gesucht.

Die Literaturrecherche für den ersten Teil erfolgte mit Hilfe der Suchmaschinen PubMed, Google scholar und Scopus und die folgenden Suchbegriffe wurden dafür verwendet: “Sommerekzem”, “sweet itch”, “sweet itch symptoms“, “sweet itch histology“, “insect bite hypersensitivity”, “equine asthma”, and “RAO”.

Für die Datensammlung im zweiten Teil wurden digitale Patientendaten im Zeitrum vom 1.Jänner 2014 bis zum 31.Mai 2022 der Klinik der veterinärmedizinischen Universität in Wien und der Klinik der veterinärmedizinischen Fakultät der Ludwig-Maximilians-Universität in München herangezogen. Den Daten wurde das Alter des Pferdes, das Geschlecht und der Vorstellungsgrund an der Klink. Desweiteren die Diagnose des Pferdes und ob es an Sommerekzem und/oder equinem Asthma litt. Die descriptiiven Statistiken und Grafiken sowie der Chi Quadrat Test wurden mit Hilfe der Computersoftware Microsoft-„EXCEL“ angefertigt. Um die Ergebnisse zu Veranschaulichen wurden sie in Kategorien eingeteilt und die Prozente berechnet. Außerdem wurde das durchschnittliche Alter berechnet.

Sommerekzem ist eine nahezu weltweit auftretende allergische Erkrankung bei Pferden. Durch das Fehlen der allergieauslösenden *Culicoides* in Island, sind Islandpferde besonders anfällig für diese Krankheit.

Die ersten Anzeichen des Sommerekzems zeigen sich zu Beginn des Frühlings, wenn die ersten *Culicoides* auftreten, und sind vorallem durch starken Juckreiz gekennzeichnet. Dieser führt zu Kratzen und Selbstverletzungen und dadurch zu Hautirritationen, Wunden und Alopecie. Neben den Hautproblemen kommen oft noch Unruhe und Rittigkeitsprobleme, verursacht durch die Anwesenheit der Insekten, hinzu.

Dem Sommerekzem liegt eine allergische Sofortreaktion, also eine Type I Reaktion, zu Grunde und eine mögliche Beteiligung einer verzögerten Reaktion, also einer Type IV Reaktion, wird diskutiert.

Zur Diagnosestellung gibt es verschiedene Möglichkeiten. Die Einfachste ist die Diagnose anhand der Krankengeschichte und des klinischen Erscheinungsbildes. Des Weiteren gibt es die Möglichkeit eines Intradermaltests und von serologischen Tests.

Obwohl das Sommerekzem zu den häufigsten Hautproblemen der Pferde zählt, gibt es noch immer keine zufriedenstellende Therapie. Es gibt zwar einerseits die Möglichkeit die Pferde mittels Decken und Insektenrepellentien vor den Insekten zu schützen und andererseits die Möglichkeit die Allergie mit Hilfe von Corticosteroiden zu behandeln, aber die bringt immer nur einen bedingten Erfolg. In letzter Zeit kommen immer mehr Impfungen, zum Beispiel gegen die Interleukine IL-5 und IL-31, auf. Diese sind allerdings noch in der Entwicklungs- und Testphase, und eine Freigabe für die Standardanwendung ist noch ausständig.

In letzter Zeit trat auch immer wieder die Frage auf, ob es einen Zusammenhang zwischen dem Sommerekzem und dem equinen Asthma gibt. Forschungen dazu ergaben, dass Pferde mit Sommerekzem eine erhöhte Prävalenz haben auch an equinem Asthma zu erkranken und umgekehrt.

Die klinische Datenanalyse lieferte in Hinsicht auf die Prävalenz vom Sommerekzem (7.7%) ein ähnliches Ergebnis wie vorhergehende Studien (6.7% bis 8%). Auch die Prävalenz vom equinem Asthma in dieser Studie (21.9%) korrelierte mit der Prävalenz in vorherergehenden Studien (über 20%). Desweiteren wurde in dieser Studie festgestellt, dass der Zusammenhang zwischen den beiden Erkrankungen signifikant ist.

So stellte sich heraus, dass trotz der ausgiebigen Forschung in den letzten Jahren die Prävalenz bei Isländern sowie die Pathomechanismen beim Sommerekzem und beim equinen Asthma noch immer nicht vollständig geklärt sind. Desweiteren sind die bisherigen Behandlungsmethoden bisher noch nicht komplett zufriedenstellend. Deshalb ist es auch weiterhin noch nötig in diesem Bereich weitere Forschung zu betreiben.

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8. List of figures and tables

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