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Review

IgE in horses: Occurrence in health and disease

Bettina Wagner*

Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA

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ABSTRACT

Since the initial characterization of IgE by Ishizaka et al. (1966), IgE was described in several mammalian species. In horses, a single gene encoding the IgE heavy chain constant region (IGHE gene) exists per haploid genome and several allelic variants of the equine IGHE gene were found. IgE occurs in its soluble form in equine serum and physiological concentrations of total IgE are around 1000-fold higher in normal horse than in normal human serum. Maternal IgE is enriched in the colostrum and transferred to the neonatal foal after birth. Foals do not produce detectable concentrations of endogenous IgE for several months after birth. IgE is also found on the surface of a small percentage of equine peripheral blood cells including basophils, and subpopulations of B-cells and monocytes, and on mast cells in various tissues such as the skin, and the submucosa of the airways and intestine. Both, the high- and low-affinity IgE receptor genes are identified in the horse suggesting binding of soluble IgE from the circulation to these receptors. Horses naturally develop type I hypersensitivities. IgE-mediated mechanisms were implicated in the pathogenesis of several allergic diseases in horses since almost 30 years. The findings were mainly based on the induction of immediate skin reactions after intradermal testing with allergen extracts. With the development of the first monoclonal antibodies to equine IgE within the past years, more insights into the pathogenesis of allergic diseases could be obtained. Today, various techniques are available to detect soluble IgE and the sensitization of mast cell or basophils with IgE in horses. An IgE-mediated allergic etiology is confirmed for skin hypersensitivity. The causing role of IgE in other diseases such as recurrent airway obstruction (RAO) still remains controversial. More recent studies did not confirm an IgE-mediated pathogenesis of RAO. This suggested that the disease is a chronic inflammatory condition with some indication for an involvement of delayed-type hypersensitivity mechanisms. In summary, our knowledge about the role of IgE during the immune response of the horse improved tremendously during the past decade. New IgE-specific tools and technologies are likely to uncover additional aspects of IgE-mediated mechanisms in equine health and disease.

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* Tel.: +1 607 253 3813; fax: +1 607 253 3440.
E-mail address: bw73@cornell.edu.

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1. Molecular organization and nucleotide sequence of the equine IGHE gene

Immunoglobulins are composed of gene products from both the immunoglobulin heavy and light chain loci. The isotype of an immunoglobulin is determined by the constant heavy chain region which is encoded by one of the genes located in the immunoglobulin heavy chain constant (IGHC) region (Wagner, 2006). In horses, the IGHG region was identified on chromosome 24 (ECA24qter; Wagner et al., 2004). The constant region of the IgE heavy chain is encoded by a single IGHE gene (Wagner et al., 1997) which is located between the IGHG5 and IGHA genes (Wagner et al., 2004). Pseudogenes for IGHE like in humans were not found in horses. Like in other mammals, the equine IGHE gene is composed of four exons each of which encodes a single immunoglobulin domain of the IgE constant heavy chain region (Fig. 1). The nucleotide sequence of the IGHE gene was investigated by various groups (Navarro et al., 1995; Marti et al., 1995; Watson et al., 1997; Wagner et al., 2001). The accumulated sequence information revealed that at least four different IGHE alleles exist in horses (Table 1). The coding nucleotide sequences of these alleles vary between 95.9% and 99.8%

homology. Most of the nucleotide sequence differences of the IGHE alleles also result in amino acid variations in the constant heavy chain region of IgE. Thus, it can be predicted that various IgE allotypes exist in horses (Fig. 2). Whether the allelic variation of the IGHE gene functionally influences the horse's immune response to parasites or during allergic diseases is unknown.

The predicted IgE constant heavy chain region has six potential N-glycosylation sites, one of which at position 174 is not conserved in the IgE*03 allotypes (Fig. 2). The N-glycosylation site at position 270 (N270, Fig. 2) is conserved between mammalian species (Wagner et al., 2002). Biochemical studies on human IgE showed that N-glycans are not directly involved in IgE receptor binding (Helm et al., 1988; Sayers et al., 1998). However, the N270 site was found to be essential for the formation of a functional IgE molecule by supporting the correct folding required for IgE receptor binding (Sayers et al., 1998). The expression of the complete equine IGHE*02 gene as part of a 4-(hydroxy-3-nitro-phenyl)acetyl (NP)-specific equimurine recombinant antibody in mammalian cells resulted in a highly glycosylated IgE chimera that mediated histamine release *in vitro* and intradermal skin reactions *in vivo* (Wagner et al., 2002).

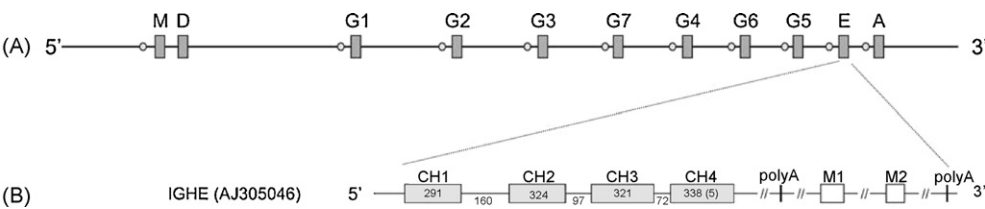


Fig. 1. The IGH locus and the genomic organization of the IGHE gene of the horse. (A) The map of the IGHG region shows the order of the eleven equine IGHG genes. The entire IGHG region has been found to be located on a minimum of two overlapping clones from the CHORI-241 Horse Bacterial Artificial Chromosome library, suggesting that the size of the IGHG region is 250–350 kb. Circles represent switch regions; boxes indicate IGHG genes; M = IGHM; D = IGHD; G1 to G7 = IGHG1 to IGHG7; E = IGHE; A = IGHA. (B) The structure of the genomic IGHE gene is shown in more detail. The GenBank accession number of the IGHE allele used for the gene map is given in parentheses. The numbers reflect the sizes (bp) of the exon (CH1–CH4) and intervening IGHE gene sequences. The secretion region of the IGHE gene is located at the 3' ends of the CH4 exon and the size is given in parentheses behind the size of the overall CH4 exon. Membrane exon sequences (M1 and M2) were not yet described for the equine IGHE gene. It is assumed from other mammalian species that they exist in the horse and this is indicated by the white boxes.

Table 1
IGHE alleles of the horse.

Allele	Coding region (bp)	GenBank accession number	Origin	Breed	Reference
IGHE*01	1272	AJ305046/AJ305047	Genomic DNA/mRNA, PBMC	Arabian/13 horses of various breeds	Wagner et al. (2001)
IGHE*02	1272	AJ305048	mRNA, PBMC	Standardbred	Wagner et al. (2001)
IGHE*03	1257	U15150	mRNA, PBMC	Thoroughbred	Navarro et al. (1995)
IGHE*04	1272	U17041	mRNA, mesenteric LNN	6 horses, breeds included Thoroughbred, Quarter Horse, Appaloosa and Standardbred	Watson et al. (1997)

The IGHE gene nomenclature conforms to the official nomenclature of the international ImMunoGeneTics database (IMGT) (<http://imgt.cines.fr>).

	CH1										80
IgE*01	VSKQAPLILP	LAACCKDTKT	TNITLGCLVK	GYFPEPVTVT	WDAGSLNRST	MTFPAVFDQT	SGLYTTISRV	VASGKWAKQK			
IgE*02	F.				R.....						
IgE*03	S.I.				G---A	P..P.					
IgE*04					I.....						
	CH2										160
IgE*01	FTCNVVSQE	TFNKTFENACI	VTFTPTTVKL	FHSSCDPGD	SHTTIQLLCL	ISDYTPGDID	IVWLIDGQKV	DEQFPQHGLV			
IgE*02											
IgE*03	G.....						E.....	TQASM			
IgE*04											
	CH3										240
IgE*01	KQEGKLASTH	SELNITQGW	ASENTYTCQV	TYK-DMIFKD	QARKCTESDP	RGVSVYLSPP	SPLDLYVSKS	PKITCLVVDL			
IgE*02								M			
IgE*03	SWPP.	N.....		DMIFN			T.....				
IgE*04				RHDLLRT	RP.....E.						
	CH4										320
IgE*01	ANVQGLSLNW	SRESGEPLQK	HTLATSEQFN	KTFESVTSLP	VDTTDWIEGE	TYKCTVSHPD	LPREVRRIA	KAPGKRLSPE			
IgE*02											
IgE*03											
IgE*04											
											400
IgE*01	VYVFLPPEED	QSSKDKVTLT	CLIQNFFPAD	ISVQWLNNV	LIQTDQATT	RPQKANGPNP	AFFVFSRLEV	SRAWEQKNK			
IgE*02											
IgE*03				R.....		D.					
IgE*04						SQW.R.					
											425
IgE*01	FACKVVHEAL	SQRTLQKEVS	KDPGK								
IgE*02											
IgE*03											
IgE*04											

Fig. 2. Amino acid sequence comparison of constant heavy chain regions of different IgE allotypes of the horse. The exon/intron boundaries of the genomic IGHE*01 gene were used to determine the sizes of the CH1 to CH4 domains. The arrows point to the first amino acid of the respective domains. Dots indicate identical amino acids, dashes show amino acid deletion. N-glycosylation sites are underlined.

2. Antibodies to equine IgE

Antibodies to equine IgE were produced by various groups and methods. First attempts to produce polyclonal antibodies were using purified IgE from equine serum for immunization (Suter and Fey, 1981; Halliwell and Hines, 1985). However, purification from serum resulted in IgE preparations with a substantial amount of contaminating IgG isotypes (Suter and Fey, 1981). Thus, the specificity of these reagents for IgE remains questionable. Other investigators used recombinant partial IgE heavy chain regions expressed in *E. coli* to produce polyclonal antibodies (Watson et al., 1997; Marti et al., 1997). Because the native IgE constant heavy chain region is heavily glycosylated (Wagner et al., 2002) and glycosylation does not occur in bacterial expression systems, the bacterial expressed proteins are likely to share only partial structural homology with the corresponding parts of the native IgE. Nevertheless, these antibodies have been used with success for ELISA, Western blotting and immunohistochemistry applications. Like for all polyclonal antibodies, the available amounts of the anti-IgE reagents were limited.

A few years ago, the first mAbs to equine IgE were developed (Wagner et al., 2003). These antibodies were produced by using complete recombinant IgE expressed in mammalian cells for immunization of mice. The mammalian expressed recombinant equine IgE was found to share a high structural and functional homology to native IgE including its binding to the high-affinity IgE receptor (Wagner et al., 2002). Monoclonal antibodies to at least two different epitopes of the IgE constant heavy chain region were identified (Wagner et al., 2003). Three years later, a second group developed additional anti-IgE mAbs using an immunization protocol that included recombi-

nant bacterial and mammalian expressed IgE heavy chain constant regions combined with purified IgE from horse serum (Wilson et al., 2006). A recent comparison of all available anti-IgE mAbs showed that the reagents produced by both groups react very similar in ELISA and immunohistochemistry (Wagner, Wilson and Marti, unpublished observation). The anti-IgE mAbs have been shown to be useful for various methods and immunological applications *in vitro* and *in vivo* (Table 2).

3. Total serum IgE concentrations, IgE⁺ cells in equine blood and tissues and receptors for IgE in adult horses

In human normal serum, IgE is detectable at concentrations in the nanogram per milliliter range, and has a short half-life of approximately 2 days. The short half-life is a result of constant removal of IgE from the circulation due to its binding to the α chain of the high-affinity IgE receptor (Fc ϵ RI) on mast cells and basophils (for review: Ravetch and Kinet, 1991). Assays for the detection of total IgE in horse serum were established after the mAbs to equine IgE became available (Wagner et al., 2003; Wilson et al., 2006). The total IgE concentration reported in horse serum is substantially higher than in humans and variations between individuals are high (Table 3). It was suggested that the higher IgE concentrations in normal horse serum are caused by generally higher parasitic loads in horses than in humans from western civilizations. This assumption was supported by findings in sheep which also have normal IgE levels in the μ g/ml range (Shaw et al., 1997). The total IgE concentration in horses affected with skin hypersensitivity was found to be increased (Table 3). However, this was not significantly higher than in clinically healthy horses kept in the same environment (Wagner et al., 2003). When horses of various breeds and

Table 2

Monoclonal antibodies to equine IgE and their successful use for different methods and applications.

Method	Anti-IgE clone	Reference
ELISA	176 for coating and 134 for detection 1C12 for coating and 3H10 for detection	Wagner et al. (2003) Wilson et al. (2006)
Flow cytometry	134, 176	Wagner et al. (2003)
Immunohistochemistry	4C2	Wilson et al. (2006)
Western blotting	134 – non-reducing conditions 176 – reducing and non-reducing conditions 3H10, 1C12, 5A2 – non-reducing conditions	Wagner et al. (2003) Wagner et al. (2003) Wilson et al. (2006)
Induction of immediate skin reaction after intradermal injection <i>in vivo</i>	134 1C12, 5A2, 7H2	Wagner et al. (2006b) Wilson et al. (2006)
<i>In vitro</i> histamine release	134	Wagner et al. (2006b)
<i>In vitro</i> leukotriene release	1C12, 5A2, 7H2	Wilson et al. (2006)
Use for affinity purification of IgE from horse serum	134 176 4C2	Wagner et al. (2003) Wagner et al. (2006b) Wilson et al. (2006)

origins were compared, a significant increase in total IgE levels was found in sera from skin hypersensitivity affected compared to healthy Icelandic horses ($p < 0.05$), but not for sera from healthy and affected horses of other breeds (Wilson et al., 2006).

A small population ($1.3 \pm 0.5\%$) of IgE⁺ leukocytes can be detected in the peripheral blood of adult horses by surface staining using anti-IgE mAbs and flow cytometric analysis (Wagner et al., 2003). The genes for the high-affinity FcεRI (McAleese et al., 2000; McAleese and Miller, 2003), and the low-affinity IgE receptor, also known as FcεRII or CD23 (Watson et al., 2000) were cloned and sequenced in the horse. The recombinant equine FcεRIα chain was found to bind to

equine IgE (McAleese et al., 2006). The distribution of IgE on the surface of peripheral blood cells suggested its binding to FcεRI and CD23 in normal horses. Double staining with anti-IgE and cell surface markers for monocytes (CD14) or B-cells (IgM) confirmed the presence of surface bound IgE on subpopulations of equine B-cells and monocytes (Wagner, unpublished). Enrichment of the IgE⁺ cells by magnetic cell sorting showed that they represent a mixed cell population of around 32% basophils, 53% monocytes and/or large lymphocytes, 13% small lymphocytes and 2% eosinophils (Wagner et al., 2003). The enrichment of basophils from around 1% in peripheral blood to more than 30% in the positive enriched fraction was likely due to high-affinity FcεRI bound IgE on the

Table 3Total IgE concentrations in equine serum and colostrum and percentages of IgE⁺ cells in peripheral blood.

Sample	horses	Clinical status/age	Total IgE (μg/ml)	IgE ⁺ cells in peripheral blood (%)	Reference
Serum	Adult	Clinically healthy	84.0 ± 90.9 ^b	1.3 ± 0.5 ^b	Wagner et al. (2003)
		Skin hypersensitivity	108.9 ± 69.0	NA	Wagner et al. (2003)
		Healthy and allergic	23.5 (0.43–82.6) ^c	NA	Wilson et al. (2006)
		Mares, day 1 p.p. ^a	254 (66–1950)	0.5 (0.07–1.3) ^c	Wagner et al. (2006a)
		Mares, day 86 p.p.	173 (39–1059)	0.9 (0.6–1.3)	Wagner et al. (2006a)
		Mares, at foaling	43.0 ± 28.5	NA	Marti et al. (2009)
	Foals	After birth, before colostrum uptake	ND	ND	Wagner et al. (2006a), Marti et al. (2009)
		Day 2	261 (54–720)	0.5 (0.08–0.8)	Wagner et al. (2006a)
		Day 5	147 (41–742)	0.5 (0.15–1.0)	Wagner et al. (2006a)
		Day 16	73 (27–73)	0.2 (0.1–0.4)	Wagner et al. (2006a)
		Day 30	41 (11–243)	0.04 (0–0.14)	Wagner et al. (2006a)
		Day 44	32 (8–112)	0.03 (0–0.19)	Wagner et al. (2006a)
		Day 58	26 (7–80)	0 (0–0.06)	Wagner et al. (2006a)
		Day 72	14 (0–58)	0 (0–0.03)	Wagner et al. (2006a)
		Day 86	15 (0–52)	0 (0–0.07)	Wagner et al. (2006a)
		5–6 months	ND	ND	Wagner et al. (2003), Wagner et al. (2006a)
		18 months	56 (50–81)	NA	Wagner et al. (2006a)
		24 hours	10.9 ± 8.1	NA	Marti et al. (2009)
	Colostrum	p.p., before suckling	773 (185–2573)	NA	Wagner et al. (2006a)
		At foaling	76.1 ± 56.2	NA	Marti et al. (2009)

NA: not available, ND: not detectable.

^a p.p. = post partum.^b Mean ± standard deviation.^c Median (range).

surface of the basophils (Wagner et al., 2003). The FcεRI is probably also expressed on equine monocytes similar to human monocytes expressing a αγγ-form of the FcεRI that mainly functions for antigen presentation and also induces the activation and release of pro-inflammatory cytokines by these cells (reviewed by Kraft et al., 2001). The IgE⁺ lymphocyte population likely bound IgE via the low-affinity IgE receptor CD23 which is mainly expressed by equine B-cells (Wagner et al., 2009).

Immunohistochemistry studies using polyclonal anti-IgE showed the presence of IgE⁺ cells in skin and lung tissue (Rüfenacht et al., 2005; Van der Haegen et al., 2005). Intradermal skin testing experiments using anti-IgE mAb 134 resulted in immediate skin reactions, confirming the presence of IgE⁺ mast cells in the normal horse skin (Wagner et al., 2006b). Other mAbs such as anti-IgE 176 did not induce immediate skin reactions, although the IgE⁺ cell staining of peripheral blood cells did not differ between anti-IgE 176 and 134. The two mAbs were found to detect different epitopes of the IgE heavy chain constant region (Wagner et al., 2003). The findings obtained by intradermal testing and *in vitro* mediator release suggested that the anti-IgE 176 did not crosslink IgE bound to FcεRI while anti-IgE 134 induced crosslinking of IgE, FcεRI signaling and mediator release from mast cells and basophils (Wagner et al., 2006b).

Despite these investigations using gene expression or anti-IgE mAbs, additional direct measurements of both receptors for IgE are still required to provide a better understanding of their expression and role during the immune response. The recent development of mAbs to equine CD23 and FcεRIα (Wagner et al., 2009) may provide new tools to shed more light on IgE receptor expression in horses.

4. IgE in foals, maternal transfer of IgE and the onset of IgE responses in horses

The analysis of IgE in foals was initiated by the finding that neither serum IgE nor IgE⁺ leukocytes could be detected in serum (foals 5–6 months) or peripheral blood (foals 2.5–5 months). The finding was unexpected because the foals spent the entire summer with their dams in an environment with high allergenic pressure. The foals were offspring of clinically healthy mares or of mares clinically affected with severe skin hypersensitivity. At least during their first six months of life, these foals did not develop an IgE immune response to *Culicoides* allergens or any other environmental antigens they were exposed to (Wagner et al., 2003). The finding was in agreement with the observation that most horses do not develop clinical signs of skin hypersensitivity before they reach 2–4 years of age, even if they are exposed to allergens from birth on (Wilson et al., 2001).

A subsequent study was designed to investigate the development of IgE in neonates and foals. It could be demonstrated that high concentrations of maternal IgE are transferred to the neonate by the colostrum. Compared to serum IgE concentrations of the same mares, the colostral IgE was 2.7-fold enriched suggesting an active transport mechanism of the IgE into the mammary gland (Table 3).

IgE was not detectable in foals before ingestion of colostrum. After colostrum uptake, IgE was found in the circulation of the foals. IgE concentrations peaked on days 2–5 after birth in foal serum and on the surface of their peripheral blood leukocytes. Then, the IgE⁺ cells and IgE serum levels rapidly decreased and became again undetectable at 2–4 months of life. The onset of the endogenous IgE production in foals was found to be delayed and occurred between 9–11 months of age depending on the individual (Wagner et al., 2006a). A second study focusing on serum IgE and detection of IgE in intestinal tissues by immunohistochemistry confirmed the initial findings on maternal IgE transfer with the colostrum, its uptake into the circulation of the neonates and the delay in the onset of endogenous IgE production in foals. The authors observed that increasing IgE serum concentrations could be detected in some foals as early as at 6 months of age (Marti et al., 2009). There is also evidence that maternal IgE binds directly to intestinal mast cells in the lamina propria of the foal after colostrum uptake (Marti et al., 2008). The incubation of isolated intestinal cells from a newborn foal with fluorescent equine IgE suggested that neonatal intestinal mast cells express high-affinity IgE receptors that subsequently bind the passively transferred maternal IgE antibodies (Wagner et al., 2009). It was suggested that maternal IgE antibodies might have an immunoregulatory effect on the immune response of the neonate (Wagner et al., 2006a). However, if and how maternal IgE antibodies influence the development of IgE responses in the offspring or allergic diseases later in life is not yet known.

5. Assays to detect allergen-specific IgE or sensitization with allergen-specific IgE

IgE is involved in type I hypersensitivity, by binding to high-affinity FcεRI on the surface of mast cells and basophils. Crosslinking of IgE by specific allergens triggers the release of inflammatory mediators from the cells. This causes an inflammatory reaction and results in the immediate onset of clinical signs of allergy.

Tests for the detection of allergen-specific IgE responses in horses are based on two principles. First, intradermal testing or *in vitro* sensitization assays are used to determine cellular sensitization with IgE to a particular allergen. Both tests are based on the release of preformed inflammatory mediators from mast cells or basophils induced by crosslinking of allergen-specific IgE bound on the cell surface. Second, serological assays are available to measure soluble allergen-specific IgE antibodies in serum. Serological assays are designed to detect the humoral immune response to a particular allergen by using either an anti-IgE antibody or the human FcεRIα chain for IgE detection.

Intradermal testing with various environmental allergens has been used to determine allergic responses in horses for over 20 years (Matthews et al., 1983; Larsen et al., 1988; Ungar-Waron et al., 1990; Anderson et al., 1993). Following allergen injection or injection of anti-IgE inflammatory mediators are released from mast cells in the skin and an immediate skin reaction characterized by the formation of a wheal at the injection site develops after

15–30 min (Wagner et al., 2006b). Horses with clinical signs of allergy were found to react more frequently to intradermal injection of allergen extracts than clinically healthy horses. This was observed for diseases like skin hypersensitivity and urticaria (Larsen et al., 1988; Fadok and Greiner, 1990; Lorch et al., 2001a). However, skin testing and the evaluation of immediate IgE-mediated reactions was not useful in horses with respiratory conditions such as recurrent airway obstruction (RAO) (McGorum et al., 1993; Jose-Cunilleras et al., 2001; Lorch et al., 2001b; Wong et al., 2005; Tahon et al., 2009). The results suggested that systemic IgE antibodies to mould or fungal antigens are not developed in RAO.

In vitro sensitization assays are performed on blood samples and are based on mediator release from peripheral blood basophils. Similar to intradermal testing the assays detect sensitization with allergen-specific IgE but they do not require the injection of allergens into the skin. It has been shown that the incubation of peripheral blood cells with anti-IgE induced histamine release (Wagner et al., 2006b). Cells from allergic and clinical healthy horses released comparable concentrations of histamine after anti-IgE stimulation confirming that the amount of IgE bound to basophils is similar in horses with and without clinical allergy (Wagner et al., 2008). Furthermore, the addition of recombinant IgE to peripheral blood cells from foals followed by anti-IgE stimulation significantly increased the induced histamine release (Wagner et al., 2002). These characteristic responses of equine blood basophils to anti-IgE suggested that *in vitro* sensitization assays can be used as an alternative to skin testing. In the laboratory, the blood cells can be incubated with various allergens and the mediator release in response to these treatments is detected. Assays to measure the release of leukotrienes (Baselgia et al., 2006) or histamine (Langner et al., 2008; Wagner et al., 2008) from basophils were described. Subsequent investigations showed that the histamine release after allergen-specific treatment of blood cells differed significantly between clinically healthy and allergic horses (Wagner et al., 2008). The histamine release assay was found to be particularly useful for measuring sensitization to *Culicoides* allergen (Langner et al., 2008; Wagner et al., 2008), the major allergen causing skin hypersensitivity in horses.

Skin testing and *in vitro* sensitization assays detect cellular sensitization with IgE which is required to develop clinical allergy. However, sensitization of mast cells or basophils with allergen-specific IgE does not necessarily mean that the horse is allergic. Several clinically healthy horses were identified by skin testing (Lorch et al., 2001a,b; Kolm-Stark and Wagner, 2002; Lebis et al., 2002) and *in vitro* sensitization assays (Wagner et al., 2008) to be clearly sensitized with IgE to *Culicoides* allergen and never showed any signs of disease. Consequently, sensitization is not always an equivalent of clinical allergy and cannot be used to diagnose the disease without considering clinical signs. Nevertheless, in horses with clinical signs the assays are helpful to identify the allergen responsible for the disease and *in vitro* sensitization assays provide a clear alternative to skin testing for this purpose (Wagner et al., 2008).

Several serological assays to detect allergen-specific IgE antibodies in serum have been described. All of them are performed by coating allergen preparations to the solid phase of the assay, most frequently an ELISA plate, to bind serum IgE to the respective allergens. Then, a variety of reagents to detect the equine IgE have been used for these assays. This includes polyclonal anti-IgE reagents (Halliwell et al., 1993; Eder et al., 2000; Kalina et al., 2003), monoclonal anti-IgE antibodies (Morgan et al., 2007; Langner et al., 2008), and the human FcεRIα chain (Frey et al., 2008). Although the principle of allergen-specific IgE detection sounds convincing, tests on the performance of serological allergen assays have shown that serum IgG antibodies also bind to almost every crude allergen preparation. Due to the much higher concentration of IgG in serum and the relatively low serum dilutions that are used for serological allergen-specific IgE detection, much higher concentrations of IgG than of IgE antibodies are bound to the solid phase of the assay (Morgan et al., 2007; Langner et al., 2008). Thus, the specificity of the anti-IgE reagent is one crucial parameter for the accurate performance of serological allergen testing. Thus far, convincing specificity to IgE has only been reported for the monoclonal anti-equine IgE antibodies (Wagner et al., 2003; Wilson et al., 2006). There is currently no reported evidence that the human FcεRIα chain which is used in one of the commercial assays does bind to equine IgE. Human and equine IgE share a low homology of only 56% (Wagner et al., 2001) and it cannot be simply assumed that the human FcεRIα chain binds to equine IgE.

Another general weakness of the current allergen-specific assays is the use of crude allergen preparations. This causes binding of many other proteins than the allergen itself to the solid phase of the assay. The results are a decreased concentration of the allergen available for the assay and also an increased risk of potential crossreactivity of serum antibodies with the non-specific proteins (Eder et al., 2000; Morgan et al., 2007). By considering both, the low specificity of some anti-IgE reagents and the poor quality of many allergen preparations, it is not surprising that many serological allergen tests result in multiple positive reactions, many of which are likely to be false positive. Overall, most authors concluded that serological allergen testing in horses performs poorly, is unreliable, and cannot be recommended using the currently available assays (Eder et al., 2000; Lorch et al., 2001c; Morgan et al., 2007; Langner et al., 2008; Frey et al., 2008). The identification of major allergens that cause allergic conditions in horses and their expression as recombinant proteins is currently ongoing. The use of pure and better defined allergens in combination with the specific monoclonal anti-IgE antibodies holds some promise for the development of improved serological assays to detect allergen-specific IgE antibodies in the horses (Morgan et al., 2007; Marti et al., 2008).

Recently, several groups compared the available sensitization tests and allergen-specific assays, including commercial services that are offered by various companies or assays for species other than the horse. Overall, these studies reported low to moderate agreements between

individual assays (Lorch et al., 2001c; Morgan et al., 2007; Langner et al., 2008; Marti et al., 2008). One major problem in comparing different allergen-specific tests is the absence of a commonly accepted 'gold standard' for allergen testing. Skin testing which is still considered to be the 'gold standard' by various groups has several well known disadvantages that make intradermal testing an inconsistent diagnostic tool. Most importantly and as discussed above, the procedure often induces positive reactions in clinically healthy horses. In addition, skin testing can be difficult to interpret and was found to be of poor repeatability (Kolm-Stark and Wagner, 2002; Lebis et al., 2002), certain extracts showed irritating characteristics (Morris and Lindborg, 2003; Lebis et al., 2002), and in accordance with the later finding several commercial allergen preparations contained detectable amounts of histamine (Wagner et al., 2009). In addition, flare as a valuable second evaluation parameter for skin testing in humans or some dogs cannot be observed in the pigmented skin of most horses which also contributes to the difficulties in interpreting immediate skin reactions. Considering these findings from various groups it must be concluded that skin testing is not a valid gold standard for allergen testing in horses, although the procedure can be valuable for the identification of responsible allergens in allergic individuals.

In summary, reliable allergen testing in horses is still difficult to perform and test interpretation is hampered by numerous often false positive test results. Sensitization assays are preferred over serological assays for allergen identification. All currently existing assays should be interpreted with precaution and in accordance with clinical signs and history of disease.

6. IgE-mediated allergies in horses

Skin hypersensitivity was the first allergic disease in horses that was assumed to be an IgE-mediated allergy (Matthews et al., 1983). The disease is known by different names, such as summer eczema, summer seasonal recurrent dermatitis, atopic dermatitis, sweet itch or insect bite hypersensitivity. Although skin hypersensitivity has been frequently described in Icelandic horses (Halldorsdottir and Larsen, 1991; Lange et al., 2005; Björnsdóttir et al., 2006), it occurs in most horse breeds including Thoroughbreds, Arabian horses, Warmbloods, Draft horses, Quarter horses, Friesian horses and ponies, and has been found in most countries all over the world (Braverman, 1988; Larsen et al., 1988; Greiner et al., 1990; Anderson et al., 1993; Littlewood, 1998; Steinman et al., 2003; Van Grevenhof et al., 2007).

More than 20 years after IgE was first assumed to be causally involved in equine skin hypersensitivity, direct evidence for IgE mediating the disease was found by transferring allergic reactions to healthy animals via IgE obtained from affected horses (Prausnitz-Küstner reaction). After challenge with *Culicoides* extract, clinically healthy recipient horses developed immediate skin reactions only at the sites of the IgE transfer from affected donors but not at the transfer sites of IgE from healthy donors (Wagner et al., 2006b).

Additional observations supporting the role of *Culicoides* midges in the pathogenesis of skin hypersensitivity were made in Icelandic horses. In Iceland, *Culicoides* midges are not endemic and the disease does not occur. Clinical signs of allergy only developed in horses after being exported from Iceland (Larsen et al., 1988; Wilson et al., 2006). Epidemiological studies confirmed that between 26% and 72% of the Icelandic horses exported to Europe developed the disease, while only 7–27% of Icelandic horses born on the continent became affected with skin hypersensitivity (Brostrom et al., 1987; Halldorsdottir and Larsen, 1991; Lange et al., 2005; Björnsdóttir et al., 2006). Comparison of extracts from various *Culicoides* species by skin testing of allergic horses showed that horses reacted to all species of *Culicoides* even if they were exposed to only some of these species (Anderson et al., 1993). This suggested that the inducing allergens of *Culicoides* are conserved between the various midge species. Western blot studies using *Culicoides* salivary gland extract antigens revealed several proteins that were detected by IgE and IgG serum antibodies from horses with skin hypersensitivity (Hellberg et al., 2006). Recently, different groups started to identify responsible allergens in *Culicoides* saliva. They revealed several allergen candidates including proteins involved in sugarmal digestion, defense and anti-coagulation (Langner et al., 2007; Wilson et al., 2008). A better characterization of the allergens inducing skin hypersensitivity is likely to improve diagnostic testing and potentially also the development of new treatment options for this important IgE-mediated allergy in horses.

Beside skin hypersensitivity where the role of IgE in the pathogenesis of disease has been confirmed, IgE-mediated mechanisms are also likely to induce urticaria, also known as hives (Lorch et al., 2001a; Jose-Cunilleras et al., 2001; Rüfenacht et al., 2005). For other diseases of the horse that were suspected to be IgE-mediated including RAO or head-shaking (Mansfield et al., 1998), a role of IgE in the pathogenesis of the diseases remains controversial.

7. Allergic components in RAO

RAO is considered to be a hypersensitivity reaction of the lower respiratory tract induced by inhaled moulds and organic dusts (Derksen et al., 1988; McGorum et al., 1993; Pirie et al., 2003). The disease is characterized by mucus production, neutrophil accumulation in the airway, bronchial hyperreactivity and bronchospasm. Inhalation of mould and fungal antigens induces the inflammatory airway response in susceptible horses within 6 h after exposure (Gerber et al., 2004). Characteristic is the up-regulation of pro-inflammatory cytokine gene expression during the induction phase of the disease and also in chronically affected horses (Franchini et al., 2000; Giguère et al., 2002; Ainsworth et al., 2003, 2006).

The involvement of IgE-mediated mechanisms in the pathogenesis of RAO remains unclear and is still controversial (Marti et al., 2008). The only support for a role of IgE in RAO was provided in studies detecting increased *Aspergillus*-specific IgE antibodies in bronchoalveolar lavage using a polyclonal anti-IgE reagent (Halliwell

et al., 1993; Schmallenbach et al., 1998). In most of the more recently performed studies, the initial findings could not be confirmed. It became also evident that environmental and genetic factors influence the IgE response to mould allergens (Eder et al., 2001; Curik et al., 2003) and a comparison of mould-, mite- or *Aspergillus*-specific IgE responses from horses living in different environments did not result in any differences between RAO-affected and control horses. The authors concluded that IgE serology is not a useful tool for the diagnosis of RAO or the determination of antigens causing the disease (Eder et al., 2000; Tahon et al., 2009). Skin testing with mould and fungal allergens in horses with RAO revealed reactions to *Aspergillus fumigatus* and other mould antigens at 4 and/or 24 h after injection. Thus, the reactivity might reflect a cell-mediated delayed-type hypersensitivity response (Jose-Cunilleras et al., 2001; Wong et al., 2005; Tahon et al., 2009). Evidence for an increase in IgE-mediated immediate skin reactions in RAO-affected horses could not be supported by these studies.

8. IgE and parasitic infection

IgE is likely to be important in mediating protective immune responses against parasites in horses (Suter and Fey, 1981). Nevertheless, studies on equine IgE responses to infection with parasites or helminths are rare. Existing reports focused on IgG responses to parasites including intestinal nematodes (Dowdall et al., 2004) or *Trichinella spiralis* (Hill et al., 2007). IgG(T) has been found to dominate the anti-nematode response to *Cyathostomins* in horses (Dowdall et al., 2004). However, it is still unknown whether IgG(T), which is composed of the two isotypes IgG3 and IgG5, shares functional similarities with IgE during anti-parasitic responses or whether IgG3 and/or IgG5 are involved in the immunoglobulin class switching pathway to IgE (Wagner, 2006). The lack of information on the role of IgE in parasitic infections was likely caused by the unavailability of reliable anti-IgE antibodies for many years. The new monoclonal anti-IgE antibodies (Wagner et al., 2003; Wilson et al., 2006) are valuable reagents to fill this gap and to obtain more information about IgE-mediated mechanisms in the equine immune response to parasites.

9. Conclusions and current research topics

Further investigations on various aspects of IgE in health and disease are required for a better understanding of the functions of this immunoglobulin isotype during effector and regulatory immune responses in domestic species. Horses naturally develop allergies and provide a valuable large animal model for studying allergic diseases. Ongoing and future research topics focusing on the influences of maternal IgE on the neonatal immune system, or early exposure to potential allergens on the development of allergy later in life have applications for both equine and human health. For equine skin hypersensitivity, the identification of *Culicoides* allergens in combination with the new IgE-specific monoclonal reagents is likely to have a positive impact on diagnostic

and therapeutic applications for allergic diseases. Additional research should be performed on the development of improved allergen tests, on the role of IgE during immune responses to parasites and other diseases of a suspected IgE-mediated etiology.

Conflict of interest statement

None.

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References

- Ainsworth, D.M., Grünig, G., Matychak, M.B., Young, J., Wagner, B., Erb, H.N., Antczak, D.F., 2003. Recurrent airway obstruction (RAO) in horses is characterized by IFN-gamma and IL-8 production in bronchoalveolar lavage cells. *Vet. Immunol. Immunopathol.* 96, 83–91.
- Ainsworth, D.M., Wagner, B., Franchini, M., Grünig, G., Erb, H.N., Tan, J.Y., 2006. Time-dependent alterations in gene expression of interleukin-8 in the bronchial epithelium of horses with recurrent airway obstruction. *Am. J. Vet. Res.* 67, 669–677.
- Anderson, G.S., Belton, P., Kleider, N., 1993. Hypersensitivity of horses in British Columbia to extracts of native and exotic species of *Culicoides* (Diptera: Ceratopogonidae). *J. Med. Entomol.* 30, 657–663.
- Baselgia, S., Doherr, M.G., Mellor, P., Torsteinsdottir, S., Jermann, T., Zurbriggen, A., Jungi, T., Marti, E., 2006. Evaluation of an in vitro sulphidoleukotriene release test for diagnosis of insect bite hypersensitivity in horses. *Equine Vet. J.* 38, 40–46.
- Björnsdóttir, S., Sigvaldadóttir, J., Broström, H., Langvad, B., Sigurdsson, A., 2006. Summer eczema in exported Icelandic horses: influence of environmental and genetic factors. *Acta Vet. Scand.* 48, 3.
- Braverman, Y., 1988. Preferred landing sites of *Culicoides* species (Diptera: Ceratopogonidae) on a horse in Israel and its relevance to summer seasonal recurrent dermatitis (sweet itch). *Equine Vet. J.* 20, 426–429.
- Brostrom, H., Larsson, A., Troedsson, M., 1987. Allergic dermatitis (sweet itch) of Icelandic horses in Sweden: an epidemiological study. *Equine Vet. J.* 19, 229–236.
- Curik, I., Fraser, D., Eder, C., Achmann, R., Swinburne, J., Binns, M., Cramer, R., Brem, G., Sölkner, J., Marti, E., 2003. Association between MHC gene region and variation of serum IgE levels against specific mould allergens in the horse. *Genet. Select. Evol.* 35, 117–190.
- Derksen, F.J., Robinson, N.E., Scott, J.S., Stick, J.A., 1988. Aerosolized *Micropolyspora faeni* antigen as a cause of pulmonary dysfunction in ponies with recurrent airway obstruction (heaves). *Am. J. Vet. Res.* 49, 933–938.
- Dowdall, S.M., Proudman, C.J., Klei, T.R., Mair, T., Matthews, J.B., 2004. Characterisation of IgG(T) serum antibody responses to two larval antigen complexes in horses naturally- or experimentally-infected with cyathostomins. *Int. J. Parasitol.* 34, 101–108.
- Eder, C., Cramer, R., Mayer, C., Eicher, R., Straub, R., Gerber, H., Lazary, S., Marti, E., 2000. Allergen-specific IgE levels against crude mould and storage mite extracts and recombinant mould allergens in sera from horses affected with chronic bronchitis. *Vet. Immunol. Immunopathol.* 73, 241–253.
- Eder, C., Curik, I., Brem, G., Cramer, R., Bodo, I., Habe, F., Lazary, S., Sölkner, J., Marti, E., 2001. Influence of environmental and genetic factors on allergen-specific immunoglobulin E levels in sera from Lipizzan horses. *Equine Vet. J.* 33, 714–720.
- Fadok, V.A., Greiner, E.C., 1990. Equine insect hypersensitivity: skin test and biopsy results correlated with clinical data. *Equine Vet. J.* 22, 236–240.
- Franchini, M., Gill, U., von Fellenberg, R., Bracher, V.D., 2000. Interleukin-8 concentration and neutrophil chemotactic activity in bronchoalveolar lavage fluid of horses with chronic obstructive pulmonary disease following exposure to hay. *Am. J. Vet. Res.* 61, 1369–1374.

- Frey, R., Bergvall, K., Egenvall, A., 2008. Allergen-specific IgE in Icelandic horses with insect bite hypersensitivity and healthy controls, assessed by FcεRIα-based serology. *Vet. Immunol. Immunopathol.* 126, 102–109.
- Gerber, V., Lindberg, A., Berney, C., Robinson, N.E., 2004. Airway mucus in recurrent airway obstruction—short-term response to environmental challenge. *J. Vet. Intern. Med.* 18, 92–97.
- Giguère, S., Viel, L., Lee, E., MacKay, R.J., Hernandez, J., Franchini, M., 2002. Cytokine induction in pulmonary airways of horses with heaves and effect of therapy with inhaled fluticasone propionate. *Vet. Immunol. Immunopathol.* 85, 147–158.
- Greiner, E.C., Fadok, V.A., Rabin, E.B., 1990. Equine *Culicoides* hypersensitivity in Florida: biting midges aspirated from horses. *Med. Vet. Entomol.* 4, 375–381.
- Halldorsdottir, S., Larsen, H.J., 1991. An epidemiological study of summer eczema in Icelandic horses in Norway. *Equine Vet. J.* 23, 296–299.
- Halliwell, R.E., Hines, M.T., 1985. Studies on equine recurrent uveitis. I. Levels of immunoglobulin and albumin in the aqueous humor of horses with and without intraocular disease. *Curr. Eye Res.* 10, 1023–1031.
- Halliwell, R.E., McGorum, B.C., Irving, P., Dixon, P.M., 1993. Local and systemic antibody production in horses affected with chronic obstructive pulmonary disease. *Vet. Immunol. Immunopathol.* 38, 201–215.
- Hellberg, W., Wilson, A.D., Mellor, P., Doherr, M.G., Torsteinsdottir, S., Zurbriggen, A., Jungi, T., Marti, E., 2006. Equine insect bite hypersensitivity: immunoblot analysis of IgE and IgG subclass responses to *Culicoides nubeculosus* salivary gland extract. *Vet. Immunol. Immunopathol.* 113, 99–112.
- Helm, B., Marsh, P., Vercelli, D., Padlan, E., Gould, H., Geha, R., 1988. The mast cell binding site on human immunoglobulin E. *Nature* 331, 180–183.
- Hill, D.E., Forbes, L., Kramer, M., Gajadhar, A., Gamble, H.R., 2007. Larval viability and serological response in horses with long-term *Trichinella spiralis* infection. *Vet. Parasitol.* 146, 107–116.
- Ishizaka, K., Ishizaka, T., Hornbrook, M.M., 1966. Physicochemical properties of reaginic antibody. V. Correlation of reaginic activity with γE-globulin antibody. *J. Immunol.* 97, 840–853.
- Jose-Cunilleras, E., Kohn, C.W., Hillier, A., Saville, W.J.A., Lorch, G., 2001. Intradermal testing in healthy horses and horses with chronic obstructive pulmonary disease, recurrent urticaria, or allergic dermatitis. *JAVMA* 219, 1115–1121.
- Kalina, V.V., Pettigrew, H.D., Gershwin, L.J., 2003. IgE ELISA using antisera derived from epsilon chain antigenic peptides detects allergen-specific IgE in allergic horses. *Vet. Immunol. Immunopathol.* 92, 137–147.
- Kolm-Stark, G., Wagner, R., 2002. Intradermal skin testing in Icelandic horses in Austria. *Equine Vet. J.* 34, 405–410.
- Kraft, S., Katoh, N., Novak, N., Koch, S., Bieber, T., 2001. Unexpected functions of FcεpsilonRI on antigen-presenting cells. *Int. Arch. Allergy Immunol.* 124, 35–37.
- Lange, S., Hamann, H., Deegen, E., Ohnesorge, B., Distl, O., 2005. Investigation of the prevalence of summer eczema in Icelandic horses in northern Germany. *Berl. Munch. Tierarztl. Wochenschr.* 118, 481–489.
- Langner, K.F., Darpel, K.E., Denison, E., Drolet, B.S., Leibold, W., Mellor, P.S., Mertens, P.P., Nimtz, M., Greiser-Wilke, I., 2007. Collection and analysis of salivary proteins from biting midge *Culicoides nubeculosus* (Diptera: Ceratopogonidae). *J. Med. Entomol.* 44, 238–248.
- Langner, K.F., Darpel, K.E., Drolet, B.S., Fischer, A., Hampel, S., Heselhäus, J.E., Mellor, P.S., Mertens, P.P., Leibold, W., 2008. Comparison of cellular and humoral immunoassays for the assessment of summer eczema in horses. *Vet. Immunol. Immunopathol.* 122, 126–137.
- Larsen, H.J., Bakke, S.H., Mehl, R., 1988. Intradermal challenge of Icelandic horses in Norway and Iceland with extracts of *Culicoides* spp. *Acta Vet. Scand.* 29, 311–314.
- Lebis, C., Bourdeau, P., Marzin-Keller, F., 2002. Intradermal skin tests in equine dermatology: a study of 83 horses. *Equine Vet. J.* 34, 666–671.
- Littlewood, J.D., 1998. Incidence of recurrent seasonal pruritus ('sweet itch') in British and German Shire horses. *Vet. Res.* 142, 66–67.
- Lorch, G., Hillier, A., Kwochka, K.W., Saville, W.A., LeRoy, B.E., 2001a. Results of intradermal tests in horses without atopy and horses with atopic dermatitis or recurrent urticaria. *Am. J. Vet. Res.* 62, 1051–1059.
- Lorch, G., Hillier, A., Kwochka, K.W., Saville, W.A., Kohn, C.W., Jose-Cunilleras, E., 2001b. Results of intradermal tests in horses without atopy and horses with chronic obstructive pulmonary disease. *Am. J. Vet. Res.* 62, 389–397.
- Lorch, G., Hillier, A., Kwochka, K.W., Saville, W.A., Kohn, C.W., LeRoy, B.E., 2001c. Comparison of immediate intradermal test reactivity with serum IgE quantitation by use of a radioallergosorbent test and two ELISA in horses with and without atopy. *J. Am. Vet. Med. Assoc.* 218, 1314–1322.
- Mansfield, J.R., Burrell, M.H., Curl, V.J., Valler, B.J., 1998. Treatment of equine allergic diseases with allergy neutralization. A field study. *J. Nutr. Environ. Med.* 8, 329–334.
- Marti, E., Szalai, G., Bucher, K., Dobbelaere, D., Gerber, H., Lazary, S., 1995. Partial sequence of the equine immunoglobulin epsilon heavy chain cDNA. *Vet. Immunol. Immunopathol.* 47, 363–367.
- Marti, E., Peveri, P., Griot-Wenk, M., Muntwyler, J., Cramer, R., Schaller, J., Gerber, H., Lazary, S., 1997. Chicken antibodies to a recombinant fragment of the equine immunoglobulin epsilon heavy-chain recognising native horse IgE. *Vet. Immunol. Immunopathol.* 59, 253–270.
- Marti, E., Gerber, V., Wilson, A.D., Lavoie, J.P., Horohov, D., Cramer, R., Lunn, P., Antczak, D., Björnsdottir, S., Björnsdottir, T.S., Cunningham, F., Derer, M., Frey, E., Hamza, E., Horin, P., Heimann, M., Kolm-Stark, G., Olafsdottir, G., Ramery, E., Russell, C., Schaffartzik, A., Svansson, V., Torsteinsdottir, S., Wagner, B., 2008. Report of the 3rd Havemeyer Workshop on Allergic Diseases of the Horse, Iceland, June 2007. *Vet. Immunol. Immunopathol.* 126, 351–361.
- Marti, E., Ehrensperger, F., Burger, D., Ousey, J., Day, M.J., Wilson, A.D., 2009. Maternal transfer of IgE and subsequent development of IgE responses in horses (*Equus caballus*). *Vet. Immunol. Immunopathol.* 127, 203–211.
- Matthews, A.G., Imlah, P., McPherson, E.A., 1983. A reagin-like antibody in horse serum. 1. Occurrence and some biological properties. *Vet. Res. Commun.* 6, 13–23.
- McAleese, S.M., Halliwell, R.E., Miller, H.R., 2000. Cloning and sequencing of the horse and sheep high-affinity IgE receptor alpha chain cDNA. *Immunogenetics* 51, 878–881.
- McAleese, S.M., Miller, H.R., 2003. Cloning and sequencing of the equine and ovine high-affinity IgE receptor beta-and gamma-chain cDNA. *Immunogenetics* 55, 122–125.
- McAleese, S.M., Brown, J.K., Macrae, A.I., Mackellar, A., Huntley, J.F., Miller, H.R., 2006. Cloning and expression of the extra-cellular part of the alpha chain of the equine high-affinity IgE receptor and its use in the detection of IgE. *Vet. Immunol. Immunopathol.* 110, 187–191.
- McGorum, B.C., Dixon, P.M., Halliwell, R.E., 1993. Evaluation of intradermal mould antigen testing in the diagnosis of equine chronic obstructive pulmonary disease. *Equine Vet. J.* 25, 257–258.
- Morgan, E.E., Miller, W.H., Wagner, B., 2007. A comparison of intradermal testing and detection of allergen-specific immunoglobulin E in serum by enzyme-linked immunosorbent assay in horses affected with skin hypersensitivity. *Vet. Immunol. Immunopathol.* 120, 160–167.
- Morris, D.O., Lindborg, S., 2003. Determination of 'irritant' threshold concentrations for intradermal testing with allergenic insect extracts in normal horses. *Vet. Dermatol.* 14, 31–36.
- Navarro, P., Barbis, D.P., Antczak, D., Butler, J.E., 1995. The complete cDNA and deduced amino acid sequence of equine IgE. *Mol. Immunol.* 32, 1–8.
- Pirie, R.S., Dixon, P.M., McGorum, B.C., 2003. Endotoxin contamination contributes to the pulmonary inflammatory and functional response to *Aspergillus fumigatus* extract inhalation in heaves horses. *Clin. Exp. Allergy* 33, 1289–1296.
- Ravetch, J.V., Kinet, J.P., 1991. Fc receptors. *Annu. Rev. Immunol.* 9, 457–492.
- Rüfenacht, S., Marti, E., von Tscharn, C., Doherr, M.G., Forster, U., Welle, M., Roosje, P.J., 2005. Immunoglobulin E-bearing cells and mast cells in skin biopsies of horses with urticaria. *Vet. Dermatol.* 16, 94–101.
- Sayers, I., Cain, S.A., Swan, J.R.M., Pickett, M.A., Watt, P.J., Holgate, S.T., Padlan, E.A., Schuck, P., Helm, B.A., 1998. Amino acid residues that influence FcεRI-mediated effector functions of human immunoglobulin E. *Biochemistry* 37, 16152–16164.
- Schmallenbach, K.H., Rahman, I., Sasse, H.H., Dixon, P.M., Halliwell, R.E., McGorum, B.C., Cramer, R., Miller, H.R., 1998. Studies on pulmonary and systemic *Aspergillus fumigatus*-specific IgE and IgG antibodies in horses affected with chronic obstructive pulmonary disease (COPD). *Vet. Immunol. Immunopathol.* 66, 245–256.
- Shaw, R.J., McNeill, M.M., Gatehouse, T.K., Douch, P.G., 1997. Quantification of total sheep IgE concentrations using anti-ovine IgE monoclonal antibodies in an enzyme immunoassay. *Vet. Immunol. Immunopathol.* 57, 253–265.
- Steinman, A., Peer, G., Klement, E., 2003. Epidemiological study of *Culicoides* hypersensitivity in horses in Israel. *Vet. Rec.* 152, 748–751.
- Suter, M., Fey, H., 1981. Isolation and characterization of horse IgE. *Zbl. Vet. Med. B* 28, 414–420.
- Tahon, L., Baselgia, S., Gerber, V., Doherr, M.G., Straub, R., Robinson, N.E., Marti, E., 2009. In vitro allergy tests compared to intradermal testing in horses with recurrent airway obstruction. *Vet. Immunol. Immunopathol.* 127, 85–93.

- Ungar-Waron, H., Braverman, Y., Gluckman, A., Trainin, Z., 1990. Immunogenicity and allergenicity of *Culicoides imicola* (Diptera: Ceratopogonidae) extracts. J. Vet. Med. B 37, 64–72.
- Van der Haegen, A., Künzle, F., Gerber, V., Welle, M., Robinson, N.E., Marti, E., 2005. Mast cells and IgE-bearing cells in lungs of RAO-affected horses. Vet. Immunol. Immunopathol. 108, 325–334.
- Van Grevenhof, E.M., Ducro, B., Heuven, H.C., Bijma, P., 2007. Identification of environmental factors affecting the prevalence of insect bite hypersensitivity in Shetland ponies and Friesian horses in The Netherlands. Equine Vet. J. 39, 69–73.
- Wagner, B., Siebenkotten, G., Leibold, W., Radbruch, A., 1997. Organization of the equine immunoglobulin constant heavy chain genes. I. C ϵ and C α genes. Vet. Immunol. Immunopathol. 60, 1–13.
- Wagner, B., Siebenkotten, G., Radbruch, A., Leibold, W., 2001. Nucleotide sequence and restriction fragment length polymorphisms of the equine C ϵ gene. Vet. Immunol. Immunopathol. 82, 193–202.
- Wagner, B., Siebenkotten, G., Leibold, W., Radbruch, A., 2002. Expression of a 4-(hydroxy-3-nitro-phenyl) acetyl (NP) specific equi-murine IgE antibody that mediates histamine release in vitro and a Type-I skin reaction in vivo. Equine Vet. J. 34, 657–665.
- Wagner, B., Radbruch, A., Rohwer, J., Leibold, W., 2003. Monoclonal anti-equine IgE antibodies with specificity for different epitopes on the immunoglobulin heavy chain of native IgE. Vet. Immunol. Immunopathol. 92, 45–60.
- Wagner, B., Miller, D.C., Lear, T.L., Antczak, D.F., 2004. The complete map of the immunoglobulin heavy chain constant gene region reveals evidence for seven IgG isotypes and for IgD in the horse. J. Immunol. 173, 3230–3242.
- Wagner, B., 2006. Immunoglobulins and immunoglobulin genes of the horse. Dev. Comp. Immunol. 30, 155–164.
- Wagner, B., Flaminio, J.B.F., Hillegas, J., Leibold, W., Erb, H.N., Antczak, D.F., 2006a. Occurrence of IgE in foals: evidence for transfer of maternal IgE by the colostrum and late onset of endogenous IgE production in the horse. Vet. Immunol. Immunopathol. 110, 269–278.
- Wagner, B., Miller, W.H., Morgan, E.E., Hillegas, J.M., Erb, H.N., Leibold, W., Antczak, D.F., 2006b. IgE and IgG antibodies in skin allergy of the horse. Vet. Res. 37, 813–825.
- Wagner, B., Childs, B.A., Erb, H.N., 2008. A histamine release assay to identify sensitization to *Culicoides* allergens in horses with skin hypersensitivity. Vet. Immunol. Immunopathol. 126, 302–308.
- Wagner, B., Miller, W.H.Jr., Erb, H.N., Lunn, D.P., Antczak, D.F., 2009. Sensitization of skin mast cells with IgE antibodies to *Culicoides* allergens occurs frequently in clinically healthy horses. Vet. Immunol. Immunopathol., doi:10.1016/j.vetimm.2009.09.015.
- Watson, J.L., Pettigrew, H.D., Wilson, L.K., Gershwin, L.J., 1997. Isolation and partial expression of a cDNA clone encoding equine IgE heavy chain and production of polyclonal antibodies to the recombinant protein. J. Vet. Allergy Clin. Immunol. 5, 135–142.
- Watson, J.L., Jackson, K.A., King, D.P., Stott, J.L., 2000. Molecular cloning and sequencing of the low-affinity IgE receptor (CD23) for horse and cattle. Vet. Immunol. Immunopathol. 73, 323–329.
- Wilson, A.D., Harwood, L.J., Björnsdottir, S., Marti, E., Day, M.J., 2001. Detection of IgG and IgE serum antibodies to *Culicoides* salivary gland antigens in horses with insect dermal hypersensitivity (sweet itch). Equine Vet. J. 33, 707–713.
- Wilson, A.D., Harwood, L., Torsteinsdottir, S., Marti, E., 2006. Production of monoclonal antibodies specific for native equine IgE and their application to monitor total serum IgE responses in Icelandic and non-Icelandic horses with insect bite hypersensitivity. Vet. Immunol. Immunopathol. 112, 156–170.
- Wilson, A.D., Heesom, K.J., Mawby, W.J., Mellor, P.S., Russell, C.L., 2008. Identification of abundant proteins and potential allergens in *Culicoides nubeculosus* salivary glands. Vet. Immunol. Immunopathol. 122, 94–103.
- Wong, D.M., Buechner-Maxwell, V.A., Manning, T.O., Ward, D.L., 2005. Comparison of results for intradermal testing between clinically normal horses and horses affected with recurrent airway obstruction. Am. J. Vet. Res. 66, 1348–1355.