

Equine IgE responses to non-viral vaccine components

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ABSTRACT

Vaccination of horses is performed annually or semi-annually with multiple viral antigens, either in a combination vaccine or as separate injections. While this practice undoubtedly prevents infection from such diseases as rabies, equine influenza, West Nile virus, and equine herpes virus, the procedure is not without repercussions. Hypersensitivity reactions, including fatal anaphylactic shock, after vaccination, although uncommon, have increased in incidence in recent years. Studies reported herein document the development of IgE antibodies against non-target antigen components of equine viral vaccines. We hypothesize that viral vaccines can induce an IgE response to non-target antigens, which could elicit an adverse response after vaccination with another viral vaccine containing the same component. In one study IgE responses to components of West Nile virus vaccine were evaluated by ELISA before and after vaccination in 30 horses. In a second five-year study 77 horses were similarly tested for IgE antibodies against bovine serum albumin (BSA), a component of most viral vaccines. Mast cell sensitization was evaluated in horses with high, moderate, and negative serum BSA specific IgE using an intradermal skin test with BSA. Over the five-year period high IgE responder horses showed gradually increasing BSA specific serum IgE levels and positive skin test reactivity, yet none had an adverse event. Sera from horses that had developed adverse vaccine reactions were also tested for IgE antibodies. Several of these horses had extremely high levels of BSA-specific IgE. These data suggest that non-essential protein components of vaccines may sensitize horses for future adverse responses to vaccination.

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1. Introduction

The practice of annual and/or semi-annual vaccination of horses with multiple vaccines is common in the equine industry. While vaccine-induced hypersensitivity is an uncommon event, fatal anaphylactic shock after routine vaccination has increased in recent years. The introduction of West Nile virus (WNV) into the USA in 1999 increased the number and frequency of vaccines administered to horses. Reports of adverse responses to vaccination caused one WNV vaccine to be withdrawn from the market [1].

Viral vaccines contain not only viral antigen, but also non-target antigens (tissue culture-derived proteins and stabilizers). Induction of an immune response to viral antigens is often accompanied by responses to these non-target antigens. Induction of IgE puts the

horse at risk for developing a type I hypersensitivity response to subsequent vaccines containing the non-target antigens.

IgE responses to human vaccines are reported for several vaccine components. Gelatin, used as a stabilizer, was implicated in anaphylaxis associated with measles, mumps, and rubella (MMR) vaccines [2]. Sensitization to gelatin by diphtheria–tetanus–acellular pertussis (DPT) vaccination was likely the cause of anti-gelatin IgE in children reacting to MMR vaccines [3,4].

Studies on influenza vaccine reactions revealed that patients with pre-existing hypersensitivity to egg proteins are at risk for anaphylaxis from egg propagated influenza vaccine. Anaphylaxis was reported following administration of live attenuated influenza nasal vaccine [5]. Patients who are RAST and skin test positive to egg proteins but do not undergo anaphylaxis after eating eggs, can be safely immunized using divided doses of vaccine [6]. Previously such patients were skin tested with influenza vaccine and only those with negative reactions were vaccinated [7]. Recent studies showed that many patients with positive skin tests and positive RAST to egg proteins can be vaccinated without induction of anaphylaxis or other adverse responses [7].

Sensitization to serum proteins and/or antigens from mammalian cell lines used to prepare vaccines has been reported in several species. Foot and mouth disease virus propagated in baby

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hamster kidney cells with horse serum caused anaphylaxis in cattle. This response was attributed to equine serum proteins present in the vaccine [8]. Equine serum proteins in swine erysipelas vaccine caused anaphylaxis in vaccinated pigs [9]. Fetal bovine serum (FBS), commonly in cell culture media, induced IgE in dogs showing type 1 hypersensitivity after vaccination with canine viral vaccines. Seven of ten reacting dogs had serum IgE specific for FBS. Gelatin and casein, used as vaccine stabilizers, reacted with IgE in serum of another reactor dog [10].

Herein we examined IgE and IgG responses to two viral vaccine non-target antigens before and after vaccination of adult horses with varied previous vaccine histories. Study one examined the IgE response of horses to Vero cell antigens in horses receiving WNV killed virus vaccine. Study two spanned five years and monitored the IgE, IgGa, and IgGc responses of horses to bovine serum albumin (BSA), the major component of FBS. A third cohort of horses, which had exhibited hypersensitivity reactions after vaccination, were evaluated for IgE reactivity to BSA and/or Vero cell antigens.

2. Materials and methods

2.1. Horse vaccination and sampling

Study one included thirty privately owned horses that varied in age, breed, gender and vaccine histories (Table 1). A 10 ml blood sample was obtained from each horse immediately before one dose of killed WNV vaccine (Innovator, Fort Dodge) was injected intramuscularly. Seven days after vaccination a second 10 ml sample of blood was collected. Serum was harvested and stored at -20°C until assayed.

Study two was initiated in 2005 with 77 horses; some became unavailable during the five years of the study; and the number of horses sampled decreased to 52 by 2009. Horses were maintained at the University of California, Davis, Center for Equine Health (CEH) and consisted of varied breeds, ages, and genders. An Institutional Animal Care and Use (IUCAC) protocol approval was in place. All horses were vaccinated at least once per year and in 2005 and 2006 semi-annually in fall and spring for WNV. Vaccines used in this study came from several companies. A vaccine history for study two horses is presented by year in Table 2.

Veterinarians submitted sera from six vaccine-induced hypersensitivity cases for Vero cell IgE testing. Sera from 23 additional reactive horses were evaluated for BSA specific IgE. For vaccine reactive horses time points for serum collection after vaccination varied; there was no pre-vaccination sample available for comparison. Adverse reactions varied in severity and presentation from urticaria to colic and anaphylactic shock.

2.2. IgE ELISA for Vero cell antigens

Vero cell antigens were prepared from African green monkey kidney cells (Vero cell) (CCL-81 ATTC) grown in Eagles Minimal Essential medium with 10% horse serum. Cells were washed, sonicated and frozen at -20°C until used to sensitize 96 well microtiter plates ($1\text{ }\mu\text{g/well}$ in $200\text{ }\mu\text{l}$ of carbonate-bicarbonate buffer pH 9.6) for 18 h at 4°C . Wells were blocked for 1 h at 37°C in the same buffer. All washes were performed six times with phosphate buffered saline-Tween 20 (PBS-Tween 20). Control and serum samples were added (1:4 dilution in PBS-Tween 20), $100\text{ }\mu\text{l}$ per well in triplicate. Negative controls: PBS, fetal horse serum (FHS), and a negative (unvaccinated for three years) serum pool. Samples and controls were incubated for 1 h at 37°C , and washed. Rabbit anti-equine IgE [11] was added ($100\text{ }\mu\text{l}$ of 1:1000) and plates were incubated for 1 h at 37°C . Horseradish peroxidase conjugated goat anti-rabbit IgG (H&L) was the detecting antibody

and the plates were developed by the addition of 1 mg/ml ortho-phenylenediamine (OPD); absorbance values were measured at 450–650 nm on a microplate reader (VERSMAX, Molecular Devices). The mean of the three well O.D. values was then used as the sample value. Data were expressed as fold increase over the negative horse pool.

2.3. ELISA for IgE antibodies specific for bovine serum albumin

Microtiter plates were sensitized with $1\text{ }\mu\text{g/well}$ of BSA (Sigma Fine Chemicals, grade V) in carbonate/bicarbonate buffer pH 9.6 for 18 h at 4°C . Wells were blocked with a 1% solution of rabbit serum albumin in the same buffer incubated at 37° for 1 h. After washing samples and controls were added ($100\text{ }\mu\text{l}$) in triplicate. These included: positive control (serum pool from horses that had shown anaphylactic reactivity to vaccination with tissue culture grown viral vaccines), negative control (serum pool from horses unvaccinated for several years), FHS, and PBS. To diminish competing IgG before adding serum to the plate each sample was treated with saturated ammonium sulfate (SAS) to create a 27.5% solution [12]. The supernatant was used as sample. All incubations were at 37°C for 1 h with 6 (PBS/Tween 20) washes between steps. Rabbit anti-equine IgE [11] was the primary antibody and the conjugate was horseradish peroxidase conjugated goat anti-rabbit IgG (H&L) (Bethyl Laboratories, TX). The substrate was o-phenylenediamine (OPD) at 1 mg/ml, and optical densities were read on a microplate reader (VERSAMAX, Molecular Devices) at dual wavelengths 450–650 nm. Results were expressed as optical densities and then converted to percentage of positive control.

2.4. ELISA for IgG antibodies specific for BSA

All serum samples collected from study two were analyzed for the presence of BSA specific IgGa and IgGc using a similar protocol as for IgE with the following exceptions: sera were not treated with SAS, but were diluted 1:10 in PBS-Tween 20 before addition to triplicate wells. Antibodies used to detect IgGa and IgGc were sheep anti-horse IgGa and sheep anti-horse IgGc (Bethyl Laboratories). Previously identified sera that were either positive or negative for BSA-specific IgGa and/or IgGc were used as controls on all plates. Values that were greater than the mean of the negative control replicates plus three standard deviations were considered to be positive for that isotype anti-BSA response.

2.5. Intradermal skin testing

Fourteen horses from study two were chosen for intradermal skin testing with BSA. IgE responder horses were grouped according to BSA IgE response level (see Section 3) and horses were chosen from each of the 4 groups for skin testing. Horses also varied in levels of IgG responsiveness. Hair was clipped from the left side of the neck and four 0.1 ml intradermal injections were made: PBS (negative control), histamine (positive control), 0.01% BSA in PBS, and 0.1% BSA in PBS. Injection sites were measured at 15 and 30 min after injection. Measurements were taken at right angles to assure the best approximation of diameter (mean of the two measurements). Sedation was not required. For each injection site the mean diameter for each wheal was expressed as a percent of the diameter of the wheal induced by histamine.

3. Results

3.1. Serum IgE responses to Vero cell antigens

The mean O.D. from triplicate samples was expressed as a ratio of the sample O.D. to the negative control O.D. (Fig. 1) for day 0 and

Table 1
Vaccination history for study one horses.

Horse ID number	Breed	Age (years)	Gender	No. of WNV vaccines ^a	Total no. of viral vaccines ^b
1	Morgan	4	Stallion	4	14
2	Morgan	4	Mare	4	14
3	Arabian	2	Mare	2	12
4	Thoroughbred (TB)	12	Mare	3	8
5	Morgan	6	Mare	4	9
6	TB	5	Mare	3	8
7	Morgan	4	Mare	4	13
8	Morgan	14	Mare	4	13
9	TB/Morgan X	4	Mare	4	15
10	Morgan	1	Mare	4	14
11	Morgan	3	Mare	4	14
12	TB/Morgan X	3	Mare	4	13
13	TB/Morgan X	2	Mare	3	12
14	Arabian	18	Mare	4	20
15	Arabian	6	Mare	4	15
16	Arabian	11	Mare	4	15
17	Arabian	10	Mare	4	12
18	TB/Morgan X	3	Mare	4	13
19	TB	11	Mare	4	6
20	Hannovarian	18		4	12
21	Warmblood X Hannovarian	5	Mare	4	12
22	Paint pony	7	Mare	4	6
23	TB	7	Gelding	4	9
24	Paint	18	Gelding	4	10
25	Morgan	19	Gelding	4	12
26	Morgan	15	Gelding	4	9
27	Morgan	9	Mare	4	5
28	Morgan	6	Mare	4	5
29	Morgan	16	Gelding	0	0
30	Morgan	26	Mare	4	7

^a Number of vaccine antigens in past two years.

^b Number of viral vaccine antigens in past two years.

Table 2
Vaccination histories for study two horses.

Year	4 way ^a	WNV/Killed	WNV/canary pox vectored	Rabies	3 way ^b	Intranasal-influenza
2003	+	+	—	—	—	—
2004	—	+ (some)	+ (some)	—	+	+
2005	—	+ (some)	+ (some)	—	+	+
2006	—	Spring: + (some) Fall: all	+ (some)	—	—	+
2007	+	+	—	—	—	—
2008	+	+	—	—	—	—
2009	—	+	—	+	+	+

^a 4 way = EEE, WEE, influenza, tetanus.

^b 3 way = EEE, WEE, tetanus.

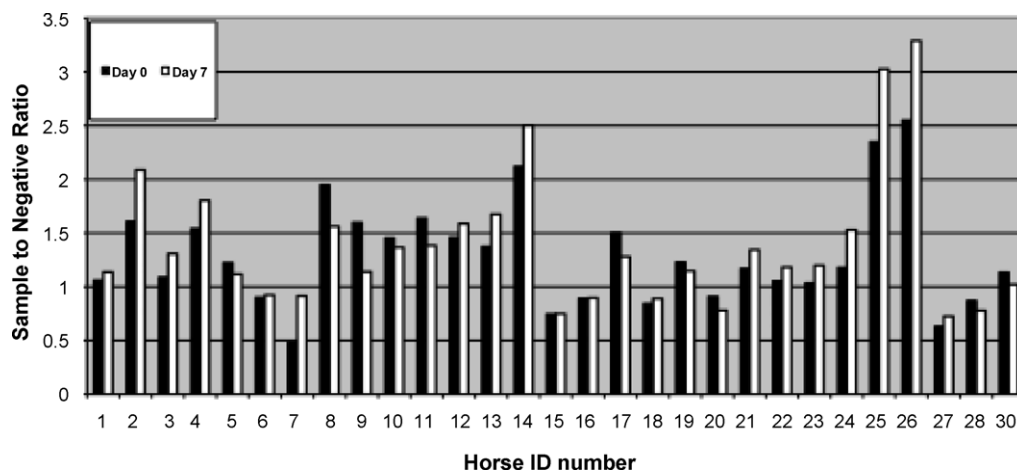


Fig. 1. IgE responses to Vero cell antigen in WNV vaccinated horses. Legend: day 0 versus day 7, $p < 0.1$.

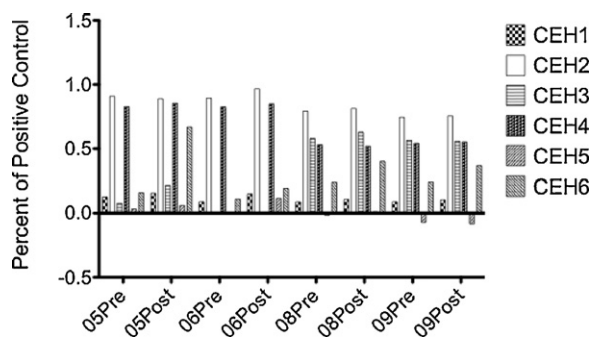


Fig. 2. IgE responses to BSA in six vaccinated horses.

day 7. Three horses (#2, #14, #25, and #26) had O.D. values greater than two times the O.D. of the negative control (Fig. 1). The overall IgE response of the group on day 7, while not reaching significance, shows a trend for an increase ($p < 0.1$). The three horses that had the highest O.D. ratios had been vaccinated with a total of 14 antigens (#2), 20 antigens (#14), 12 antigens (#25), and 9 antigens (#26) in the past two years (Table 1). Two clinical reactor horses showed a twofold increase over negative control for the O.D. response to Vero cell antigen. The remaining four reactor horses did not show a specific response to Vero cell antigen.

3.2. Serum IgE responses to BSA

In study two the IgE response of each horse was calculated as % of positive control and the response over time was evaluated as either positive or negative for IgE production. To determine the cut off for positive the mean O.D. plus 3 standard deviations of the negative serum pool (18% of the positive control pool) was used in conjunction with the mean pre-vaccine BSA specific IgE O.D. for the 2005 horses (25% of the positive pool). Positive horses were grouped in one of three response categories: (1) pre-vaccine and/or post-vaccine level $>60\%$ of positive control, (2) pre-vaccine level $<60\%$, but post-vaccine level at least twofold greater than pre-vaccine level, and (3) pre- or post-vaccine level $>40\%$ and $<60\%$. Horses not falling into any of the three responder categories were considered to be non-responders (IgE negative). Thus a horse that had a 25% value pre-vaccination would be considered positive at level 2 if the post-vaccination value was at least 50%. As seen in Table 3A at no time were there more than 34% of horses tested considered positive responders by any of the three criteria.

Fig. 2 illustrates different patterns of IgE response before and after vaccination during four years of study two for 6 horses. Ages of these horses at the beginning of the study were: CEH1 (15 years), CEH2 (14 years), CEH3 (15 years), CEH4 (22 years), CEH5 (15 years), and CEH (14 years).

Table 3A
BSA specific IgE in horses sampled before and after vaccination.

Year	Total # horses sampled	Responder level 1 ^a	Responder level 2 ^b	Responder level 3 ^c	Negative for BSA IgE ^d
2005 K ^e	76	24 (32%)	3 (4%)	13 (17%)	36 (47%)
2005 CPV ^f	64	10 (16%)	3 (5%)	11 (17%)	40 (62%)
2005 O ^g	50	17 (34%)	0	11 (22%)	22 (44%)
2006	38	13 (34%)	2 (5%)	13 (34%)	10 (26%)
2008	50	15 (30%)	0	11 (22%)	24 (48%)
2009	49	12 (25%)	3 (6%)	7 (14%)	27 (55%)

^a IgE responder level 1 – pre- and/or post-vaccine O.D. values $\geq 60\%$ positive control.

^b IgE responder level 2 – pre-vaccine O.D. $<60\%$, but post-vaccine O.D. at least twofold greater than pre-vaccine O.D.

^c IgE responder level 3 – pre- or post-vaccine O.D. is $>40\%$ and $<60\%$ of positive control.

^d Negative – O.D. values $\leq 40\%$ of the positive control.

^e Horses boosted with killed WNV vaccine.

^f Horses boosted with recombinant canary pox vectored vaccine.

^g Horses boosted with other vaccines (not WNV).

In 25 horses sampled for an adverse clinical response to vaccination one horse had a serum level that was 160% of the positive control, another was 120%, two were 80%, two were 60% and the remainder were less than 60% for reactivity to BSA.

3.3. Serum IgGa and IgGc responses to BSA

Several response patterns for IgG emerged: negative before vaccination but positive after (–/+), negative before vaccination and negative after (–/–), and positive before and after vaccination (+/+). Results of IgGa and IgGc subclass responses to BSA are shown in Tables 3B and 3C respectively. The mean plus 3 standard deviations of the negative serum pool was used to establish the lower limit for positive (45%) IgG responses to BSA.

Seventy two percent of the 26 reactive horses were positive for BSA specific IgGa. These sera were not tested for IgGc due to insufficient amounts. Horses that were positive for IgE were also positive for IgGa. In addition several horses negative for IgE were positive for IgGa. Some horses were negative for both BSA specific IgE and IgGa.

3.4. Intradermal skin testing with BSA

Intradermal skin testing results with 0.1% BSA are shown (Table 4). Four of six IgE negative horses were negative on intradermal skin tests with both 0.01% and 0.1% BSA. The remaining two negative horses had wheals varying from 0 to 25% of the histamine control with one or both of the antigen concentrations. Horses in response level #1 had strongly positive intradermal wheal reactions, which were greatest with the 0.1% BSA solution. One horse in response level 2 had a very strong wheal response (70% histamine) with both 0.1% and 0.01% BSA, while the other had a wheal response of 60% histamine with the 0.1% BSA at 30 min, but was negative with the 0.01% BSA at 30 min. The two horses in response level 3 showed wheal diameters greater than 50% of the histamine control with both BSA concentrations. Responder group 1 horses showed a very significantly ($p = 0.0019$) greater skin test response to BSA than horses that were negative for BSA specific IgE. IgG response patterns varied amongst skin test positive and negative horses.

3.5. Statistical analysis

Differences between group means were evaluated using Graph Pad Prism and Statfast 3 software; non-parametric statistics used included Mann–Whitney Test and Spearman's correlation coefficient.

Table 3B

BSA specific IgGa in horses sampled before and after vaccination.

Year	Total # horses sampled	O.D. $\geq 45\%$ of + control	O.D. $> 90\%$ of + control	O.D. $< 45\%$ (negative)
2005 K ^a	77	40 (52%)	13 (17%)	37 (48%)
2005 CPV ^b	64	44 (69%)	12 (18%)	20 (31%)
2005 O ^c	50	27 (54%)	4 (13%)	23 (46%)
2006	64	27 (42%)	11 (17%)	37 (58%)
2008	50	22 (44%)	16 (32%)	28 (56%)
2009	52	28 (54%)	17 (31%)	24 (46%)

Lower limit for positive was determined using mean O.D. + 3 S.D. of negative serum pool.

^a Horses boosted with killed WNV vaccine.^b Horses boosted with recombinant canary pox vectored vaccine.^c Horses boosted with other vaccines (not WNV).**Table 3C**

BSA specific IgGc in horses sampled before and after vaccination.

Year	Total # horses sampled	O.D. $\geq 45\%$ of + control	O.D. $> 90\%$ of + control	O.D. $< 45\%$ (negative)
2005 K ^a	77	42 (55%)	17 (22%)	35 (45%)
2005 CPV ^b	64	36 (56%)	14 (22%)	28 (44%)
2005 O ^c	50	23 (46%)	4 (8%)	27 (54%)
2006	64	27 (42%)	11 (17%)	37 (58%)
2008	50	25 (50%)	1 (2%)	25 (50%)
2009	52	18 (35%)	0 (0%)	34 (65%)

Lower limit for positive was determined using mean O.D. + 3 S.D. of negative serum pool.

^a Horses boosted with killed WNV vaccine.^b Horses boosted with recombinant canary pox vectored vaccine.^c Horses boosted with other vaccines (not WNV).

4. Discussion

Immune responses to components of vaccines unrelated to the immunizing antigen are frequent in regularly vaccinated horses. IgE responses to BSA occurs in a small subset of vaccinated horses; IgG responses are more common. It is well recognized that the propensity to produce IgE is under genetic control [13]. This variability is illustrated in Fig. 2 as horses CEH1 and CEH5 showed no IgE response over time; whereas horse CEH3 showed a considerable increase during five years. Horse CEH2 had a high IgE response to BSA present on initial testing and it remains high throughout the five years. Horse CEH4 was also consistently positive, but levels declined gradually over the five years. Horse CEH6 responded to vaccination each year and was positive throughout the five years, yet only exceeds the high response level (60% of positive control) in the final post vaccination sample. All of these horses were 14 years old or greater when the study began.

In humans, dogs, mice and horses the IgE response is genetically controlled [14,15]. Data presented herein demonstrates this variability in IgE responsiveness and is supported by the low incidence of anaphylactic type vaccine reactions in horses. A significant effect of genetics on allergen specific IgGa and IgE has been shown in horses with recurrent airway obstructive disease [15]. Insect bite hypersensitivity, caused by an IgE mediated gnat response, also has a genetic association as seen in several breeds [16,17].

Some horses have a measurable serum IgE response to BSA and do not show clinical signs of hypersensitivity after vaccination; while other horses that are highly positive for serum BSA-specific IgE respond to vaccination with mild to severe clinical signs. Horse CEH2 has consistently high BSA IgE and did react to vaccination with a local wheal on at least one occasion. Some horses with demonstrated clinical reactivity did not test positive for IgE to either BSA or Vero cell antigens. Allergic to other vaccine antigens, such as the common stabilizers gelatin and casein, is a likely cause.

Table 4

Intradermal skin test and serum antibody levels.

Horse number	BSA IgE responder level	BSA ^A IgGa	BSA ^A IgGc	30' skin test (%histamine)
1	1 ^a	+/+	+/+	42%
2	1 ^a	+/+	+/+	104%
3	1 ^a	-/-	-/-	95%
4	1 ^a	+/+	+/+	82%
5	2 ^a	-/-	-/-	70%
6	2 ^a	+/+	+/+	67%
7	3 ^a	+/+	+/+	57%
8	3 ^a	-/-	-/-	78%
9	Negative ^b	-/-	+/+	0
10	Negative ^b	-/+	+/+	25%
11	Negative ^b	-/+	-/+	0
12	Negative ^b	-/-	-/-	0
13	Negative ^b	-/-	-/-	0
14	Negative ^b	+/+	+/+	0

IgE responder level 1 – pre- and/or post-vaccine O.D. values $\geq 60\%$ positive control, IgE responder level 2 – pre-vaccine O.D. $< 60\%$, but post-vaccine O.D. at least twofold greater than pre-vaccine. IgE responder level 3 – pre- or post-vaccine O.D. is $> 40\%$ and $< 60\%$ of positive control. Negative – O.D. values $\leq 40\%$ of the positive control.

Mean for a > b, $p = 0.0019$.

^A For IgG subclasses the lower limit for positive was determined using mean O.D. + 3 S.D. of negative serum pool. IgG subclass status before vaccination/IgG status after vaccination.

Grouping of CEH horses by IgE responder status facilitated evaluation of skin test results in relation to the magnitude of the serum IgE and IgG responses. The relationship between positive IgE (all 3 levels) and positive 30 min skin test response was highly significant.

The serum half-life of IgE is considerably shorter than the duration of time IgE remains fixed to tissue mast cell receptors [18]. Thus, serum taken within several weeks of stimulation by vaccination should correlate well with skin test reactivity, while serum taken months later may be low for circulating IgE while concurrent skin testing would reveal a positive response [19]. As expected all of the horses that were positive for BSA specific IgE developed positive skin tests with BSA.

The presence of IgE specific for BSA in serum of recently vaccinated horses and the lack of clinical reactivity poses a question about the threshold reactivity required for clinical signs to occur. Horses classified as level one IgE reactors (>60% of the positive control) with positive skin tests did not show adverse clinical signs after vaccination. There may be a mast cell IgE saturation level that is required for demonstration of a systemic response. Other factors to consider include the level of mast cell receptor saturation in lung and intestine, the shock organs for anaphylaxis in the horse. The time required for antigen to reach such receptors may also be modulated by the nature of the adjuvant and whether or not there was an interruption of integrity of blood vessel walls during the injection process. Nonetheless horses with positive skin and/or serum reactivity should be closely monitored as the data show yearly vaccination increases IgE levels in IgE responder horses over time.

The IgG response to BSA varied among horses and did not appear to influence either skin test positivity or clinical reactivity. An IgG homocytotropic antibody has not been described in horses and data presented herein support the conclusion that IgG antibodies made against BSA did not sensitize mast cells. However, the presence of IgG against BSA in IgE negative horses such as CEH9, CEH11, and CEH14 with negative skin test reactivity to BSA could represent a population of horses in which yearly exposure to BSA in vaccines has induced regulatory T cells to selectively down-regulate the IgE response [20]. Immunotherapy for allergy is thought to induce IgG antibodies, and to down-regulate specific IgE by induction of T regulatory cells [20]. Studies on Icelandic horses with Culicoides-induced insect hypersensitivity have shown that IgE positive allergic horses are more likely to also have specific IgG in their serum than non-allergic, but similarly exposed horses [21].

The role of adjuvants in induction of adverse responses to vaccination has been examined and a syndrome called “ASIA” was defined to describe autoimmune/inflammatory syndrome induced by adjuvants [22]. While this syndrome is broader than the hypersensitivity responses that are the topic of this research it is important to remember that the combination of genetics, environment, and adjuvant may induce a variety of adverse immune responses. The use of viral vectored vaccines, such as canary pox, that lack fetal bovine serum proteins may decrease responses to BSA; however avian proteins have been associated with IgE production [7].

Overall the data presented herein supports the hypothesis that annual or semi-annual vaccination of horses with several viral vaccines enhances the likelihood that they will make an immune response to non-target proteins shared among the vaccines. Some horses respond by making IgE antibodies and develop type 1 hypersensitivity, but these IgE antibodies can be present in serum and on dermal mast cells without causing a hypersensitivity response.

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