Investigation of Bovine Serum Albumin specific IgE expression in horses

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March 17, 2023

Abstract

Background: Neonatal foals are born essentially agammaglobulinemic and therefore must ingest colostrum or receive immunoglobulins to maintain health. Failure of passive transfer treatment involves administration of equine colostrum, plasma or commercial powdered colostrum (CPC). Anecdotal reports suggest a risk of anaphylaxis associated with plasma transfusion in neonates that received CPC prior to gut closure. Bovine serum albumin (BSA) in CPC may serve as a target for BSA-specific immunoglobulin E (IgE) in donor equine plasma. Objectives: To determine presence of BSA-specific IgE in samples collected post-routine vaccination in healthy horses, horses experiencing adverse vaccine reactions and commercial equine plasma. Study Design: Prospective Observational Methods: Serum was collected from 65 healthy horses at day 0, 14, 28, 90, 180, 270 and 365 post-vaccination, 26 horses after vaccine reaction at day 1, 180 or 270 post-vaccination, 4 horses not vaccinated and 10 horses from a commercial plasma donor herd. BSA-specific IgE was determined using enzyme-linked immunosorbent assay (ELISA). Results: BSA-specific IgE was not detected in non-vaccinated horses and was identified in all vaccinated horses. Younger horses demonstrated higher fold changes in post-vaccination BSA-specific IgE expression compared to older horses. No significant difference in BSA-specific IgE levels between commercial plasma donors and healthy horses was identified. No significant difference in post-vaccination anti-BSA IgE levels between reactor and healthy horses at day 180 and 270 post-vaccination were identified. Main Limitations: Small number of reactor horses at day 180 and 270 post-vaccination with most samples being collected 24 hours. There were no healthy horse samples for 24 hours post-vaccination; therefore, it was not possible to compare the two groups at this timepoint. Conclusions: Horses may express BSA specific IgE following vaccination. There may be risk of hypersensitivity type reaction when veterinarians administer commercial plasma to neonatal foals that have consumed CPC prior to gut closure.

Original Article

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Keywords: horse; vaccine; plasma; IgE; bovine serum albumin

Summary
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Objectives : To determine presence of BSA-specific IgE in samples collected post-routine vaccination in healthy horses, horses experiencing adverse vaccine reactions and commercial equine plasma.

Study Design: Prospective Observational

Methods: Serum was collected from 65 healthy horses at day 0, 14, 28, 90, 180, 270 and 365 post-vaccination, 26 horses after vaccine reaction at day 1, 180 or 270 post-vaccination, 4 horses not vaccinated and 10 horses from a commercial plasma donor herd. BSA-specific IgE was determined using enzyme-linked immunosorbent assay (ELISA).

Results : BSA-specific IgE was not detected in non-vaccinated horses and was identified in all vaccinated horses. Younger horses demonstrated higher fold changes in post-vaccination BSA-specific IgE expression compared to older horses. No significant difference in BSA-specific IgE levels between commercial plasma donors and healthy horses was identified. No significant difference in post-vaccination anti-BSA IgE levels between reactor and healthy horses at day 180 and 270 post-vaccination were identified.

Main Limitations : Small number of reactor horses at day 180 and 270 post-vaccination with most samples being collected 24 hours. There were no healthy horse samples for 24 hours post-vaccination; therefore, it was not possible to compare the two groups at this timepoint.

Conclusions : Horses may express BSA specific IgE following vaccination. There may be risk of hypersensitivity type reaction when veterinarians administer commercial plasma to neonatal foals that have consumed CPC prior to gut closure.

Clinical Relevance

- It has been demonstrated that horses can produce BSA-specific IgE following vaccination and this may contribute to the presence of BSA-specific IgE in commercial plasma products.
- Neonatal foals receiving CPC supplementation within 24 hours of birth may be at risk of developing an adverse reaction to plasma transfusion if BSA-specific IgE is present in the administered plasma product.
- Veterinarians should follow all label recommendations for USDA licensed Ig products, specifically including avoidance of administering multiple products to neonatal foals.

INTRODUCTION

Prevention of infectious disease is an important component of maintaining equine host health. American Association of Equine Practitioner (AAEP) vaccination guidelines are available to help equine veterinarians implement safe and effective vaccine protocols (AAEP 2020). In most cases, vaccine administration results in immunologic protection against administered disease antigens with low risk to the host. In select cases, adverse reactions may occur and be associated with triggering the innate immune system and not just stimulating the adaptive, antigen-specific arm of the immune system (Tizard 2018). In a previous investigation, the etiology of allergic reactions among horses following routine vaccination was explored (Gershwin et al. 2012). Findings from this investigation revealed that vaccine-induced hypersensitivity reactions may be related to IgE responses towards non vaccine-target, culture-derived proteins (Gershwin et al. 2012). One such protein associated with this response is bovine serum albumin (BSA), the major constituent of fetal bovine serum (Gershwin et al. 2012).

Fetal bovine serum has been used for decades during vaccine manufacturing serving as an integral part of cell line cultivation. Previous work has demonstrated that BSA is associated with both milk and beef allergies.
in people, and there is evidence that vaccine-associated hypersensitivity reactions in people occur secondary to BSA hypersensitivities (Restani et al. 2004, Shoenfeld et al. 2011, Loughney et al. 2014, Silva et al. 2017). Due to concerns for life-threatening anaphylaxis, the World Health Organization requires BSA concentration to be less than 50 ng per human vaccine dose (Loughney et al. 2014). Although used less commonly in veterinary vaccines, historically BSA was used during the manufacturing process of certain vaccines for animal use without restrictions. Evidence from the report by Gershwin et al. (2012) supports the suggestion that repeated annual or semi-annual vaccination of horses with certain vaccines could enhance the likelihood that an immune response to non-target proteins, that include BSA, could occur.

In addition to the risk for immediate hypersensitivity to non-target proteins upon vaccination, risk can exist for adverse reactions to biologic product administration sourced from hyperimmunized horses. Specifically, commercial plasma manufacturers provide a precautionary label statement that equine plasma should not be administered to neonatal foals if they received powdered colostrum or milk replacer product prior to gut closure due to risk for possible hypersensitivity reaction. Foals are born agammaglobulinemic and therefore require ingestion of maternal colostrum for adequate absorption of circulating immunoglobulins, specifically IgG (Lewis et al. 2008, Felippe 2016, Tizard 2018). In cases where mare maternal colostrum is not available, commercial powdered colostral products may be administered (Gapper et al. 2007). Most commercial powdered colostral products are of bovine origin containing BSA. During the first approximately 24 hours of life, while the equine neonatal small intestine is capable of mediating absorption of intact immunoglobulins through pinocytosis, macromolecule protein absorption also takes place (Felippe 2016). In a case where a neonatal foal may ingest bovine-sourced colostral products, BSA can also be absorbed through the small intestine and enter general circulation. While previous studies have shown the administration of hyperimmune equine plasma to foals to be relatively safe (Wilson et al. 2009; Francesca et al. 2017), anecdotal reports among equine clinicians provides evidence that when foals have received powdered colostral or milk replacer products prior to gut closure and receive a subsequent plasma transfusion, unexpected and at times fatal hypersensitivity reactions have occurred. The current study reports such a case demonstrating the need for providing evidence to clinical equine veterinarians of possible adverse and even fatal outcomes in foals receiving plasma transfusions following administration of powdered colostral or milk replacers.

Anaphylaxis is a serious and potentially life-threatening reaction mediated predominately by IgE (Kalina et al. 2003; Achatz et al. 2008). Although many factors can contribute to the development of immediate hypersensitivity reactions, the primary objectives of the current study were 1) to determine if BSA-specific IgE expression occurred in healthy horses following routine annual vaccination and 2) if BSA-specific IgE expression differed in horses with reported vaccine reactions. Additionally, because commercial plasma harvested from hyperimmunized horses may contain BSA-specific IgE and commercial plasma is a commonly administered therapeutic agent in equine neonates, a secondary aim of the study was to determine BSA-specific IgE expression in commercial plasma products through testing of plasma samples from commercial plasma donors used for plasma transfusion.

MATERIALS AND METHODS

Index case supporting the need to identify BSA-specific IgE levels in commercial plasma.

A colt of unknown gestational age was born to a malnourished dam with little to no udder development nor colostrum/milk production. The owner administered a commercial powdered bovine/multi-species colostrum supplement orally within the first 2 hours of life. Subsequently, the colt was examined and evaluated for transfer of passive immunity status. An IgG test was performed at 12 hours of age, which revealed complete failure of transfer of passive immunity (0 mg/dL IgG). The owners elected to continue supplementing the foal on the farm with the powdered colostrum product despite recommendations that it was unlikely to result in adequate transfer of passive immunity. At 24 hours of age a recheck IgG test measured an IgG level of 400 mg/dL IgG, consistent with ongoing partial failure of transfer of passive immunity. Once these results were provided, the owners elected to have the attending clinician perform a plasma transfusion with 1L (22 ml/kg) of commercial high immunoglobulin equine plasma. Plasma was thawed according to the label instructions while an intravenous catheter was placed into the left jugular vein. Initial physical examination performed was
within normal limits. Plasma administration was initiated at a very slow rate initially of 0.05 ml/kg/minute (1 drop/2-3 seconds). Temperature, heart and respiratory rates were measured at 3-5 minute intervals. Signs of colic, dyspnea and urticaria were monitored for continuously. With no evidence of an adverse reaction, the administration rate was slowly increased to 0.15 ml/kg/minute (1 drop/second) over the next 5-10 minutes. At 15 minutes the rate was increased to approximately 0.38 ml/kg/minute (2-3 drops/second), at this point the foal quickly began to show colic signs. The plasma infusion was stopped immediately. Crackles were auscultated in all lung fields and pink-tinged foamy nasal discharge became evident bilaterally suggestive of pulmonary edema. The foal became apneic after one minute. Intravenous epinephrine\(^5\) (0.02 mg/kg) was administered along with furosemide\(^6\) (1 mg/kg) and dexamethasone\(^7\) (0.15 mg/kg). The foal did not respond to emergency medications or attempts at field cardiopulmonary resuscitation and died within 3 minutes following discontinuation of the plasma transfusion.

Based on the history and response of this foal to plasma transfusion, it was suspected that this foal suffered from BSA-associated hypersensitivity. This outcome was consistent with previous anecdotal reports of foals consuming powdered colostrum followed by IV commercial plasma infusion, which has been reported to result in fatal reactions. The remaining commercial plasma administered to this foal was saved at -80\(^\circ\) C for further analysis.

**Sample Collection**

All procedures performed on horses during the study were approved by Kansas State University Institutional Animal Care and Use Committee (IACUC #4003).

**Blood collection procedure**

After cleansing of the haired skin surface with 70% isopropyl alcohol, 20 mL of blood was collected through an 18 g vacutainer needle directly into a red topped tube via jugular venipuncture. Blood collection was performed on days 0 (baseline), 14, 30, 90, and 180 after vaccination. A subset of horses also had blood collected on either or both days 270 and 360 after vaccination. Blood samples were stored at 4\(^\circ\)C immediately after collection for less than 48 hours prior to being centrifuged at 2200-2500 RPM for 15 minutes. Serum was removed and frozen at -18\(^\circ\)C for a maximum of 14 days. Serum was thawed at 4\(^\circ\)C for 24 hours, after which it was aliquoted into 2 mL plastic collection tubes and stored at -80\(^\circ\)C. Serum samples were batched for testing and were only thawed once on the morning of testing to avoid freeze thaw degradation, thus maintaining protein integrity for testing.

**Enrollment of healthy horses following routine vaccination**

Horses were selected based on health status and historical information that they had been on an AAEP-approved vaccine protocol for annual vaccinations. Enrollment criteria included that the owners reported routine annual vaccines had been administered and confirmed no previous adverse vaccine reactions observed. Information was collected via a client questionnaire for all enrolled animals (supplemental information). Horses were determined to be healthy and able to receive routine annual vaccinations following physical examination by a veterinarian (CAB). All horses received the same type of vaccine that were administered by the same veterinarian (CAB). Vaccines administered included a combination against Eastern equine encephalitis (EEE), Western equine encephalitis (WEE), tetanus, West Nile virus (WNV), equine influenza, and equine herpesvirus-1 and -4 (EHV-1/-4)\(^8\) and a separate vaccine against rabies.\(^9\) All vaccines were administered intramuscularly following label instructions and site of administration was recorded in individual medical records.

Sixty-five healthy horses were enrolled in the study. Horses were either client-owned with client consent or part of the Kansas State University College of Veterinary Medicine (KSU-CVM) teaching herd horses. A convenience sample of client-owned horses and all KSU-CVM teaching herd horses that were due for their annual booster vaccinations during the months of March through July were enrolled into the study. Healthy horses were comprised of Quarter horses (n=39), Thoroughbreds (n=9), Warmbloods (n=5), Appaloosas (n=3), Paints (n=3), Morgan (n=1), Miniature (n=1), Mustang (n=1), Draft (n=1), Haflinger (n=1), and
a Tennessee Walking Horse (n=1). All horses were between 3 and 32 years of age. Thirty-three were mares, 31 were geldings and 1 was a stallion.

All 65 healthy horses had blood samples obtained on days 0, 14, 30, 90 and 180 after vaccination. Nineteen of the 65 horses also had blood samples obtained on day 270 after vaccination. Twenty-one of the 65 horses also had blood samples obtained on day 365 after vaccination. Eight of the 65 horses had blood samples collected at both 270 and 365 days after vaccination in addition to the 0, 14, 30, 90, and 180 day time points.

In an effort to determine if there was an age-associated response to BSA-specific IgE expression following vaccination, horses were grouped by age. Subject grouping included horses as young adult (3-9 years), adult (10-14 years), middle aged (15-19 years) and geriatric (20-32 years) as detailed in Table 1.

Enrollment of horses that demonstrated severe adverse vaccine reactions (reactors)

A group of 21 horses were accepted into the study for sample submission purposes. These samples were defined as a horse that had experienced a severe adverse reaction after vaccination either previously or during the course of the current study. A questionnaire was completed either by the owner or primary veterinarian (supplemental information). Clinical signs exhibited by these 21 horses included severe swelling at the injection site, fever, colic, laminitis, lethargy, and markedly stiff neck. Blood was collected and processed as previously described. Fourteen horses had blood drawn within 24 hours, two at 180 days, three at 270 days and two at unknown times after vaccination. Horses ranged from 2 to 15 years of age while age was not documented for 3 horses. There were 13 Warmbloods, 4 Quarter horses and 3 horses did not have their breed reported. Thirteen were geldings and 8 were mares.

Enrollment of unvaccinated horses

A group of 4 Quarter horses between 6-12 months of age were utilized in the study that had never been vaccinated previously. Blood was collected at a single time-point, processed and stored as described above. These samples were used as negative controls.

Utilization of plasma samples from donor horses from a commercial plasma manufacturer

Based on the reaction of the neonatal foal to a commercial plasma product, the decision was made to test additional aliquots of samples from commercial plasma donor horses. Plasma samples were provided from 10 horses among the donor herd. Two 10 mL plasma samples from each donor horse were collected and shipped to Kansas State University. These samples were aliquoted into 2 mL vials and stored in a -80°C freezer. No information on the plasma donors was provided other than that they were routine donor horses used for commercial biologic product collection.

Utilization of serum from index case prior to plasma administration

A single serum sample was obtained from the one-day old neonatal colt that received commercial powdered colostrum and experienced a fatal anaphylactic reaction within the first 15 minutes of plasma administration. The serum sample that was collected during the first 12 hours of life was stored at -80°C prior to analysis.

Utilization of commercial plasma administered to the index case

An aliquot of the commercial plasma that had been administered to the index case described previously, which apparently contributed to a fatal anaphylactic reaction was available for analysis. This sample was aliquoted into 10 mL and 2 mL vials and stored at -80°C.

Indirect ELISA to detect BSA-specific IgE

This protocol was adapted from Gershwin et al. 2012 with modifications. ELISA plates were coated with 1 ug/well BSA in bicarbonate-carbonate buffer pH 9.6 overnight. ELISA wells were blocked with 0.5% rabbit serum albumin (RSA) in coating buffer. Each well in a 96 well plate was filled with 200 µL of 0.5% RSA for blocking. The plates were incubated at 4°C overnight. After incubation concluded, the wells were washed with 300 µL of PBS (PBS + 0.1% Tween-20) by an electronic plate washer 3 times. Plates were coated and
blocked in bulk and stored at 4°C until use within one week. The commercial plasma that was administered to the index case was used as a positive control. Unvaccinated horse serum was used as a negative control. PBS was used as a blank to establish background absorbance. Two wells were designated as the positive control, two as the negative control and two as the blank. Undiluted serum samples (100 μL) were added to each remaining well in duplicate and incubated at room temperature for 2 hours. A standard curve was not available and optical density (OD) values were used to analyze the data.

Plates were washed as described above. Anti-horse IgE horse radish peroxidase (HRP)\textsuperscript{10} was diluted out to 1:500 with wash buffer, added to each well (100 μL) and incubated for 1 hour at room temperature. The plates were washed again. Tetramethylbenzidine (TMB) (100 μL) was added to each well and left to incubate for 30 minutes at room temperature protected from the light. After 30 minutes 100 μL of sulfuric acid (2 M SO\textsubscript{2}H\textsubscript{4}) was added to each well to terminate the reaction. Plates were read with an electronic plate reader at 450 nm and recorded to obtain the optical density.

All horse samples including 0, 14, 30, 90, 180, 270, and 365 day post-vaccination, if available, were tested by way of ELISA for the presence of BSA-specific IgE. All horses classified as a reactor were tested by ELISA. Plasma from the plasma donor samples from commercial source were all tested by ELISA. All plates contained the same positive control, negative control and blanks. The OD value for blank wells was averaged for each plate and then subtracted from each sample well to correct for background absorbance. The adjusted duplicate sample OD values were then averaged together for each horse.

**Statistical analysis**

Statistical analysis was completed with Prism (v.8) on the BSA-specific IgE ELISA data. Horses in the healthy vaccine group had their post-vaccine Ig OD measurements compared with baseline values. Means were determined for each timepoint for all age groups. Differences between groups at specific timepoints were determined with t-tests. Significance was defined as a $p$-value $<0.05$. Data was then compiled in Microsoft Excel as a spreadsheet function.

**RESULTS**

**ELISA BSA-specific IgE**

The OD value for the positive control mean across all plates was 0.340. The OD value for the negative control mean across all plates was 0.062. The OD value for the blank control mean across all plates was 0.071. The OD value for the positive control mean was significantly different from the negative control mean ($p$ value $<0.001$). The OD value for the negative control mean was significantly different from the OD value blank control mean ($p$ value = 0.008).

**Healthy Horses**

The mean OD value +/- standard deviation for day 0 BSA-specific IgE in all healthy horses (0.108 +/- 0.089) was not significantly different when compared to the mean OD value +/- standard deviation (SD) for 14 days post-vaccination BSA-specific IgE in all healthy horses (0.124 +/- 0.118) ($p$ value = 0.43) (Figure 1). The mean OD value +/- SD for day 0 BSA-specific IgE in all healthy horses (0.108 +/- 0.089) was significantly higher when compared to the mean OD value +/- SD for 180 days post-vaccination BSA-specific IgE in all healthy horses (0.073 +/- 0.069) ($p$ value = 0.007) (Figure 1).

When comparing the fold change between day 0 BSA-specific IgE OD values and 14 days post-vaccination BSA-specific IgE OD values, 63% of horses between the ages of 3 and 9 years had a fold increase greater than 1.5 (Figure 2 and Table 1). The percentage of horses displaying a fold change greater than 1.5 decreased with age across age groups. Only 29% displayed a fold change greater than 1.5 in the 10-14 year old age group, 0% in the 15-19 year old age group and 5.6% in the 20-32 year old age group (Table 1).
The mean BSA-specific IgE OD values +/- SD at time points 0, 14, 30, 90, 180, 270 and 365 days after vaccination were compared across age groups (Table 1). The mean BSA-specific IgE OD value +/- SD for horses 3-9 years of age at day 0 was 0.072 +/- 0.086 and at 14 days post-vaccination had increased to 0.125 +/- 0.09 (p-value = 0.013) (Table 1). The mean BSA-specific IgE OD value +/- SD decreased to 0.070 +/- 0.070 at 180 days post-vaccination with days 0 and 180 being significantly different (p-value = 0.035) (Table 1). All other timepoints compared to day 0 within this age group were not significantly different. When comparing all other age groups’ mean BSA-specific IgE OD values at 14 days after vaccination and day 0, the differences were not significant. When comparing all other age groups’ mean BSA-specific IgE OD values at day 0 and 180 days after vaccination, the differences were significant for age groups 10-14 years (p value = 0.001) and 20-32 years (p value = 0.028). In all of these age groups, the mean BSA-specific IgE OD values decreased at 180 days post-vaccination compared to time 0 and 14 days post-vaccination (Table 1). No other time points in any age group were significantly different when compared to day 0.

Anamnestic response of BSA-specific IgE expression in response to annual vaccination in healthy horses was most common in younger horses, aged horses rarely demonstrated a > 1.5 fold change in BSA-specific IgE expression within 14 days of annual booster vaccination (Table 1).

Among young adult horses that were evaluated over the course of a year, two-thirds of the horses demonstrated a greater than 1.5 fold increase in BSA-specific IgE expression without adverse event (Figure 2). Among horses greater than 10 years of age, it was uncommon to have a marked increase in BSA-specific IgE expressed following booster vaccination (Table 1).

**Horses demonstrating adverse vaccine reaction (Reactors)**

Horses with a history of severe adverse vaccine reaction were evaluated for BSA-specific IgE expression at the time of evident vaccine reaction (Figure 3). At the time of initial IgE assessment following a vaccine reaction, mean BSA-specific IgE OD +/- SD for all reactors was 0.286 +/- 0.08. Comparing the mean BSA-specific OD values for reactors and healthy horses at similar time points, there was a trend for higher OD values, but the differences were not statistically significant (Figure 3). ELISA measurement effectively allowed for an assessment of immunoglobulin expression.

**Comparison among all samples**

Figure 4 provides a comparison of mean OD values for BSA-specific IgE among all samples that were tested. The index case serum at 12 hours of age and unvaccinated horses were among the lowest values measured. Healthy horses and commercial plasma donor horses’ values were intermediate. The highest values were observed from reactor horses and the commercial plasma sample administered to the index case.

**DISCUSSION**

Data from the current investigation as well as from previous work demonstrate that horses express BSA-specific IgE following routine vaccination. These findings are consistent with post-vaccination studies in both humans (Hardefeldt et al. 2010; Silva et al. 2017) and horses (Loughney et al. 2014). As expected, horses that had never been vaccinated did not demonstrate notable levels of BSA-specific IgE. Among horses tested, the younger age group (3-9 years of age) had more horses displaying a greater than 1.5-fold increase in BSA-specific IgE at 2 weeks post-vaccination compared to baseline. This suggests that younger horses develop a greater immune response to BSA than older horses. Forty-six of the 65 healthy horses demonstrated an increase in BSA-specific IgE during at least one time point after vaccination compared to baseline. Sixteen of the 65 healthy horses showed a greater than 1.5-fold increase in BSA-specific IgE at 14 days post-vaccination compared to baseline. Six of these 16 horses had a greater than 4-fold increase in BSA-specific IgE at 14 days post-vaccination. All of these 6 horses were between 3 and 11 years of age. Interestingly, none of these 65 horses developed clinical signs associated with an adverse vaccine reaction. This is suggestive that the presence of BSA-specific IgE or the rise in BSA-specific IgE does not directly correlate with the development of an adverse vaccine reaction, which is consistent with the previous report from Gershwin et al. (2012).

Horses that had either historically or currently experienced an immediate severe adverse vaccine reaction all...
had detectable levels of BSA-specific IgE on ELISA. The majority of these horses had blood samples collected within 24 hours post-vaccination. Ten out of the 12 reactor horse samples at 24 hours post-vaccination had an BSA-specific IgE OD value greater than 0.200 (83.3%). Whereas only 9 out of the 65 healthy horses had an BSA-specific IgE OD value greater than 0.200 at 14 days post-vaccination (13.8%). Twenty-four-hour post-vaccination blood samples from horses not experiencing a vaccine reaction could be investigated by ELISA to determine a reference interval for BSA-specific IgE in healthy horses. A very small subset of these reactor horses had blood samples from 180- and 270-days post-vaccination. While their BSA-specific IgE levels appeared to be higher than the healthy horses in this study, these values were not statistically different from healthy horses. A larger sample of horses is needed in order to further investigate whether reactor horses have a significantly increased BSA-specific IgE level at different time points post-vaccination. With this information, horses developing a vaccine reaction could be tested in order to determine if they have elevated BSA-specific IgE levels. With reference intervals available, it could be possible to screen horses prior to vaccination to determine if they are at a higher risk for developing a vaccine reaction specifically against BSA.

Horses used as commercial plasma donors had BSA-specific IgE levels similar to healthy, non-reactor horses. The history of these horses was unknown. Important information would include the age of these horses, how frequently they were vaccinated, when their last vaccine was administered and how long they had been in the program for plasma collection.

The index foal had serum collected at 12 hours of life which was analyzed for BSA-specific IgE and was similar to a non-vaccinated horse. Commercial plasma administered to the index foal was analyzed for BSA-specific IgE and was found to have higher BSA-specific IgE compared with healthy horses, commercial donor plasma or vaccine reactor horses. Based on this finding, it appears that at least an individual commercial plasma sample had elevated BSA-specific IgE levels and this could occur in other donor horses although it appears uncommon. In the described index case, the authors presume interaction of the high BSA-specific IgE administered in plasma interacted with circulating BSA absorbed from the powdered colostral product, which led to an immediate hypersensitivity and anaphylactic reaction due to mast cell degranulation and acute release of histamine and serotonin with resulting effects on the shock organs of horses: respiratory and gastrointestinal tracts.

Two commercial plasma products administered to equine neonatal patients are USDA-licensed for administration in equine neonates for the treatment of failure of transfer of passive immunity. USDA-licensed plasma is guaranteed to have an IgG concentration of at least 2800 mg/dL. When plasma is administered to a foal that has been exposed to BSA in commercial powdered colostrum, it has the possibility to lead to a fatal, idiosyncratic reaction as described above. Among available commercial plasma products, manufacturers explicitly state that the product should not be administered to neonatal foals that have received any commercial powdered Colostral or milk replacer product for this specific reason.

Further investigation is indicated to determine the frequency of elevated BSA-specific IgE among commercial plasma donor horses as well as the kinetics of administered BSA-specific IgE in foals and when or if it may be safe to administer plasma products to foals that have received powered colostral products in their first 24 hours of life. Further investigation is also warranted in larger number of reactor horses to determine if BSA-specific IgE may be predictive of adverse vaccine reactions. This study additionally brings up the practice of vaccine manufacturing using BSA and its safety.

This study supports the theory that horses can mount an immune response to BSA in vaccines by producing BSA-specific IgE. Based on the current report, there is an indication to use caution upon administration of commercial plasma to a neonate that has consumed powdered colostrum supplementation prior to complete gut closure. Collectively, USDA licensed plasma manufacturer guidelines should be followed to avoid administration of greater than any one commercial IgG supplement to neonatal foals.

**Author’s declaration of interests**

No conflicts of interest have been declared
Ethical animal research

Written consent was obtained from each owner prior to participation in the study which was approved by Kansas State University Institutional Animal Care and Use Committee (IACUC #4003).

Source of funding

MCAT College of Veterinary Medicine Intramural Grant Program Kansas State University Grant

Acknowledgements

The authors would like to acknowledge Dr. Ann Rowlands, Ph. D. and President of Lake Immunogenics, Inc. for providing plasma samples from donor horses used for harvesting plasma products. Dr. Robert Larson, KSU-CVM, was extremely helpful in directing the statistical analysis of the data. We would also like to acknowledge Kara Smith (KSU-CVM) for help procuring experimental supplies and Misty Bear (KSU-CVM) for laboratory assistance with running ELISA plates. Finally, we would like to acknowledge Dr. Jason Grady and Dr. Laurie Beard for assistance with sample acquisition in the field.

Authorship

ERP was involved in experimental design, grant proposal, sample collection, experimental data collection and analysis, and manuscript drafting and revisions. KMD and CAB were involved in experimental and study design, grant proposal, sample collection, data analysis, and manuscript drafting and revisions. KK, NLS, and EGD were all involved in experimental and study design, grant proposal, experimental data collection and analysis, and manuscript revisions. All authors reviewed the manuscript and approved the final version.

Manufacturers’ addresses

1. HiGamm Plasma, Lake Immunogenics, Inc. 348 Berg Rd, Ontario, NY 14519, USA
2. MG Biologics, Inc. 2366 270th St, Ames, IA 50014, USA
3. Manna Pro Products LLC, 707 Spirit 40 Park Dr, Chesterfield, MO 6300, USA
4. Snap Foal IgG test, IDEXX Laboratories, Inc. 1 IDEXX Drive, Westbrook, Maine 04092, USA
5. Adrenaline, Par Pharmaceutical, 1 Ram Ridge Rd., Chestnut Ridge, NY 10977
6. VetOne, MWI Animal Health, 3041 Pasadena Dr., Boise, ID 83705
7. VetOne, Sparhawk Laboratories Inc., 12340 Santa Fe Trail Dr., Lenexa, KS 66215
8. Vetera Gold, Boehringer-Ingelheim Pharmaceuticals, Inc. 900 Ridgebury Rd., Ridgefield, CT 06877, USA
9. EquiRab, Merck & Co., Inc. 2000 Galloping Hill Road, Kenilworth, NJ 07033
10. Anti-EQ IgG – HRP, Bio-Rad Laboratories, 1000 Alfred Nobel Dr., Hercules, CA 94547

References


**Table Legends**

Table 1: BSA-specific IgE fold changes according to age group. Within each age group average fold change (Day 0 compared to 14 day post-vaccine and 180 day post-vaccine) is provided. The percentage of horses in each age group that displayed a fold change greater than 1.5 (Day 0 compared to 14 day post-vaccine) is also provided.

Table 2: BSA-specific mean OD values at day 0, 14 and 180 days after vaccination according to age group. The difference between these time points is listed as a p-value. A * indicates a significant difference between the two timepoints (p value < 0.05)

**Figure Legends**

Figure 1: BSA-specific IgE OD values for all healthy horses for a 1-year sampling period. Mean OD at each time point is represented by red X and standard deviation by red horizontal line. Each individual horse is represented by a circle in gray scale. Symbol * represents a time point that differed significantly from day 0 (p value <0.05).
Figure 2: BSA-specific IgE values for healthy horses aged 3-9 years. Each individual horse is labeled A-L, and colored bars represent time points of BSA-specific IgE measurement after vaccination. A blue star indicates post-vaccination time point where the fold change in anti-BSA IgE was greater than 1.5 when compared to Day 0.

Figure 3: BSA-specific IgE for reactor horses. Individual reactor horses are represented as blue circles for each of the time points when collected. Purple triangles depict the mean ELISA OD BSA-specific IgE value for the reactor horses at each time point. Red X depict the mean ELISA OD BSA-specific IgE value for healthy horses at each similar time point.

Figure 4: BSA-specific IgE mean OD values of the index case at 12 hours of life (purple bar), unvaccinated horses (blue bar), healthy horses at Day 0 (green bar), commercial donor horses (N=10) (yellow bar), reactor horses at ~ 24 hours post-vaccination (red bar), and the commercial plasma treatment administered to the index case (orange bar).

Table 1:

BSA-specific IgE fold changes according to age group. Within each age group average fold change (Day 0 compared to 14 day post-vaccine and 180 day post-vaccine) is provided. The percentage of horses in each age group that displayed a fold change greater than 1.5 (Day 0 compared to 14 day post-vaccine) is also provided.

<table>
<thead>
<tr>
<th>Age groups (years)</th>
<th>Horses (n)</th>
<th>Day 0 BSA-specific IgE mean +/- standard deviation</th>
<th>Day 14 BSA-specific IgE mean +/- standard deviation</th>
<th>0-14 day fold change mean</th>
<th>0-14 day fold change &gt;1.5%</th>
<th>Day 180 BSA-specific IgE mean +/- standard deviation</th>
<th>0-180 day fold change mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-9</td>
<td>12</td>
<td>0.072 +/- 0.0125</td>
<td>3.359 +/- 0.0125</td>
<td>7/11 (63.6%)</td>
<td>0.048 +/- 0.0125</td>
<td>0.048 +/- 0.0125</td>
<td>0.482 +/- 0.0125</td>
</tr>
<tr>
<td>10-14</td>
<td>24</td>
<td>0.101 +/- 0.135</td>
<td>1.511 +/- 0.121</td>
<td>7/24 (29.2%)</td>
<td>0.069 +/- 0.0125</td>
<td>0.069 +/- 0.0125</td>
<td>0.615 +/- 0.0125</td>
</tr>
<tr>
<td>15-19</td>
<td>12</td>
<td>0.151 +/- 0.121</td>
<td>0.845 +/- 0.121</td>
<td>9/12 (0%)</td>
<td>0.097 +/- 0.0125</td>
<td>0.097 +/- 0.0125</td>
<td>0.691 +/- 0.0125</td>
</tr>
<tr>
<td>20-32</td>
<td>17</td>
<td>0.104 +/- 0.122</td>
<td>1.027 +/- 0.182</td>
<td>1/18 (5.56%)</td>
<td>0.070 +/- 0.0125</td>
<td>0.070 +/- 0.0125</td>
<td>0.836 +/- 0.0125</td>
</tr>
</tbody>
</table>
Table 2: BSA-specific mean OD values at day 0, 14 and 180 days after vaccination according to age group. The difference between these time points is listed as a p-value. * indicates a significant difference between the two timepoints (p value < 0.05)

<table>
<thead>
<tr>
<th>Age groups (years)</th>
<th>Horses (n)</th>
<th>Day 0 anti-BSA IgG mean</th>
<th>Day 14 anti-BSA IgG mean</th>
<th>Day 180 anti-BSA IgG mean</th>
<th>Day 0 vs. 14 P value</th>
<th>Day 0 vs. 180 P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-9</td>
<td>12</td>
<td>0.072</td>
<td>0.0125</td>
<td>0.048</td>
<td>0.0125*</td>
<td>0.0352*</td>
</tr>
<tr>
<td>10-14</td>
<td>24</td>
<td>0.101</td>
<td>0.135</td>
<td>0.069</td>
<td>0.0647</td>
<td>0.0012*</td>
</tr>
<tr>
<td>15-19</td>
<td>12</td>
<td>0.151</td>
<td>0.121</td>
<td>0.097</td>
<td>0.2582</td>
<td>0.0866</td>
</tr>
<tr>
<td>20-32</td>
<td>17</td>
<td>0.104</td>
<td>0.122</td>
<td>0.0700</td>
<td>0.5018</td>
<td>0.0276*</td>
</tr>
</tbody>
</table>