Effects of serial harvest of plasma on total plasma protein and total immunoglobulin G concentrations in donor horses involved in a plasmapheresis program

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Objective—To determine the effects of intensive serial plasmapheresis on total plasma protein and total IgG concentrations in donor horses involved in a plasmapheresis program.

Animals—18 horses (13 mares and 5 geldings; 13 Belgians, 3 Percherons, 1 Standardbred, and 1 warmblood) ranging from 7 to 14 years of age (mean ± SD, 10 ± 3 years) and weighing 822 ± 128 kg.

Procedures—Horses from which 22 mL of plasma/kg of donor body weight was harvested at 14-day intervals for a minimum of 8 consecutive plasmapheresis donations were retrospectively selected for use in the evaluation. Automated plasmapheresis procedures were performed by use of 2 modified plasmapheresis instruments/donor horse. Plasma samples were obtained at each donation and used for determination of total protein and total IgG concentrations. Total plasma protein concentrations were determined via refractometry. A commercially available ELISA was used to determine total equine IgG concentrations.

Results—The 18 donor horses were used in 8 to 19 serial donations (mean ± SD, 13 ± 3 donations) during the study. Donor horses had significant decreases in both plasma protein and IgG concentrations over the study period.

Conclusions and Clinical Relevance—Serial plasmapheresis procedures caused significant decreases in both plasma protein and IgG concentrations in donor horses; however, decreases were not physiologically relevant. Performing plasmapheresis in horses in accordance with the evaluated automated plasmapheresis procedures did not result in a critical decrease in total plasma protein or total IgG concentrations. (Am J Vet Res 2012;73:770–774)
Currently, instruments are used by researchers and commercial operations to perform automated plasmapheresis procedures in horses. These instruments connect to the donors via sterile tubing sets, which form a closed-loop collection system. Automated instruments withdraw whole blood and infuse it with anticoagulant at a controlled rate. Anticoagulated whole blood is fractionated via centrifugation or filtration techniques. Isolated plasma is diverted to an attached collection bag or bottle. Simultaneously, the concentrated cells are returned to the equine donors.

Research facilities and commercial operations repeatedly perform plasmapheresis procedures on horses at 14- to 30-day intervals and harvest a maximum of approximately 20 mL of plasma/kg of donor body weight during each procedure. Although a number of facilities use automated plasmapheresis procedures to harvest a high volume of sterile plasma from horses, only a few studies have been conducted to examine these procedures and their effects over time. In one study, investigators evaluated the effects of automated plasmapheresis procedures on clinical, hematologic, biochemical, and coagulation variables in horses after plasmapheresis procedures on clinical, hematologic, and coagulation variables in horses after plasmapheresis.

The purpose of harvesting equine plasma is to obtain valuable proteins, including IgG. Equine plasma collection facilities strive to harvest high-volume, high-quality plasma as frequently as possible without causing sustained protein depletion in donor horses. The purpose of the study reported here was to determine the effects of intensive serial plasmapheresis on total plasma protein and total IgG concentrations in donors throughout the duration of their involvement in a plasmapheresis program.

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Materials and Methods

Animals—Eighteen horses (13 mares and 5 geldings, 13 Belgians, 3 Percherons, 1 Standardbred, and 1 warmblood) that were 7 to 14 years old (mean ± SD age, 10 ± 3 years) with a mean body weight of 822 ± 128 kg were used as donors. All research procedures were conducted with approval by the Auburn University Institutional Animal Care and Use Committee.

The horses were healthy as determined on the basis of results of physical examination, a CBC, and serum biochemical analysis. The horses had negative results when tested for equine infectious anemia by use of an agar gel immunodiffusion test and for equine herpes virus-1 by use of a PCR assay. Horses were naive to the plasmapheresis procedure or had not been subjected to plasmapheresis procedures for a mean ± SD of 132 ± 32 days before participation in the study. During the study period, horses were maintained on mixed-grass pastures and had ad libitum access to water and alfalfa hay. In addition, approximately 2 kg of pelleted feed was provided daily to each horse.

During the study, horses received moxidectin and were vaccinated annually against tetanus, rabies, equine influenza, equine herpes virus-1, equine herpes virus-4, and Streptococcus equi. Horses were vaccinated twice annually against Eastern equine encephalomyelitis, Western equine encephalomyelitis, and West Nile virus. Donor horses were also routinely vaccinated against a project-specific immunogen during the study period. It was determined prior to the study reported here that single and repeated vaccination with the immunogen did not impact total equine IgG concentrations; therefore, information regarding the schedule of administration for the immunogen was not included.

Plasmapheresis procedures—Horses were approved for use in automated plasmapheresis procedures as often as once every 14 days. Prior to the collection procedure, each potential donor was weighed and underwent a physical examination. Horses approved for plasmapheresis procedures were deemed healthy with a rectal temperature < 39.5°C and total plasma protein concentration > 3.5 g/dL, as determined via refractometry. Hair was clipped from a 20 × 12-cm area over each jugular vein. The areas were prepared for catheterization by washing with 2% chlorhexidine acetate and a 1-step antiseptic sponge. Approximately 1.5 mL of 2% lidocaine hydrochloride was injected SC over each catheterization site, and a small skin incision was made with a No. 15 scalpel blade in the anesthetized areas. A 10-gauge, 76-mm catheter was inserted through each skin incision and directed ventrally into each jugular vein of donor horses. The catheters were capped and then secured in place with 2-0 sutures.

Plasmapheresis was performed with horses standing in stocks. The head of each horse was placed in a sling made from padded saddle girths. The catheters of each donor horse were connected to a modified disposable collection set, which was installed on 2 simultaneously operating plasmapheresis instruments. A tourniquet consisting of an elastic wrap was placed around the neck of donors and was designed to put pressure on only the right jugular vein to prevent venous collapse and ensure adequate blood flow through the catheter during the procedure. Whole blood was continuously extracted from the outflow catheter and infused with sodium citrate solution at a controlled rate, which resulted in an anticoagulant-to-whole blood ratio of 1:16. The anticoagulated whole blood passed through the modified disposable collection set toward the separation device associated with each instrument. The separation device acted as a rotating membrane filter to separate plasma from cellular components of blood. Plasma exited the bottom port of each separation device, passed through the instrument’s refractometer, and entered the 20-L-capacity collection bag. Simultaneously, concentrated blood cells exited the side port of each separation device, then passed through the cell pump and immediately back into the donor via the inflow catheter. Each instrument processed approximately 135 mL of whole blood/min.

Automated plasmapheresis procedures were terminated by the project director or on the basis of the fol-
lowing conditions: a target of 22 mL of plasma/kg of donor body weight was harvested, the 20-L capacity of the bag was reached, the donor’s health appeared in jeopardy, the instruments were unable to maintain plasma separation, or there was power loss in the facility. The procedure was ended by pressing the stop button on both instruments. Tubing of the modified disposable collection set was heat sealed to maintain a closed collection system. This allowed the donor and plasma collection bag to be separated from the disposable set; the disposable collection set then was discarded as medical waste.

The collection bag was transported to a separate room for further processing. There, the plasma was homogenized and aseptically placed into 1-L high-density polyethylene bottles. Appropriate labels were applied to the bottles; bottles were stored at −35°C in a continuously monitored freezer.

Donor horses were closely observed throughout the plasmapheresis procedures. Pulse rate, respiratory rate, characteristics of the mucous membranes, and an ECG were recorded at approximately the midpoint and rate, characteristics of the mucous membranes, and an ECG were recorded at approximately the midpoint and end of each plasmapheresis procedure. Donors were administered detomidiner (3 to 6 µg/kg, IV) or detomidine and butorphanols (3 to 6 µg of each/kg, IV) as needed; sedatives were administered IV through an infusion port located in the modified disposable collection set.

At the end of the plasmapheresis procedure, the tourniquet was removed from the neck of each horse. Each horse then received 15 L of fluids IV via gravity flow. Catheters were removed from the jugular veins after fluid administration; hemostasis at the catheterization sites was achieved by use of 4 × 4-inch gauze sponges and slight manual pressure. The donors then were washed, visually inspected, and returned to their designated pastures.

Measurement of total plasma protein concentration—Total plasma protein concentration was determined for each donor within 24 hours before the automated plasmapheresis procedure and 14 days after the procedure, the latter of which corresponded to the evaluation preceding the next donation. A 7-mL blood sample was collected from the jugular vein of each donor into an evacuated blood-collection tube that contained 12 mg of EDTA. Plasma samples from these tubes were used to determine the total plasma protein concentration via refractometry. One refractometer was used to measure all samples throughout the study. The refractometer was calibrated and compared against a known concentration each day before use.

Measurement of total IgG concentration—Plasma samples were obtained from the collection bag of each donor on the day of plasmapheresis. Plasma samples were obtained by use of a 3-mL evacuated blood-collection tube that contained no additives. Plasma samples were evaluated at 14-day intervals (ie, the same interval as for the plasmapheresis procedures). Samples were stored frozen at ≤–20°C until analysis of IgG concentrations. Samples were thawed in a water bath at 37°C for analysis. Total equine IgG concentration was determined by use of a commercially available ELISA kit, which was validated for repeatability and reproducibility. The equine IgG standard, which was derived from purified equine IgG, was validated for accuracy against an equine IgG reference serum. All samples and standards were analyzed in duplicate on a microplate reader with data analysis software.

Statistical methods—Statistical methods for analysis of data in the study included a mixed model for repeated-measures ANOVA and a Dunnett test to compare means. Mean total protein and IgG concentrations determined for subsequent plasmapheresis donations were compared with the mean concentrations determined at the first donation (baseline). Values of P < 0.05 were considered significant. Analysis was conducted by use of statistical software.

Results

Plasmapheresis was performed on the 18 treatment horses at a mean ± SD interval of 15 ± 3 days. During the 5-year study period, a mean of 21 ± 1 mL of plasma/kg was harvested from each donor horse during each plasmapheresis procedure. The automated plasmapheresis procedures lasted between 2 and 6 hours, with most sessions lasting 4 to 5 hours. Donor horses participated in 8 to 19 (mean, 13 ± 3) serial plasmapheresis procedures over the study period. No horses were deferred from participation in plasmapheresis sessions because of a rectal temperature ≥39.5°C or total protein concentration ≤5.5 g/dL. The plasmapheresis procedure was tolerated well by the donor horses, except for 1 restless and anxious horse. Donor horses typically remained calm and infrequently required administration of sedatives during the plasmapheresis procedure. Donors remained in good condition immediately following automated plasmapheresis sessions and during intervals between plasmapheresis sessions.

Table 1—Mean ± SD total plasma protein and total IgG concentrations for 18 donor horses that underwent a minimum of 8 serial plasmapheresis procedures at 14-day intervals.

<table>
<thead>
<tr>
<th>No. of plasmapheresis procedures</th>
<th>No. of donor horses</th>
<th>Total plasma protein (g/dL)</th>
<th>Total IgG (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18</td>
<td>7.49 ± 0.59</td>
<td>6,668 ± 2,160</td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td>7.37 ± 0.41</td>
<td>5,967 ± 2,096</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>7.12 ± 0.34*</td>
<td>5,690 ± 2,144</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>6.99 ± 0.37*</td>
<td>5,242 ± 2,083</td>
</tr>
<tr>
<td>5</td>
<td>18</td>
<td>6.91 ± 0.61*</td>
<td>4,985 ± 2,075*</td>
</tr>
<tr>
<td>6</td>
<td>18</td>
<td>7.16 ± 0.48*</td>
<td>4,884 ± 2,152*</td>
</tr>
<tr>
<td>7</td>
<td>18</td>
<td>6.98 ± 0.48*</td>
<td>5,143 ± 2,571*</td>
</tr>
<tr>
<td>8</td>
<td>18</td>
<td>6.99 ± 0.35*</td>
<td>5,253 ± 2,498</td>
</tr>
<tr>
<td>9</td>
<td>17</td>
<td>6.92 ± 0.46*</td>
<td>5,297 ± 2,138</td>
</tr>
<tr>
<td>10</td>
<td>17</td>
<td>7.02 ± 0.23*</td>
<td>5,047 ± 2,223*</td>
</tr>
<tr>
<td>11</td>
<td>12</td>
<td>6.92 ± 0.45*</td>
<td>5,554 ± 2,318</td>
</tr>
<tr>
<td>12</td>
<td>11</td>
<td>6.84 ± 0.47*</td>
<td>4,993 ± 2,602</td>
</tr>
<tr>
<td>13</td>
<td>8</td>
<td>6.89 ± 0.40*</td>
<td>4,971 ± 2,323</td>
</tr>
<tr>
<td>14</td>
<td>5</td>
<td>6.94 ± 0.13*</td>
<td>4,283 ± 1,454*</td>
</tr>
<tr>
<td>15</td>
<td>4</td>
<td>7.10 ± 0.43</td>
<td>5,187 ± 2,243</td>
</tr>
<tr>
<td>16</td>
<td>4</td>
<td>7.25 ± 0.35</td>
<td>5,745 ± 2,194</td>
</tr>
<tr>
<td>17</td>
<td>3</td>
<td>7.13 ± 0.12</td>
<td>7,914 ± 1,906</td>
</tr>
<tr>
<td>18</td>
<td>2</td>
<td>7.00 ± 0.71</td>
<td>7,479 ± 1,710</td>
</tr>
<tr>
<td>19</td>
<td>2</td>
<td>7.00 ± 0.00</td>
<td>6,397 ± 134</td>
</tr>
</tbody>
</table>

*Value differs significantly (P < 0.05) from the mean concentration at plasmapheresis procedure No. 1.
Mean ± SD total plasma protein concentration for horses was 7.49 ± 0.59 g/dL at the time of the initial plasmapheresis procedure (Table 1). Mean total plasma protein concentration was significantly (P = 0.01) decreased at the third plasmapheresis procedure (7.12 ± 0.34 g/dL). Mean total plasma protein concentration remained significantly decreased through the 14th procedure. The largest decrease in mean total plasma protein concentration was detected at the 12th plasmapheresis procedure, when the concentration decreased significantly (P < 0.001) by 0.65 g/dL to a mean of 6.84 ± 0.47 g/dL.

The mean ± SD total IgG concentration for donor horses was 6,666 ± 2,160 mg/dL at the time of the initial plasmapheresis procedure (Table 1). Compared with this baseline concentration, there was a significant decrease in equine IgG concentration at plasmapheresis procedures 5 (P = 0.02), 6 (P = 0.02), 7 (P = 0.04), 10 (P = 0.04), and 14 (P = 0.03).

**Discussion**

The horses evaluated in the present study represented a subset of horses described in another study conducted by our research group. These particular donors were retrospectively selected for use in the evaluation because their history of participation in the plasmapheresis program best fit the rigorous criteria of the present study. These horses consistently donated the maximum of 22 mL of plasma/kg of donor body weight per harvest, it seemed logical to evaluate horses that consistently donated the plasma protein concentration best fit the rigorous criteria of the present study. Donors were frequently added and removed from the plasmapheresis schedule on the basis of demand to fill orders from the manufacturing company with which we had a contract for the plasmapheresis procedure. Donors occasionally received medical treatment for conditions unrelated to the plasmapheresis procedure. Administered medications were assigned specific withdrawal times, which prevented horses from being used for plasma harvest during this period. Therefore, these donors had longer intervals between successive plasmapheresis procedures. Large horses often yielded enough plasma to fill the collection bag to its maximum capacity (20 L of plasma), which resulted in harvest of < 22 mL of plasma/kg for these horses. Horses selected for inclusion in the study were naive to the procedures or had been rested from plasmapheresis procedures for an extended period. Active donors were rarely ever rested for an extended period.

The automated plasmapheresis procedure was tolerated well by the donor horses. Despite the fact it typically required 2 to 6 hours to complete plasma harvest, only restlessness, foot stomping, signs of mild anxiety, and head shaking were observed. This is in agreement with results reported in other studies on automated plasmapheresis procedures in horses. In those studies, investigators reported only mild restlessness toward the end of the procedure. In contrast to the methods used in those 3 studies, sedatives were administered as needed during the plasmapheresis procedures of the present study to induce mild sedation. Sedation allowed restless, anxious horses to relax and reduce the incidence of stomping and head shaking.

Total plasma protein concentrations in donor horses decreased significantly by the third plasmapheresis procedure and remained significantly decreased through the 14th procedure. Although the decrease in total protein concentration was significant, total protein concentration was always well within the reference interval of 6.0 to 8.5 g/dL for horses. Even more meaningful, the donors’ mean total protein concentration never decreased by ≥ 2 SDs, compared with the baseline concentration. Therefore, it is unlikely that the decrease in total protein concentration was physiologically important. These findings suggest that the 14-day interval between plasmapheresis sessions provided sufficient time for donors to replace lost proteins. This is in contrast to results of a study in which investigators used automated plasmapheresis procedures to remove a similar volume of plasma from first-time donor horses.

Total protein concentration of the donor horses in that study decreased significantly and remained below the reference interval 21 days after a single plasma harvest. The difference in outcome may have been attributable to the age, breed, body weight, diet, exercise level, or geographic location of the horses used in the studies. Mean total protein concentration in horses of the present study was significantly decreased by the third plasmapheresis procedure; however, the plasma protein concentration did not continue to decrease further with successive plasmapheresis sessions. Instead, it remained at the same slightly decreased concentration through the 14th plasmapheresis procedure. Perhaps the donor horses did not have a continued further decrease because the frequent removal of plasma proteins stimulated an increase in synthesis and decrease in catabolism of these proteins, as has been suggested in another study. In addition, donors may have become more efficient at shifting extravascular protein stores to the intravascular space.

Mean IgG concentration in the donor horses initially decreased significantly by the fifth plasmapheresis session. Compared with the baseline IgG concentration, the IgG concentration decreased by 1,701 mg/dL to a mean ± SD concentration of 4,965 ± 2,073 mg/dL. A similar decrease in concentration was detected at plasmapheresis sessions 6, 7, and 10, with decreases of 1,782, 1,523, and 1,619 mg/dL, respectively. The largest decrease in IgG concentration was at the 14th plasmapheresis session, with a decrease of 2,403 mg/dL.

Despite significant decreases in IgG concentration, donors did not have an increase in disease incidence after removal of immunoglobulins. Although the horses had significant decreases in IgG concentration, the total IgG concentration never decreased below the reference interval of 1,000 to 1,500 mg/dL for healthy adult horses. In fact, mean total IgG concentrations for the donor horses remained well above the reference interval throughout the study period. More notably, mean total IgG concentration of donor horses never decreased by
≥ 2 SDs, compared with the baseline concentration. It is reasonable to conclude that the evaluated plasmapheresis program allowed time for donor horses to replace lost IgG and did not place them at an increased risk for development of infectious disease.

For the study reported here, we concluded that automated plasmapheresis procedures in horses performed in accordance with the described protocol did not result in continued further depletion of total plasma protein or total IgG concentrations over time. This plasmapheresis program was used to harvest high-quality, IgG- and protein-rich plasma throughout the entire 5-year study period.

a. Life Design Prime 14, Nutrena, Minneapolis, Minn.
b. Quest gel, Fort Dodge Animal Health, Fort Dodge, Iowa.
c. West Nile Innovator + EWT, Fort Dodge Animal Health, Fort Dodge, Iowa.
d. RabVac 3, Fort Dodge Animal Health, Fort Dodge, Iowa.
e. Fluvac Innovator EHV-4/1, Fort Dodge Animal Health, Fort Dodge, Iowa.
f. Pinnacle IN, Fort Dodge Animal Health, Fort Dodge, Iowa.
g. West Nile Innovator + EW, Fort Dodge Animal Health, Fort Dodge, Iowa.
h. Nolvasan surgical scrub, Fort Dodge Animal Health, Fort Dodge, Iowa.
i. Chloraprep One-Step antiseptic sponge, Medi-Flex Hospital Products, Overland Park, Kan.
j. Hospira Inc., Lake Forest, Ill.
k. Baxter-Fenwal, Lake Zurich, Ill.

References