Establishment of reference values for various coagulation tests in healthy Florida manatees (*Trichechus manatus latirostris*) and evaluation of coagulation in debilitated manatees during rehabilitation

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**Objective**—To establish reference ranges for coagulation parameters in healthy Florida manatees (*Trichechus manatus latirostris*) and compare results with those for debilitated manatees undergoing treatment at a rehabilitation facility.

**Design**—Prospective study.

**Animals**—29 healthy manatees and 45 debilitated manatees with various diseases.

**Procedures**—Manatees considered healthy on the basis of results of physical examination, CBC, and serum biochemical analysis underwent coagulation testing including measurement of prothrombin time, partial thromboplastin time, d-dimer concentration, platelet count, and fibrinogen concentration to establish reference ranges. For comparison, a group of manatees undergoing rehabilitation was also tested, and the results were compared. Thromboelastography was also performed on some animals.

**Results**—Values for d-dimer concentration were significantly higher in debilitated versus healthy animals. There was no significant difference for prothrombin time, partial thromboplastin time, platelet count, or fibrinogen concentration between groups. Thromboelastography was performed on 8 healthy animals.

**Conclusions and Clinical Relevance**—Reference ranges were established for various tests of coagulation that may assist clinicians during the initial evaluation and rehabilitation of Florida manatees. Future research to evaluate the effect of specific disease processes on the coagulation cascade is recommended. (*J Am Vet Med Assoc* 2015;247:1048–1055)
maintained in healthy animals. In response to tissue trauma, procoagulant components dominate to form a stable clot. As tissues heal, anticoagulant and fibrinolytic components dominate to dissolve the clot, allowing restoration of normal blood flow and nutrient delivery to cells. In certain disease processes, this system is compromised, and the imbalance between procoagulant and anticoagulant components may favor either a hypercoagulant or hypocoagulant state. Hypercoagulation can result in widespread thrombus formation, leading to tissue or organ hypoxia and subsequent organ damage or death. Hypocoagulation can predispose to or cause uncontrolled hemorrhage, resulting in severe illness or death. Congenital and acquired clotting factor deficiencies, sepsis, hemolysis, renal disease, neoplasia, inflammation, infectious diseases, toxin ingestion, cardiac disease, and metabolic and nutritional disturbances have all been correlated with the development of coagulopathies in other species.

Cold stress syndrome is a disease process that affects manatees following prolonged exposure to cold water (eg, weeks). Affected animals are lethargic and become emaciated, with varying severity of gastrointestinal, epidermal, and pulmonary disease. Animals with brevetoxinosis become obtunded and develop seizures following exposure to brevetoxins, which are neurotoxins produced naturally by a species of marine plankton called Karenia brevis during algal blooms (ie, red tide) that occur most often in spring. Both disease processes are poorly characterized, but have become leading causes of death in manatees in recent years.

We suspect that coagulopathies exist in manatees with cold stress syndrome and brevetoxinosis on the basis of previous research and clinical experience, and unpublished histopathology records. Given the lack of information on disease processes in Florida manatees and the difficulty associated with applying diagnostic and therapeutic options to such large, wild, aquatic animals, care is largely supportive, with administration of thromboelastography.

**Materials and Methods**

Animals and study design—This study was reviewed and approved by the Animal Care and Use Committee at Tampa’s Lowry Park Zoo and was performed in compliance with institutional guidelines for research on animals.

**Healthy animals**

Twenty-nine healthy Florida manatees underwent various diagnostic tests related to coagulation between August 31, 2011, and October 31, 2013, at Tampa’s Lowry Park Zoo approximately 1 month prior to release back into the wild. All manatees either had recovered from a disease process or were housed at the hospital during rehabilitation of a related individual (cow with injured calf or call with injured cow). Animals were included in the study if they were considered healthy on the basis of results of a physical examination, CBC, and serum biochemical analyses, which were all performed at the same time as the coagulation tests, and if animals were released back into the wild. Animals transferred to other institutions were excluded from the healthy data set because of difficulty in collecting the necessary samples prior to their release back into the wild, a process that often takes years. Wild manatees were excluded from the study because of permit requirements; difficulty in processing, storage, and transfer of samples; lack of information on individual health prior to and following collection of the samples; or a combination of these factors.

**Debilitated animals**

Forty-five manatees undergoing rehabilitation for various disease processes were enrolled in the study from August 31, 2011, through October 31, 2013. Samples were collected from all animals arriving at the hospital during this time period at triage and were included in this study. Some animals were included in both the healthy and debilitated groups because all released animals (healthy group) were sampled at triage (debilitated group). Some animals contributed multiple samples to the debilitated set if they died or were euthanized because they were sampled at triage and at a time point prior to death while still debilitated. Healthy animals contributed to the data set only once. Not every coagulation test was performed on every animal at each sampling. Some debilitated animals contributed several samples used for platelet counts but only 1 sample used for a coagulation profile (PT and PTT) or multiple samples used to measure D-dimer concentration as follow-up to abnormal results.

**Sample collection—**Serum biochemical profiles and CBCs were compared with established reference ranges for Florida manatees. All samples (from both the debilitated and healthy groups) were obtained from the brachial plexus of the left or right foreflipper following standard sterile preparation (alternating alcohol and betadine scrubs). An 18-gauge, 1.5-inch needle or a 22-gauge, 1-inch needle (depending on animal size) connected to a plastic extension set and evacuated container was inserted on the medial aspect of the flipper just dorsal to the ulna for blood collection. Blood was collected into an additive-free evacuated tube or serum separator tube first for banking and serum biochemical analysis. This also allowed clearing of the line and removal of excess tissue factor from the initial veni-
puncture sample. An evacuated tube containing sodium citrate was filled next, followed by an evacuated tube containing liquid sodium–EDTA. All samples were placed on ice and submitted to a commercial veterinary laboratory in the same day for processing, except for the sample used for thromboelastography, which was processed immediately following collection. All tests were performed at the same reference laboratory, except for the α-dimer quantitative test, for which frozen citrated plasma samples were shipped to a specialty laboratory.

Because of scheduling logistics and the need to process samples for thromboelastography within 5 minutes after collection, thromboelastography was not performed for every animal. Instead, healthy animals were chosen at random dependent upon when thromboelastography was available.

**Thromboelastography**—For thromboelastography analysis, 340 μL of citrated, nonactivated whole blood was pipetted into each of 2 thromboelastography cups that had been loaded with 20 μL of calcium chloride, and the test was initiated at 37°C (98.6°F) in accordance with the manufacturer’s instructions. A 5-minute rest time at room temperature (approx 22°C) was used because of logistic difficulties (thromboelastography analyzer and reagents were only available at that facility). Because of scheduling logistics and the need to process samples for thromboelastography within 5 minutes after collection, thromboelastography was not performed for every animal. Instead, healthy animals were chosen at random dependent upon when thromboelastography was available.

Thromboelastography was performed simultaneously on both channels of a single analyzer with associated software to obtain thromboelastographic parameters: R (reaction time [indicates time from activation to the initiation of clot formation]), K (clot formation time), α-angle, MA, and G (global clot strength). The parameters R, K, and α-angle represent the speed of clot formation after initiation, whereas MA represents overall strength and stability of the clot. Global clot strength is calculated from the MA:

\[ G = 5,000 \times \frac{MA}{100 – MA} \]

Although controversial, G can be used as a single measurement to define normocoagulability, hypercoagulability, and hypocoagulability. Each tracing was run for 120 minutes, and then the mean for each measured parameter was calculated from the paired samples for analysis.

**Statistical analysis**—For the healthy population, histograms of all parameters were initially evaluated to help identify outliers. Far outliers were identified by means of the Horn algorithm with Tukey interquartile fences to identify multiple outliers located at the upper and lower extremes. The far outliers identified by this method were then eliminated and reference intervals were calculated. Because of a robust method according to American Society for Veterinary Clinical Pathology Quality Assurance and Laboratory Standards Committee Guidelines for the Determination of Reference Intervals in Veterinary Species, 90% reference intervals were then calculated. Reference intervals were created on the basis of the healthy data set so that results obtained could be used by clinicians for individual animals at rehabilitation facilities. Mean ± SD and 95% CIs for α-dimer concentration, PT, PTT, fibrinogen concentration, and platelet count were all calculated for the debilitated population and for the individual disease processes as a comparison. A comparison of the means for the healthy and debilitated data sets was performed by means of Mann-Whitney–Wilcoxon tests with the aid of software.

Because of the small number of animals in which thromboelastography was performed, thromboelastography data were not statistically analyzed.

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**Table 1**—Results of various tests of coagulation in 29 healthy manatees being released into the wild and 45 debilitated manatees undergoing rehabilitation for various disease processes.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Healthy</th>
<th>Reference interval</th>
<th>Debilitated</th>
<th>Reference interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-dimer (ng/mL)</td>
<td>134.41</td>
<td>42 (0–953)</td>
<td>497.51 ± 688.53</td>
<td>268 (0–3,309)</td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td>383.25</td>
<td>337 (263–516)</td>
<td>309.83 ± 368.05</td>
<td>368 (129–800)</td>
</tr>
<tr>
<td>PT (s)</td>
<td>9.53 ± 1.90</td>
<td>9.2 (7.7–17.4)</td>
<td>9.21 ± 1.45</td>
<td>8.9 (6.5–14.2)</td>
</tr>
<tr>
<td>PTT (s)</td>
<td>15.94 ± 1.63</td>
<td>12.1 (8.1–100)</td>
<td>12.29 ± 3.52</td>
<td>11.4 (8.8–120)</td>
</tr>
<tr>
<td>Platelet count (× 10³/µL)</td>
<td>333.13 ± 220.31</td>
<td>267 (154–992)</td>
<td>418.66 ± 247.69</td>
<td>453 (61–1,125)</td>
</tr>
</tbody>
</table>

**Table 2**—Results of various coagulation tests in debilitated Florida manatees undergoing rehabilitation categorized according to diagnosis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cold stress syndrome</th>
<th>Brevetoxinosis</th>
<th>Trauma (from any source)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-dimer (ng/mL)</td>
<td>830.13 ± 397.12</td>
<td>304 (0–3,300)</td>
<td>705 (45–540)</td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td>383.14 ± 195.07</td>
<td>333.06 ± 430.23</td>
<td>342 (200–484)</td>
</tr>
<tr>
<td>PT (s)</td>
<td>4.96 ± 1.43</td>
<td>4.7 (12.7–19.6)</td>
<td>3.97–15.13</td>
</tr>
<tr>
<td>PTT (s)</td>
<td>10.13 ± 1.40</td>
<td>11.4 (10.8–12.8)</td>
<td>5.8 ± 7.41</td>
</tr>
<tr>
<td>Platelet count (× 10³/µL)</td>
<td>329.21 ± 271.62</td>
<td>583 (360–520)</td>
<td>NA</td>
</tr>
</tbody>
</table>

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**Notes:**

- IQR = Interquartile (25th to 75th percentile) range.
- NA = Not applicable.
- See Table 1 for remainder of key.
Results

Twenty-nine healthy manatees and 45 debilitated animals were included in the study. The total length of the animals ranged from 120 to 334 cm (median, 258 cm), and body weight ranged from 40 to 1,081 kg (88 to 2,378.2 lb; median 262 kg [576.4 lb]). There were 17 adults (6 males and 11 females), 6 subadults (1 male and 5 females), and 22 calves (15 males and 7 females), on the basis of the US Geological Survey’s Sirenia Project age classification guidelines (animals with a straight length > 265 cm were considered adults, animals between 235 and 265 cm were considered subadults, and animals < 235 cm were considered calves).

Coagulation testing results for both healthy and debilitated animals were summarized (Table 1). True reference intervals were calculated for the healthy animals, whereas mean ± SD and 95% CIs are reported for the debilitated animals and their related disease processes. For the healthy animals, because of some individual outlier values for D-dimer, PT, and PTT in particular, the mean for these parameters exceeded the 90% upper reference limit. The debilitated set was further divided to display values for specific disease processes (Table 2). A nonparametric test was used to compare the healthy and debilitated populations, and a significant \( P < 0.001 \) difference in D-dimer concentration between healthy and debilitated animals was found, with higher concentrations observed in the latter. A significant difference was not found for PT, PTT, platelet count, or fibrinogen concentration for healthy versus debilitated animals (Table 3). Thromboelastography was performed on 8 samples from 8 healthy animals (Table 4; Figure 1).

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Median</th>
<th>Interquartile (25th–75th percentile) range</th>
<th>Z approximation</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-dimer concentration (ng/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>29</td>
<td>42</td>
<td>0.7–155.7</td>
<td>–3.83</td>
<td>0.001</td>
</tr>
<tr>
<td>Debilitated</td>
<td>43</td>
<td>268</td>
<td>101.3–626.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>22</td>
<td>337</td>
<td>319.8–379.5</td>
<td>–0.49</td>
<td>0.622</td>
</tr>
<tr>
<td>Debilitated</td>
<td>22</td>
<td>352</td>
<td>304.6–385.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PT (s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>29</td>
<td>9.2</td>
<td>8.5–9.8</td>
<td>0.26</td>
<td>0.786</td>
</tr>
<tr>
<td>Debilitated</td>
<td>37</td>
<td>8.9</td>
<td>8.27–10.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTT (s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>28</td>
<td>12.1</td>
<td>10.9–13.35</td>
<td>0.51</td>
<td>0.609</td>
</tr>
<tr>
<td>Debilitated</td>
<td>37</td>
<td>11.4</td>
<td>10.37–13.53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet count ( (\times 10^3 \text{ platelets/µL}) )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>13</td>
<td>267</td>
<td>171.7–470.0</td>
<td>–1.18</td>
<td>0.239</td>
</tr>
<tr>
<td>Debilitated</td>
<td>32</td>
<td>468</td>
<td>224–583.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are the mean of 2 test results. — = Not available. R = Reaction time to initial fibrin formation K = Kinetic time for clot formation. \( \alpha \) Angle = Angle from baseline to slope of tracing (represents clot formation). MA = Maximum amplitude of tracing (measures platelet number and function). G = Global clot strength. LY30 = Percentage of clot lysis at 30 minutes after maximum amplitude (MA). LY60 = Percentage of clot lysis at 60 minutes after MA.

Canine reference intervals\(^{36} \) (95% CI) are included for comparison. Only 8 healthy animals underwent thromboelastography because of limited availability of equipment.

Table 3—Comparison of values for various tests of coagulation in healthy released manatees and debilitated manatees undergoing rehabilitation.

Table 4—Thromboelastography results run in duplicate for 8 healthy manatees.
In the present study of coagulation parameters in healthy and debilitated Florida manatees, reference ranges were established for various tests that can be used to evaluate animals for coagulopathies at triage and during rehabilitation. A significant difference in D-dimer concentration was found between healthy and debilitated animals. To our knowledge, there is only 1 previous report\(^2\) that describes any aspect of coagulation in manatees. Medway et al\(^2\) compared the concentrations of individual clotting factors from 10 West Indian manatees (Trichechus manatus) with those of domestic dogs. That study\(^2\) found that the clotting factor activities of the intrinsic and extrinsic systems were higher and lower than those of dogs, respectively. Medway et al\(^2\) also noted that biologists reported sirenian blood clotted more rapidly than blood of other species. We have made the same observation. Elephants, the closest relative of manatees, have also been reported to have higher clotting factor concentrations detected on assay compared with humans.\(^3\,^7\)

Rehabilitation centers for endangered Florida manatees have been established throughout the state. Although there is a paucity of antemortem literature on manatee health, pre- and postmortem cases of disease processes with a propensity to cause coagulopathies including sepsis, renal disease, neoplasia, inflammation, infectious diseases, and metabolic and nutritional disturbances have been reported in manatees.\(^4\,^6\,^20\,^21\,^38\,^41\)

Inflammation, infectious disease, and metabolic and nutritional disturbances are hallmarks of cold stress syndrome in manatees.\(^5\,^11\,^46\) Inflammation and hemorrhage are the predominant findings on postmortem examination of manatees that die of brevetoxicosis, with hemorrhage also observed in live animals.\(^20\,^43\,^45\) Both cold stress syndrome and brevetoxicosis were determined to be leading causes of death in recent years.\(^2\,^3\) Karenia brevis blooms (ie, red tide) in the southwest region of Florida have led to declaration of 6 unusual mortality events since 1996, with the largest brevetoxicosis-related die-off on record occurring in 2013.\(^1\) In that year, 36.3% (170/466) of all manatee deaths in which a cause of death was able to be determined were attributed to brevetoxicosis.\(^1\) Furthermore, unusual mortality events were declared following the extremely cold winters of 2010 and 2011 after cold stress syndrome was determined to be the cause of death in an outstanding number of animals (44.1%; 145/329 deaths in which a cause of death could be determined).\(^2\) Given the increase in manatee deaths from both disease processes in recent years, we suggest that research into these diseases including the underlying pathophysiological mechanism should be a priority. Understanding the potential role of a coagulopathy in these diseases has implications in both understanding the pathophysiology and identifying possible therapeutic options for animals at rehabilitation facilities.

In the present study, data for animals deemed healthy on the basis of results of physical examination, a CBC, and serum biochemical analysis were used to create reference ranges for various coagulation tests. These reference intervals were then compared with findings in animals debilitated because of illnesses identified at triage (Tables 1–3). Only D-dimer concentrations were significantly different between healthy and debilitated animals. The 95% CIs for D-dimer concentration established for each disease process, excluding brevetoxicosis, did not overlap with the reference intervals. D-dimer concentration is a measurement of fibrin degradation and is elevated in hypercoagulable states.\(^46\) Increased D-dimer concentration has also been used as an early marker for sepsis and disseminated intravascular coagulation in domestic species.\(^46\,^47\) A cross-comparison of each disease process with the normal data set is beyond the scope of this study. However, it should be noted that the D-dimer concentration was lowest in animals with brevetoxicosis, where hemorrhage, not thrombus formation, has been identified on pre- and postmortem evaluations and in other species with brevetoxicosis.\(^20\,^22\) Bossart et al\(^2\) found that 52% of 149 animals determined to have died from brevetoxicosis had evidence of hemorrhage and congestion on necropsy. Hemolysis has also been described in various species with brevetoxicosis and has been shown to be a risk factor for the development of coagulopathies.\(^10\,^22\,^43\,^45\) Interestingly, the highest D-dimer concentrations in the present study were observed in animals with cold stress syndrome. This aligns with our suspicion (which is based on clinical signs and postmortem findings) that hypercoagulation and possible disseminated intravascular coagulation exist in those animals.\(^45\)

Fibrinogen concentration, platelet count, PT, and PTT were not found to significantly differ between healthy and debilitated animals in the present study. In human patients, it has been reported\(^9\) that fibrinogen
concentration may remain normal even in the most severe thromboembolic states such as disseminated intravascular coagulation. It has also been described as an insensitive marker of inflammatory disease processes in manatees.52 Platelet count increases in the presence of chronic inflammation; platelets release micrbidial proteins in addition to providing scaffolding for transmigration of WBCs.46,47 Interestingly, no overlap was observed between the reference intervals for platelet counts of healthy animals and those with cold stress syndrome in this study. Animals with cold stress syndrome had consistently higher platelet counts, compared with healthy animals, a finding that may be a direct result of the chronic inflammation associated with cold stress syndrome. Further research into cold stress syndrome is suggested to evaluate whether platelet count significantly differs in sick animals, compared with that in healthy animals, and whether an identified difference is clinically relevant.

Prothrombin time and PTT are generally used exclusively for identifying disorders of hypocoagulation and monitoring response to anticoagulant therapy.50 Prolongation of PT or PTT is often associated with abnormalities in coagulation, leading to bleeding disorders.50,51 Traditionally, a decreased PTT is attributed to inappropriate sample collection, handling, or storage.50,51 More recently, it has been reported that decreased PTT may correlate with a number of other disease processes, such as neoplasia, diabetes, thromboembolic events, and myocardial infarctions.50,51 Thus, understanding PTT as it relates to specific disease processes may be crucial in understanding coagulation in manatees. Prothrombin time and PTT are limited in that they represent only distinct parts of a very complex coagulation pathway.51,52 They are also measured to test clotting ability in relation to external reagents in the absence of cellular blood components and vascular endothelium, and they provide no information on clot strength or resolution.52

Thromboelastography provides information on many aspects of coagulation in whole blood, including clot formation, clot strength, and clot dissolution, and may therefore be a more useful diagnostic test for identifying clinical hypercoagulation, compared with traditional coagulation tests such as PT and PTT. Briefly, whole blood is placed in a cup and added to a thromboelastography machine so that a pin approximates the center of the sample. The cup is gently oscillated, and as fibrin strands form within the sample, they cause movement of the pin that is translated into a graph.58 Although few animals (n = 8) underwent thromboelastography in this study, preliminary data were suggestive of hypercoagulability, compared with published reference ranges59–61 for humans and dogs53 (Figure 1; Table 4). Thromboelastography was performed without an activator in samples obtained from manatees after only 5 minutes of incubation. In dogs, kaolin activation leads to hypercoagulability during tracing.51 However, in companion animals, kaolin or tissue factor activation is recommended for thromboelastography analysis to reduce interassay variability unless thromboelastography is performed in duplicate,64 as was done in this study. Our prior experience with thromboelastography in manatees led to the modification in rest time and the decision to use blood without an activator because we had difficulty with overly quick and robust initial clot formation when longer rest times and activators were used. We have theorized that this is due to the relatively high concentration of factor XII present in manatees,59 but this requires more investigation. Further studies are needed to determine whether thromboelastography is a good choice in a clinical setting for identifying disorders of coagulation and fibrinolysis in manatees, and standardized protocols for analysis should be created.

This study had several important limitations. Ideally, > 40 animals would be used to establish reference intervals64; however, because of the nature of working with wild species, this was not practical for our study. Furthermore, validation of coagulation assays has not been established for Florida manatees. Assays developed for use in humans have been used successfully in various mammalian species, and evidence exists that coagulation functionality is considerably conserved among mammals,56–59 yet the results reported here must be interpreted with caution. To mitigate the effects a small sample size has on reference intervals, the guidelines established by the American Society for Veterinary Clinical Pathology34 were closely followed. Potential subjects were selected on the basis of strict criteria, sampling procedures were standardized, and all samples were handled carefully and submitted to the same reference laboratory.

Future research should focus on further characterizing coagulation in animals with various disease states, compared with coagulation in healthy animals. We are currently working on identifying the concentration of various clotting factors and continuing to collect data on various tests of coagulation in both rehabilitated and debilitated manatees. This research could lead to more effective therapies for manatees with coagulopathies and a better understanding of the pathophysiology of the most common disease processes encountered at rehabilitation facilities.

References
AQUATIC ANIMALS


51. Lippi G, Salvagno GL, Ippolito L, et al. Shortened activated par-
AQUATIC ANIMALS


From this month’s AJVR

Iron metabolism following intravenous transfusion with stored versus fresh autologous erythrocyte concentrate in healthy dogs
Virginie A. Wurlod et al

Objective—To determine effects of IV transfusion with fresh (3-day-old) or stored (35-day-old) autologous erythrocyte concentrate on serum labile iron concentration, iron-binding capacity, and protein interaction with iron in dogs.

Animals—10 random-source healthy dogs.

Procedures—Dogs were randomly assigned to receive autologous erythrocyte concentrate stored for 3 days (n = 5) or 35 days (5). One unit of whole blood was collected from each dog, and erythrocyte concentrates were prepared and stored as assigned. After erythrocyte storage, IV transfusion was performed, with dogs receiving their own erythrocyte concentrate. Blood samples were collected from each dog before and 5, 9, 24, 48, and 72 hours after transfusion. Serum was harvested for measurement of total iron, labile iron, transferrin, ferritin, hemoglobin, and haptoglobin concentrations.

Results—For dogs that received fresh erythrocytes, serum concentrations of the various analytes largely remained unchanged after transfusion. For dogs that received stored erythrocytes, serum concentrations of total iron, labile iron, hemoglobin, and ferritin increased markedly and serum concentrations of transferrin and haptoglobin decreased after transfusion.

Conclusions and Clinical Relevance—Transfusion with autologous erythrocyte concentrate stored for 35 days resulted in evidence of intravascular hemolysis in healthy dogs. The associated marked increases in circulating concentrations of free iron and hemoglobin have the potential to adversely affect transfusion recipients. (Am J Vet Res 2015;76:996–1004)