Published in final edited form as:
J Vet Intern Med. 2011 ; 25(4): 927–933. doi:10.1111/j.1939-1676.2011.0737.x.

In Vitro Lysis and Acute Transfusion Reactions with Hemolysis Caused by Inappropriate Storage of Canine Red Blood Cell Products

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Abstract

Background—Transfusion of red blood cell (RBC) products carries considerable risk for adverse reactions, including life-threatening hemolytic reactions.

Objective—To report the occurrence and investigation of life-threatening acute transfusion reactions with hemolysis in dogs likely related to inappropriate blood product storage.

Animals—Four dogs with acute transfusion reactions and other recipients of blood products.

Methods—Medical records were reviewed from 4 dogs with suspected acute hemolytic transfusion reactions after receiving RBC products at a veterinary clinic over a 1-month period. Medical records of other animals receiving blood products in the same time period also were reviewed. Blood compatibility and product quality were assessed, subsequent transfusions were closely monitored, and products were diligently audited.

Results—During or immediately after RBC product transfusion, 4 dogs developed hemolysis, hemoglobinuria, or both. Two dogs died and 1 was euthanized because of progressive clinical signs compatible with an acute hemolytic transfusion reaction. Blood type and blood compatibility were confirmed. RBC units from 2 blood banks were found to be hemolyzed after storage in the clinic's refrigerator; no bacterial contamination was identified. After obtaining a new refrigerator dedicated to blood product storage, the problem of hemolyzed units and acute transfusion reactions with hemolysis completely resolved.

Conclusions—Acute life-threatening transfusion reactions can be caused by inappropriate storage of RBC products. In addition to infectious disease screening and ensuring blood-type compatibility, quality assessment of blood products, appropriate collection, processing, and storage techniques as well as recipient monitoring are critical to provide safe, effective transfusions.

Keywords

Anemia; Blood banking; Shock; Transfusion medicine

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Ensuring the safety of blood products is of utmost importance in both human and veterinary blood banking and transfusion medicine. There are several different causes of transfusion reactions, and the clinical manifestations of these reactions vary from acute to delayed, immune-mediated or nonimmune-mediated, and hemolytic or nonhemolytic. Most transfusion reactions occur during or shortly after transfusing a patient and range from subclinical and transient to severe and life threatening. The successful management of transfusion reactions can be challenging because no established successful protocols exist in human or veterinary medicine. Transfusion reactions can be minimized by performing blood typing and compatibility testing between donor and patient, infectious disease screening of donors, and by instituting appropriate blood collection, processing, storage, and administration techniques.[1–7] Storage conditions and length of blood product storage can substantially impact product quality and potentially lead to increased red blood cell (RBC) fragility with in vitro and in vivo hemolysis.[8–12]

We report 4 dogs seen at the Animal Specialty Center (ASC) in Yonkers, NY, that were transfused within a month of one other and developed acute, life-threatening transfusion reactions with hemolysis, either during or shortly after RBC transfusions.

Methods

Study Design

The initial investigation was a simple retrospective review of medical records from all dogs that received transfusions during the time period in which an increased incidence of transfusion reactions occurred. The medical records from 4 dogs suspected to have experienced acute hemolytic transfusion reactions were reviewed in depth and are reported here. The apparent clustering of 4 cases of suspected acute hemolytic transfusion reactions within 4 weeks of one other triggered a more thorough investigation involving the Transfusion Laboratory at the University of Pennsylvania's School of Veterinary Medicine (PennGen), and included a thorough evaluation of in-house transfusion materials, techniques, and product quality control. PennGen performed additional diagnostic testing on blood samples from stored blood units as well as a blood sample from 1 patient drawn posttransfusion to determine whether bacterial contamination or immunologic reactions had occurred. Fluid pumps[a,b] (2 of each type used to deliver blood products at ASC; total of 4 pumps), transfusion sets,[c] filters,[d] and fluid lines were evaluated for the possibility of damaging RBCs during blood delivery. Conditions in the refrigerator used to store blood were examined, and all stored blood units were rigorously monitored and evaluated closely before being transfused into patients. In addition, more rigorous monitoring of patients was instituted during and after transfusion.

Diagnostic Investigation of Potential Transfusion Reactions

Laboratory Analysis—At initiation of the investigation, all 3 remaining canine blood units stored at ASC, as well as serum and heparinized whole blood from the most recent case (Case 1, collected several hours posttransfusion), were submitted overnight (chilled) to PennGen for further analysis, including blood type compatibility (gel column typing method[e,13] and Coombs-enhanced major crossmatch test[14]), bacterial contamination (blood smear and 16S bacterial RNA polymerase chain reaction [PCR]), and in vitro lysis by assessing plasma hemoglobin concentration after microcentrifugation in capillary tubes.[f,15]

[a]Vet-Pro VIP 2000, Caesarea Medical Electronics Ltd, Lichtenstein, Germany
[b]Heska Vet IV 2.2, Heska, Loveland, CO
[c]Hospira Blood Set, Lake Forest, IL
[d]Hemonate filters, Utah Medical, Midvale, UT
[e]DiaMed, Cressier, Switzerland

In-house prothrombin time (PT) and partial thromboplastin time (PTT) were performed on an SCA 2000 [g] with normal reference ranges of 11–17 and 70–102 seconds, respectively.

Quality Control

Blood Products—At the time of the potential transfusion reactions, ASC obtained its blood products from only 1 commercial blood bank.[h] The blood bank regularly sent blood products (stored whole blood [SWB] and packed red blood cells [pRBCs]) to ASC, which then were stored in a refrigerator until used or discarded when past the stated expiration date (for canine products 35 days from collection as labeled by the manufacturer). All RBC products were stored in the bottom crisper drawer of a multipurpose refrigerator, [i] in which drugs and laboratory supplies also were stored, and to which all hospital staff had access. Blood units were laid flat, frequently stacked on top of one another, and occasionally kept separately in unsealed plastic bags.

Until the cause of the acute hemolytic reactions could be identified and corrected, blood products were obtained from the Penn Animal Blood Bank (PABB) and stored in the same refrigerator used for the blood products suspected in the acute hemolytic reactions. Blood product storage was moved from the crisper drawer to the middle shelf and blood units were kept upright and separated by cardboard. Temperature on this shelf and in the crisper drawer was monitored and logged every 2 hours for a 48-hour period. Routine temperature monitoring occurred twice daily for the remainder of the study period.

More rigorous surveillance of blood products was initiated, including evaluating the units and tube segments (pigtails) for any gross in vitro lysis (centrifuging aliquots in a microcentrifuge) before their administration. If gross hemolysis was noted, the unit was removed from inventory and submitted to PennGen for testing.

Transfusion Materials and Techniques—Administration materials and technique, including fluid pumps[a],[b],[j] used for blood administration, were evaluated for potential mechanical injury to RBCs. Stored units of pRBCs were run through pumps at variable rates to mimic a typical transfusion while being handled and processed through the same type of tubing and filters as were typically used at ASC (standard transfusion sets[c] for volumes > 50 mL; Hemonate filters[d] for volumes ≤ 50 mL). Samples were evaluated for visual evidence of gross hemolysis after centrifugation of a microhematocrit tube at 15-minute intervals throughout the procedure.

Patient Monitoring—Routine monitoring of all animals receiving blood products (heart rate, respiratory rate, and rectal temperature every 15 minutes) was continued. Transfusions were to be discontinued and the patient further evaluated if reactions were suspected (increase of rectal temperature ≥ 2°F or any other adverse signs were observed). In addition, 1 hour into and 1 hour after each transfusion, EDTA blood from the patient was obtained, centrifuged in a microhematocrit tube and evaluated grossly for hemolyzed plasma. If gross hemolysis was present, the transfusion was to be discontinued and the remainder of the unit submitted to PennGen for further testing.

[f] Hemocue, Lake Forest, CA
[g] SCA 2000, Synbiotics, Kansas City, MO
[h] Animal Blood Bank, Dixon, CA
[i] Whirlpool 18 cu. ft. Top Mount Refrigerator, Whirlpool Corp, Benton Harbor, MI
[j] Medfusion 2001, NOVATEK Medical, Effingham, IL

Results

Case Studies

Case 1—A 10-year-old female spayed (FS) Siberian Husky underwent exploratory laparotomy with liver lobectomy to remove 2 hepatocellular carcinomas. Before surgery, PCV, PT, and PTT[g] were within the normal reference range and excessive hemorrhage was not noted during surgery. The abdomen was lavaged thoroughly, and a closed suction drain was placed. The dog was postoperatively stable but progressive anemia (PCV 19%) necessitated transfusion the next day. The dog was determined to be DEA 1.1-positive by an in-house card typing method,[k] which was confirmed by the gel column typing method[e],13 at PennGen. Because the previous transfusion history was unknown, a major crossmatch[l] was performed and the patient was transfused 10 mL/kg of DEA 1.1-negative, crossmatch-compatible pRBCs over 4 hours with a regular transfusion administration set.[c] The compatible crossmatch results later were confirmed at PennGen with a Coombs-enhanced major crossmatch test.[14] Routine monitoring showed the dog to be stable and normothermic throughout the transfusion. The PCV 1-hour posttransfusion increased appropriately (PCV of 26%) and no hemolysis was observed. Five hours after completion of the transfusion, the dog's temperature increased from 102 to 104.5°F (38.9–40.2°C), pigmenturia and hemolyzed plasma were noted, and the patient's PCV was again 19%. A few hours later, the patient developed melena, hematemesis, and hemorrhage around the IV catheter sites, warranting reevaluation of coagulation parameters. The PT and PTT were now prolonged (21 seconds [reference range, 11–17 seconds] and 179 seconds [reference range, 70–102 seconds], respectively) and the patient was estimated to be thrombocytopenic (approximately 40–60,000/μL) based on microscopic blood smear evaluation. Differential diagnoses included surgical complications or a hemolytic transfusion reaction. The patient received 10mL/kg fresh frozen plasma, 2 mg/kg diphenhydramine IM, and 0.25 mg/kg dexamethasone IV. The PT and PTT normalized by the next day; however, the patient's clinical status continued to decline. The dog developed progressive azotemia and hypoxemia (thoracic radiographs disclosed bilateral diffuse pulmonary infiltrates) that was refractory to oxygen supplementation. Thirty-six hours after receiving the pRBCs, the patient suffered cardiopulmonary arrest.

Case 2—A 12-year-old FS Dachshund with a history of hypothyroidism and hyperadrenocorticism developed hematochezia. Medications included levothyroxine 0.1mg PO q12h and selegiline HCl 10mg PO q24h. Hematologic and biochemical analyses revealed pan-hypoproteinemia, spherocytic, regenerative anemia (PCV 12%), marked polychromasia, and thrombocytopenia (19,000/μL). Before referral, the patient received a fresh whole blood transfusion (patient and donor blood type and transfusion volume unknown). On presentation to ASC, the dog was hemodynamically stable and had a PCV of 22% with straw-colored plasma. Immune-mediated thrombocytopenia with anemia secondary to gastrointestinal hemorrhage or Coombs-negative immune-mediated hemolytic anemia and concurrent immune-mediated thrombocytopenia (Evans syndrome) were differential diagnoses. Despite treatment with prednisone, cyclosporine, and famotidine, the dog became progressively more anemic (PCV 16%) with persistent but improving thrombocytopenia (platelet count 40,000/μL) by day 3. The patient was administered two 10mL/kg transfusions of DEA 1.1-negative and crossmatch-compatible pRBCs on days 3 and 4 through a filter[d] with a syringe pump,[j] resulting in the expected increase in PCV and no overt clinical reactions.

[k]RapidVet-H Canine DEA 1.1, DMS Laboratories, Flemington, NJ
[l]RapidVet-H CM, DMS Laboratories

On day 6, the dog developed acute worsening of hematochezia, progressive anemia (PCV 14%), and thrombocytopenia (3,000/μL). The dog was transfused with 30mL/kg DEA 1.1-negative, crossmatch-compatible SWB. During the transfusion, the dog developed severe hyperthermia (105°F; 40.5°C), which persisted despite discontinuation of the transfusion and administration of 2.2mg/kg diphenhydramine IM. Although the dog's hemodynamic status remained stable, hemolyzed plasma and pigmenturia were noted shortly thereafter. Two hours after cessation of blood administration, the dog developed hypoxemia secondary to pulmonary infiltrates, which was only partially oxygen and furosemide responsive. Despite continued supportive care, the dog developed hyphema, generalized petechiation, and ecchymoses, as well as progressive dyspnea and was euthanized 36 hours after receiving the last transfusion.

Case 3—A 10-year-old FS Toy Poodle was evaluated for vomiting, regenerative anemia (PCV 18%), severe thrombocytopenia (5,000/μL), marked hyperbilirubinemia (12.1 mg/dL; reference range, 0.1–0.4 mg/dL), severely increased serum liver enzyme activity and hypoalbuminemia (1.9 g/dL; reference range, 2.7–4.0 g/dL), but normal PT and PTT. Ultrasonographically, the liver appeared enlarged and diffusely mottled with a distended gall bladder and hyperechoic mesentery in the surrounding area; no other abnormalities were found. A presumptive diagnosis of immune-mediated hemolytic anemia and thrombocytopenia was made, and the dog was treated supportively with famotidine, ursodiol, Denamarin,[m] vitamin K_1, and immunomodulatory therapy (dexamethasone and cyclosporine).

By day 7, the thombocytopenia had resolved (238,000/μL) and the anemia improved (PCV 26%) without transfusion. Exploratory laparotomy identified a severely distended, erythematous, and edematous gall bladder, with dilated cystic and common bile ducts, a large firm green-discolored liver, and an infarcted, ischemic spleen. Splenectomy, cholecystectomy, and hepatic biopsy were performed without complication. Postoperatively, the dog developed hematochezia and worsening anemia (PCV 16%) and was given approximately 12mL/kg of DEA 1.1-negative SWB through a filter[d] with a syringe pump.[j] No crossmatch was performed because the dog had not been previously transfused. Routine monitoring showed no evidence of an adverse reaction during blood product administration.

Approximately 10 hours after completing the transfusion, the dog became hypoxemic, tachycardic, hyperthermic (103°F; 39.4°C), and developed frequent vomiting refractory to antiemetic therapy. Evidence of intravascular hemolysis was observed (hemoglobinemia and hemoglobinuria). The PT was within the normal reference range (16 seconds), but the PTT was prolonged (138 seconds). Despite aggressive supportive care with fluids, oxygen, and antiemetics, the dog's clinical status continued to decline and cardiopulmonary arrest occurred 24 hours after the SWB transfusion.

Case 4—A 10-year-old male castrated mixed breed dog was diagnosed with chronic pure red cell aplasia (PCV 14%, erythroid hypoplasia on bone marrow evaluation). The patient had no prior transfusion history and received 10 mL/kg of DEA 1.1-negative pRBCs without a crossmatch. Routine monitoring showed no evidence of an adverse reaction during blood product administration. Approximately 30 minutes after transfusion, the dog collapsed and became minimally responsive, hyperthermic (103.8°F; 39.9°C), and tachycardic. At that time, the PCV was 21% and the plasma appeared hemolyzed. The dog was resuscitated with a 20 mL/kg crystalloid fluid bolus IV, dexamethasone (0.25 mg/kg IV), and diphenhydramine (2 mg/kg IM). The dog recovered uneventfully and was discharged the

[m]Nutramax Laboratories Inc, Edgewood, MD

next day. At time of discharge the PCV was 20% and serum bilirubin concentration (previously normal) was increased at 3.4 mg/dL with no other clinically relevant changes on the serum biochemistry panel. Reevaluation 1 week after the incident showed a stable nonregenerative anemia (PCV 21%), mildly increased hepatocellular enzyme activities similar to pretransfusion values, and a normal serum bilirubin concentration.

Diagnostic Investigation Findings

At PennGen, the 3 pRBC units from the initial source were confirmed to be DEA 1.1-negative by gel column typing method.[e],13 The posttransfusion blood smear from Case 1 revealed few crenated RBCs and neutrophilia with few toxic changes, but no bacteria were seen. Furthermore, 16S bacterial RNA PCR tests of the recipient's blood and the 3 units were negative for bacterial contamination.[15] In-house evaluation of centrifuged microhematocrit tubes from blood run through the filters and pumps used at ASC did not reveal any gross evidence of lysis. Free hemoglobin concentrations of the 3 submitted pRBC units were increased at 0.6, 1.0, and 1.5 g/dL, respectively (reference range, < 0.4 g/dL).[7] Moreover, plasma from Case 1 collected shortly after the transfusion reaction appeared grossly hemolyzed, and its free plasma hemoglobin concentration was 1.9 g/dL, suggesting in vitro or intravascular hemolysis of the donor (or less likely recipient) erythrocytes or both.

Over the 2-month time period surrounding these events, 6 additional dogs received 9 transfusions (7 pRBC, 2 SWB units) at ASC, without any adverse reactions. Each dog demonstrated clinical improvement and had the projected increases in PCV posttransfusion. In the same time period, 4 cats received 6 pRBC transfusions, also without any apparent adverse reaction, and demonstrated appropriate clinical improvement and increases in PCV posttransfusion. However, no additional analyses of these specific units or the recipients' blood or plasma samples were performed.

The pRBC units subsequently purchased from PABB also were found to be grossly hemolyzed after 14 days of storage on a middle shelf in the original refrigerator at ASC. These units were not hemolyzed before shipment to ASC and had not reached their expiration date by the time lysis was noted. In contrast to the original units, they were kept upright, separated by cardboard and were gently rotated every other day. Stored pigtails from these units kept at PABB from the time of collection also were analyzed when the units were returned and did not show any hemolysis. When analyzed, the temperature in the initial blood storage refrigerator at ASC was found to be between 3 and 5°C (37.4–41°F) on the shelf and 1.5–3°C (34.7–37.4°F) in the crisper drawer; additional fluctuations could not be ruled out because there was no continuous monitoring device attached to the unit.

A refrigerator[i] dedicated to the storage of blood products then was purchased, and improved maintenance and storage protocols were implemented. The new refrigerator is kept locked and opened only to access or survey blood products. A thermometer is positioned on the bottom shelf of the refrigerator to monitor internal temperature, which is evaluated daily. The temperature varies between 3 and 5°C (37.4 and 41°F). All blood products are stored upright and separated by cardboard on refrigerator shelves, rather than being laid flat in drawers. All blood units are rotated and gently mixed every other day. Since instituting these RBC product storage changes, as well as vigilant pretransfusion blood type and compatibility testing when indicated, 108 RBC units from the original blood bank[h] have been stored and transfused to 82 dogs at ASC over a 1-year period. Neither hemolyzed units nor hemolytic transfusion reactions have been observed.

Discussion

This clinical report describes acute, fatal, or life-threatening, nonimmune-mediated transfusion reactions with hemolysis in 4 dogs over a short period of time, most likely because of an RBC product storage problem. All of the recipient dogs in this report showed evidence of markedly hemolytic plasma and pigmenturia during or within several hours after a blood transfusion, supporting a diagnosis of acute hemolytic transfusion reaction. The presence of free hemoglobin in plasma and urine indicate that either lysed RBCs were transfused or intravascular hemolysis (either fragile cells or in vivo antibody and complement mediated hemolysis) occurred after transfusion of the cells, however, hemolysis secondary to underlying disease could have contributed.

Acute hemolytic transfusion reactions generally are the most severe transfusion reactions and usually are because of blood type incompatibilities. Such reactions generally are only observed in previously transfused dogs (≥ 4 days) receiving RBC products of mismatched blood type.[2,16–18] In the cases reported here, only DEA 1.1-negative blood was transfused and a crossmatch was performed in all recipients that had been transfused previously or if transfusion history was unknown, to ensure compatibility before transfusion. Thus, blood type incompatibility seemed to be an unlikely cause of the hemolysis. A blood type incompatibility reaction cannot completely be ruled out because this recipient had many prior transfusions, possibly increasing sensitization to blood groups not routinely tested for, but the pretransfusion crossmatch test was compatible. No further investigations were done after the transfusion reaction occurred, because appropriate samples were not available.

Acute transfusion reactions with hemolysis that do not have an immunologic basis include those caused by RBC damage from aging, bacterial contamination, osmotic injury, mechanical injury, or thermal injury.[19,20] RBCs are sensitive to osmotic forces and will rapidly lyse if exposed to hypotonic fluids. Thus they should be delivered separately or with isotonic solutions such as 0.9% NaCl, and not in hypotonic, glucose-, or calcium-containing solutions.[2] In the cases described here, no other fluids or medications were administered through the same line during the transfusion. Thus, such a technical error was not thought to contribute to the cluster of adverse transfusion events.

Mechanical injury secondary to inappropriate blood collection or transfusion techniques also can lead to RBC damage or lysis. Kinked, narrow, or twisted tubing, and small bore needles or catheters all can cause shear injury to RBCs.[7,20] In addition, filters that have too narrow a pore size or become clogged, or blood that is delivered too rapidly or with too much force, can cause considerable injury to RBCs.[7,20] Some fluid pumps also have been shown to lyse RBCs.[21] One of the pumps used at ASC is approved for use[e] with blood products, but the other has not been approved[f] because of inaccurate volumetrics (not damage to RBCs). There should be no concerns with syringe pumps because the tubing is not compressed during use. By re-creating a typical transfusion set up with the transfusion techniques and equipment used at ASC, we did not discover any visible hemolysis in centrifuged microhematocrit tubes collected after passing blood through our pumps and filters; thus mechanical damage from fluid pumps or excessive pressure during delivery was considered as an unlikely cause of the reactions. Although a kinked catheter, kinked tubing, or clogged filter could cause RBC damage in individual cases, this was not considered a likely cause of the cluster of reactions because the ensuing investigation discovered hemolyzed blood bags after storage before administration.

Bacterial contamination of blood products can induce damage to RBCs and in vitro lysis.[15] RBC units from the same blood bank source, stored in a similar fashion to the units that caused the above described reactions did not show any evidence of bacterial contamination

by PCR techniques or blood smear. In addition, the RBC units did not turn black, which can be a sign of bacterial contamination. Thus bacterial contamination seemed to be an unlikely cause of the in vitro lysis observed.[15]

Blood products containing RBCs (pRBC or SWB) have a short lifespan and are marked with a maximal expiration date (usually 28–35 days for canine RBCs), at which point storage lesions may compromise the viability of the cells.[22,23] Even with additional preservative solutions and optimal storage conditions, the quality of RBC products declines with time.[8,24,25] There is a direct relationship between storage time and the amount of free hemoglobin in RBC products.[26] Some data even suggest that the age of blood has an impact on outcome of critically ill or injured recipients.[9–12] The amount of free hemoglobin should not exceed 0.4 g/dL or 0.8% of total hemoglobin, even at the end of storage time.[7] At ASC, any unused RBC products are discarded at the time of the expiration date (as determined by the supplying blood bank) in order to ensure that expired blood is not transfused into any hospitalized animals. In the investigation reported above, the RBC units started to show gross hemolysis well before their expiration date of 35 days from date of collection. The patients that had the severe reactions likely were transfused similarly damaged or hemolyzed blood products, although most of these units were not available for testing to confirm this suspicion. In Case 1, the fact that there was no hemolysis present on the patient's centrifuged PCV tube 1-hour posttransfusion is hard to explain if the blood was hemolyzed in the blood bag. This unit may not have been hemolyzed in the bag, but rather damaged enough to cause hemolysis or rapid clearance in vivo shortly after transfusion.

As in human medicine, animal blood banks have stringent quality control procedures to assure high quality products that include thorough donor screening for systemic and infectious disease as well as aseptic collection techniques with closed human blood collection systems.[15,27,28] Moreover, special insulated shipment containers and expedited shipment (usually overnight) are used to minimize damage from environmental conditions. Based on our investigation and the standard operating procedures at both blood banks, neither collection nor shipment seemed to be a contributing factor to the transfusion problems.

The fact that blood from 2 different sources lysed during initial storage conditions in a standing refrigerator, but remained in good quality after acquiring a separate refrigerator dedicated to blood storage strongly suggests that the conditions in the original all-purpose refrigerator resulted in in vitro lysis, potentially because of temperature cycling. Irreversible membrane damage that results in impaired deformability and increased osmotic fragility occurs if RBCs are heated to temperatures ≥ 40°C (104°F), which ultimately leads to lysis or rapid clearance of damaged RBCs by the reticuloendothelial system of the spleen and liver.[4,6] In practice, similar effects can be seen if unmonitored warming baths are used to bring RBC units to room temperature before transfusion, but warming baths are not routinely used at ASC. Conversely, freeze-thaw cycling of RBC products without a cryoprotective agent such as glycerol or DMSO can cause membrane damage secondary to intracellular ice crystal formation.[29] When measured, the internal temperature of the refrigerator was within the acceptable range for blood products both in the crisper drawer and on the shelves.[23] However, because this analysis was retrospectively performed it may not accurately reflect conditions in the refrigerator at the time the reactions were occurring. Variations in temperatures in the refrigerator (both because of freeze-thaw cycling and constant opening and closing of the door) could have directly led to in vitro lysis or RBC membrane fragility resulting in additional in vivo hemolysis. Blood that was stored for longer periods in these conditions would likely have suffered more RBC membrane damage than newer units. Unfortunately, expiration dates of transfused units were not available in the records of the cases reported here, and therefore the age of blood in patients that had

fatal reactions could not be compared with that of patients that received blood without apparent reactions. Furthermore, the possibility that expired units were transfused into these patients cannot be completely ruled out.

There are few reports of accidental transfusions of hemolyzed blood in the human literature.[30,31] In 1 case, 2 units of inadvertently frozen pRBCs were administered to a man with sideroblastic anemia who later developed hemoglobinemia and hemoglobinuria, but did not experience any blood type incompatibility or other adverse reactions.[30] In another report, 2 separate cases of intraoperative autologous transfusion resulted in delivery of hemolyzed blood.[31] Both patients developed hemoglobinemia and hemoglobinuria, and 1 also developed severe consumptive coagulopathy; both patients survived to discharge.

How lysed blood can cause a transfusion reaction still is not well understood. The effects of lysed blood on systemic and pulmonary arterial blood pressure were studied in healthy dogs. Marked but transient increases were noted in pulmonary systolic and diastolic pressure, while systemic arterial pressure showed a marked but transient decrease, mainly in the diastolic component.[32] In a canine hemorrhagic shock model (induced by phlebotomy), 1 group served as a control, while the other received 2 mL/kg of hemolyzed (frozen and thawed) autologous blood.[33] Both groups were resuscitated after the study period in the same manner, but only the dogs in the control group survived. The dogs that died after receiving hemolyzed blood had prolonged PT, PTT, and lower platelet counts when compared with control dogs, suggesting a consumptive coagulopathy, but the precise cause of death was not determined. In the cases reported here, all recipients displayed serious transfusion reactions and 3 of 4 dogs died or were euthanized. Interestingly, coagulation parameters were abnormal posttransfusion in 2 of 4 dogs, similar to the dogs in the hemorrhagic shock model. Although the patients reported here received blood with a presumably lesser degree of hemolysis than in the hemorrhagic shock model, they did all have serious underlying illnesses that may have contributed to the severity of the reactions.[10–12,33,34] Likewise, it is unclear why these 4 dogs had severe reactions whereas 15 other transfusions were completed in the same time period without any obvious adverse reactions. As with major blood type incompatibilities in humans, there can be great variation in the degree of reaction in dogs and cats, from no overt signs to anaphylaxis and death. It is possible that other units also were lysed when administered but did not cause serious clinical signs or such signs were mistaken for those of the recipients' underlying illness. Additionally, although we were not able to evaluate age of blood units transfused to these dogs, it is possible that they received older units than other patients that did not experience reactions. Longer exposure to inappropriate storage conditions may have resulted in increased levels of hemolysis in blood units transfused to these dogs. Regardless of the underlying mechanism or mechanisms, the fact that no additional reactions were seen once blood storage conditions were modified with use of a dedicated refrigerator and regular monitoring and turning of units, suggests that inappropriate storage conditions led to the cluster of severe transfusion reactions.

Much has been written in veterinary medicine about preventing acute hemolytic transfusion reactions by minimizing blood type incompatibilities, but this report is the first to implicate blood product storage as a cause of severe, acute transfusion reactions, and it highlights the importance of diligence in handling blood products from collection to transfusion.

To decrease the risks associated with improper storage, the authors recommend adhering to strict storage guidelines. According to the American Association of Blood Banks Technical Manual and standards for animal blood products, RBC products should be stored in a refrigerator that is kept between 1 and 6°C (34–42°F).[23,28] Ideally, the refrigerator should be dedicated exclusively to blood product storage and have a continuous temperature

monitoring device with an alarm. If this is not possible, a dedicated space in a high quality refrigerator used for other purposes may be acceptable in some circumstances; however, it is crucial to keep blood separated from other products so as to avoid chemical and biological contamination. If the refrigerator does not provide a continuous monitoring device, a reliable nonmercury thermometer should be placed inside the refrigerator to allow monitoring of the actual internal temperature. The refrigerator should be opened as infrequently as possible to prevent temperature fluctuations and damage to RBC products. Blood units should be stored upright in a vertical position (or hung) with airspace between each bag and units should be gently rotated every other day to allow mixture of RBCs with nutrients in anticoagulant media.[23,27,28] Blood products should be stored on refrigerator shelves and never in crisper drawers. Lastly, expired blood products should be promptly removed from the storage unit and detailed logs should be kept of all inventories with expiration dates, dates of blood administration, and recipient name. At ASC as well as PABB, blood bag color and plasma color (centrifuged PCV) are monitored before transfusing RBC units and 1 hour into the transfusion as additional quality control measures.

Acknowledgments

The authors acknowledge the clinicians and nursing staff at ASC as well as the PABB nurses for their management of patients and assistance with the investigations.

Abbreviations

ASC	Animal Specialty Center
FS	female spayed
PABB	Penn Animal Blood Bank
PCR	polymerase chain reaction
PennGen	School of Veterinary Medicine Transfusion Laboratory, University of Pennsylvania
pRBC	packed red blood cells
PT	prothrombin time
PTT	partial thromboplastin time
RBC	red blood cell
SWB	stored whole blood

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