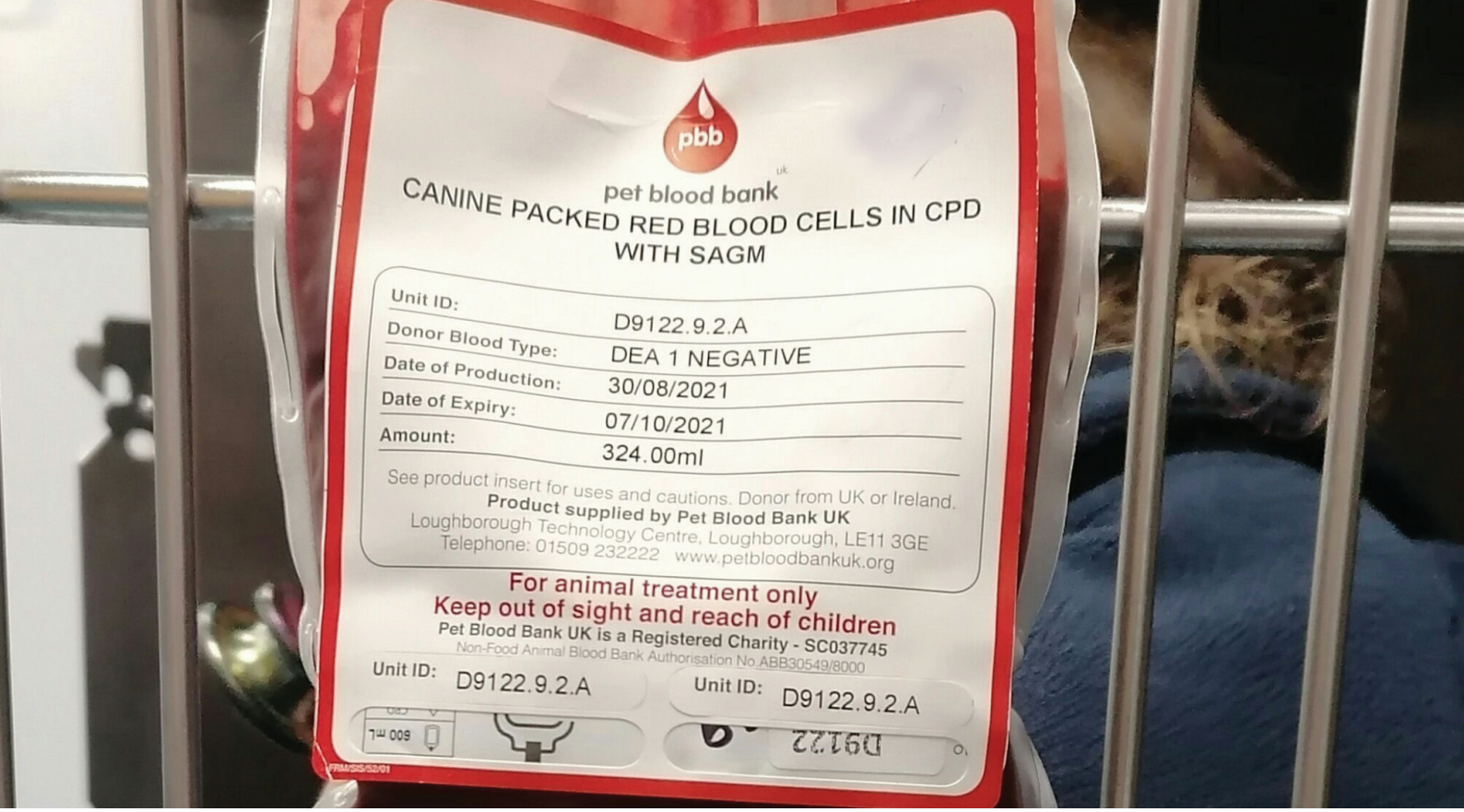

The RVN's role in reducing the risk of packed red blood cell transfusion reactions in dogs

Katie Peates, RVN, BSc (Hons), DipHE, CVN, DipAVN (SA)

The College of Animal Welfare and Southern Counties Veterinary Specialists

katie.peates@scvetspecialists.co.uk

Katie graduated with a first-class honours degree in Veterinary Nursing from Middlesex University (in partnership with The College of Animal Welfare) in 2015, before joining the field of referral nursing where she specialised in internal medicine. In 2019, she gained her Advanced Veterinary Nursing diploma with distinction from Myerscough College. Katie's main clinical interests include transfusion medicine, hepatobiliary and endocrinology. She is also passionate about teaching and now teaches veterinary nursing full time on the diploma and degree pathways at The College of Animal Welfare, as well as working as a senior wards RVN in a referral practice at the weekend.

ABSTRACT The author carried out a literature review to analyse research into packed red blood cell (PRBC) transfusions and associated reactions, with the aim of updating practice protocols on the safe handling, storage and administration of PRBCs to patients. The information examined how RVNs can assist in reducing the risk of transfusion reactions by keeping up to date with current research. In this article, the author reviews three key causes of blood-transfusion reactions (blood typing and cross-matching, storage and handling, and storage lesion), with the intention of increasing awareness among RVNs so they can prioritise and implement care that optimises patient wellbeing.

Keywords blood transfusion, packed red blood cells, cross-matching, storage lesion, erythrocyte antigens, transfusion medicine

Introduction

Transfusion medicine has evolved greatly and RVNs are heavily involved in the storage (**Table 1**), handling and administration of packed red blood cells (PRBCs). PRBC transfusions can save the lives of patients suffering from symptomatic anaemia, but they are not without risk (**Figure 1**, overleaf).

Among canine patients, 3–28% have a fatal reaction caused by the administration of a blood product (Blois, 2016). Whether this is immunological or non-immunological, the body will respond in one way or another (**Table 2**).

RVNs can take appropriate steps to avoid transfusion risks to their patients by staying up to date with evidence-based practice, implementing updated strategies and understanding how blood and its components work.

What is blood?

Blood is a fluid connective tissue found within the blood vessels of the circulatory system. It consists of blood cells and plasma, which is itself comprised of water, mineral salts and plasma proteins. They each carry out a specific task contributing to the overall role of blood (Aspinall & Cappello, 2009).

Plasma is composed of 90% water, within which many substances (including carbon dioxide, glucose, mineral salts and amino acids) are dissolved and then transported around the body. Together, they act as buffers to maintain the osmotic balance and internal pH of the blood, which allows the body's processes to function effectively.

In addition, plasma proteins (large proteins unable to filter out of circulation) ensure fluid is retained within the blood vessels, allowing regulation of osmotic pressure by osmosis. Plasma proteins play a major role in the control of blood pressure and blood volume.

Table 1. Storage of blood products.

Blood product	Storage temperature	Duration of storage	Additional notes
Whole blood	2–6°C	8 hours maximum	
Packed red blood cells	2–6°C	42 days (or until expiry date)	• Gently agitate bag once a day to ensure sufficient mixing of red blood cells and anticoagulant and preservative solutions • Do not store in plastic bags
Fresh frozen plasma	–18°C	1 year (then stored as frozen plasma)	• Store in a protective padded bag (usually provided with the unit) to prevent damage
Frozen plasma	–18°C	4 years	• As above

Table 2. Classifications of transfusion reactions that can occur during or after a PRBC transfusion.

Acute immunological	Acute non-immunological
• Anaphylaxis (type I hypersensitivity) • Acute haemolytic (type II hypersensitivity) • Febrile, non-haemolytic • Transfusion-related acute lung injury (TRALI)	• Haemolysis (e.g. due to incorrect storage of blood unit, or administration – incorrect giving set use) • Embolism • Citrate toxicity • Bacterial contamination • Transfusion-associated circulatory overload (TACO)
Delayed immunological	**Delayed non-immunological**
• Haemolytic (3–5 days post transfusion)	• Blood-borne Infectious agents (more likely in feline patients, e.g. FIV/FeLV)

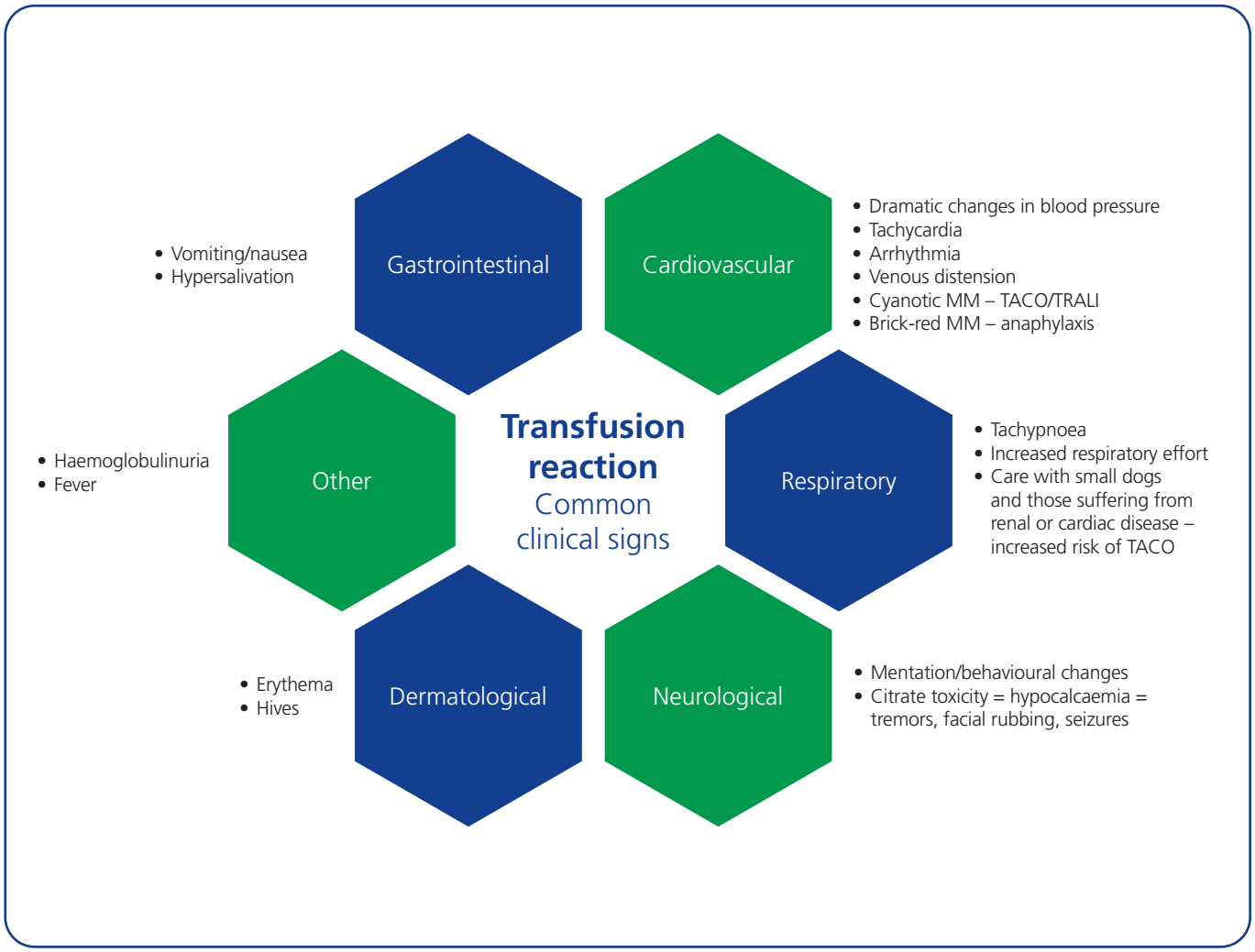

Figure 1. Transfusion reaction – common clinical signs. MM, Mucous membranes; TACO, transfusion-associated circulatory overload; TRALI, transfusion-related acute lung injury.

Table 3. Potential causes of anaemia (Mills, 2000). RBC, Red blood cell.

Increased RBC loss	Increased RBC destruction (haemolysis)	Decreased production of RBCs
• Acute/chronic haemorrhage	• Immune-mediated haemolytic anaemia • Pyruvate kinase deficiency • Onion ingestion • Zinc toxicosis • Snake bite • Disseminated intravascular coagulopathy (DIC) • Metastatic tumour • Heartworm • Babesiosis	• Nutritional deficiencies • Drugs (e.g. some cytotoxic drugs) • Chronic disease • Leukaemia • Multiple myeloma • Renal disease • Hepatopathies • Endocrine disease

The solid components of blood are the blood cells, known as erythrocytes, leukocytes and thrombocytes. Erythrocytes, also referred to as red blood cells (RBCs), are the most abundant blood cell and are responsible for the transport of oxygen to the tissues. A decline in the number of circulating erythrocytes is defined as anaemia and ongoing loss will eventually lead to decreased tissue oxygen consumption (Garcia & South-Bodiford, 2012). In current practice, PRBC transfusions are commonly used to treat patients that are anaemic, whether this is from immune-mediated disease, acute haemorrhage or decreased production of erythrocytes (Walker, 2016) (**Table 3**). It should be noted that deciding to transfuse a patient with PRBCs based upon laboratory results alone is not recommended. However, a transfusion may be necessary if the patient shows signs of hypoxia due to anaemia (Yagi, 2021) (**Table 4**).

Table 4. Clinical signs of anaemia (Yagi, 2021).

Clinical signs of anaemia
• Tachycardia
• Tachypnoea
• Increased respiratory effort
• Pale mucous membranes
• Weakness/lethargy
• Dull mentation

Leukocytes are less numerous compared with erythrocytes but they play a major role in the body's defence against infection, and function closely with the body's immune system (Aspinall & Cappello, 2009). The immune system's function is to protect the body against infectious organisms and, for it to perform effectively, recognition molecules must recognise the presence of a pathogen by analysing a potential invader and distinguishing it as being either self or non-self.

Through evolution, the immune system has developed to allow for adaptation, so species and individuals can create their own recognition molecules, tailored by the pathogens they have already encountered (Playfair & Bancroft, 2013). This is carried out by a type of leukocyte termed a lymphocyte. Once a lymphocyte has recognised an antigen, it will proliferate and instruct a response, which can include the release of antibodies or cytokines, or cytotoxicity. Once complete, a number of these proliferated cells will remain circulating throughout the blood and tissues as memory cells, allowing the individual to respond faster and more aggressively during repeat exposure to the specific antigen.

Blood typing and cross-matching

Blood typing identifies the presence of a specific antigen on the surface of the animal's RBCs. Cross-matching assesses whether any components of a recipient's blood will react with any of the donor's blood. Blood typing and cross-matching recipients against potential donors can prevent immunological transfusion reactions by avoiding the administration of incompatible blood products (Walton & Tappin, 2017). Type I and type II hypersensitivity and acute haemolytic transfusion (AHR) reactions are examples of immunological responses in which a patient's immune system triggers antibody release against the mismatched antigen (Yagi, 2021). Cross-matching is achieved by visually inspecting for agglutination during sample mixing (Webb, 2019).

DEA ANTIGEN

In dogs, the most significant blood types have been assigned the prefix DEA (dog erythrocyte antigen) (Kendon & McMichael, 2020). Webb (2019) details that it is the antigen DEA 1 that is most likely to cause an AHR in patients that are originally DEA 1 negative and is therefore the most clinically significant. As a result, in-house canine blood-typing kits have been developed to determine the presence of the DEA 1 antigen, allowing practices to administer initial blood transfusions safely depending on the patient's DEA 1 status (**Figure 2**).

Figure 2. Canine blood-typing kit.

Naturally occurring alloantibodies to any blood type are rare in canine patients and, even if they do exist, their risk of causing an AHR is low, so it is common practice to use the term 'universal donor' when describing patients with a DEA 1 negative status. However, because patients' immune systems will develop alloantibodies to all foreign antigens in the days after an initial transfusion, consequently increasing the risk of delayed reactions or serious complications during subsequent transfusions, use of the term 'universal donor' should be void (Zaremba et al., 2019).

THE DAL ANTIGEN

In 2007, Blais et al. (2007) suspected a new RBC antigen not yet described in veterinary medicine, as a result of a dalmatian developing specific antibodies due to a sensitisation of unknown origin during an initial transfusion. The new antigen was named the Dal antigen. Blais et al. (2007) theorised dalmatians to be the only breed to test negative for the Dal antigen and predisposed to acute transfusion reactions if transfused blood from a Dal-positive donor.

This theory was recently disputed by Goulet et al. (2017) in a clinical trial that set out to determine the mode of inheritance and prevalence of the Dal antigen among other dog breeds in North America. Their study consisted of 1,130 dogs: 128 dalmatians, 432 dobermanns, 21 shih tzus and 549 dogs of other breeds. This was an extremely sizeable sample, representing a large number of breeds to eliminate the risk of chance on concluding results (Crombie, 1996). The results found an increased incidence of dalmatians (12%), dobermanns (42%) and shih tzus (57%) testing negative for the Dal antigen. Furthermore, three of the 122 mixed-breed dogs also tested negative. Sex, coat colour and DEA 1 status did not have any effect on the Dal status of the patient. However, within this study, geographical area did vary, in particular with dobermanns, which coincides with an additional discovery by Goulet et al. (2017) of the Dal antigen being of a dominant inheritance.

The Goulet et al. (2017) study raised some challenges relating to Dal-negative blood donors, of which RVNs should be cautious. First, of the 1,130 dogs tested within the study, 228 of them were already enrolled on a blood donation programme. However, 227 of the 228 dogs tested positive for the Dal antigen, making them unsuitable donors for Dal-negative patients. Second, none of the core breeds testing negative for the Dal antigen (dalmatians, dobermanns and shih tzus) make ideal candidates for blood donating, due to either their size, temperament or predisposition to diseases such as von Willebrand disease and cardiomyopathies. Therefore, blood banks and veterinary practices may find it difficult to recruit Dal-negative blood donors, amplifying the risk of these patients developing acute transfusion reactions at subsequent blood transfusions if not cross-matched beforehand.

There are currently no in-house blood-typing test kits available for the Dal antigen so, for the safety of a patient receiving successive transfusions, cross-matching is highly recommended. This is supported by the Goulet & Blais (2018) clinical study, which set out to better characterise anti-Dal antibodies in terms of their rate of production and agglutination titres following sensitisation. Two Dal-negative beagles received a unit of Dal-positive blood. Post transfusion, one of the dogs developed antibodies against the Dal antigen after 21 days, the other after only 4 days. This supports current recommendations (Pet Blood Bank, 2021) to cross-match patients a minimum of 4 days after receiving a transfusion in order to detect levels of antibodies sufficient for a cross-match result that is as accurate as possible. Neither of the two beagles displayed signs of a transfusion reaction. However, immune-mediated transfusion reactions are rarely seen during the first transfusion, due to dogs not possessing naturally occurring blood antigen antibodies (Hohenhaus, 2004), so a reaction was unlikely to be seen. To determine the real clinical effect of developing antibodies to the Dal antigen in these beagles, it would require a second transfusion to be given. However, this would be unethical due to the theorised risk based on previous blood antigen-antibody related transfusion reactions.

Further research is required to study the genuine clinical significance of Dal antibodies and their risk of provoking a transfusion reaction. However, as RVNs, we must promote the new evidence found in the field of canine transfusion medicine and negate the use of the term ‘universal donor’ as this is no longer true. RVNs involved in administering PRBCs should be aware of breeds that may be more susceptible to blood transfusion reactions, due to the formation of blood antigen-antibody complexes.

KAI 1 AND KAI 2 ANTIGENS

In addition to the Dal antigen, two new RBC antigens – Kai 1 and Kai 2 – are currently being investigated in regard to their clinical importance. Again, this is after the development of an unanticipated blood-transfusion reaction. Much like the Dal antigen, no commercial test kits are yet available to test for Kai 1 or Kai 2, and therefore cross-matching is strongly recommended. The ongoing improvement in technology will ultimately result in more erythrocyte antigens being discovered, which only amplifies the necessity for RVNs to keep up to date with evidence-based research to ensure they provide the best care to their patients.

Storage and handling

The integrity and efficacy of a unit of PRBCs is reliant on the holding temperature, aseptic handling during storage and the age of the unit post donation (Mansell & Boller, 2016) (**Figure 3**). Currently, canine PRBC units are stored for up to 42 days. This is in accordance with human-medicine guidelines from the US Food and Drug Administration (FDA) because, at present, there are no legal stipulations on storage duration for canine PRBCs in the UK (Obrador et al., 2015). Refrigeration of PRBC units reduces the rate of RBC metabolism. This can increase the length of storage time by reducing RBC fragility and decreasing bacterial growth (Kisielewicz, 2016). The risk of bacterial contamination in human PRBC products is low, but donor bacteraemia and contamination during processing and storage has been documented (Hillyer et al., 2003).

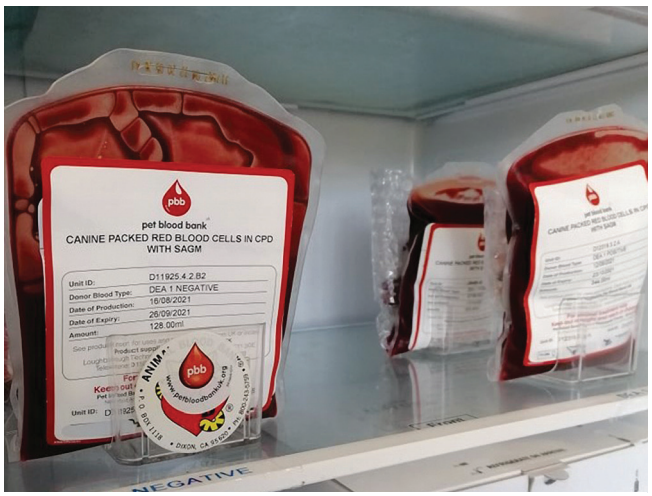

Figure 3. Storage of PRBCs.

Although little research in this area has been conducted within veterinary literature, it is presumed that these mechanisms are similar with canine patients, reiterating the importance of following strict aseptic techniques during the blood donation process and wearing gloves at all times when handling blood products. The presence of bacteria within a blood unit at collection is concerning but the proliferative rate of the bacteria while in storage, which could potentially lead to septic complications if administered, should also be contemplated (Hillyer et al., 2003).

Miglio et al. (2016) conducted a study to identify bacterial microorganisms in whole blood products and revealed that low amounts of bacteria had no clinical significance or effect on triggering a transfusion reaction. Nevertheless, the authors suggested that storing blood products for shorter periods of time would benefit patients by reducing the amount of time bacteria are given to proliferate.

Of the units that tested positive for bacterial contamination in the Miglio et al. (2016) study, none showed any gross visual changes. In contrast, in a study conducted by Stefanetti et al. (2016), all four contaminated PRBC units showed distinct colour changes from light red to dark red and almost black appearance. These units tested positive for the bacteria *Serratia liquefaciens* and *Pseudomonas* spp. (both of which are commonly found in the environment and on patients' skin) on the 20th, 31st and 32nd day of storage – which are towards the latter end of the recommended storage time.

The contrast in these two studies clearly demonstrates that visual changes are not always apparent, so RVNs need to remain vigilant and should regularly inspect units in an attempt to detect any changes. The findings of bacteria in older units also suggests that the current guidelines of storing PRBC units for 42 days may need evaluating as, even though these studies suggest that low-yield bacteria do not induce a transfusion reaction, older units still have the potential to induce post-transfusion bacterial sepsis, which would be detrimental to patients. If at all concerned, test units for bacterial contamination prior to transfusing.

Storage lesions

In human and veterinary medicine, there have been growing concerns about the age of PRBC units and the risk of a transfusion reaction due to RBC storage lesions (SLs). These are biochemical and biomechanical changes that occur during PRBC storage, which compromise function and stability when transfused to a patient (Hann et al., 2014). Wilson et al. (2017) aimed to describe the biochemical changes that occur in canine PRBCs during a 42-day storage period. Unfortunately, the study had a very small sample size, weakening the significance of its findings. However, many of the findings did correlate with current human review literature (D'Alessandro et al., 2015). Continually increasing concentrations of both ammonia and lactate were seen during storage, alongside a decrease in pH and glucose. Conversely, the concentration of potassium was considerably lower in canine PRBCs compared with human PRBCs. Nevertheless, all concentrations of the tested analytes changed significantly, many within the first 21 days of storage, which may have considerable effects if transfused to a critically ill patient.

The clinical impact of these changes in certain disease conditions is yet to be fully understood. However, due to hepatic involvement in the clearance of lactate from the body and the conversion of ammonia into the less toxic urea, veterinary surgeons and RVNs should be cautious about administering older PRBC units to patients with hepatic disease. It may be sensible to transfuse fresher blood to these patients, as well as to patients with metabolic disturbances, to reduce the risk of worsening the condition by transfusing blood with increased concentrations of lactate.

Within the past 10 years, two retrospective studies have sought to determine whether the age of PRBC units, and possible SL processes within, increase the risk of a patient suffering an acute transfusion reaction and whether they have an effect on mortality and morbidity rates. Hann et al. (2004) retrospectively analysed 3,095 dogs that had received PRBC for various clinical conditions and found no difference in mortality rates of patients that received fresher versus older PRBC units in the overall canine population. However, it was noticed that patients with pre-occurring haemolysis (e.g. immune-mediated haemolytic anaemia), were more at risk of not surviving to discharge ($P = 0.024$). This coincides with the more recent study by Maglaras et al. (2017), which, in addition, suggests that patients receiving older units are more at risk of developing transfusion-related reactions, with fever (12.3%) and transfusion-related haemolysis (6.3%) being the most common. These did not necessarily lead to death of the patient.

Interestingly, both studies found an association between older units and an increased risk of progressive coagulopathies. Maglaras et al. (2017) theorised that this may be due to increased levels of free-circulating haemoglobin within the unit during storage, which can lead to an increased risk of thrombosis and vasculitis, both of which can cause consumption of coagulation factors (Garcia & South-Bodiford, 2012). This is yet to be determined but warrants further investigation as thrombotic disease could have damaging effects on a patient's outcome. Both studies also found a link between larger volumes of transfused PRBCs and an increased risk of multiple organ dysfunction syndrome (MODS), along with an increased transfusion reaction risk. Maglaras et al. (2017) questioned whether a more conservative transfusion strategy should be implemented to decrease these risks, but it should be queried why these patients are receiving larger units. It may be that patients requiring larger transfusion volumes are more at risk of developing conditions such as MODS due to their underlying conditions. This will, undoubtedly, increase the risk of a transfusion reaction due to the high numbers of circulating inflammatory factors already present (Playfair & Bancroft, 2013).

Further research is required to determine new methods of storing PRBC units and reducing SL. In the interim, RVNs should be aware of the changes that could occur in PRBC units while in storage and ensure that any effects of SL are promptly noticed and treated to improve the outcome for their patient. In some cases, RVNs may still question whether, ethically, PRBC products that have undergone substantial metabolic changes should be transfused to patients that are already physiologically compromised.

Recommendations for further study

It is clear from the research reviewed that further research into transfusion medicine is warranted to further protect patients from fatal transfusion reactions. There is a risk of bacterial-contamination of PRBC units, which can induce sepsis, even if an aseptic technique has been performed (Miglio et al., 2016; Stefanetti et al., 2016), so further research is required to evaluate the safest level of bacteria that can be administered in a transfusion without inducing a transfusion reaction.

In addition, to optimise patient safety, consideration should be given to updating guidelines on monitoring blood units while in storage. It is clear from the aforementioned studies that the regularly documented gross colour changes observed with bacterial contamination are not see as often as originally presumed, and perhaps bacteria causing colour changes differ depending upon the type of blood product stored (e.g. whole blood vs PRBC). It is also possible that the current recommended storage period increases the risk of bacterial contamination, as bacteria are given more time to reproduce. The 42-day storage period also provides more time for further biochemical changes to occur. However, shortening storage times could lead to veterinary practices reducing their stock levels and blood banks shortening expiration dates on their products, which could lead to excessive wastage and stock shortages. Current literature on the effects of storage length, SL and their involvement in blood transfusion reactions are all retrospective studies, so original clinical studies are needed to assess the true relationship between these variables.

The newly discovered Dal antigen and its dominant genetic aspect and geographical significance within North America warrants additional studies in other geographical areas, including the UK and other parts of Europe, to determine the prevalence of the Dal antigen in these areas. In addition, further studies into the clinical significance of Dal, Kai 1 and Kai 2 antibodies and their effect on provoking a transfusion reaction are warranted.

Recommendations for veterinary practice

Emerging research demonstrates that correct storage and monitoring of PRBCs is key to reducing the risk of a transfusion reaction. Therefore, veterinary practices that store blood products should ensure the daily implementation of protocols for the monitoring of storage temperature and duration, and the visual checking of products. The correct use of personal protective equipment (PPE) should be included in these guidelines to minimise the risk of bacterial contamination.

Due to advancements within transfusion medicine, it is not unheard of for a patient to have a transfusion history. However, with new RBC antigens being discovered, a patient's risk of reaction to subsequent blood transfusions increases, and current in-house blood-typing kits may not be enough to reduce the risk. Practices should therefore update their protocols to make cross-matching mandatory for all subsequent PRBC transfusions and familiarise themselves with how to carry out this procedure.

There are gaps in research into the clinical significance of the development of antibodies due to sensitisation to an unknown antigen and their role in eliciting a transfusion reaction. On completion of studies in this area, blood banks may have to revise their protocols for testing the blood antigen status of blood donors, to ensure all blood groups are accommodated. Due to the possibility of a breed predisposition among the newly discovered RBC antigens, a shortage of donors testing negative for either Dal, Kai 1 or Kai 2 may occur. There could be a reliance on littermates and family members to donate blood, as research suggests a strong dominant gene inheritance for these antigens. It may become difficult for veterinary practices to purchase safe, cross-matched units for their patients so they may need to create their own blood donor register, listing donors by antigen status to ensure safe transfusions.

Conclusion

PRBCs are a valuable resource and are life-saving in many medical situations. However, their administration has the potential to cause transfusion reactions that can have a serious negative impact on a patient's recovery.

Risks can be reduced by RVNs through careful PRBC storage, aseptic handling and by performing blood typing and cross-matching on patients prior to transfusion. RVNs with an awareness of the clinical signs of a transfusion reaction, and an understanding of risk-elevating factors such as breed type or disease status, will be able to respond more rapidly in the event of an emergency, improving the overall prognosis of their patient.

Guidelines surrounding the storage and administration of blood products are constantly being updated and RVNs involved in transfusion medicine should make a conscious effort to keep up to date with new research, and be prepared to change practice protocols if they feel it is necessary to protect their patients.

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