Case Report

Acute normovolaemic haemodilution in a Clydesdale gelding prior to partial resection of the left ventral concha under general anaesthesia

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Summary

A 16-year-old Clydesdale gelding underwent oral extraction of tooth 210 under standing sedation with partial resection of the left ventral concha under general anaesthesia 4 days later. Due to the highly vascular nature of the surgical site, significant intraoperative haemorrhage was anticipated. A lack of compatible donor horses confirmed by crossmatching prompted an autologous donation of 6 l of blood and acute normovolaemic haemodilution using a gelatine solution immediately prior to surgery. Intraoperative haemorrhage was estimated at 5% circulating volume and autologous transfusion was commenced once haemostasis was confirmed. The horse recovered uneventfully from general anaesthesia and surgery. To our knowledge this is the first report of acute normovolaemic haemodilution used in a clinical equine case.

Introduction

Equine whole blood transfusion is a well-recognised procedure and may be required in circumstances of severe intraoperative blood loss. In some types of surgery (e.g. sinus surgery) significant haemorrhage may be anticipated and provision made for appropriate replacement products to be available (Durham 1996). Donor horses may be prepared for blood collection or alternative blood products such as modified bovine haemoglobin or packed red cells may be sourced for use. Most equine erythrocyte antigens are weakly immunogenic and repeated transfusions are usually required to increase their immunogenic potential. Exceptions to this are the Aa, Ca and Q antigens, which are reported to be the most antigenic. Ideally, horses intended to be used as blood donors should be blood typed and determined to be negative for these antigens (Tocci and Ewing 2009). Generally a single allogenic blood transfusion is tolerated in horses without the need for pretransfusion testing, but major and minor crossmatching is indicated if time allows or if serial transfusions are anticipated (Hart 2011). In cases where equine allogenic blood transfusion is unavailable or incompatible, the administration of modified bovine haemoglobin may be considered but is financially costly in mature horses. Autologous transfusion in the emergency setting using blood salvaged at the haemorrhage site has been successful in dogs (Hirst and Adamantos 2012) and horses (Finding *et al*. 2011; Fouché *et al*. 2014) but is contraindicated in the presence of infectious or neoplastic disease and may require specialised equipment.

In human medicine, the technique of preoperative autologous donation (PAD) is used in patients scheduled for surgery where anticipated blood loss is likely to require blood cell replacement. This involves donation and subsequent storage of one or more units of blood preoperatively, which is then available for administration during or after surgery (Henry *et al*. 2010). More recently, acute normovolaemic haemodilution (ANH) has gained popularity in human medicine and involves preoperative removal of a predetermined amount of whole blood from a patient while restoring the circulating volume with a synthetic colloid or crystalloid fluid (Goodnough 2002; Klein *et al*. 2007). The blood volume collected preoperatively is most commonly calculated using an equation published by Gross (1983) (**Fig 1**). ANH is tolerated in most healthy animals to a packed cell volume (PCV) of 25% (Seeler 2007) and 27–33% in human patients undergoing cardiac surgery (Van der Linden and Sakr 2005). Blood is collected in standard blood bags containing anticoagulant and stored at room temperature until required for transfusion in

The following equation, published by Gross (1983) was used to estimate the volume of blood that could be collected preoperatively.

$$V = EBV \times \frac{(PCV_i - PCV_t)}{PCV_{av}}$$

where: V = volume of blood to be collected, EBV = estimated blood volume (l; based on 80 ml/kg bwt; Durham 1996). PCV_i = initial packed cell volume (PCV; %) PCV_t = target PCV PCV_{av} = average PCV.

For the 752 kg horse, aiming to reduce the PCV to 29%, to maintain PCV within normal limits for the breed (24–44%; Corley and Stephen 2008) and avoid excessive haemodilution, the following calculation was made:

$$V = 60.16 \times \frac{0.32-0.29}{0.305}$$

V = 5.92 litres.

Fig 1: Calculation of the volume of blood collected preoperatively.

the operating room after major blood loss has ceased, or sooner if indicated (Duguid 1999). It is a technique reserved for elective surgical procedures where severe haemorrhage is expected and may avoid or reduce allogenic blood requirement (Segal *et al*. 2004). Preoperative blood donation and ANH with autologous transfusion have not yet been reported in clinical veterinary cases (Kisielewicz and Self 2014). This case report describes the use of preoperative autologous donation and ANH in a horse scheduled for sinus surgery that exhibited incompatibility with 6 hospital blood donor horses.

Case history

A 16-year-old 752 kg Clydesdale gelding was admitted to the Philip Leverhulme Equine Hospital with inspiratory stertor and fetid left sided nasal discharge.

Clinical findings, treatment and outcome

Standing computed tomography (CT) revealed obliteration of the left nasal passage due to infection and mineralisation of the ventral concha and apical infection of tooth 210. The following day, a 24 mm internal diameter temporary tracheostomy tube[1] was placed and tooth 210 extracted orally under sedation (80 µg/kg bwt i.v. romifidine [Sedivet][2] and 0.15 mg/kg bwt i.v. butorphanol [Torbugesic])[3] and a left sided maxillary nerve block (10 ml 0.5% levobupivacaine [Chirocaine])[4]. General anaesthesia to permit surgical resection of the concha via a nasal flap was scheduled for 4 days later. Due to the anticipated blood loss, crossmatching with 6 hospital blood donor horses was carried out. Erythrocytes were washed 4–6 times in saline and minor and major crossmatching procedures indicated incompatibility with all 6 donor horses. Preoperative autologous donation and acute normovolaemic haemodilution was proposed. Physical examination reported a heart rate of 36 beats/min, respiratory rate of 8 breaths/min, patent airflow via the tracheostomy tube, pink moist mucous membranes with capillary refill time of <2 s and a rectal temperature of 37.8°C. Packed cell volume was 32%. A 12 gauge 80 mm cannula (Intraflo 2)[5] was placed in the left jugular vein and 6 l of blood collected into commercially available plasma collection bags (Plasma Collection Kit)[6] containing acid-citrate dextrose anticoagulant (**Fig 2**). The volume of blood collected preoperatively was calculated according to Gross (1983) (**Fig 1**). The blood was initially stored in a refrigerator at 4°C and then allowed to warm to room temperature at the onset of surgery. Collection was completed in <40 min and followed by infusion of an equal volume of gelatine solution (Geloplasma)[7] completed within a further 30 min. During blood donation and subsequent infusion of gelatine solution, heart rate, respiratory rate, pulse quality and capillary refill time were assessed every 15 min and remained unchanged within normal limits. The horse was moved to the induction box and PCV and total protein (TP) measured prior to preanaesthetic medication were 27% and 70 g/l respectively. Phenylbutazone 2.2 mg/kg bwt (Equipalazone)[6] was administered orally on the morning of surgery and procaine penicillin 12 mg/kg bwt (Depocillin)[8] was administered i.m. one hour prior to the onset of surgery.

Premedication was carried out 75 min after completion of ANH and consisted of romifidine 40 µg/kg bwt i.v. (Sedivet)[2] and morphine 0.2 mg/kg bwt i.v. (Morphine Sulphate)[9]. Ten minutes later, induction of general anaesthesia was achieved

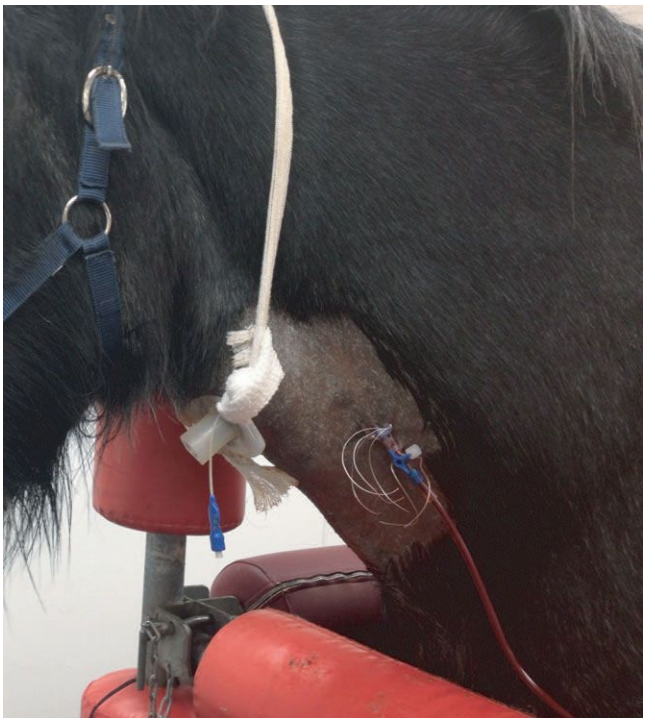

Fig 2: Preoperative blood collection.

using ketamine 2.2 mg/kg bwt i.v. (Ketaset)[3] and diazepam 0.05 mg/kg bwt (Diazepam)[10]. The horse made a smooth transition to recumbency, the patency of the tracheostomy tube was re-confirmed and the cuff inflated prior to moving the horse to the operating table. The horse was positioned in right lateral recumbency and a large animal circle breathing system (LAVC 2000)[11] was connected to the tracheostomy tube and used to deliver isoflurane (Isoflo)[4] in 100% oxygen to maintain general anaesthesia. Intermittent positive pressure ventilation was delivered via a pressure-limited flow-controlled ventilator (Mark 7 Bird Servo)[12]. End-tidal carbon dioxide tension was maintained at 38–44 mmHg with a peak inspiratory pressure of 24 cmH_2O. No audible or palpable leak could be detected associated with the tracheostomy tube. Hartmann's solution (Vetivex 11)[6] was infused intravenously (4 ml/kg bwt/h) throughout anaesthesia. Instrumentation included electrocardiography, capnography, pulse oximetry, end-tidal and fractional inspired concentration of isoflurane and oxygen and invasive blood pressure measurement (Datex-Ohmeda S/5)[13] via a cannula placed in the left dorsal metatarsal artery. A left maxillary nerve block was performed using 10 ml 0.5% levobupivacaine (Chirocaine)[4]. Dobutamine 0.25–1.0 µg/kg bwt/min (Dobutamine)[9] was infused to effect to maintain mean arterial pressure above 70 mmHg throughout the duration of anaesthesia. Arterial blood gas analysis was carried out using an automated blood gas analyser (Radiometer ABL77)[14]. After 20 min of surgery, mild haemorrhage was noted and blood loss was estimated by measuring blood suctioned from the surgical site and floor (**Fig 3**) and weighing blood-soaked swabs. During the period of haemorrhage, there were no significant changes in heart rate or arterial blood pressure; however, these changes may have been negated by the concurrent infusion of dobutamine. Estimated blood loss at the end of surgery was

Fig 3: Intraoperative haemorrhage.

3 l or 5% circulating volume (80 ml/kg bwt [Durham 1996]). After resection of the concha and confirmation of haemostasis at the surgical site, a second cannula (12 gauge 80 mm) was placed in the left jugular vein and autologous blood transfusion was commenced with one litre of blood being administered prior to the end of surgery. No fluctuations in heart rate or arterial blood pressure were associated with the autologous transfusion. Towards the end of surgery PCV was 26% and TP 68 g/l. At the end of surgery, the right nostril was suctioned and a small amount (200 ml) of serosanguinous fluid was removed. The horse was placed in right lateral recumbency in the recovery box and spontaneous breathing resumed within 2 min. Supplemental oxygen was supplied via the tracheostomy tube using a demand valve and a nasal tube was introduced into the right nostril. The horse recovered uneventfully and the tracheostomy tube cuff was fully deflated once the horse was standing.

The remaining 5 l of whole blood was infused at 2.2 ml/kg bwt/h post operatively, the transfusion being completed within 4 h. The following day, PCV was 29% and TP 70 g/l. An indwelling lavage catheter allowed daily sinus lavage and repeat endoscopy at 48 h confirmed a patent left nasal passage. Forty-eight hours post operatively, there was significant tracheostomy site infection and necrosis which necessitated tube removal. Left nasal passage airflow was restored and the tracheostomy site was allowed to heal by secondary intention. Phenylbutazone 2.2 mg/kg bwt (Equipalazone)[6] and trimethroprim-potentiated sulfonamide 15 mg/kg bwt (Trimediazine Oral Powder)[15] were administered orally twice daily for 14 and 12 days, respectively. The horse made a full recovery and returned home 7 days after surgery.

Discussion

This case report describes the management of a horse where intraoperative haemorrhage was anticipated but due to haematological incompatibility with 6 donor horses identified preoperatively, an alternative to allogenic transfusion was sought.

The identification of 7 equine blood groups (A, C, D, K, P, Q and U) and >30 equine erythrocyte antigens (Johnson and Parish 2009; Tocci and Ewing 2009) means that donor and recipient are unlikely to have identical erythrocyte phenotype (Durham 1996). However, in many cases, a single allogenic blood transfusion is tolerated in previously unsensitised horses without the need for pretransfusion testing (Morris 1989). Prospective blood donors should be free from infectious blood-borne agents, have normal erythrocyte indices and plasma protein concentrations (Durham 1996) and ideally be negative for Aa, Ca and Q antigens (Tocci and Ewing 2009). In the current report, to the authors' knowledge, the donor horses were not blood typed and since time allowed, crossmatching was carried out. Although, crossmatching in horses has been associated with false negative and false positive results (Durham 1996), the apparent incompatibility with all 6 blood donors indicated that an alternative source of blood or blood substitute would be necessary.

In human medicine, Fantus (1937) first reported the use of PAD, now widely used in transfusion medicine (Duguid 1999). Advantages include a longer lifespan of the transfused erythrocytes and minimised risk of transfusion reactions (Mudge *et al*. 2004). Transfusion reactions can be immune-mediated or nonimmune-mediated, immediate or delayed and are more common with whole blood (16%, Hurcombe *et al*. 2007) compared to plasma (0–10%, Hardefeldt *et al*. 2009; Wilson *et al*. 2009). The lifespan of transfused autologous erythrocytes is significantly longer than their allogenic counterparts, although lifespan is influenced by storage duration. Allogenic red blood cells had a mean post transfusion half-life of 20 days after only 24 h of storage (Mudge *et al*. 2012) compared to 50 days for autologous blood transfused immediately after collection (Owens *et al*. 2010). It is known that median age of transfused red cells negatively correlates with survival of patients in human intensive care (Purdy *et al*. 1997).

With improved planning, PAD could have been carried out avoiding the need to perform ANH immediately prior to surgery. Preoperatively, however, time was spent continuing the search for a compatible blood donor. Theoretically fewer red cells are lost in the event of intraoperative haemorrhage following ANH, while the volume of preoperatively collected blood (with a higher PCV) can be subsequently infused. The blood was stored for 3 h and infused once haemostasis was confirmed. The collection bags available contained acid-citrate-dextrose anticoagulant. It is reported that citrate phosphate dextrose with supplemental adenine is a superior medium for storing equine whole blood and results in better preservation of 2,3-diphosphoglycerate concentrations, which favours peripheral tissue oxygenation since 2,3-diphosphoglycerate modulates the oxygen affinity of haemoglobin (Mudge *et al*. 2004). Significant changes in potassium, pH and lactate may be seen with both storage media after 2 weeks; however, these changes are unlikely to be clinically significant for metabolically stable patients receiving low dose transfusions (Mudge *et al*. 2004). In the current case, storage in acid-citrate-dextrose was considered sufficient due to the short storage time, which probably induced minimal erythrocyte storage lesions.

In the current case, haemorrhage was less than predicted and once haemostasis was confirmed and the volume of

autologous blood transfused exceeded the estimated volume lost at surgery. It is difficult to predict transfusion requirements accurately, resulting in underestimation, and the need for allogenic blood, or overestimation and wasted autologous blood. Transfusion of the remaining autologous volume aimed to replenish erythrocytes lost to haemodilution and haemorrhage. This may result in transient hypervolaemia or circulatory overload; however, considering the short-lived (1–3 h) plasma expansion effect of succinylated gelatines (Salmon and Mythen 1992) and the elapsed time period (4 h) since colloid infusion in the current case, it is unlikely that hypervolaemia occurred. Although rare, potential adverse events including autologous transfusion reaction and blood contamination cannot be discounted (Domen 1998) and in cases where transfusion criteria are not met and volume overload poses a significant clinical risk, the surplus ANH blood should be discarded (Shander 1999). In the medical literature, remaining autologous blood is normally transfused at the end of surgery once haemostasis is confirmed even if minimal haemoglobin concentration or PCV transfusion triggers are not reached (Matot *et al*. 2002; Brecher 2011). Alternative methods to replace oxygen carrying capacity in the event of haemorrhage include the use of ultrapurified polymerised bovine haemoglobin (Oxyglobin; OPK Biopure, Netherlands). The polymerisation process slows renal excretion, which acts to prolong half-life, and the lack of stroma or erythrocyte membranes makes transfusion reactions unlikely (Hollis and Corley 2011). Ultrapurified bovine haemoglobin has been used successfully in a mature horse where incompatibility with 7 blood donors was identified (Hollis and Corley 2011); however, the cost in a mature horse is considerable (£15,000 for a 500 kg horse administered at a rate of 10 ml/kg bwt [Finding *et al*. 2011]). In the current case, the cost associated with ANH was <£500.

Cell salvage involves collection of blood at sites of surgical haemorrhage, washing and filtration of the blood before autotransfusion, using specialist equipment. Finding *et al*. (2011), however, reported bypassing this process in one horse, with direct collection of blood from a haemoabdomen into plasma collection bags before re-transfusion, apparently without detrimental consequences. Documented adverse effects of cell salvage include air embolism, infection, nephrotoxicity and coagulation abnormalities such as disseminated intravascular coagulation (Duguid 1999). The lack of specialist equipment and potential for infection or neoplastic dissemination prevented the use of cell salvage in the current case.

Overall, the horse recovered uneventfully from surgery and appeared to suffer no detrimental consequences as a result of ANH. In this case, surgical haemorrhage was less than anticipated but in similar cases where allogenic transfusion is not an option, the acquisition of autologous blood via ANH technique may represent a valuable source of transfusion either as a sole agent or adjunct to other blood substitutes.

Authors' declaration of interests

No conflicts of interest have been declared.

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[Correction added on 19 March 2015, after first online publication: This information was omitted in the previous version and has now been added.]

Manufacturers' addresses

[1]Bivona Tracheostomy Tubes. Smith's Medical International Ltd, Ashford, Kent, UK.
[2]Boehringer Ingelheim Limited. Bracknell, Berkshire, UK.
[3]Zoetis UK Limited, London, UK.
[4]Abbott Laboratories, Maidenhead, Berkshire, UK.
[5]Vygon, Ecouen, France.
[6]Dechra Veterinary Products Limited, Shrewsbury, Shropshire, UK.
[7]Fresenius-Kabi, Runcorn, Cheshire, UK.
[8]MSD Animal Health, Walton, Milton Keynes, UK.
[9]Wockhardt UK, Wrexham, Clwyd, UK.
[10]Hameln Pharmaceuticals, Brockworth, Gloucester, UK.
[11]Eickemeyer, Tuttlingen, Germany.
[12]JD Medical Distribution Company, Phoenix, Arizona, USA.
[13]GE Healthcare, Chalfont St Giles, Buckinghamshire, UK.
[14]Radiometer Medical, Brønshøj, Denmark.
[15]Vétoquinol, Great Slade, Buckingham, UK.

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