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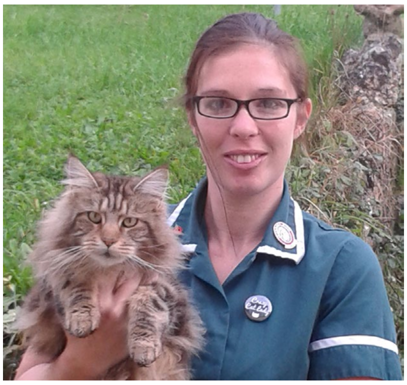
A delayed blood transfusion reaction in a border collie

Angela Jonas BSc (Hons), CertSAN, CertFN, DipAVN (Small animal), RVN
Nute Veterinary Surgery, Higher Trenant, Wadebridge, Cornwall, PL27 6HB, UK



Angela Jonas BSc (Hons), CertSAN, CertFN, DipAVN (Small animal), RVN

Anji qualified as an RVN in 2005 and achieved her DipAvn via Harper Adams University in 2016. She has a keen interest in Medical nursing and Laboratory diagnostics and has completed nurse training at Axiom and IDEXX Laboratories. Anji works in a mixed first-opinion practice, is a clinical coach and also lectures and teaches lab techniques. In practice she is often found behind a microscope getting overexcited about an unusual blood smear or infecting students with her enthusiasm.

Email: anjijonas@gmail.com

ABSTRACT: Blood transfusions are undertaken in most first-opinion practices and RVNs are often required to support patients both during and after a transfusion. Knowledge of the mechanisms behind transfusion reactions and the ability to recognise them are useful to any RVN supporting such patients. Development of laboratory skills and confidence to provide patient side information to the attending VS improves clinical care and is a great asset within any Practice team.

In November 2016, a two-year-old entire female border collie was admitted to the author's first-opinion practice due to uncharacteristic lethargy and an episode of collapse.

Patient assessment

On examination, the patient was quiet with pale mucus membranes and tachycardia (HR 128). Her body condition score was 3/9 and she weighed 18.25 kg. Her owners reported that this was a marked deviation from her normal behaviour as a working sheepdog. The owners had recently distributed rodenticide on their farm and they were concerned that ingestion of rodenticide was the cause of the symptoms (**Figure 1**).

Initial diagnostics and treatment

Blood was taken for in house biochemical and haematological analysis. The haematology results showed marked anaemia (1.7×10^{12}/l) with a haematocrit (HCT) of 11.4%, elevated granulocytes (13.3×10^{9}/l) and low haemoglobin (4.3 g/dl). Biochemistry revealed elevated alkaline phosphatase (120 U/l) and urea (12.3 mmol/l). The veterinary surgeon (VS) diagnosed rodenticide toxicity based on these findings and the clinical history and proceeded with a blood transfusion as a first-line treatment.

A blood donor was selected from the practice database who fulfilled the criteria described by Helm and Knottenbelt (2010) (**Box 1**). Whole blood (440 ml) was collected from the donor and transfused immediately to the patient. A discussion of the transfusion procedure is beyond the scope of this article, but is covered extensively by Helm and Knottenbelt (2010). Phytomenadione 2.2 mg/kg (Vitamin K1 Laboratoire TVM, Dechra) was also administered to the patient subcutaneously in several sites. Following the transfusion, the patient

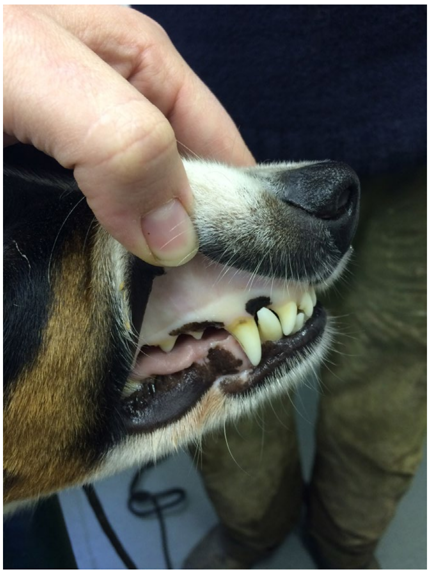


Figure 1. The patient on first presentation exhibiting pale mucus membranes
Source: author's own.

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received 100 ml of crystalloid intravenous fluid solution (Hartmanns, Vetivex 11) at 66 ml/h for the following 15 h to correct hypovolaemia.

Box 1. Canine blood donor criteria (Helm and Knottenbelt 2010)

Canine blood donor criteria

- Healthy and friendly
- Normal on physical examination
- Lean bodyweight of over 25 kg
- 1–8 years old
- Nulliparous and females should be neutered
- PCV of at least 35%
- Fully vaccinated and wormed

The following morning, the patient was bright, active and eating well. She was discharged with 1.1 mg/kg phytomenadione (Vitamin K1 Laboratoire TVM, Dechra) to be given orally twice daily for three weeks and her owners were instructed to provide high-quality food and strict rest for a month.

Follow-up outpatient care

Twenty-four hours later, the patient was represented as her lethargy had returned and appetite declined. On examination, she was tachycardic (HR 128) and hyperthermic (102F). The VS attributed this to initial transfusion metabolism and instructed the owners to continue with the rest and tempting her appetite.

Four days later, the patient returned for a check-up and her owners reported that she was eating and behaving normally. Blood was drawn for haematological evaluation and although the HCT was still low (25%), smear evaluation showed marked anisocytosis, polychromasia and the presence of nucleated erythrocytes, indicating a regenerative response to the anaemia. The VS prescribed three weeks further rest before a final check-up prior to returning to work. At the final check the patient was clinically normal and in-house haematology results were all within normal ranges. The VS confirmed that the patient could return to working sheep.

On day 45, the patient was represented to the VS as she had once again become depressed, lethargic and anorexic. The owners confirmed that all rodenticide had been removed from their premises. Examination revealed pale mucous membranes and a manual packed cell volume (PCV) assessment returned a value of 25%. A slide agglutination test was positive for autoagglutination and blood smear review indicated a leucocytosis with a mild eosinophilia (**Figure 2**). A blood sample preserved in ethylene diamine tetra acetic acid (EDTA) and a fresh blood smear were referred to an external laboratory for a more detailed haematology review.

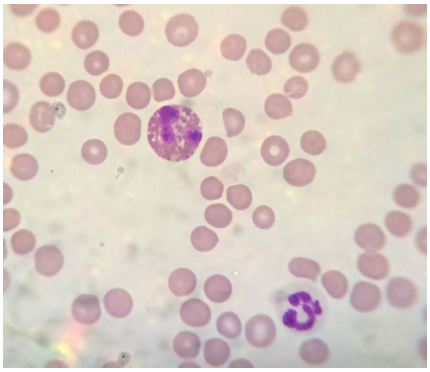

Figure 2. Eosinophil seen at ×100 magnification
Source: author's own.

The external laboratory confirmed the low HCT and elevated leucocyte count, while the differential leucocyte count showed neutrophilia and eosinophilia. The pathologist review of the blood smear reported moderate polychromasia, marked anisocytosis and moderate spherocytosis. An absolute reticulocyte count was also performed, which returned a value of 160 × 10^9/l indicative of erythrocyte regeneration. In consultation with the veterinary pathologist, the VS concluded that although the patient was mounting a regenerative response, the transfused erythrocytes were being prematurely destroyed at an accelerated rate, leading to the redevelopment of anaemia and deterioration in the patient's clinical condition.

Over the following week, the patient was brought to the practice regularly for blood sampling to monitor her anaemia and regenerative response (results in **Table 1**). At first recheck the PCV had fallen so the VS administered an immunosuppressive dose of intravenous 0.1 mg/kg dexamethasone (Dexadreson, Intervet). This was repeated on days 46 and 47 and then followed up with oral prednisolone (Prednidale, Dechra) 1 mg/kg daily. By day 56 the PCV was within normal range and the owners reported that the patient was once again behaving normally and had a normal appetite. The VS prescribed further oral prednisolone (Prednidale, Dechra) 0.5 mg/kg daily for 7 days before dropping to 0.5 mg/kg every other day for a final 7 days. Ideally, this patient would have been hospitalised and closely monitored for the redevelopment of severe anaemia and its associated problems.

Table 1. Timeline of significant case events

Date	Day of presentation	Visit notes	PCV % or HCT%
26/11/16	1	First presentation & blood transfusion	11.4 HCT
27/11/16	2	Send home	
28/11/16	3	Represent - mild transfusion reaction	
2/12/16	7	First recheck	25 PCV
21/12/17	26	Return to work	43.5 PCV
9/1/17	45	Anaemia redevelops	25 HCT
10/1/17	46	Dexadreson administration	13 PCV
11/1/17	47	Dexadreson administration	13 PCV
12/1/17	48	Dexadreson administration	17 PCV
13/1/17	49	Follow up	21 PCV
16/1/17	52	Follow up	25 PCV
20/1/17	56	Final visit to practice	33 PCV

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However, cost was a major limiting factor, so the owners opted for outpatient care.

Case discussion

Second-generation anticoagulant rodenticides such as Bromadiolone render patients unable to activate vitamin K to produce clotting factors (Rock, 2007). Therefore, once existent clotting factors are used, patients exhibit coagulopathies leading to development of anaemia. Administration of active vitamin K allows the recommencement of clotting factor production within an hour of administration (Rock, 2007). Ideally, vitamin K is administered on the basis of a prothrombin time assessment, but in this case cost was a limiting factor; therefore, treatment was based on known toxin exposure and clinical signs.

A whole-blood transfusion provides the patient with fluid volume, erythrocytes and thrombocytes which are lost via microhaemorrhages and provides clotting factors to restrict further bleeding. Due to the urgency of this patient's need and cost issues, blood typing was not performed; however, this patient had never received a transfusion before and was nulliparous, so a reaction to a first transfusion was thought to be unlikely. A slide crossmatch was performed in house (**Box 2**) which included a major crossmatch to check for antibodies in the recipient against donor red cell antigens and a minor crossmatch to detect antibodies in the donor against recipient red cells. No reaction was observed.

Box 2. Slide crossmatch protocol adapted from McMichael (2014)

- Collect 2 ml of EDTA-anticoagulated blood from donor and recipient.
- Centrifuge blood samples for 5 min and remove plasma to labelled tubes.
- Make a 2% red cell suspension of each specimen in physiological saline (0.85% NaCl) solution. This can be done by placing 0.02 ml of concentrated red cells in 0.98 ml of saline solution; mixing, centrifuging, and washing three times; and resuspending washed red cells in an equal volume of saline solution.
- Place two drops of the donor's RBC solution with two drops of recipient plasma on a slide (major crossmatch). On a second slide, place equal volumes of donor's plasma and recipient's RBC solution (minor crossmatch). To check for autoagglutination, set up controls in the manner by mixing the donor's red cells with its own plasma and follow the same procedure with the recipient's red cells and plasma.
- If no agglutination is observed after 15 min examine under the low power of the microscope. Significant haemolysis and/or agglutination in one or both of the crossmatched slides but not in the controls indicates incompatibility.

The donated blood was transfused immediately and initiated at a rate of 0.5 ml/min and the patient was closely monitored for changes in HR, RR, rectal temperature and any swelling indicative of a transfusion reaction.

The PCV of the donated blood was 48% and following transfusion it was estimated that the patient's PCV would be 23%. Although still low, this would provide adequate support to the patient until her own regeneration had begun. A post-transfusion PCV of over 30% can cause the erythrocyte regeneration response, thus hindering the patient's recovery (Balch and Mackin 2007).

The continuation of oral vitamin K therapy on discharge is to guard against the lasting activity of this type of rodenticide because effects of up to 30 days' duration are reported (Rock 2007). Oral administration with a fatty meal increases the bioavailability of vitamin K.

Although a slide crossmatch showed no incompatibility, it is likely that when the patient represented 48 h after transfusion exhibiting lethargy and anorexia, she was experiencing a mild transfusion reaction. These reactions are usually mild and are often clinically undetectable although human patients describe feeling feverish and experiencing back pain (Nall, 2016).

By day 7 the patient was responding to the anaemia, her haematocrit had fallen from the initial post-transfusion value but blood smear evaluation revealed a marked regenerative response (**Figure 3**). Blood smears were prepared directly from fresh blood to prevent EDTA from producing artefactual changes in erythrocyte morphology. The following indicators of regeneration were noted:

- Anisocytosis – variation in RBC size

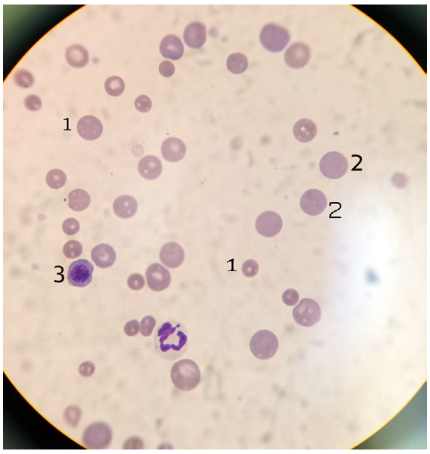

Figure 3. Blood smear showing anisocytosis (1), polychromatophils (2) and nucleated erythrocyte (3)
Source: author's own.

- Polychromatophils – immature RBCs which are large and blue staining
- Nucleated erythrocytes – RBCs containing a nucleus prematurely released from bone marrow

At day 26 when the patient was declared fit for a return to working sheep, her haematocrit had reached 33%. On evaluation of the smear, the erythrocytes were now a mature population, indicating that the regenerative response had stabilised.

The decline of the patient on day 45 was unexpected and investigations were restricted by cost. A manual PCV was performed in-house alongside a slide agglutination test and a smear review. The sample was then referred to an external laboratory for specialist review. There were several morphological changes evident on the blood smear and confirmed by the external laboratory that were relevant to the physiological processes occurring within the patient.

- Spherocytosis – Small RBCs with no central pallor
- Leucocytosis – Increased numbers of WBCs
- Eosinophilia – Increased numbers of eosinophils

These changes may be attributable to an immune-mediated process or a delayed haemolytic transfusion reaction. Such reactions are attributed to the presence of antibodies against dog erythrocyte antigen (DEA) 3, 5 and 7 or the recipient developing antibodies against other antigens on transfused erythrocytes causing them to be prematurely removed from the circulation. It has been proposed that even dogs with no history of blood transfusion should be typed and crossmatched prior

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to transfusion, as a study of transfusion crossmatching showed 17% of recipients were incompatible with at least one potential donor (Odunayo *et al.*, 2017). Crossmatching individuals does not rule out the development of delayed transfusion reactions. Incompatibility causes erythrocytes to be prematurely destroyed and removed from the circulation, as was seen in this case. Dexadreson administration dampens the immune response directed at the erythrocytes by suppressing lymphocyte activity and the phagocytic activity of macrophages. Three intravenous doses were administered to establish a loading dose and the continuation of oral medication maintained the effect.

Due to cost restrictions, the patient was managed as an outpatient. In some cases, patients with anaemia can become hypoxic and require oxygen therapy (Balch and Mackin 2007), so the owners were instructed to closely monitor and report any changes to her condition. Further blood transfusion was withheld, because the risk of acute transfusion reaction increases markedly with subsequent untyped transfusions (Balch and Mackin 2007).

Each time the patient returned to the practice thereafter, she was seen by the Registered Veterinary Nurse (RVN) and the VS in combination. The RVN was responsible for discussing progress and general observations with the owner, drawing blood for in-house testing, performing a manual PCV assessment and examining a blood smear for indicators of regeneration. The results were then presented to the VS who discussed them with the owner and formulated an ongoing treatment plan.

PCV was assessed in combination with total solids measured on the refractometer. This was important as PCV was being used an indicator of progress and is impacted by levels of hydration. Checking that the total solids value was within normal ranges each time allowed confident comparison between PCV values taken at 24-h intervals. The PCV values indicated that the steroid administration had been successful in slowing the rate of erythrocyte destruction and the blood smear evaluation indicated a regenerative response.

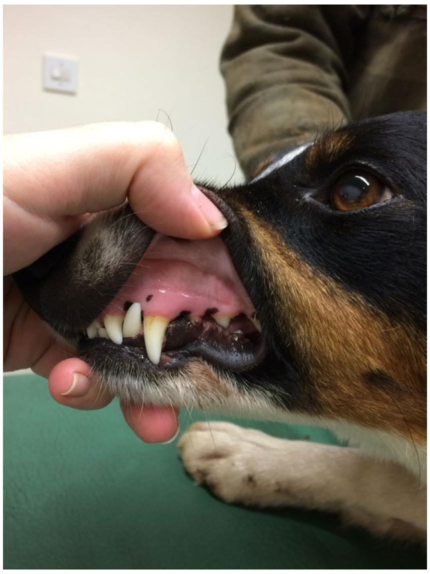

Figure 4. The patient on day 56 exhibiting normal mucus membrane colour
Source: author's own.

On day 47 a manual reticulocyte count was performed in-house (**Box 3**) which returned a value of 50%, indicative of a strong regenerative response.

Box 3. Manual reticulocyte count protocol (Valenciano et al., 2014)

Manual reticulocyte count protocol

- Place equal amounts of EDTA blood and New Methylene Blue Stain in a test tube for 20 min.
- From this mixture, make a blood smear.
- Count 1000 RBCs, recording the number of reticulocytes observed.
- Divide the number of reticulocytes counted by 10 to give % reticulocytes.

The final time the patient was seen in the clinic was 56 days after the initial presentation for rodenticide toxicity. She was still receiving 0.5 mg/kg of prednisolone daily. Her PCV was now within the normal range (33%), her blood smear was still showing indicators of regeneration and she was clinically normal. Figure **3** shows the normal salmon pink mucus membranes of the patient on day 56. She was discharged from close supervision, with the owners instructed to tailor down the steroid dose over the following two weeks and return to the practice if there were any signs of clinical deterioration. Steroid should not be stopped abruptly when used for immunosuppression because relapse may occur (**Figure 4**).

Conclusion

Delayed transfusion reactions such as this are rare, but highlight the importance of follow-up and monitoring of all patients following blood transfusions. This patient is now fully recovered and has resumed her role as a working sheepdog.

Overall, this case is a good example of the diagnostic support which can be provided even in cases of limited funds by maximising the practice in-house laboratory and the skills of the RVN team. Determination of PCV and blood smear preparation are day one competencies for all RVNs. Identification of blood smear abnormalities allows RVNs to identify cases in need of referral to external pathologists and provide an in-house monitoring service in cases such as this. Laboratory skills should not be overlooked as an area of specialism for RVNs in first-opinion practice, as in addition to the satisfaction of providing patient-side information vital for clinical decision-making, blood smears are awesome!

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