Prospective evaluation of the utility of crossmatching prior to first AB matched transfusion in cats: 101 cases

Objectives: To: 1) assess the frequency of crossmatch incompatibility in naïve feline blood transfusion recipients using two crossmatching methods, 2) measure the effect of crossmatch incompatibility on packed cell volume increase following transfusion, 3) assess the frequency of acute transfusion reactions and errors in blood transfusions in cats and 4) assess the impact of crossmatch incompatibility on the likelihood of transfusion reactions.

Methods: Cats being administered a first AB-matched transfusion in a veterinary teaching hospital were prospectively recruited for this observational study. Major and minor crossmatching were performed using a slide agglutination method and a commercial test. Packed cell volume increase at 12 hours post transfusion relative to the mass of red blood cells given per recipient bodyweight (ΔPCV_{norm}) and occurrence of transfusion reactions were recorded.

Results: 101 cats were recruited. Crossmatch incompatibility was common when using the slide agglutination method (27% and 10% major and minor incompatibility, respectively),

but less common with the commercial test (major and minor incompatibility both 4%). Crossmatch incompatibility (with any method) was not associated with decreased ΔPCV_{norm}. Transfusion reactions occurred in 20 cats, most commonly febrile non-haemolytic transfusion reactions (n=9) and haemolytic transfusion reactions (n=7). The commercial test appeared to be most specific for predicting haemolytic transfusion reactions.

Conclusions and clinical relevance: Transfusion reactions were fairly common but were not associated with increased mortality. Use of crossmatch compatible blood did not lead to a greater increase in packed cell volume at 12 hours but the commercial test may predict a haemolytic transfusion reaction.

Key words: Blood type, Transfusion reaction, Mik, Packed red blood cells, whole blood, blood types, blood incompatibilities, hemolytic transfusion reaction.

Introduction

It is well recognised that recipients of feline whole blood (WB) or packed red blood cells (pRBCs) need to be administered AB type-matched products (Giger and Akol 1990, Barfield and Adamantos 2011, Giger, 2014). There is also general consensus that crossmatching should be performed prior to a subsequent transfusion of red blood cell (RBC) containing products 3-5 days after the first transfusion of a blood product (Jagodich and Holowaychuk 2016). However, there has been debate about the necessity to perform crossmatching prior to first transfusion in cats being administered AB matched blood (McClosky *et al* 2018; Sylvane *et al* 2018; Tasker *et al* 2014; Weltman *et al* 2014; Weinstein *et al* 2007). Crossmatching prior to first transfusion allows detection of serological incompatibility between the donor and recipient resulting from pre-formed antibodies against non-AB erythrocyte antigens. A compatible crossmatch result should decrease the likelihood of a haemolytic transfusion reaction (HTR). Although this was initially a theoretical concern in cats, Weinstein *et al* (2007) reported a novel feline non-AB RBC antigen, Mik, and described 3 cats which had crossmatch findings consistent with naturally occurring anti-Mik antibodies. A retrospective study suggested that crossmatched feline blood was more efficacious in raising recipient packed cell volume (PCV) than non-

crossmatched blood and this was postulated to be due to pre-existing recipient antibodies to Mik and possibly other non-AB erythrocyte antigens (Weltman *et al* 2014). However, a recent prospective randomised trial compared administration of non-crossmatched and crossmatched pRBCs to cats (with 24 cats in each group) and found no difference in PCV increase or the rate of transfusion reaction between the groups (Sylvane *et al* 2018). A retrospective study of 300 cats also found no difference in PCV increase but noted an increased rate of febrile non-haemolytic transfusion reactions (FNHTR) in the cats administered non-crossmatched pRBCs (McClosky et al 2018).

Complications and errors during blood product administration, alongside the frequency and types of transfusion reaction, are recorded in human haemovigilance monitoring schemes (Poles *et al* 2018). This allows repeated problems to be identified and possible solutions devised. Clinical governance is a developing area in veterinary medicine, but a prospective assessment of the frequency and type of transfusion reactions, complications and errors in administration of blood products has not been reported before to the authors' knowledge.

69 The aims of this study were therefore to: 1) assess the frequency of crossmatch
70 incompatibility in a large cohort of naïve feline blood transfusion recipients, 2) compare
71 the results of a commercially available feline crossmatch kit with crossmatches performed
72 by a clinical laboratory, 3) assess the effect of crossmatch incompatibility on the change in
73 PCV seen post feline blood donation and 4) assess the frequency of acute transfusion
74 reactions, complications and errors in cats receiving crossmatch compatible and non-
75 crossmatch compatible blood transfusions.
76
77 Materials and methods
78 This was a prospective observational study performed at a veterinary teaching hospital
79 aiming to recruit 100 cats receiving either a fresh WB or stored pRBC transfusion that had
80 not previously received a RBC product. Informed consent for participation in the study
81 was obtained from both recipient and donor owners and the study was approved by the
82 hospital Clinical Research Ethical Review Board. Cats were blood typed using ethylene
83 diamine tetraacetic acid (EDTA) anticoagulated blood and a commercially available kit[A]
84 and AB type-matched blood was administered. All donors were healthy and were checked
85 prior to each donation for feline leukaemia virus antigen and feline immunodeficiency

virus antibodies. Blood anticoagulated in EDTA was submitted for polymerase chain reactions to detect DNA from *Candidatus Mycoplasma hemominutium*, *Mycoplasma hemofelis* and *Candidatus Mycoplasma turicensis* annually. Blood was obtained prior to transfusion from the recipient and also from the donor if fresh whole blood was administered, or if stored pRBCs were used, then a crossmatch segment was obtained. A major and a minor crossmatch (minorly modified versions of those described by Abrams-Ogg, 2016) were performed by trained personnel (hereafter referred to as the laboratory method) as follows. Recipient and donor blood samples were spun at 664 x G for 5 minutes and plasma and the erythrocyte pellet were then separated. The erythrocytes were washed using an automated cell washing instrument[B] and a 2% erythrocyte suspension was made using 980 µL of sterile saline and 20 µL of washed erythrocytes. Two drops of recipient plasma and 2 drops of donor erythrocyte suspension were placed and gently mixed in an Eppendorf tube[C] for the major crossmatch. Two drops of donor plasma and 2 drops of recipient erythrocyte suspension were placed and gently mixed in an Eppendorf tube for the minor crossmatch. Control tests (whereby donor plasma and donor erythrocyte suspension and recipient plasma and recipient erythrocyte suspension were mixed) were also performed. The solutions of plasma and RBC were incubated at

room temperature for 30 minutes. The solutions were then resuspended via tube inversion and a small drop (~10 µL) of the suspension was placed onto a slide and immediately examined under the microscope at x10 and x20 objectives. Any sign of agglutination was then recorded as a positive agglutination for that crossmatch. If the recipient control was agglutinating then an agglutinating minor crossmatch was deemed uninterpretable. If the donor control was agglutinating then an agglutinating major crossmatch was deemed uninterpretable. A commercially available crossmatch kit[D] was also used to assess major and minor crossmatch compatibility. A crossmatch was deemed incompatible if a line of cells was present on the top of the serum gel. Crossmatches were not routinely performed prior to first transfusion in this hospital, so these results were not consulted prior to transfusion.

Signalment, weight, blood type, whether WB or pRBCs were administered, age of pRBCs, PCV prior to transfusion and as close to 12 hours after the end of the transfusion, diagnosis, administration of additional RBC containing blood products and survival to discharge were recorded for the recipients. Blood type, PCV and volume of donation were recorded for the donors.

120

121 The recipient increase in PCV at 12 hours post donation was normalised relative to the
122 amount of RBCs administered to the recipient cat and their bodyweight (ΔPCV_{norm}) using a
123 novel formula:

124 $\Delta PCV_{norm} = (PCV_{post} – PCV_{pre}) \ / ((BDV \times PCV_{donor}) / Wt_{recip})$

125 Where:

126 PCV_{post}: The PCV of the recipient at 12 hours after the end of the transfusion in %

127 PCV_{pre} : The PCV of the recipient prior to transfusion in %

128 BDV : The blood donation volume in ml (without anticoagulant)

129 PCV_{donor} : The PCV of the donor in %

130 Wt_{recip} : The weight of the recipient in kg

131

132 The recipient transfusion monitoring sheets (involving a minimum of hourly temperature,
133 pulse and respiratory rate measurement) and kennel sheet medical records were
134 reviewed to assess for the presence of a transfusion reaction. As there are no current
135 veterinary definitions for transfusion reactions human guidelines were adapted for the
136 purposes of this study (NHSN, 2018). Acute development of urticaria or pruritis during the

transfusion was classified as an allergic reaction. If a recipient had an increase in rectal temperature of greater than 1°C from baseline at the beginning of the transfusion, non-pathological reasons for the increase e.g. external warming, recovery from general anaesthesia were considered by the study authors on a case by case basis. If no such reason was found a HTR was diagnosed if there was evidence of haemolysis in plasma or urine or an otherwise unexplained increase in total bilirubin concentration post transfusion alongside a rapid decrease of PCV post transfusion (a HTR was also diagnosed if these factors were fulfilled without a pyrexia during the transfusion). If there was no evidence of a HTR, cytological examination of the blood product was performed to assess for the presence of bacteria and the blood product date and appearance were checked. If abnormalities were noted, or if the pyrexia did not spontaneously resolve after cessation of blood product administration, a suspected septic transfusion was recorded and a culture of the blood product was performed. If neither a septic nor a HTR were suspected then a FNHTR was recorded. Transfusion associated circulatory overload (TACO) was recorded if a cat developed respiratory distress (defined as increased effort and tachypnoea) or novel pleural fluid or radiographic changes consistent within volume overload within 24 hours of the transfusion alongside echocardiographic changes

compatible with volume overload or if the patient was treated with furosemide. Transfusion related acute lung injury (TRALI) was recorded if the recipient developed acute respiratory distress within 24 hours of the transfusion, with radiographic or computed tomography evidence of bilateral pulmonary infiltrates and no evidence of congestive heart failure on echocardiography.

Age, recipient PCV before and after transfusion, and ΔPCV_{norm} were assessed for normality using a Shapiro–Wilk test. Descriptive statistics were produced for the study population. Concurrence between crossmatching techniques was assessed using Cohen's unweighted Kappa. The mean/median ΔPCV_{norm} was calculated for all cats and separately for those cats which received pRBCs and WB and those with and without major crossmatch incompatibility results. Results were then compared using a t-test or Mann–Whitney U test as appropriate.

The frequency and type of transfusion reactions in all cats and those in each crossmatch incompatible group were also calculated. Rates of FNHTR and HTR for cats receiving

pRBCs and WB and survival rates for cats that had transfusion reactions were compared to those that did not via Chi-squared tests.

Results

Recipient population

One hundred and one cats were recruited to the study between May 2016 and September 2018, with an extra cat being recruited before it was noted that a sufficient number had been reached. There were 45 female neutered, 54 male neutered, and 2 female entire cats. There were 56 domestic short hair cats, 10 domestic long hair cats, 5 Persians, 5 British short hairs, 4 Burmese, 4 Russian blues, 3 Bengals, 2 Siamese, 2 British blues and 1 each of Abyssinian, Burmese cross, domestic medium hair, exotic short hair, Havana, Maine coon, Norwegian forest, ragdoll, Tonkinese and Turkish van breeds. There were 87 type A cats, 10 type B cats and 4 type AB cats. The median age was 81 months (interquartile range (IQR) 44-113 months). The patient's underlying disease processes were classified as anaemia due to lack of RBC production (23 cats), RBC destruction (45 cats) or loss of RBCs (33 cats). Sixty-five cats received pRBCs and 36 received fresh WB.

Seventy-eight cats survived to discharge from the hospital, 17 were euthanased and 6 died during their hospitalisation period.

The median PCV prior to transfusion was 12% (IQR 9-15%) and after transfusion was 19% (IQR 15-21%). A post transfusion PCV was not obtained for one cat as it was unstable and venepuncture was not possible prior to cardiopulmonary arrest which occurred 12.25 hours after the end of the transfusion. The median time the PCV was obtained after the end of the transfusion was 12 hours (IQR 10.5-13 hours).

Crossmatch compatibility and PCV increase

A high frequency (27%) of major crossmatch incompatibility was found with the laboratory method with a lower frequency (10%) for the minor crossmatch laboratory method. Both major and minor crossmatch incompatibility was less frequent (both 4%) with the commercial test method (Table 1). Agreement between the laboratory and commercial crossmatching methods is shown in Tables 2 and 3. Unweighted Kappa agreement between the methods was found to be poor for the major crossmatch ((Kappa statistic 0.1351, 95% CI 0-0.5057, n=68) and fair for the minor crossmatch (Kappa statistic

0.3645, 95% CI 0-0.9254, n=43) . The recipient control was reported to be agglutinating in 18/96 cats (19%), and in 9 of these cases, the minor crossmatch was also agglutinating and was therefore deemed to be uninterpretable. The donor control was reported to be agglutinating in 1/98 cats (1%) and in this case the major crossmatch was also agglutinating and was therefore deemed to be uninterpretable.

The median ΔPCV_{norm} was 0.01279kg/ml (IQR 0.00567-0.02100). The ΔPCV_{norm} did not differ significantly between cats receiving pRBCs (median 0.01207, IQR 0.00926-0.02248) and WB (median 0.01413, IQR 0.00509-0.01972) (p=0.24). The ΔPCV_{norm} for major crossmatch compatible and incompatible blood for each test is shown in Table 4. There were no significant differences between ΔPCV_{norm} for crossmatch compatible and crossmatch incompatible blood for either crossmatching method (table 4).

Transfusion reactions and crossmatch compatibility

Transfusion reactions occurred in 20/101 cats. Nine cats had FNHTRs, 7 cats had HTRs, 3 cats had TACO, 2 developed hypothermia during their transfusion and 1 cat had a transfusion transmitted infection (the cat was transfused with *Mycoplasma*

haemominutium positive blood and was found be PCR positive for the organism post transfusion). Although all feline donors are checked for feline *Mycoplasma spp*, the result was not available prior to transfusion as this was an emergency fresh WB transfusion to the recipient. Complications occurred in 2 transfusions. One cat was administered approximately 10-15 mL of her transfusion subcutaneously due to intravenous cannula displacement. One cat was initially typed as an AB cat and was administered type A pRBCs due to a lack of immediate type AB blood availability. The cat had a HTR and on repeat blood typing, it was found that the cat was actually blood type B. Both the laboratory and commercial test major crossmatches were incompatible with both minor crossmatches compatible for this cat as would be expected.

Seven/65 ((11%) cats receiving pRBCs had a FNHTR compared to 2/36 (6%) cats receiving WB. Five/65 (8%) cats receiving pRBCs had a HTR compared to 2/36 (6%) receiving WB. These proportions were not significantly different.

The laboratory crossmatch suggested incompatibility for 3/7 (43%) cats that had a HTR (2 cats had major crossmatch incompatibility and one had both major and minor

incompatibly); this compared to an incompatibility rate of 30/90 (33%) for cats that did not have a HTR. The commercial test crossmatch suggested incompatibility in 2/4 (50%) cats (one major crossmatch incompatibility and one minor crossmatch incompatibility) compared to 2/68 (3%) that did not have a HTR. The 2 HTR cats that did not have incompatibility noted by the commercial test did not have minor crossmatches performed with this method.

Of the 9 cats with FNHTRs, the laboratory major crossmatch suggested incompatibility for 2 cats and the laboratory minor crossmatch suggested incompatibility for no cats. The commercial test was used in 7 of the FNHTR cats and all major and minor crossmatches were compatible for these cats.

Recipient outcome

Nineteen cats had at least one further WB or pRBC transfusion after their first transfusion. Two of the 6 cats (33%) that had a HTR required a further transfusion compared to 15 of the 92 (16%) cats that did not. This was not statistically significantly different. Survival to discharge was 66% for cats that had a HTR compared to 78% in cats that did not and 89%

for cats that had a FNHTR compared to 76% in cats that did not (neither difference was statistically significantly different).

Discussion

The first and second aims of this study were to determine the frequency of crossmatch incompatibility in cats which had not previously been administered a blood product and to compare the results of two crossmatching methods. It was shown that the frequency differed markedly between the two techniques studied, with a relatively high level of crossmatch incompatibility reported using a laboratory method and a much lower level of incompatibility reported using the commercial test. This finding concurs with an investigation comparing a laboratory method with the same commercial test used in this study in dogs (Guzman *et al* 2016). In that study it was concluded that the commercial test was inaccurate, but in this study, the clinical follow up of the crossmatched cats suggest that the laboratory method may actually be the less useful method as incompatibility was not associated with a detectable HTR in most cases. Guzman *et al* (2016) noted that interpretation of the commercial test could be difficult and it should be noted that in this study a simplified approach to identification of an incompatible

crossmatch result was used which differs slightly from that recommended by the manufacturers. Other studies looking at major crossmatching in cats prior to first transfusion report report similar incompatibility rates between 14.9 and 17% (McCloskey et al 2018, Sylvane et al 2018, Weltmand et al 2014) between the laboratory rate of 27% and the commercial test rate of 4% reported here.

The Kappa agreement between the crossmatching methods was very poor, suggesting they are not interchangeable. There are very wide confidence intervals for the Kappa agreement due to the relatively low overall number of cases when both crossmatching methods were used and the lower level of incompatibility reported by the commercial test.

Our third aim was to assess the effect of crossmatch incompatibility on the change in PCV seen post-blood donation. We found that administration of crossmatch incompatible blood in transfusion naïve cats was not associated with a lower retention of RBCs at 12 hours when compared to administration of crossmatch compatible blood (for both

method of crossmatching). However, it could be argued that sampling PCV at 12 hours may have been too early to detect the effects of a HTR, and the fall in PCV may occur later. Weingart *et al* (2004), in a large retrospective study, described several cats that had clinical signs consistent with HTRs when their total bilirubin concentrations increased 1-5 days after transfusion and their PCV rise was lower than expected at 16-24 hours post transfusion. Similarly, a Mik-negative cat administered presumed Mik-positive blood was described to have an increase in serum bilirubin and haemoglobinemia 24-48 hours post transfusion (Weinstein *et al*, 2007). It was therefore important that this study monitored the progression of the cats throughout their hospitalisation time and assessed them for a HTR which may not have been noted by assessing PCV at the 12-hour mark.

The commercial test found incompatibility in 2/4 HTR cases. Although not all HTRs were detected by this method of crossmatching, this may at least in part have been because minor crossmatches were not performed in the 2 cats where no incompatibility was detected. This study suggests that as a minimum, the commercial test may be a useful method for assessing compatibility. If this test suggests compatibility, then a HTR is unlikely. The laboratory crossmatch method did not appear as useful in the detection of

HTR patients where 43% of the cats had either major or minor (or both) crossmatch incompatibility reported, compared to 35% for those that did not have a HTR.

Several reasons can be postulated as to why the laboratory method was not reliable for the prediction of HTRs in transfusion naive cats. Firstly, the method is subjective, and although technicians were trained in assessment for agglutination, human error is possible (Abrams-Ogg, 2016). Secondly a large proportion of the cats in the study had immune mediated haemolytic anaemia and many had spontaneous agglutination. The laboratory method included cell washing, but agglutination is still possible after this procedure. Finally, it is possible that the laboratory method was detecting incompatibilities that were present, but that were not clinically relevant and did not result in an appreciable HTR.

Although crossmatch incompatibility would suggest a HTR was more likely rather than any other transfusion reaction , FNHTR were also examined in this study as McClosky *et al* (2018) found that FNHTR were more common in their non-crossmatched cats compared to their group administered crossmatch compatible blood. This was not the case in this

study, with low levels of incompatibility with both crossmatching methods noted for cats that had FNHTRs. It is possible that in the McClosky *et al* (2018) study, the patients classified as having FNHTR may have actually been having HTRs and this was hard to detect given the retrospective nature of the study. It is probably important to note that primary clinicians caring for the patients in this study did not always recognise the occurrence of a transfusion reaction as clinical signs were sometimes mild in nature.

The final aim of the study was to assess the frequency of transfusion reactions and complications and errors. The frequency of transfusion reactions in this population was high at approximately 1 in 5 cats. It is much higher than that reported in several previous studies (Castellanos *et al* 2004; McClosky *et al* 2018; Weingart *et al* 2004). A study in dogs reported a much higher transfusion reaction rate of 15% with reactions being more common with pRBC transfusions compared to other blood products (Bruce *et al* 2015). In people, transfusion reactions are well defined and rates of between 0.2 and 3.8% have been reported, with variation between studies and blood products administered (Kato et al 2013; Kato et al 2015; Negi et al 2015).

However veterinary studies reporting transfusion reactions are hampered by the lack of clear guidelines of what constitutes an transfusion reaction. Also, this was a prospective study where the cats were specifically being monitored for transfusion reactions and so it is likely that cases were recorded which could otherwise have been missed. This is especially true for HTRs, where close monitoring was required to detect the increase in serum bilirubin as this was often not marked and often did not result in clinical icterus. The transfusion reaction rate in this study is similar to that described in another prospective study where a frequency of 23% transfusion reaction was noted, with the majority being FNHTR, as with this study (Sylvane *et al* 2018).

HTRs are classified as acute if they result from pre-formed antibodies and delayed if the antibodies develop post transfusion (Strobel 2008). In this study it is suspected that the HTRs noted were acute. Although only one of the cats developed pyrexia during the transfusion, in all cases evidence of haemolysis occurred with 24 hours, when signs of a delayed HTR are expected after 24 hours (National Healthcare Safety Network 2018). This suggests that pre-formed non-AB antibodies, such as anti-Mik antibodies, were present in several cats in this study. There was no difference in the need for further blood products

or survival to discharge noted in the cats with a HTR however. Although this does not mean that the cats with a HTR had no difference in morbidity when compared to those without, it does suggest that the effect was not marked.

Definitive proof that these were genuine transfusion reactions was not possible to obtain given the clinical nature of the study. Even the transfusion-transmitted infection case with the administration of blood from a *Mycoplasma haemominutium* infected donor was not a definite transfusion reaction, as the recipient was not assessed for the presence of the organism prior to transfusion. Ideally, to confirm a patient had a HTR, a direct antiglobulin test should be performed both before and after the transfusion to assess for the presence of anti-erythrocyte antibodies and whether there is an increased reaction post transfusion as in human medicine (Strobel 2008). The diagnosis of FNHTR is made by discounting all other possible causes of pyrexia, which was attempted during the study, but it is possible that HTRs could have been misdiagnosed as FNHTR if they were mild although most of the HTRs in this study were not associated with a pyrexia. It is difficult in the clinical situation to state that development of hypothermia or respiratory distress is definitely secondary to transfusion given the multitude of other treatments being

administered in these critical patients. However, the guidelines, based on human guidelines for the diagnosis of transfusion reactions, described in the methods were used to maximise the likelihood of genuine diagnosis.

This study has many limitations. Firstly, there was insufficient data in the literature when the study was planned to perform a sample size calculation to determine the number of cases. Moreover, this study was aimed at assessing the agreement in performance of 2 tests for the detection of a reaction, not to assess the frequency of a disease. In light of these issues, a convenience sample of 100 cats was chosen to provide a sufficiently large population that we hoped would detect a difference if one was present and that was also achievable to allow recruitment over 2 years, but given the results obtained, it is likely the study was under-powered. Secondly, as noted above, the timing of blood sampling post transfusion may have been too early to detect the results of a HTR. However, given the dynamic nature of many of these patients' disease processes, leaving sampling too long may have meant it was difficult to assess the impact of the transfusion. This clinical aspect of the trial is a strength, as it allows assessment of the impact of crossmatching and transfusion reactions in the clinical situation. However, it also means the recipients were

very variable, transfusion administration was not standardised and the impact of general anaesthesia, dehydration and volume status could all have affected the PCV alongside on-going haemorrhage and RBC lysis depending on the underlying disease process. Also, some patients died during or shortly after transfusion administration, meaning that possible transfusion reactions may have been missed. Thirdly, sufficient blood to run each crossmatch was not available for each patient as they were often unstable prior to transfusion. Therefore, a commercial test major crossmatch was only performed on approximately 2/3 of the study population and a commercial test minor crossmatch on approximately ½. Only 2 methods of crossmatching were tested and other commercial and non-commercial methods are available which may have differing results. Finally, although every effort was made to monitor for transfusion reactions, they may have been missed as the clinical nature of the patients meant that treatment and blood sampling was not standardised.

In summary, this study showed no advantage in crossmatching patients prior to first transfusion when assessing increase in PCV at 12 hours and survival to discharge which is consistent with the findings of previous large studies (McClosky *et al* 2018; Sylvane *et al*

2018). Interestingly, these studies have differing conclusions with Sylvane *et al* (2018) suggesting that their results do not support the use of crossmatching prior to first transfusion in cats, whereas McCloskey *et al* (2018) state that the prevalence of naturally occurring non-AB incompatibilities they detected is sufficiently high to justify the recommendation to perform a crossmatch prior to first RBC transfusions in cats. Our study has found that cats can have a HTR on first transfusion and that this is not uncommon. Although the laboratory method seems less useful at predicting these, when the commercial test suggests incompatibility, a HTR appears to be more likely. Although a negative impact of HTR could not be demonstrated in this study, that could be due to low case numbers and lack of sufficient monitoring. Ultimately, in the authors' opinion, a pragmatic approach is probably best. It could be argued that if there are multiple donors available, then crossmatching prior to first transfusion, and use of a compatible donor is optimal, although if this is not feasible, this study suggests that transfusion without prior crossmatching can be safe and effective.

Footnotes

A: QuickTest Blood Typing, Alvedia, Limonest, France

B: Rotalavit, Hettich Lab Technology, Tuttlingen, Germany

C: 5ml Eppendorf tubes, VWR Inernational , LLC Radnor, Pennsylvania

D: RapidVet-H Crossmatch kits, DMS Laboratories, Flemington, New Jersey

Conflicts of Interest: The cross matching kits assessed in this study were supplied by the manufacturers but the company had no input into the content of this manuscript.

Acknowledgements: With thanks to Charlotte Russo, Claire Marshall and Susie Hunt for their help with sample collection, to David Arnfield and Paul Barber for their performance of the crossmatch tests and to Geraint James for his statistical and mathematical help.

Funding: This study was kindly sponsored by a grant provided by the PetPlan Charitable Trust.

441 References:

442 Abrams-Ogg ACG (2016) Feline Recipient Screening In: Manual of Veterinary Transfusion
443 Medicine and Blood Banking. Eds K Yagi and M Holowaychuk. Wiley Blackwell, Iowa USA.
444 Pp. 129-154

445 Barfield D and Adamantos S (2011) Feline blood transfusions: A pinker shade of pale.
446 Journal of feline medicine and surgery 13, 11-23

447

448 Bruce JA, Kriese-Anderson L, Bruce AM *et al* (2015) Effect of premedication and other
449 factors on the occurrence of acute transfusionreactions in dogs. *Journal of Veterinary*
450 *Internal Medicine* 25, 620-30

451

452 Castellanos I, Couto CG and Gray TL. (2004) Clinical use of blood products in cats: a
453 retrospective study (1997--2000). *Journal of Veterinary Internal Medicine* 18, 529-32

454

455 Giger U and Akol KG. (1990) Acute hemolytic transfusion reaction in an Abyssinian cat
456 with blood type B. *Journal of Veterinary Internal Medicine* 4, 315-6

457

458 Giger (2014) Blood Typing and Crossmatching to Ensure Blood compatibility In: Kirk's
459 Current Veterinary Therapy XV. Eds J D Bonagura and D C Twedt. Elsevier Saunders,
460 Missouri USA e143

461

462 Goy-Thollot I, Giger U, Boisvineau C *et al* (2017) Pre- and Post
463 Transfusion Alloimmunization in Dogs Characterized by 2 Antiglobulin-Enhanced Cross-
464 match Tests. *Journal of Veterinary Internal Medicine* 31, 1420-1429

465

466 Guzman LR, Streeter E and Malandra A. (2016) Comparison of a commercial blood cross-
467 matching kit to the standard laboratory method for establishing blood transfusion
468 compatibility in dogs. *Journal of Veterinary Emergency and Critical Care* 26, 262-268

469

470 Jagodich TA and Holowaychuk MK. (2016) Transfusion practice in dogs and cats: an
471 Internet-based survey. *Journal of Veterinary Emergency and Critical Care* 26, 360-72

472

473 Kato H, Uruma M, Okuyama Y *et al* (2013) Incidence of transfusion-related adverse
474 reactions per patient reflects the potential risk of transfusion therapy in Japan. *American*
475 *Journal of Clinical Pathology* 140, 219-24
476
477 Kato H, Nakayama T, Uruma M *et al* (2015) A retrospective observational study to assess
478 adverse transfusion reactions of patients with and without prior transfusion history. *Vox*
479 *Sang.*108, 243-50

480 McClosky ME, Cimino Brown D, Weinstein NM *et al* (2018) Prevalence of naturally
481 occurring non-AB blood type incompatibilities in cats and influence of crossmatch on
482 transfusion outcomes. *Journal of Veterinary Internal Medicine* 32, 1934-1942

483 National Healthcare Safety Network (2018) National Healthcare Safety Network
484 Biovigilance Component Hemovigilance Module Surveillance Protocol.
485 [https://www.cdc.gov/nhsn/pdfs/biovigilance/bv-hv-protocol-current.pdf] Accessed 17th
486 September 2019

487 Negi G, Singh Gaur D *et al* (2015) Blood transfusion safety: A study of adverse reactions at
488 the blood bank of a tertiary care center. *Advances in Biomedical Research* 4, 237

PHB Bolton-Maggs (Ed) D Poles et al. (2018) on behalf of the Serious Hazards of Transfusion (SHOT) Steering Group. The 2017 Annual SHOT Report

Strobel E. (2008) Hemolytic transfusion reactions. *Transfusion Medicine and Hemotherapy* 35, 346-353

Sylvane B, Prittie J, Hohenhaus AE *et al* Effect of cross-match on packed cell volume after transfusion of packed red blood cells in transfusion-naïve anemic cats. *Journal of Veterinary Internal Medicine* 32, 1077-1083

Tasker S, Barker EN, Day MJ et al. (2014) Feline blood genotyping versus phenotyping, and detection of non-AB blood type incompatibilities in UK cats. *Journal of Small Animal Practice* 55, 185-9

Weingart C, Giger G and Kohn B. (2004) Whole blood transfusions in 91 cats: a clinical evaluation. *Journal of Feline Medicine and Surgery* 6, 139-48

Weinstein NM, Blais MC, Harris K, *et al* (2007) A newly recognized blood group in domestic shorthair cats: the Mik red cell antigen. *Journal of Veterinary Internal Medicine* 21, 287-92

Weltman JG, Fletcher DJ and Rogers C. (2014) Influence of cross-match on posttransfusion packed cell volume in feline packed red blood cell transfusion. *Journal of Veterinary Emergency and Critical Care* 24, 429-36