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STANDARD ARTICLE

Prevalence of naturally occurring non-AB blood type incompatibilities in cats and influence of crossmatch on transfusion outcomes

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Background: Recognition of the feline red blood cell (RBC) antigen *Mik* and the presence of naturally occurring anti-*Mik* antibodies resulting in acute hemolytic transfusion reactions prompted the recommendation to perform a crossmatch before a cat's first RBC transfusion, but this guideline has not yet become a standard practice.

Objective: To determine the prevalence of naturally occurring non-AB alloantibodies detectable by tube crossmatch, and to compare transfusion outcomes in cats with and without a crossmatch performed.

Animals: Three hundred cats that received an RBC transfusion, with or without a major crossmatch performed.

Methods: Retrospective study.

Results: Major crossmatch incompatibilities were documented in 23 of 154 transfusion-naive cats (14.9%) and in 15 of 55 previously transfused cats (27%; $P = 0.042$). Type-specific packed RBCs (pRBCs) were administered to 167 and 82 cats with and without a crossmatch, respectively. Median volume of pRBCs administered during the first transfusion was 5.3 mL/kg (range, 2.4-18 mL/kg). Median change in PCV scaled to dose of pRBCs was +0.8%/mL/kg; administration of crossmatch-compatible pRBCs was not associated with a greater increase in PCV. Febrile transfusion reactions occurred more often in cats that received non-crossmatched (10.1%) compared to crossmatched (2.5%) pRBCs ($P = 0.022$). Seventy-six percent of cats that received pRBC transfusions survived to hospital discharge. A crossmatch was not associated with improved survival to discharge or at 30 or 60 days posttransfusion.

Conclusions and Clinical Importance: The prevalence of naturally occurring non-AB incompatibilities is sufficiently high to justify the recommendation to perform a crossmatch before all (including the first) RBC transfusions in cats.

KEYWORDS

crossmatch incompatibility, *Mik* antigen, packed red blood cells, transfusion reaction

1 | INTRODUCTION

The clinical importance of the feline AB blood group system with naturally occurring anti-A and anti-B alloantibodies is well recognized. Investigations of feline red blood cell (RBC) alloantibodies worldwide have shown that all type B cats (after 6-8 weeks of age) have anti-A antibodies that are typically high-titered (64-1024) hemagglutinins and hemolysins.[1–4] In contrast, type A cats have relatively weak anti-B antibodies, with hemagglutinin and hemolysin titers generally ≤32 and often ≤8.[1–4] The percentage of type A cats reported to have

Abbreviations: FWB, fresh whole blood; pRBCs, packed red blood cells; RBC, red blood cell

detectable anti-B antibodies is highly variable, ranging from 16.4% to 100%, with some differences potentially attributable to geographic location and methodology.[1–4] The clinical relevance of strong anti-A alloantibodies is readily apparent from experimental and clinical reports of severe, acute hemolytic transfusion reactions (characterized by hypotension, bradycardia, apnea, urination, defecation, vomiting, hemoglobinemia, and hemoglobinuria) in type B cats receiving type A blood.[5–8] Although weaker anti-B antibodies produce a mild reaction (slight hemoglobinemia, hemoglobinuria, and bilirubinuria) in type A cats that receive type B RBCs, such type-mismatched transfusions are associated with shortened survival of transfused RBCs, with a mean half-life of 2 days.[7,8] Therefore, there is universal agreement that all feline blood donors and recipients should be blood typed (for A, B, or AB antigens) before an RBC transfusion. Point-of-care feline blood-typing kits allow for rapid and accurate determination of type within the AB blood group system in clinical practice.[9–11]

In 2007, a new feline RBC antigen, *Mik*, was recognized after a type A-matched RBC transfusion resulted in an acute hemolytic transfusion reaction in a *Mik*-negative cat with anti-*Mik* alloantibodies that received blood from a *Mik*-positive donor.[12] Anti-*Mik* alloantibodies are naturally occurring but could develop after an RBC transfusion in a *Mik*-negative cat.[12] *Mik* appears to be a common RBC antigen, with few *Mik*-negative cats identified thus far; however, typing for the *Mik* antigen is restricted because of a lack of typing reagent (ie, serum from a *Mik*-negative cat having anti-*Mik* alloantibodies).[12] Although the standard recommendation has been to perform a crossmatch for cats (and dogs) that are to receive blood >4 days after their first transfusion,[11,12] documentation of naturally occurring anti-*Mik* alloantibodies and the possibility of other non-AB, non-*Mik* alloantibodies in cats has prompted the recommendation to consider a crossmatch for cats before their first RBC transfusion.[12] Furthermore, administration of type-specific, crossmatch-compatible pRBCs to cats has been associated with a significantly greater increase in PCV posttransfusion compared to cats receiving typed, non-crossmatched pRBCs.[13]

Addition of a crossmatch to pretransfusion testing increases client cost and potentially delays the start of a transfusion. However, patient safety and transfusion efficacy are of paramount importance. The primary objective of our retrospective study was to determine the prevalence of naturally occurring, non-AB blood type incompatibilities in cats having a crossmatch performed in anticipation of a blood transfusion or renal transplantation. A secondary objective was to compare transfusion outcomes in cats with and without a pretransfusion crossmatch.

2 | MATERIALS AND METHODS

2.1 | Data collection

Medical records of all cats that received an RBC transfusion with or without a major crossmatch or had a major crossmatch performed without subsequent RBC administration at the Matthew J. Ryan Veterinary Hospital of the University of Pennsylvania from January 1, 2013, to December 31, 2016, were reviewed retrospectively. Our institution's clinical laboratory feline crossmatch logbook and blood bank transfusion logbook were reviewed to identify cats for this study. Cats were excluded if the medical record was missing or incomplete.

The following information was recorded: signalment, blood type, transfusion history, major and minor crossmatch results, RBC product administered (pRBCs or fresh whole blood [FWB]), volume of pRBCs (approximately 20 mL pRBCs per unit, to which 9 mL of RBC additive solution [Optisol, AS-5; Terumo, Tokyo, Japan] was added; volume administered was calculated based on pRBCs without additive solution), FWB (approximately 40 mL per unit) administered or some combination of these, reason for transfusion (eg, hemolysis, blood loss, ineffective erythropoiesis), pretransfusion and posttransfusion PCV, adverse events, and patient outcome (defined as survival to discharge, euthanasia, or in-hospital death). Thirty- and 60-day survival times were obtained from the medical record or by contacting the referring veterinarian. Duration of pRBC storage was determined based on the blood collection date recorded in the blood bank transfusion logbook. For pRBC units obtained from a commercial animal blood bank (Animal Blood Resources International, Dixon, CA), the collection date was determined by subtracting 35 days (maximum storage duration recommended by the commercial animal blood bank) from the expiration date listed on the unit and recorded in the blood bank transfusion logbook.

2.2 | Blood donors

Feline pRBCs and FWB were obtained primarily from our institution's blood bank. During times of increased demand or shortage of blood products, pRBCs were purchased from a commercial animal blood bank (Animal Blood Resources International). Our institution's donor cats were owned by students or hospital staff or maintained in the hospital colony. Donor health screening included history, physical examination, and blood hemoglobin concentration performed before each donation, and an annual CBC, serum biochemistry panel, and testing for blood-borne pathogens, as outlined in the updated consensus statement of American College of Veterinary Internal Medicine.[14]

2.3 | Transfusions and patient monitoring

The decision to administer an RBC transfusion was at the discretion of the primary clinician. The main cause of anemia was classified as blood loss, hemolysis, or ineffective erythropoiesis. The pretransfusion PCV was defined as that measured closest to the start of the RBC transfusion. The posttransfusion PCV was defined as that recorded closest to the end of the RBC transfusion, typically within 1-2 hours posttransfusion. The change in PCV, hereafter referred to as ΔPCV, was calculated as the difference between the posttransfusion and pretransfusion PCV. Patients were monitored during the transfusion by a standard protocol that includes evaluating vital parameters, mucous membrane color, pulse quality, blood pressure, and mentation every 15 minutes during the transfusion. A febrile transfusion reaction was defined as an increase in body temperature ≥ 2 °F during or within 4 hours posttransfusion if active rewarming was not used. An acute hemolytic transfusion reaction was defined as a development of fever, hemoglobinuria, hemoglobinemia, and a lack of increase in PCV

posttransfusion during or within 24 hours posttransfusion. If cats were anesthetized or hypothermic for other reasons, fever was not required as a criterion for an acute hemolytic transfusion reaction. If urine was not available for analysis, the diagnosis of an acute hemolytic transfusion reaction was based on the presence of fever (if it could be evaluated), hemoglobinemia, and lack of increase in PCV (not attributable to another cause, such as ongoing blood loss). Medical records were reviewed for other potential transfusion-associated adverse events.

2.4 | Blood type and crossmatch procedures

Blood typing was performed with a commercially available immunochromatographic test kit (Quick Test A+B; Alvedia, Limonest, France). Crossmatch (major, minor, and auto control to evaluate for RBC agglutination) was performed using a standard tube method, as previously described,[12] by experienced technical staff of our institution's clinical laboratory or, after hours, by emergency service personnel or the primary clinician on the case. The following scale was used: 4+, single large agglutinate of RBCs; 3+, few large agglutinates of RBCs; 2+, large agglutinates of RBCs amid many smaller RBC clumps; 1+, many small RBC agglutinates amid a background of free cells; and negative, no granularity.[12] Hemolysis was also recorded if noted. The number and identification of donors were evaluated; the number and degree of major and minor incompatibilities, and the duration of storage of the donors' pRBC sample used in the crossmatch were recorded.

For a small subset of cats, a crossmatch was performed using both the tube method and the gel column method, as previously described,[12] in a prospective comparison of these procedures. Gel crossmatches were performed by a single experienced laboratory technician who was not blinded to results of the tube crossmatch. Degree of RBC retention in gel corresponded to degree of incompatibility: 4+, all RBCs at the top of the gel; 3+, RBC agglutinates throughout and on top of gel; 2+, RBC agglutinates dispersed throughout the gel; and 1+, few RBC agglutinates in the lower half of the gel but most RBCs at the bottom of the gel. For a compatible crossmatch, all RBCs were at the bottom of the gel column.

2.5 | Statistical analysis

Statistical analyses were performed using standard statistical software (STATA 13.1; Stata Corporation, College Station, TX). Descriptive statistics were calculated. Continuous variables were described with means and standard deviations (SD) if normally distributed and median values and ranges if not. Categorical variables were described as proportions and frequencies. Because of non-normality of the data, the Mann-Whitney *U* test was used to determine whether there was a difference in ΔPCV (including scaled to dose of pRBCs administered) between cats that did and did not have a pretransfusion crossmatch performed, and whether there was a difference in ΔPCV depending on the cause of anemia. Pearson's chi-square test was used to determine if storage time of donor RBC samples was associated with major crossmatch incompatibilities. Fisher's exact test was used to determine whether there was a difference in frequency of adverse events and patient outcome between cats that did and did not have a pretransfusion crossmatch performed. A P value <0.05 was considered significant.

3 | RESULTS

3.1 | Patient characteristics

A total of 331 cats was identified, but 31 were excluded because of missing or incomplete medical records. The median age was 9 years (range, 5 weeks to 20 years). Fifteen breeds were represented, but the majority were domestic shorthair (n = 255) and domestic longhair (n = 17) cats. There were 120 spayed and 8 intact females, and 164 castrated and 8 intact males. The median body weight was 4.1 kg (range, 0.6-8.3 kg). The blood-type frequencies were 96% type A (n = 287), 3% type B (n = 10), and 1% type AB (n = 3).

3.2 | Transfusion history

History regarding RBC transfusions was available for 277 cats. Of those, 220 (79%) were transfusion-naive, and 57 (21%) had been transfused previously. A crossmatch was performed for 157 of the transfusion-naive cats and for all 57 previously transfused cats. The median number of previous RBC transfusions was 1 (range, 1-9). If previously transfused, the median time since first RBC transfusion was 25 days (range, 1-3666 days). Two cats had a history of receiving both renal transplant and pRBC transfusion 3-4 weeks before evaluation of subsequent crossmatch results.

3.3 | Major and minor crossmatch

Two-hundred eighteen cats, including 157 transfusion-naive cats, 57 previously transfused cats, and 4 cats with an unknown transfusion history, were screened with a major crossmatch to a median of 3 donors (range, 1-5 donors): 1 donor (n = 16 cats), 2 donors (n = 66 cats), 3 donors (n = 120 cats), 4 donors (n = 5 cats), and 5 donors (n = 7 cats). The number of donors was unavailable for 4 cats. A minor crossmatch also was performed for 210 of the 218 cats. Two hundred crossmatches (major and minor; 91.7%) were performed by the clinical laboratory staff, 17 major (and 10 minor) crossmatches were performed by emergency service personnel or by the primary clinician after hours and 1 major crossmatch was performed by the commercial animal blood bank (Animal Blood Resources International) for a type B cat before purchasing a pRBC unit. Results of the crossmatch could not be interpreted in 2 cats (1 transfusion-naive and 1 previously transfused) because of persistent RBC autoagglutination. Results of major crossmatches performed after hours for 3 cats (2 transfusion-naive and 1 with unknown transfusion history) and by the commercial blood bank for 1 cat did not include sufficient information in the medical record to comment on the number of donors tested or compatibility with all donors, but a compatible pRBC unit was identified for each of the 4 cats. Therefore, compatibility data were available for 212 cats.

The majority of cats (167 of 212, or 79%) were compatible with all donors on the major and minor crossmatch (Table 1). Forty cats had major crossmatch incompatibilities. For the transfusion-naive cats, 23 of 154 (14.9%) showed some degree of incompatibility (1+ to 3+)

TABLE 1 Non-AB RBC incompatibilities detected via tube major crossmatch in transfusion-naive and previously transfused cats

Number of major incompatibilities/ number of donors tested	Transfusion-naive cats (n = 154)	Previously transfused cats (n = 55)	Unknown transfusion history (n = 3)
All compatible	131	40	1
1/1	0	1	
1/2	2	4	
1/3	9	4	1
1/4	1	0	1
1/5	2	0	
2/2	1	0	
2/3	4	0	
3/3	1	5	
3/5	1	0	
4/4	0	1	
4/5	1	0	
5/5	1	0	

Abbreviation: RBC, red blood cell.

on the major crossmatch to ≥1 donor (Table 1). No difference was observed in the prevalence of RBC incompatibilities among crossmatches performed by the clinical laboratory staff and emergency service personnel (P = 0.66). For the 3 remaining transfusion-naive cats for which a crossmatch was performed (3 of 157 cats), compatibility data were not available because of autoagglutination (n = 1) or insufficient information recorded during after-hours testing (n = 2). Of the 55 cats previously transfused, 15 cats (27%) were incompatible (1+ to 2+) on the major crossmatch to ≥1 donor (Table 1), significantly more than in the transfusion-naive group (P = 0.042). In addition, 2 cats with unknown transfusion history had an incompatibility (1+ to 2+) on the major crossmatch. All of the incompatibility reactions observed on both major and minor crossmatches were manifested as agglutination; hemolysis was not reported for any crossmatch.

Eleven cats had minor crossmatch incompatibilities (1+ to 2+) to ≥1 donor, with 6 of these cats (4 transfusion-naive and 2 previously transfused) also having major crossmatch incompatibilities (Table 2). The 5 cats with minor incompatibility only all were transfusion-naive. Thirteen feline blood donors were involved with these minor incompatibilities. During the 4-year study period, the number of minor crossmatches performed using plasma from each of these 13 donors ranged from 1 to 19 (median, 7), with plasma from only 1 donor responsible for causing a minor incompatibility in >1 cat. Plasma from this donor was used in minor crossmatches for 19 cats but produced an incompatibility reaction in only 2 cats.

3.4 | Influence of storage time of donor RBC sample on major crossmatch compatibility

With major crossmatches performed for 212 cats, most cats crossmatched to >1 donor, and 51 cats having subsequent crossmatches performed during the study period, 683 major crossmatches were performed. Results were available for 674 crossmatches; compatibility could not be determined in 9 crossmatches because of RBC autoagglutination. There were 586-compatible and 88-noncompatible major crossmatches. Donor RBC samples used for compatibility testing for renal transplantation screening were fresh, with the crossmatch performed the same day as sample collection. There were 498 donor RBC samples stored for less than 10 days ("fresh"), with 65 (13%) major crossmatch incompatibilities noted, and 176 donor RBC samples stored for more than 10 days ("old"), with 23 (13%) major crossmatch incompatibilities, with no difference between groups (P = 0.996). Furthermore, no difference was observed in degree of major crossmatch incompatibility when comparing fresh and old donor RBC samples (P = 0.412).

3.5 | RBC transfusion characteristics and influence of crossmatch on outcome

Red blood cell transfusions were administered to 249 cats for anemia because of ineffective erythropoiesis (n = 118), blood loss (n = 118), and hemolysis (n = 13). The majority of cats (n = 235) received pRBCs

TABLE 2 Non-AB RBC incompatibilities detected via tube minor crossmatch in transfusion-naive and previously transfused cats

Number of minor incompatibilities/ number of donors tested	Transfusion-naive cats (n = 152)	Previously transfused cats (n = 55)	Unknown transfusion history (n = 3)
All compatible	143	53	3
1/2	3	0	0
1/3	3	1	0
1/5	2	0	0
2/4	1	0	0
3/3	0	1	0

Abbreviation: RBC, red blood cell.

only, whereas 11 cats received both pRBCs and FWB, and 3 cats received FWB only. One-hundred eighty-eight of 246 cats (76.4%) were given a single pRBC transfusion, whereas 41 cats (16.7%) received 2 pRBC transfusions and 13 cats (5.3%) received 3 pRBC transfusions. Two cats were given 4 pRBC transfusions, and 1 cat each had 5 and 10 pRBC transfusions during a single hospitalization. Of the 14 cats that received FWB, 12 received a single unit and 1 cat each received 2 (in combination with 4 units of pRBCs) and 3 units of FWB. A total of 352 units of pRBCs was administered during the study period, with 280 prepared by our institution's blood bank and 72 purchased from a commercial animal blood bank (Animal Blood Resources International).

A crossmatch was performed for 167 of the 249 cats (67%) before their first RBC transfusion, whereas the remaining 82 cats received type-compatible, non-crossmatched RBCs. Fifty-one cats had a crossmatch performed without subsequent RBC administration, 42 for anticipated blood loss, persistent anemia, or both, and 9 as part of the screening process for renal transplantation.

For the first pRBC transfusion, the median pretransfusion PCV (n = 243) was 15% (range, 5%-40%), with no difference between cats with (PCV, 15.5%) and without (PCV, 15%) a crossmatch performed ($P = 0.19$). The median volume of pRBCs administered during the first transfusion was 5.3 mL/kg (range, 2.4-18 mL/kg), with a median of 5.1 mL/kg for cats without a crossmatch and 5.4 mL/kg for cats with a crossmatch, also with no difference between groups ($P = 0.704$). The median posttransfusion PCV (n = 231) was 20% (range, 10%-55%) and ΔPCV was +5% (range, −17% to +21%). For cats that did not have a pretransfusion crossmatch performed, the median ΔPCV (n = 77) was +6% (range, −5% to +21%), whereas the median ΔPCV (n = 154) for cats that had a pretransfusion crossmatch performed was +5% (range, −17% to +14%) ($P = 0.019$). The median ΔPCV scaled to the dose of pRBCs administered was +0.8%/mL/kg (range, −1.7 to +5.25%/mL/kg). For cats that did not have a pretransfusion crossmatch performed, the median scaled ΔPCV was +0.97%/mL/kg (range, −1.56 to +5.25%/mL/kg), whereas the median scaled ΔPCV for cats that had a pretransfusion crossmatch performed was +0.76%/mL/kg (range, −1.7 to +5.18%/mL/kg; $P = 0.042$). Cause of anemia did not influence ΔPCV. The median total volume of pRBCs administered during hospitalization was 6.5 mL/kg (range, 2.4-38.5 mL/kg). For cats that did not have a pretransfusion crossmatch performed, the median total volume of pRBCs administered was 5.7 mL/kg (range, 2.5-26.4 mL/kg), whereas the median total volume of pRBCs administered to cats that had a pretransfusion crossmatch performed was 6.7 mL/kg (range, 2.4-38.5 mL/kg; $P = 0.04$).

Transfusion monitoring data associated with the first pRBC transfusion were available for 240 cats. Twelve cats (5%) developed fever, without evidence of hemolysis, during the transfusion, occurring more often in cats that received typed, non-crossmatched pRBCs (8 of 79; 10.1%) than in cats given crossmatch-compatible pRBCs (4 of 161; 2.5%; $P = 0.022$). In addition, 2 cats developed suspected transfusion-associated adverse events; neither had a pretransfusion crossmatch performed. One cat received a pRBC transfusion for blood loss anemia secondary to severe flea infestation and experienced respiratory followed by cardiac arrest 36 hours later. Transfusion-associated lung injury or pulmonary thromboembolism was suspected by the primary clinician. The second cat received pRBCs at the start of a hemodialysis procedure for lily toxicity, partially dislodged its dialysis catheter during treatment, and then experienced cardiopulmonary arrest. After resuscitation, hemoglobinuria, hyperbilirubinemia, and a lack of increase in PCV were noted. Post-event crossmatching to investigate the possibility of RBC alloantibodies leading to an acute hemolytic transfusion reaction was not performed. Necropsy was declined in both cases.

Of the 246 cats that received pRBC transfusions, 188 (76.4%) survived to hospital discharge. A pretransfusion crossmatch was not associated with improved survival to discharge ($P = 0.15$) or 30 ($P = 0.208$) or 60 ($P = 0.052$) days posttransfusion. In addition, there was no difference in euthanasia ($P = 0.191$) or in-hospital death ($P = 0.6$) between cats that received crossmatched as compared to non-crossmatched pRBCs.

3.6 | Development of non-AB incompatibilities posttransfusion

During the study period, 43 cats had a crossmatch performed within 1-15 days after the initial pRBC transfusion in preparation for a second transfusion. Some degree of incompatibility (1+ to 3+) was observed on the major crossmatch for 11 cats (25.6%), and 7 of these cats (16.3%) had no incompatibility on their first crossmatch to 2 or 3 donors tested. Four of these 7 cats developed an incompatibility to the same donors to which they previously had been compatible, and 3 of the 4 cats had received only 1 pRBC transfusion before the second crossmatch (ie, they were transfusion-naive at the start of the study). The time to detection of new non-AB incompatibilities (1+) in these 3 cats was 2 (n = 1) to 3 (n = 2) days posttransfusion. The fourth cat had received a pRBC transfusion 16 days before its first crossmatch during the study period and developed a 1+ and 2+ incompatibility to 2 donor cats only 1 day after the initial crossmatch and pRBC transfusion. Pretransfusion crossmatches for all 11 cats were performed by the clinical laboratory; posttransfusion crossmatches were performed by the clinical laboratory for 9 cats, including the 4 cats with new non-AB incompatibilities, and by the emergency service for 2 cats.

3.7 | Administration of type-matched, crossmatch-incompatible pRBCs

Six cats, 5 of which had been transfused previously, were crossmatch-incompatible to all pRBC units tested (3 units, n = 4; 4 units, n = 1; 2 units, n = 1) and were given the least incompatible unit. Five cats received pRBCs to which there was a 1+ major incompatibility, and 1 cat was given a pRBC unit to which there was a 1+ minor incompatibility. None of these cats developed an obvious febrile or acute hemolytic transfusion reaction, although 4 of 6 cats received the pRBC unit as a hemodialysis prime, which complicated transfusion monitoring. The median ΔPCV was +4% (range, +1% to +13%), and the median scaled ΔPCV was +0.86%/mL/kg (range, +0.19 to +2%/mL/kg). The 2 non-dialysis cats, 1 with a minor and 1 with a major RBC incompatibility, survived to >60 days posttransfusion. Of the 4 cats that underwent hemodialysis, 1 cat being treated for a massive overdose of

vincristine died in the hospital 2 days posttransfusion, 2 cats were discharged but survived <30 days, and 1 cat survived >60 days posttransfusion.

3.8 | Comparison of tube and gel column crossmatch

Blood crossmatches were performed using both the tube and gel column method for 10 cats. The number of pRBC units tested ranged from 1 to 7 for each recipient, for a total of 31 major and 21 minor crossmatches available for comparison. One cat was crossmatched to 7 different donors during an 8-day period. On the cat's first 5 crossmatches, a 4+ mixed field agglutination (a line of RBCs at the top of the gel column [4+ reaction] and an RBC button at the bottom of the gel [negative]) was noted in the auto control and major crossmatch performed using the gel method, but no agglutination was noted in either the auto control or major crossmatch using the tube method. Microscopic review of a blood smear and saline dilution test by a board-certified clinical pathologist (N.M. Weinstein) confirmed robust rouleaux formation. On day 8, only slight rouleaux formation was observed and the last 2 crossmatches performed had the same results among the auto control, major crossmatch (n = 2), and minor crossmatch (n = 1) using the tube and gel method. Results differed in the minor crossmatch from 1 donor, with a 1+ incompatibility noted with the gel method and a compatible result with the tube method. For the remaining 9 recipient cats and 24 major and 20 minor crossmatches (all compatible, except for 2 major incompatibilities [1+ and 3+] and 2 minor incompatibilities [both 2+]), results were the same for the tube and gel methods, with the exception of 1 cat that had a 2+ major incompatibility with the tube method and a 3+ major incompatibility with the gel method to the same donor.

4 | DISCUSSION

Our study indicates that the prevalence of non-AB blood type incompatibilities, likely representing naturally occurring alloantibodies against RBCs outside of the AB system, in 154 transfusion-naive cats was approximately 15%. In contrast to a previous study,[13] administration of type- and crossmatch-compatible pRBCs was not associated with a greater increase in posttransfusion PCV compared to transfusion of type-specific, non-crossmatched pRBCs. However, febrile transfusion reactions were more common in cats that received AB type-specific pRBCs without a crossmatch. A pretransfusion blood crossmatch did not appear to have an effect on patient outcome with regard to discharge from the hospital or survival to 30 and 60 days after administration of AB type-compatible pRBCs.

Recognition of the feline RBC antigen *Mik* and naturally occurring anti-*Mik* alloantibodies in 2007 prompted the recommendation to consider performing a crossmatch before a cat's first RBC transfusion.[12] With 3 of 65 blood donor cats having naturally occurring anti-*Mik* alloantibodies,[12] the prevalence of naturally occurring, non-AB RBC alloantibodies would be expected to be at least 5%, not taking into account the possibility of alloantibodies to RBC antigens other than *Mik*. In a prospective, randomized, controlled study of 48 transfusion-naive cats in the United States, 24 cats had a pretransfusion crossmatch performed, with 7 cats (29%) incompatible to ≥1 type-specific pRBC unit.[15] In a study evaluating transfusion practices in cats during a 3-year period at a university teaching hospital in Germany, pretransfusion crossmatching of 60 cats identified a major crossmatch (non-AB blood type) incompatibility in 1 transfusion-naive cat.[16] Crossmatch screening of 112 cats in the United Kingdom failed to detect any non-AB blood type incompatibilities.[17] In a study of 20 hospitalized cats in Germany undergoing serial crossmatching to detect alloimmunization to transfused RBCs, major crossmatch incompatibility was not found in any transfusion-naive cats.[18] The reason for the higher prevalence of non-AB blood-type incompatibilities in transfusion-naive cats in our and another US study[15] is unclear, but potential explanations include geographical variation (the United States versus Germany and the United Kingdom), larger sample population (154 transfusion-naive cats in our study), subtle differences in crossmatch technique, and subjective interpretation of low grade (1+) agglutination reactions, of which the clinical relevance is uncertain. Of the 23 transfusion-naive cats in our study with non-AB blood type incompatibilities, 17 cats (74%) had a 1+ major incompatibility, whereas 6 cats (26%) had 2+ to 3+ incompatibility on the major crossmatch. Previously documented anti-*Mik* alloantibodies have resulted in 2+ and 3+ major crossmatch incompatibilities.[12] Testing of the transfusion-naive cats for the *Mik* antigen was not possible because of the lack of typing reagent, and it is uncertain if any of the non-AB blood type incompatibilities were caused by naturally occurring anti-*Mik* alloantibodies.

Development of RBC alloantibodies posttransfusion is an anticipated event across many species and is the basis for the long-standing recommendation to perform a blood crossmatch for recipients given a blood transfusion ≥4 days before the planned transfusion, but, to our knowledge, data to support this specific recommendation for cats are lacking. The prevalence of non-AB RBC incompatibilities in previously transfused cats has been reported to be approximately 25%.[13,18] Pretransfusion screening of 43 cats, most of which were known to have been transfused >3 days previously, identified 11 cats (25.6%) with major crossmatch incompatibility to ≥1 pRBC unit.[13] Similarly, 5 of 20 (25%) cats, all transfusion-naive, with a pretransfusion-compatible crossmatch were documented to have a major crossmatch incompatibility developed 2-10 days (median, 5 days) after receiving AB-compatible whole blood.[18] In our study, 15 of 55 (27%) previously transfused cats had major crossmatch incompatibilities, 11 cats with 1 + incompatibility and 4 cats with 2+ incompatibility. Forty-three of 214 cats had a second crossmatch performed 1-15 days after the initial transfusion during the study period, with 10 cats (23%) having a 1 + to 3+ major crossmatch incompatibility, including 6 cats that were previously compatible to 2-3 donors tested. Four of 6 cats developed an incompatibility to donors to which they were previously compatible; 3 of the 4 cats had received only 1 pRBC transfusion, and the time to detection of incompatibility was 2-3 days, similar to the previously cited prospective study.[18] Although our study was not designed to evaluate time to alloimmunization posttransfusion, the finding of non-AB RBC incompatibilities in cats as early as 2 days after transfusion in 2 studies warrants reconsideration of the general guideline to perform a crossmatch ≥4 days after an initial blood transfusion.

Minor crossmatch incompatibilities are considered less clinically relevant than major crossmatch incompatibilities with regard to recipient safety and transfusion efficacy, particularly with pRBC transfusions in which a negligible amount of donor plasma is administered. Nevertheless, the finding of plasma from 13 donor cats, none of which had ever received a blood transfusion, resulting in minor crossmatch incompatibilities with 11 recipient cats suggests that these donors had naturally occurring non-AB RBC alloantibodies. Although plasma from the 13 donors was used in minor crossmatches for multiple recipients (median, 7; range, 1-19), only plasma from 1 donor resulted in a minor incompatibility with >1 recipient, with the donor used for 19 crossmatches being incompatible with 2 recipients. Therefore, none of the donors likely had naturally occurring antibodies to a common RBC antigen, such as *Mik*.

In a previous retrospective study evaluating the influence of crossmatch on posttransfusion PCV in cats, administration of type-specific, crossmatch-compatible pRBC transfusions was associated with a significantly greater increase in PCV (scaled to the dose of pRBCs administered, +1.02 ± 0.51%/mL/kg) when compared to administration of typed, non-crossmatched pRBCs (+0.75 ± 0.65%/mL/kg)[13] but, the clinical relevance of this finding is uncertain. A subsequent prospective study evaluating the change in PCV (scaled to the dose of pRBCs) at 4 time points posttransfusion did not detect a difference between cats with (+0.60 ± 0.66%/mL/kg at 1 hour posttransfusion) and without (+0.74 ± 0.53%/mL/kg at 1 hour posttransfusion; $P = 0.43$) a pretransfusion crossmatch.[15] Similarly, in our study, cats receiving crossmatch-compatible pRBCs did not have a greater increase in PCV posttransfusion compared to cats without a pretransfusion crossmatch. Rather, statistical analysis indicated that cats receiving pRBCs without a pretransfusion crossmatch had a greater increase in median scaled ΔPCV (+0.97%/mL/kg) compared to cats with a crossmatch (+0.76%/mL/kg), a finding for which no logical explanation is apparent. Many factors other than crossmatch results can influence ΔPCV, including ongoing patient blood loss or hemolysis, concurrent administration of other fluids, and timing of posttransfusion PCV. In our retrospective study, we could not control for these variables, but there was no significant difference in ΔPCV between cats with blood loss or hemolysis compared to other causes of anemia. However, in the prospective study,[15] none of the cats received IV fluids during the pRBC transfusions, and PCVs were checked at set time points (0, 1, 12, and 24 hours) posttransfusion. The lack of difference in efficacy (defined as mean change in PCV posttransfusion scaled to the dose of pRBCs) between crossmatched and non-crossmatched pRBCs administered to transfusion-naive cats (along with no difference in adverse events) prompted the authors to conclude that there is no support for pretransfusion crossmatching to increase efficacy and decrease adverse events associated with RBC transfusions in AB-typed transfusion-naive cats.[15]

The frequency of adverse events associated with RBC transfusions in cats has been reported to range from 1.2% to 20.8%, with febrile transfusion reactions being most common.[15,16,19,20] Febrile, nonhemolytic transfusion reactions were noted in 5% of cats in our study, with cats receiving AB type-specific, non-crossmatched pRBCs having significantly more febrile reactions (8 of 79 cats) compared to cats receiving crossmatch-compatible pRBCs (4 of 161 cats). Because febrile nonhemolytic transfusion reactions are believed to be caused by accumulation of proinflammatory white blood cell (WBC) and platelet-derived cytokines during storage, by interactions between WBCs and WBC antibodies present in donors and patients, or both,[21] there is no obvious explanation for the finding of febrile reactions occurring more often in cats that were not crossmatched. In a prospective study of transfusion-naive cats, no significant difference was found in the incidence of febrile, nonhemolytic transfusion reactions between cats with (3 of 24; 13%) and without (7 of 24; 29%) a pretransfusion crossmatch.[15] Two other cats in our study that received non-crossmatched pRBCs were suspected to have transfusion-associated adverse events (one of which could have been an acute hemolytic transfusion reaction) and died, but neither necropsy nor posttransfusion compatibility testing was performed for either cat. Given the variability in degree of patient monitoring during and after transfusions, the recipient's underlying condition (eg, hemolytic anemia, hepatobiliary disease), as well as the potential impact of general anesthesia, surgery, and hemodialysis on recipient parameters evaluated, it is possible that transfusion-associated adverse events were under-recognized in our study. Likewise, some of the febrile reactions could have been a consequence of the patient's underlying disease process rather representing a transfusion-associated adverse event, but this would have been the case for both cats with and without a pretransfusion crossmatch.

In a situation in which a recipient cat is in need of oxygen-carrying support but is crossmatch-incompatible to all donors tested, veterinarians typically select the least incompatible RBC unit, but the safety and efficacy of such a transfusion is uncertain. In a study in which crossmatch testing was performed after whole blood transfusions had already been given, blood had been administered to 5 cats (receiving 7 transfusions) despite incompatible major crossmatch results (ranging from microscopic 1+ to macroscopic 3+ agglutination), with no obvious clinical transfusion reactions noted in any of the cats and with an increase in hematocrit noted after 5 of 7 transfusions.[16] In our study, 5 previously transfused cats were incompatible on the major crossmatch to all donors tested and received pRBC units to which there was a 1+ incompatibility. Although no obvious adverse events were noted, 4 of the 5 cats received the pRBC unit as a hemodialysis prime, complicating monitoring. The median ΔPCV was +4% (median scaled ΔPCV of +0.86%/mL/kg), suggesting that the transfused RBCs were not immediately lysed or removed from the circulation. It is not possible to conclude from our study that administration of feline RBC units to which there is a 1+ major incompatibility will be a safe and efficacious transfusion.

Several blood crossmatch techniques have been used to evaluate RBC compatibility in cats: tube method,[12,13,22] gel column method,[12] and microtitration system.[17] In a comparison of crossmatch results using the tube and gel methods for a feline renal transplant recipient with anti-*Mik* alloantibodies, there was an agreement between the 2 methods in identifying 3 incompatible donors and 2 compatible donors, but the grading of agglutination tended to be higher using the gel method (3+) as compared to the tube method (weak to 2+).[12] Similarly, a comparison of the tube and gel methods for evaluation of RBC compatibility in horses indicated a high correlation between results of the 2 methods.[23] In our study, a prospective comparison of

crossmatch results of the tube and gel methods for 10 recipient cats (31 major and 21 minor crossmatches) found overall agreement between the 2 methods, with the exception of robust rouleaux formation resulting in a 4+ mixed field agglutination in the gel method in 1 cat; the tube method yielded a compatible result. Because of the potential for rouleaux formation in cats, the tube method could be considered preferable to the gel method in this species. As in the previous study of cats comparing the tube and gel methods,[12] the gel method gave a higher grade of agglutination compared to the tube method in 1 major crossmatch (3+ versus 2+). In addition, in 1 minor crossmatch, a 1+ incompatibility was noted with the gel method, whereas the tube method yielded a compatible result. Although only 10 of 218 cats in our study had pretransfusion crossmatching performed with both the tube and gel methods, based on the general agreement between these methods, we would expect the prevalence of non-AB RBC incompatibilities to be similar, regardless of which method was used. Furthermore, if low-grade agglutination would be detected only by the gel method (eg, 1+ incompatible in gel and compatible in tube), the prevalence of non-AB RBC incompatibilities among cats in our geographical area could be >15%. A previous study comparing crossmatches in cats performed using a standard saline gel column and an antiglobulin-enhanced gel column indicated that the latter was more sensitive in identifying RBC incompatibilities, with 81 of 143 observed incompatibilities appreciated only in the antiglobulin-containing gel columns (Seth M, Jackson KV, Giger U. A gel column based antiglobulin test to detect erythrocytic auto- and alloantibodies in cats. *J Vet Intern Med.* 2008;22:740 [abstract]). Similar to 1+ RBC incompatibilities noted in the tube method in our study, the clinical relevance of incompatibilities noted only in the antiglobulin-enhanced gel column crossmatch is unclear.

A study evaluating the effect of blood storage time on crossmatching in horses documented that donor RBC samples stored for 1-4 weeks resulted in a higher number of major incompatibility agglutination reactions compared to fresh RBC samples.[24] For practical purposes, in small animal transfusion medicine, the storage time of the donor RBC sample is the same as the duration of storage of the pRBC unit. Our study was not designed specifically to evaluate the effect of storage time of donor RBC samples on crossmatching in cats, but we did not detect an association between duration of RBC storage and major crossmatch incompatibilities. The pRBC unit stored for 40 days (which is beyond the recommended storage time) was an anomaly caused by incorrect calculation of the collection date for a unit purchased from a commercial animal blood bank. Nonetheless, a major crossmatch to this aged pRBC unit was compatible, but the unit was not administered.

The limitations of our study include its retrospective nature, with inherent difficulties in obtaining an accurate transfusion history and detecting transfusion-associated adverse events, as well as lack of standardization in posttransfusion monitoring. The largest possible data set was collected and evaluated. However, in those instances where statistical significance was not found, it is possible that a type II error existed and that significance might be identified with a larger data set. Although the majority of crossmatches were performed by trained clinical laboratory technicians, approximately 8% were performed after hours by the primary or emergency clinician, potentially adding an element of variability to interpretation of results. In addition, the gel crossmatches were performed by an experienced clinical laboratory technician who was not blinded to the results of the corresponding tube crossmatch, and thus a component of interpretation bias cannot be excluded although it is considered unlikely. Although anti-*Mik* alloantibodies have been documented previously to elicit acute hemolytic transfusion reactions,[12] the clinical relevance of the non-AB incompatibilities detected in our study is uncertain because crossmatch-compatible pRBC units were selected for transfusion when available (in 35 of 40 cats with major crossmatch incompatibilities). Finally, testing of cats with major crossmatch incompatibilities for the absence of the *Mik* antigen was not performed because of the lack of typing reagent, as well as the retrospective evaluation of these cats.

Red blood cell incompatibility noted in 15% of transfusion-naive cats suggests that the prevalence of naturally occurring non-AB RBC alloantibodies is sufficiently high to justify the recommendation to perform a major crossmatch before all (including the first) RBC transfusions in cats in a non-emergent setting. However, one could argue that crossmatching all cats would result in additional cost and unnecessary delay in time to transfusion. Although the clinical relevance of major crossmatch incompatibilities detected in transfusion-naive cats is unknown, previous documentation of anti-*Mik* RBC alloantibodies causing acute hemolytic transfusion reactions in transfusion-naïve cats suggests erring on the side of caution.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflicts of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

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