Incidence of incompatible crossmatch results in dogs admitted to a veterinary teaching hospital with no history of prior red blood cell transfusion

Adesola Odunayo DVM
Kayode Garraway DVM
Barton W. Rohrbach VMD, MPH
Amanda Rainey DVM
Jennifer Stokes DVM

From the Departments of Small Animal Clinical Sciences (Odunayo, Garraway, Rainey, Stokes) and Biomedical and Diagnostic Sciences (Rohrbach), College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37996. Dr. Garraway's present address is Department of Internal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA 50011.

Address correspondence to Dr. Odunayo (aodunayo@utk.edu).

OBJECTIVE
To determine the incidence of incompatible crossmatch results in dogs without a history of prior RBC transfusion and to evaluate changes in Hct following RBC administration for transfusion-naïve dogs that did and did not have crossmatching performed.

DESIGN
Retrospective study.

ANIMALS
169 client-owned dogs.

PROCEDURES
Information obtained from the medical records included signalment, pretransfusion Hct or PCV, and crossmatching results where applicable. Dogs that underwent major crossmatching (n = 149) as part of pretransfusion screening were each crossmatched with 3 potential donors. Donor blood was obtained from a commercial source and tested negative for dog erythrocyte antigens (DEAs) 1.1, 1.2, and 7 but positive for DEA 4. Mean change in Hct after transfusion was compared between crossmatch-tested dogs (57/91 that subsequently underwent RBC transfusion) and 20 other dogs that underwent RBC transfusion without prior crossmatching by statistical methods.

RESULTS
25 of 149 (17%) dogs evaluated by crossmatching were incompatible with 1 or 2 of the 3 potential donors. All 149 dogs were compatible with ≥ 1 potential donor. Mean ± SD change in Hct after transfusion was significantly higher in dogs that had crossmatching performed (12.5 ± 8.6%) than in dogs that did not undergo crossmatching (9.0 ± 4.3%).

CONCLUSIONS AND CLINICAL RELEVANCE
Results indicated immunologic incompatibility can exist between first-time transfusion recipients and potential blood donor dogs. The clinical importance of these findings could not be evaluated, but considering the potential for immediate or delayed hemolytic transfusion reactions or shortened RBC life span, the authors suggest veterinarians consider crossmatching all dogs prior to transfusion when possible. (*J Am Vet Med Assoc* 2017;250:303–308)

Transfusion of RBCs, either as PRBCs or whole blood, is a common procedure in critically ill human and veterinary patients.[1–4] The frequency of canine RBC transfusion in veterinary medicine has increased over the past decade, likely owing to increased awareness by veterinarians as well as the availability of RBCs from commercial blood bank facilities.[5,6] However, RBC transfusions in veterinary patients are associated with a risk for potentially severe transfusion reactions, including acute or delayed hemolytic reactions. Proper prescreening, including blood typing and crossmatching to exclude immunologic incompatibility between a donor and recipient, can help minimize these reactions.[7] Crossmatching is a serologic test designed to determine compatibility between the donor and the recipient. The major crossmatch identifies antibodies in recipient serum or plasma against donor RBC antigens.[7]

There are 7 internationally recognized RBC antigens in dogs, and all 7 are categorized under the DEA system (DEA 1.1, 1.2, 1.3, 3, 4, 5, and 7),[7,8] although at least 13 canine blood groups have been described.[9,10] Naturally occurring alloantibodies against various canine RBC antigens have been described in dogs not known to have received transfusions. The most common naturally occurring alloantibody is against DEA 7, although naturally occurring alloantibodies against DEAs 3 and 5 have also been reported.[11,a] These antibodies in dogs are thought to be unlikely to cause acceler-

ABBREVIATIONS

CI	Confidence interval
DEA	Dog erythrocyte antigen
IMHA	Immune-mediated hemolytic anemia
PRBCs	Packed RBCs

ated destruction of transfused cells, but might result in delayed transfusion reactions. The DEA 1.1 is the most immunogenic antigen and is most commonly associated with acute hemolytic transfusion reactions in previously sensitized dogs.[8] In a preliminary study[a] that involved screening of 2,500 canine blood samples to assess the prevalence of alloantibodies against DEAs 1.1, 3, 5, and 7, the prevalence of anti-DEA 1.1 antibodies was found to be low (0.3%). An alloantibody such as DEA 1.1 that decreases RBC lifespan by accelerated destruction (hemolysis) of transfused RBCs is considered clinically important (commonly termed clinically significant).[7,12] However, delayed removal of transfused RBCs can occur with or without hemolysis.

Owing to the reported infrequent occurrence of clinically important alloantibodies in dogs, crossmatching prior to a canine patient's first blood transfusion has been considered optional or unnecessary by many veterinary practitioners.[13–15] In fact, the results of an online survey of participants from 73 veterinary referral or teaching hospitals indicate that only 11 of 73 (15%) performed a crossmatch evaluation for all dogs prior to transfusion.[16] Results from that study[16] suggest that veterinarians in most clinics do not routinely perform this evaluation for dogs that have not previously undergone a transfusion (ie, naïve recipients), although 72 of 73 (99%) respondents reported that they perform a crossmatch 3 to 5 days after the first transfusion has been given to a dog.

To the authors' knowledge, no studies have been performed in which immunologic compatibility with potential donor dog RBCs among dogs that have not undergone a previous transfusion was assessed by means of crossmatching. The objective of the study reported here was to determine the incidence of incompatible crossmatch results in transfusion-naïve dogs. A secondary aim was to compare the posttransfusion change in Hct between naïve recipient dogs that received a crossmatch-compatible RBC transfusion and those that underwent transfusion without prior crossmatching as a means of assessing the clinical value of performing the test for such dogs. The hypothesis of the study was that a low incidence of incompatible crossmatch results would be found in this population.

Materials and Methods

Case selection

Electronic medical records at the University of Tennessee Veterinary Medical Center were reviewed to identify dogs that received a first-time blood transfusion (PRBCs or whole blood) with or without prior crossmatching from March 1, 2008, through August 30, 2013. Transfusion-naïve dogs for which a major crossmatching test was performed, as well as dogs that did not have crossmatching performed prior to RBC administration, were included in the study. Dogs were determined to be first-time blood recipients at the time of the evaluated transfusion on the basis of information available in the medical records and verbal confirmation with the owner. Dogs were excluded if they had previously received other blood products (eg, plasma), if the medical records were incomplete, or if prior medical history was unknown.

Medical records review

Information collected from the medical records included age; weight; breed; sex; whether crossmatching was performed and results, if applicable; blood product administered (PRBCs or whole blood); reason for transfusion; and pre- and posttransfusion Hct, volume of blood transfused, and patient outcome (survival to hospital discharge, death, or euthanasia). The posttransfusion Hct was recorded as the first Hct measured ≤ 24 hours after the blood transfusion, although the standard practice at the study institution was to measure posttransfusion Hct 2 hours after a blood transfusion. When Hct results were not available but PCV was recorded, the latter data were used in place of Hct information.

Crossmatching procedure

All animals requiring a transfusion at the University of Tennessee Veterinary Medical Center had crossmatching performed, except on rare occasions when the recipient was not considered stable enough for a crossmatch analysis or when immediate transfusion was required. Crossmatching for dogs in the study was performed by an immunologist or a medical laboratory technologist at the Immunology Laboratory at the University of Tennessee College of Veterinary Medicine. Donor erythrocytes were obtained from the so-called pigtails of the PRBC or whole blood unit. For each dog that underwent crossmatching, blood from 3 potential donors was obtained from a commercial blood bank facility.[b] All donors tested negative for DEAs 1.1, 1.2, and 7 but positive for DEA 4.

The crossmatch procedure was performed as follows. Recipient blood was collected in an EDTA-containing tube and a serum separator tube. The blood in the serum separator tube was allowed to clot, and the serum was removed after a 2-minute centrifugation at 1,000 × *g*. Next, the serum was transferred and held in a separate tube. For the purpose of creating controls for the test, recipient and donor RBCs from the remaining precipitate, pigtails of the unit, or the EDTA-containing tube were washed 3 times with phosphate-buffered blood bank saline (0.85% NaCl w/v) solution, and then a 3% suspension was created with additional buffered blood bank saline solution.

Major crossmatching was performed by combining 0.1 mL of the recipient serum and 0.1 mL of donor RBC suspension in a test tube. Donor and recipient controls were set up with 0.1 mL of donor serum and 0.1 mL of washed donor RBCs (for the donor control) and 0.1 mL of recipient serum and 0.1 mL of washed

recipient RBCs (for the recipient control). All tubes were gently mixed. Two sets of each type were used; one set of tubes was allowed to incubate at room temperature (approx 71.6°F [22°C]), and the other set was incubated at 98.6°F (37°C) for 30 minutes. The tubes were then centrifuged at 500 × *g* for 30 seconds and visually evaluated for gross macroagglutination with and without use of a concave lens.[c] An incompatible crossmatch was identified when RBCs were lysed and hemolysis was present or when RBCs were observed to agglutinate after tapping the tube 15 times at either room temperature or at 37°C. Only a small number of the recipient dogs in the study were tested for infectious diseases (eg, rickettsial or fungal diseases) that could lead to the development of anti-RBC antibodies; however, all samples had to have a control for autoagglutination. Any sample that had evidence of autoagglutination underwent RBC washing multiple times until the autoagglutination was eliminated. The crossmatching was then performed after elimination of the autoagglutination.

Statistical analysis

A commercial software program[d] was used to summarize continuous and categorical data. Categorical data are presented as a number, proportion, and 95% CI around the estimate. Continuous data are presented as mean ± SD or median and range, depending on the distribution of data. The test statistic of Shapiro-Wilk was used determine the fit of continuous data to a normal distribution.

The effect of crossmatch status, adjusted for the volume of blood administered, on the difference between pre- and posttransfusion Hct was evaluated with a mixed-model ANOVA.[e] Independent variables included in the model were blood volume administered per kilogram of body weight and crossmatch status. The –2 log likelihood ratio was used to assess the fit of the model to the data, and the Shapiro-Wilk test was used determine the fit of residuals from the model to a normal distribution. Values of $P < 0.05$ were accepted as significant.

Results

Crossmatching, RBC transfusion, or both were performed for 496 dogs during the study period. Of these, 119 dogs were excluded because it could not be determined whether they had a prior transfusion, and 192 dogs were excluded because of a history of prior fresh frozen plasma or RBC or plasma transfusion. An additional 36 dogs were excluded from analyses for the primary objective because, although they had no history of a prior transfusion, crossmatching was not performed before the RBCs were administered.

Thus, 149 dogs (73 females [64 spayed and 9 sexually intact] and 76 males [60 castrated and 16 sexually intact]) were included in the analyses for crossmatching results. Median age of these dogs at the time of crossmatching was 9 years (range, 2 to 17 years). Of the 149 crossmatch-tested dogs, 115 were purebred, with Miniature Schnauzers (n = 11), Labrador Retrievers (9), Dachshunds (8), and Golden Retrievers (8) most commonly represented; 34 dogs were of mixed breeds.

The reasons RBC transfusion was considered for dogs that underwent crossmatching included anemia due to causes other than IMHA (n = 72), blood loss during surgery (55), IMHA (13), and trauma (6). The reason was not documented in the records of 3 dogs. Causes for anemia other than IMHA included immune-mediated thrombocytopenia (n = 23), hemoabdomen (before surgery; 9), chronic nonregenerative anemia of undetermined cause (9), chronic nonregenerative anemia secondary to renal disease (6), hemorrhage secondary to rodenticide toxicosis (5), coagulopathy other than rodenticide toxicosis (4), hemolytic anemia of unknown cause (4), chronic nonregenerative anemia secondary to suspected neoplasia (4), pancytopenia (2), hematuria (2), hemothorax (2), *Ancylostoma caninum* infestation (1), and garlic-associated hemolysis (1).

The median Hct for all dogs that had crossmatching performed and a (pretransfusion) measurement documented (n = 123) was 20% (range, 6% to 58%). Crossmatch evaluation with 3 donors each revealed that 25 of 149 (17%; 95% CI, 11.1% to 23.7%) dogs were not compatible with 1 or 2 potential donors, and 5 (3.4%; 95% CI, 0.5% to 6.3%) were not compatible with 2 potential donors **(Table 1)**. Most (124/149 [83.2%]; 95% CI, 77% to 89%) dogs were compatible with all 3 potential donors, and none were incompatible with all 3.

Of the 149 dogs that underwent crossmatching, 91 subsequently had an RBC transfusion. Most (89) dogs received PRBC transfusions, and 2 received whole blood. All dogs that did not undergo crossmatching received a PRBC transfusion.

Evaluation for the change in Hct following transfusion included 57 of the 91 dogs that underwent pretransfusion crossmatching and 20 of the 36 dogs that had RBC transfusion without prior crossmatching. The remaining 50 dogs did not have sufficient information available in the medical records for the analysis. The mean ± SD pretransfusion Hct for the 57 dogs that underwent crossmatching was 16.1 ± 5.3%, whereas that for the 20 dogs that did not have crossmatching performed was 16 ± 5.1%; mean ± SD posttransfusion Hcts for these groups were 28.6 ± 7.4% and 25.0 ± 5%, respectively. The mean ± SD relative blood volume administered for dogs that underwent crossmatching (14.85 ± 6.4 mL/kg) did not differ significantly ($P = 0.089$) from that of dogs that did not have crossmatching performed (12.14 ± 4.76 mL/kg). However, dogs that underwent crossmatching before the transfusion had a significantly ($P = 0.026$) greater mean increase in Hct after transfusion (12.5 ± 8.6%) than did dogs that did not undergo crossmatching (9.0 ± 4.3%).

Table 1—Characteristics of 149 hospitalized dogs grouped by results for crossmatching with 3 potential blood donors during assessment prior to transfusion.

Variable	No incompatibility (n = 124)	Incompatible with 1 or 2 donors (n = 25)	Incompatible with 2 donors (n = 5)
Age (y)	10 (2–17)	12 (6–15)	8 (6–11)
Sex and reproductive status			
Female			
Sexually intact	7 (6)	2 (8)	1 (20)
Spayed	58 (47)	6 (24)	2 (40)
Male			
Sexually intact	13 (10)	3 (12)	
Castrated	46 (37)	14 (56)	2 (40)
Breed category			
Purebred	95 (77)	20 (80)	5 (100)
Mixed breed	29 (23)	5 (20)	
Hct at assessment* (%)	20 (6–58)	20 (12–44)	37 (9–39)
Reason for transfusion†			
Anemia (other than IMHA)	64 (53)	7 (35)	1 (20)
Blood loss at surgery	45 (37)	9 (45)	1 (20)
IMHA	11 (9)	1 (5)	1 (20)
Trauma	1 (1)	3 (15)	2 (40)
Survived to hospital discharge	87 (70)	14 (70)	3 (60)

Values are shown as number (% of compatibility group) or median (range). All dogs were compatible with ≥ 1 donor; 91 subsequently received transfusions with RBCs.

*Data were missing for 22, 4, and 0 dogs incompatible with 0, 1 or 2, and 2 donors, respectively. †Data were missing for 3, 0, and 0 dogs incompatible with 0, 1 or 2, and 2 donors, respectively.

Discussion

To the authors' knowledge, this is the first report describing the frequency of incompatible crossmatch results in transfusion-naïve dogs. Unlike cats, dogs are not thought to have clinically important, naturally occurring alloantibodies, which are responsible for producing acute hemolytic transfusion reactions and decreased RBC lifespan. Instead, it is thought that most alloantibodies develop in dogs that have been sensitized by a previous transfusion. Thus, staff at many veterinary hospitals perform crossmatches in dogs only 3 to 5 days after the first transfusion or in those with an unknown transfusion history, but do not test routinely before the first transfusion.[16]

In 1913, Ottenberg et al[17] first demonstrated what was likely naturally occurring isoagglutinins and hemolysins in dogs. They described the agglutination and hemolysis to be weaker than that typically found in rats, rabbits, and people.[17] Specific alloantibodies were not identified in that study, and the study methodology was not well described. In the present study, none of the patients with an incompatible crossmatch result received a blood transfusion from a source deemed incompatible. Therefore, the clinical implications of administering RBCs to naïve recipients deemed incompatible remained unknown. However, there is a potential for transfusion of blood deemed incompatible by crossmatching to lead to increased RBC destruction and clearance and to produce life-threatening reactions.

Several other studies[11,18,a] have identified naturally occurring alloantibodies in dogs. The prevalence of naturally occurring alloantibodies against DEA 1.1 in one study[a] was 0.3%, whereas the prevalence of naturally occurring alloantibodies against DEA 7 ranged from 9.8% to 50% in others.[11,18] Another study[18] found naturally occurring alloantibodies in low titer in 22 of 145 (15%) included dogs, but the specificity of the alloantibodies was not determined. Because of its retrospective nature, the present study did not evaluate all RBC transfusion recipients for the presence of alloantibodies. However, 25 of 149 (17%) dogs tested had an incompatible crossmatch result with ≥ 1 potential donor and likely had preexisting alloantibodies. It is possible that a higher percentage of dogs included in the study had previously formed alloantibodies but did not have crossmatching results indicating immunologic incompatibility either because they were crossmatched with a compatible unit or because the donors were screened and tested negative for DEA 1.1, 1.2, and 7.

One reason for the high incidence of crossmatch incompatibility in the present study might have been the interpretation of the crossmatch test. At the facility's immunology laboratory, any agglutination at room temperature or at 37°C is considered to represent a positive test result. In human blood, most clinically important antibodies are warm agglutinins, meaning they react best at close to 37°C. Conversely, most clinically unimportant antibodies are cold agglutinins, reacting at < 37°C. The argument for evaluating antibodies only at 37°C is that clinically relevant antibodies are more likely to be physiologically active at that temperature. However, antibody screening of human patients has become common, and immediate-spin crossmatching at room temperature is typically used only for patients who test negative for clinically important antibodies through an antibody screening test.[19] Our laboratory deemed agglutination at either temperature as incompatible and did not differentiate between reactions at room temperature and those observed in samples at 37°C. The clinical relevance of antibodies active at room temperature versus 37°C in pretransfusion crossmatching of veterinary patients requires further evaluation.

Another important potential contributor to the high incidence of incompatible crossmatches in the study might have been incomplete owner knowledge of dogs' previ-

ous transfusion history. For example, an owner might have been unaware that, prior to its adoption, a dog had received a blood transfusion as a very young puppy for treatment of severe hookworm infestation. In the authors' opinion, such possibilities make it more important to critically consider the value of a crossmatch evaluation, even in situations where owners do not report a history of a prior transfusion.

On the basis of results of previous studies,[11,a] it is possible that most of the incompatible crossmatch responses in the present study involved clinically unimportant alloantibodies, which do not typically cause acute hemolytic transfusion reactions. However, these antibodies can be associated with delayed hemolytic transfusion reactions, which would lead to a shortened RBC lifespan.[15] The DEA 3, 4, 5, and 7 antigen-antibody interactions in vivo result in RBC sequestration, RBC loss, and increased RBC clearance 3 to 5 days after transfusion.[9,15,18] To the authors' knowledge, the incidence of delayed hemolytic transfusion reactions in canine patients is not currently known. In a study by Hann et al,[20] the incidence of acute hemolytic transfusion reactions was 7 of 3,261 (0.2%), although this value was thought to be likely underestimated owing to the retrospective nature of the study. None of the 7 dogs with acute reactions in that study[20] had a prior transfusion, so crossmatching was not performed before administration of RBCs. In another study,[21] all dogs that had a hemolytic transfusion reaction (4/558 [0.7%]) were transfusion-naïve dogs that did not have a crossmatch evaluation before the transfusion was administered. Because our study was retrospective and some medical records were incomplete, the incidence of transfusion reactions in the present study could not be properly evaluated.

Commercial blood banks might only screen potential donors for DEA 1 alleles. In addition, practitioners commonly use donor dogs with unknown RBC antigens for transfusion to naïve recipient dogs.[22] Transfusion of blood positive for DEA 7 to a naïve recipient with preexisting anti-DEA 7 antibodies might lead to RBC sequestration and clearance, and it is speculated that transfusion of blood positive for DEAs 3 and 5 to a naïve recipient with antibodies against those antigens may also lead to reduced RBC lifespan.[9,15,18] Considering the possible risks of acute or delayed transfusion reactions, it is prudent to consider crossmatching before transfusion to a naïve recipient dog, especially when the donor blood antigen type is unknown.

It is, however, important to emphasize that an immunologically compatible crossmatch result does not eliminate the possibility that transfusion reactions will occur. This is because false-negative results can occur if an alloantibody titer is not high enough to detect.[23] Other transfusion reactions, including allergic reactions, transfusion-related acute lung injury, febrile nonhemolytic transfusion reaction, and delayed hemolytic transfusion reactions, can occur despite a compatible crossmatch result.

An inherent limitation of the present study was its retrospective design. It would have been ideal to identify the specific antibodies causing incompatible crossmatch results and to evaluate how many of those results were associated with clinically important antibodies. In addition, it would have been preferred to compare results for samples at room temperature and at 37°C to determine whether some samples reacted at both temperatures.

As mentioned, the clinical relevance of immunologically incompatible crossmatch results found in the present study could not be evaluated because almost all patients requiring transfusions received PRBCs from crossmatch-compatible donors. Weltman et al[24] evaluated the influence of major crossmatching in cats prior to transfusion on posttransfusion PCV. Those results indicated that crossmatch-compatible PRBC transfusions resulted in significantly greater increases in posttransfusion PCV, compared with results when typed PRBCs were administered without crossmatching. The present study included evaluation of posttransfusion changes in Hct in dogs that did and did not undergo pretransfusion crossmatching. Similar to the findings described for cats, dogs in this study that received a crossmatch-compatible transfusion had a significantly greater change in Hct after transfusion than did dogs that did not have crossmatching performed before the transfusion. Although these data supported that there are benefits to crossmatching, there were severe limitations to this comparison, including the possibility of continued RBC destruction or other losses after the time of the posttransfusion Hct measurement. Also, the time of posttransfusion Hct measurement was not standardized in all dogs and could have affected the results obtained. In addition, because it is commonly thought that most preexisting alloantibodies in transfusion-naïve dogs are not clinically important, future studies that include evaluation of the transfused RBC lifespan as well as the incidence of delayed transfusion reactions will be useful to determine the efficacy of the transfusion in such dogs.

Although the data presented herein indicated that immunologic crossmatch incompatibility can occur in transfusion-naïve dogs, the clinical importance of these findings is still uncertain, and further research is needed. However, considering that transfusion of crossmatch-incompatible RBCs could potentially lead to immediate or delayed hemolytic transfusion reactions or reduced RBC lifespan, the authors recommend that crossmatch evaluation should be considered for naïve recipient dogs when possible.

Footnotes

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From this month's *AJVR*

Effects of a combination of acepromazine maleate and butorphanol tartrate on conventional and two-dimensional speckle tracking echocardiography in healthy dogs

Giorgia Santarelli et al

OBJECTIVE
To determine effects of a combination of acepromazine maleate and butorphanol tartrate on conventional echocardiographic variables and on strain values obtained by use of 2-D speckle tracking echocardiography (STE) in healthy dogs.

ANIMALS
18 healthy medium- and large-size adult dogs.

PROCEDURES
Transthoracic echocardiographic examination (2-D, M-mode, color flow, spectral Doppler, and tissue Doppler ultrasonography) and high-definition oscillometric blood pressure measurement were performed before and after dogs were sedated by IM administration of a combination of acepromazine (0.02 mg/kg) and butorphanol (0.2 mg/kg). Adequacy of sedation for echocardiographic examination was evaluated. Circumferential and longitudinal global and segmental strains of the left ventricle (LV) were obtained with 2-D STE by use of right parasternal short-axis and left parasternal apical views. Values before and after sedation were compared.

RESULTS
The sedation combination provided adequate immobilization to facilitate echocardiographic examination. Heart rate and mean and diastolic blood pressures decreased significantly after dogs were sedated. A few conventional echocardiographic variables differed significantly from baseline values after sedation, including decreased end-diastolic LV volume index, peak velocity of late diastolic transmitral flow, and late diastolic septal mitral and tricuspid annulus velocities, increased ejection time, and increased mitral ratio of peak early to late diastolic filling velocity; global strain values were not affected, but 1 segmental (apical lateral) strain value decreased significantly.

CONCLUSIONS AND CLINICAL RELEVANCE
Results indicated that acepromazine and butorphanol at the doses used in this study provided sedation adequate to facilitate echocardiography, with only mild influences on conventional and 2-D STE echocardiographic variables. (*Am J Vet Res* 2017;78:158–167)

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