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Alloimmunization in dogs after transfusion: A serial cross-match study

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Abstract

Background: Cross-matching is performed to determine the serological compatibility of donor and recipient blood. Current guidelines recommend that cross-matching should be performed in dogs when an initial transfusion was performed more than 4 days ago or when the transfusion history is unknown.

Hypothesis: Determination at what time point alloantibodies are detected in dogs after transfusion. The hypothesis was that dogs would form alloantibodies within 4 days after a transfusion.

Animals: Twenty-one anemic dogs were transfused and monitored for at least 4 subsequent days. Exclusion criteria were persistent red blood cell (RBC) agglutination and a previous transfusion.

Methods: Prospective observational study. Cross-matching was performed before the initial DEA 1-compatible transfusion and on days 1, 2, 3, and 4 and if possible, between day 5 and 28, using the tube method without enhancement (major cross-match, recipient controls); recipients were monitored for transfusion reactions.

Results: In 12/21 dogs a positive cross-match (microscopic degree of agglutination [AD] 1+ to 2+) was observed within 4 days after the transfusion. In a nonlinear regression model, no significant association was detected between type of anemia ($P = .41$), RBC storage time ($P = .44$), immunosuppressive treatment ($P = .75$) nor transfusion volume ($P = .70$) and the occurrence of positive cross-matches within 4 days after transfusion. Another 4 dogs developed a positive cross-match (microscopic AD 1+ to 2+) after 6 to 13 days.

Conclusions and Clinical Importance: Because production of alloantibodies was detected as early as 1 day after transfusion, cross-matching should be performed before every subsequent transfusion.

KEYWORDS
antibody, antigen, compatibility testing, formation of alloantibodies, transfusion medicine

Abbreviations: AD, degree of agglutination; DEA, dog erythrocyte antigen; EDTA, ethylen-diamin-tetra-acetat; PBS, phosphate buffered saline; PCV, packed cell volume; pRBC, packed red blood cells; RBC, red blood cell.

1 | INTRODUCTION

Blood transfusions have been used in veterinary medicine since the 1950s and have become an important aspect of intensive care and emergency treatment in dogs.[1-7] Although transfusions are often a life-saving measure, they are not without risk. To ensure that transfusions are as safe as possible and to prevent potential complications, guidelines and testing procedures for compatibility testing have been developed.[2-10]

More than 12 blood group systems have been identified in dogs, and additional previously unknown blood groups exist.[11,12] These are of varying clinical relevance. DEA 1 is the blood group with the highest antigenicity and can cause severe acute hemolytic transfusion reactions after sensitization by administration of an incompatible transfusion.[13] Natural alloantibodies have only been detected for the blood groups DEA 3, 5, and 7.[14,15] However, their titers are relatively low (<1:2), meaning that their clinical relevance is questionable.[16] Although acute transfusion reactions are not triggered by these alloantibodies during an initial transfusion, transfusion of foreign antigens could lead to an increase in the antibody titer and thus to a reduced lifespan of the transfused erythrocytes or, in the case of a second transfusion, also to acute transfusion reactions.[17,18]

The formation of transfusion related alloantibodies is a common complication.[11,17-23] Therefore, in order to minimize the risk of a transfusion reaction, cross-matching is recommended in addition to blood typing.[11,17,18] Cross-matching is an in vitro assay that tests the serological compatibility of the recipient and donor blood.[3] According to the recommendations of the Association of Veterinary Hematology and Transfusion Medicine (AVHTM), cross-matching is indicated when the transfusion history is unknown or the dog has been transfused more than 4 days ago.[24] The time point at which alloantibodies are formed in dogs as a result of blood transfusion has been investigated in only one study immediately after transfusion: 2 dogs were cross-matched daily in the first week and weekly thereafter after receiving a Dal-incompatible transfusion. One dog developed incompatibility as early as day 4, and 1 dog on day 21.[25] In other studies, alloimmunization in dogs has been investigated only at larger time intervals after transfusion. By performing serial cross-match tests, alloimmunization after transfusion has been demonstrated in 44% of dogs between day 26 and 126 and in 71% between day 13 and 99 in previous studies.[11,21] The prevalence of potential delayed transfusion reactions is difficult to determine because of the influence of the dog's particular disease on blood variables.[26]

The aim of this prospective study was to investigate the frequency and time point at which dogs develop alloantibodies after transfusion. The hypothesis was that dogs would form alloantibodies within the first 4 days after a transfusion.

2 | MATERIAL AND METHODS

2.1 | Study group

The study group included anemic dogs transfused during their hospital stay at the Clinic for Small Animals of the Freie Universität Berlin and examined on at least 4 subsequent days. Exclusion criteria were persistent erythrocyte agglutination and previous transfusions. Informed owner consent was obtained for the use of samples for research purposes (Ethics Committee Approval Protocol Number: O 0246/19).

2.2 | Transfusion

The decision to transfuse was made by the respective treating veterinarian, based on clinical and hematological variables. Before each transfusion, a complete blood cell count, including PCV, blood chemistry and, if necessary, other diagnostic procedures were performed to elucidate the cause of anemia. Any pretreatment or treatment with immunosuppressants was recorded. The DEA 1 blood group was determined in both the recipient and the donor using an immunochromatographic procedure (Lab Test Blood Typing; Alvedia, Limonest, France). Transfusion was performed by using gravity via a 200 µL in-line filter (Sangofix, B. Braun, Melsungen, Germany) into a peripheral venous catheter or into the jugular vein via a central venous catheter. During transfusion, heart rate, respiratory rate, rectal temperature, and mucosal color and moisture were assessed every 15 minutes. Subsequently, that is, 3 to 8 hours after transfusion, the PCV was measured. An increase of 1% per 1 mL/kg of transfused packed red blood cells (pRBCs) or 2 mL/kg of transfused whole blood was expected.[6]

2.3 | Sample material

One blood sample each from donor and recipient was required to perform cross-matching. At first 1 EDTA sample was obtained for initial cross-matching. Further, 1 sample was taken on each of the next 4 subsequent days and beyond up to 5 samples were taken over the further study period for follow-up cross-match testing, depending on the individual disease. This was used immediately, if possible, but at the latest after 72 hours of storage at 4°C. The donor sample was preserved from the blood bag (including pRBC with PAGGSM as an anticoagulant preservative solution) into a tube. 3.5 mL of donor blood was collected via a sterile syringe with a 20G needle either during the preparation of the pRBCs or directly before transfusion. Donor blood samples were stored at 4°C until transfusion and beyond until the end of the study period.

2.4 | Cross-matching

Cross-matching was performed before transfusion and on days 1, 2, 3, and 4, and whenever possible during follow-up between days 5 and 28 using the tube method. Therefore, the same donor-recipient pairings could be used for each cross-match performed throughout the study period. For the cross-match tests, 200 µL whole blood from the recipient and 100 µL pRBCs from the donor were used. In addition to each major cross-match test, a recipient-control was performed, as described.[15] Testing was always performed by the same person.

Recipient whole blood and donor pRBCs were centrifuged (Häma Pico 17; Heraeus; Thermo Scientific GmbH, Schwerte, Germany) at 2000 rpm for 2 minutes. The recipient plasma was stored in another tube for cross-matching. The sample was examined for signs of hemolysis or icterus. Donor and recipient erythrocytes (RBCs) were washed 3 times. For this, 500 μL of PBS solution was added to the RBCs and centrifuged again at 2000 rpm for 1 minute. The supernatant was taken up with a pipette and the procedure was repeated twice. To prepare a 3% to 5% RBC suspension, 500 μL PBS was mixed with 20 μL RBCs. For the major cross-match, 2 drops (50 μL) of recipient plasma were mixed with 1 drop (25 μL) of donor RBC suspension. To prepare the recipient-control, 2 drops (50 μL) of recipient plasma were mixed with 1 drop (25 μL) of recipient RBC suspension. The test tubes were then sealed and incubated at 37°C for 15 minutes before centrifugation (Hettich Eba 20; Tuttlingen, Germany; 15 seconds at 1000 rpm). For macroscopic evaluation, the supernatant was first examined for hemolysis. Subsequently, the sample was resuspended by tapping against the tube. One drop of this sample was examined on a microscope slide. The degree of macroscopic agglutination was graded as 0 to +++ (0, none; +, weak; ++, moderate; +++, high). In the subsequent microscopic assessment, erythrocyte agglutination was examined within 60 seconds, first at ×100 magnification and finally at ×500 magnification. Here, the degree of agglutination was described as 0 to ++++ (0, no agglutination; +, many small agglutinates with RBCs in suspension; ++, some larger agglutinates with many small agglutinates; +++, several large agglutinates with clear plasma; ++++, 1-2 large agglutinates with clear plasma) (Figure 1). A cross-match test result was considered positive at a microscopic agglutination level of ≥1+. Cross-match test results were documented photographically and assessed blinded by a second person.

2.5 | Statistical analyses

To investigate the 4-day rule, the alternative hypothesis was that 5% of dogs would form alloantibodies to the transfused red cells by day 4 after transfusion. We calculated a study group of 21 transfused dogs to test this hypothesis. Statistical analysis was performed using IBM SPSS Statistics 27 (IBM Corp Released 2020 IBM SPSS Statistics for Windows, version 27.0; IBM Corp, Armonk, New York). Descriptive data analysis was performed with the following variables: Signalment, disease/indication for transfusion, treatment with immunosuppressants, blood group, cross-match results, storage time and transfusion volume (mL/kg), incidence of transfusion reactions, PCV before transfusion, and PCV increase because of transfusion.

The correlations between the difference in the increase of the PCV, the transfusion volume, and the storage time were assessed using the Spearman rho test. The Mann-Whitney *U* test was used to examine differences in the increase of the PCV between the various types of anemia (blood loss anemia compared with other types of anemia). The Mann-Whitney *U* test was also used to determine the influence of the storage duration of blood units or donor blood samples on the cross-match results. The influence of the type of anemia, the transfusion volume, the storage duration of the blood unit, and the administration of immunosuppressants at the time of transfusion on the probability of alloimmunization within the first 4 days was examined using a multivariable logistic regression. Also, the influence of the type of anemia, the transfusion volume, the storage duration of the blood unit, and the administration of immunosuppressants on the decrease of agglutination was examined using a multivariable logistic regression. All *P*-values ≤.05 were considered significant.

3 | RESULTS

3.1 | Study group

During the study period from October 2019 to December 2020, 99 dogs received 163 red blood cell transfusions. Twenty-one dogs were enrolled in the study. The dogs were 3 months to 14 years (median 9 years) old and weighed 4.5 to 30 kg (median 12.5 kg); 9 dogs were female, 12 male. Nine mixed-breed and 12 purebred dogs were represented.

The most common indications for blood transfusion were blood loss anemia (n = 12), hemolytic anemia (n = 8), and anemia because of ineffective erythropoiesis (n = 1). Causes of hemorrhage included gastrointestinal bleeding (n = 7), severe thrombocytopenia (n = 2), trauma (n = 1), blood loss during surgery (n = 1), and hemoabdomen because of ruptured hemangiosarcoma (n = 1). Five dogs were diagnosed with immune-mediated hemolytic anemia. One dog had hemolysis because of *Babesia canis* infection, and in 2 dogs the cause of hemolysis was unclear.

Based on the diagnosis, 9 dogs (with immune-mediated hemolytic anemia [n = 5], hemolysis of unknown etiology [n = 1], ineffective erythropoiesis [n = 1], blood loss anemia because of immune-mediated thrombocytopenia [n = 1], chronic inflammatory enteropathy with bleeding [n = 1]) were treated with immunosuppressants. Three dogs were already pretreated with immunosuppressive agents before transfusion. Therapy was maintained in 2 dogs, in 1 dog treatment was interrupted and then continued 10 days after transfusion. In another 3 dogs, therapy was started on day 0, in 1 dog on day 2, and in 2 dogs on day 3 after transfusion. Six dogs received monotherapy with prednisolone (1.9-2.5 mg/kg per day) and 3 dogs a combination therapy (prednisolone 0.8-2 mg/kg per day) with cyclosporin (n = 2; 4-10 mg/kg per day) alone, mycophenolate mofetil (n = 2; 9.2-12.5 mg/kg per day) alone or both medicaments in combination.

3.2 | Transfusion

The PCV before transfusion was 10% to 38% (median 16%). Nine dogs were DEA 1 negative, 12 DEA 1 positive. All dogs were transfused with pRBC. The transfusion volume ranged from 6.1 to 20 mL/kg (median 10 mL/kg). The storage period of the blood units varied from 0 to 28 days (median 7 days), and in 3 transfusions the blood units had been stored for >14 days.

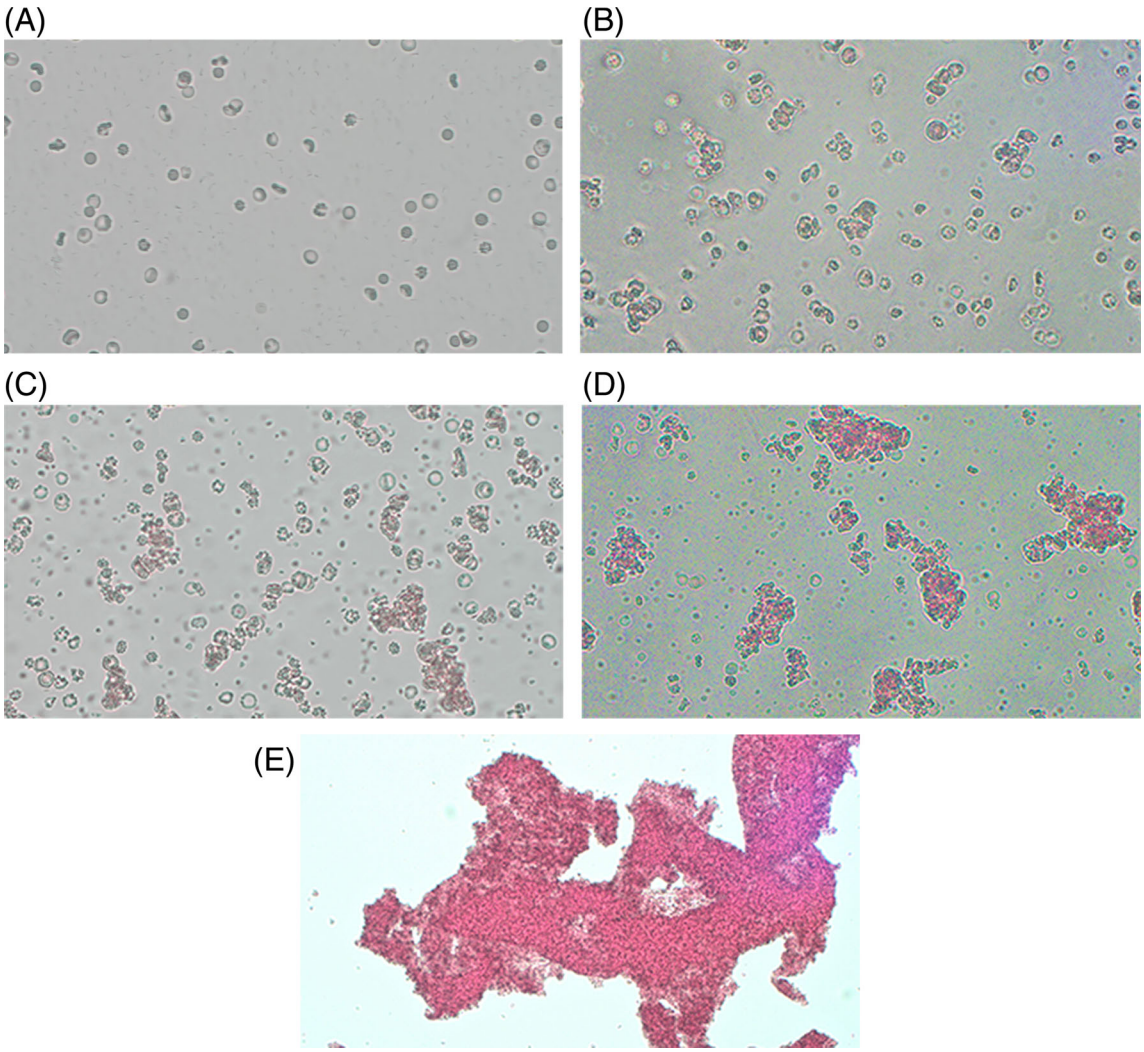

FIGURE 1 Microscopic cross-match evaluation at ×500 magnification: (A) microscopic negative cross-match, (B) microscopic positive cross-match (degree of agglutination 1+), (C) microscopic positive cross-match (degree of agglutination 2+), (D) microscopic positive cross-match (degree of agglutination 3+), (E) microscopic positive cross-match (degree of agglutination 4+)

Acute transfusion reactions were not observed. After transfusion, a PCV of 16% to 43% (median 24%) was achieved. The increase in PCV after transfusion ranged from 3% to 14% (median 7%). The difference of actual to calculated PCV increase was −11% to 5% (median −2%) with a SD of 4%. There was no significant difference in the distribution of the difference in PCV increase between the types of anemia ($P = .97$, Mann-Whitney U test). Possible effects of transfusion volume ($P = .08$, Spearman rho) or storage time of blood units ($P = .82$, Spearman rho) were also not detected.

3.3 | Cross-matching

A total of 141 cross-match tests were performed. One hundred and five cross-match tests were performed in all 21 dogs before and up to the 4th day after transfusion, and in 18 dogs a total of 36 additional cross-match tests were performed in the further study period between the 5th and 23rd day (median day 11) after transfusion. This was 1 cross-match test in all dogs before the transfusion, 4 cross-match tests in all dogs in the first 4 subsequent days after the transfusion. In 18 dogs, 1 to 5 cross-match tests per dog (median 2 cross-match tests) were performed additionally in the further study period. All cross-match tests were performed with the same donor-recipient pairs at different time points before and after the transfusion. Before transfusion, all recipient-controls and major cross-match tests were negative. After transfusion, 44 major cross-match tests (37%) and 6 recipient-controls (5%) were positive in the microscopic evaluation at different time points. Macroscopic agglutination or hemolysis did not occur. At least 1 microscopically weak positive major cross-match test was observed in 16 dogs (67%). These dogs first developed a microscopically weak positive major cross-match test between days 1 and 13 (median day 4), in 1 dog even within 12 hours, after transfusion.

3.3.1 | Cross-match test results within the first 4 days after transfusion

Alloimmunization was detected in 12/21 dogs (57%) within the first 4 days after transfusion, as indicated by a microscopically weak positive major cross-match test. The first positive cross-matches occurred in 2 dogs on day 1 (AD 1+; in 1 dog already after 12 hours), in 1 dog on day 2 (AD 1+), in 3 dogs on day 3 (AD 1+), and in 6 dogs on day 4 (AD: 1+, 1+, 1+, 1+, 2+). Overall, 23/84 cross-match tests (27%) were weakly positive with a degree of agglutination (AD) from 1+ to 2+ on microscopic evaluation during this study period. Type of anemia ($P = .15$), transfusion volume ($P = .22$), blood storage duration ($P = .50$), or immunosuppressive treatment at the time of transfusion ($P = .91$) did not affect the alloimmunization rate by day 4 after transfusion, using logistic regression.

3.3.2 | Cross-match test results after transfusion (>day 4)

Additional cross-match tests were performed in 8/9 dogs that did not have positive cross-match test results by day 4 after transfusion. In 4 dogs, there were no positive cross-match test results during a 5-18 day period (median day 11) after transfusion. Positive cross-match test results were noted in 4 dogs: microscopically weak positive cross-match test results in 1 dog each on days 6 (AD 2+), 8 (AD 1+), 9 (AD 2+), and 13 (AD 1+) after transfusion.

3.3.3 | Cross-match test results throughout the study period

While an increase in RBC agglutination was observed within 3 days in 2 dogs, a decrease occurred 3 to 14 days (median 5 days) after the initial appearance of agglutination in 9 dogs and a resolution occurred 5 to 11 days (median 5.5 days) in 6 of these dogs. Possible associations between the type of anemia ($P = .47$), transfusion volume ($P = .07$), storage duration ($P = .31$) or treatment with immunosuppressants ($P = .52$) with the decrease of agglutination during the follow-up period were not detected in the logistic regression model.

3.3.4 | Results of the recipient controls after transfusion

In total, a microscopically weak positive recipient-control (AD as 1+) was detected in 4 dogs (6 cross-match tests). In 1 dog, a microscopically weak positive recipient-control (AD 1+) occurred on the 3rd and 4th day after transfusion in addition to a microscopically weak positive major cross-match test (AD 1+). Subsequently, resolution of agglutination occurred in both tests. One dog developed a microscopically weak positive recipient-control (AD 1+) and a microscopically weak positive major cross-match test (AD 1+ and 2+, respectively) simultaneously on day 2 and another dog on day 4 after transfusion. While the agglutination in the recipient-control resolved in the further course of the study, the agglutination in the major cross-match test remained. In another dog, after an initially positive major cross-match test result on day 3 after transfusion, microscopically weak agglutination was detected in the recipient-control on the last day of the study period (day 5).

3.3.5 | Sample material: Storage duration of donor blood samples

Overall, the storage duration of the donor blood samples ranged from 0 to 39 days (median 12 days). Within the first 4 days after transfusion, this corresponded mostly to the storage period of the blood unit (0-28 days), which showed no association with the cross-match test result ($P = .37$, Mann-Whitney U test). During the rest of the study period, the storage duration of the donor blood samples ranged from 6 to 39 days (median 19 days). Again, no association with the cross-match test result was found ($P = .96$, Mann-Whitney U test).

4 | DISCUSSION

This study describes serial cross-match evaluation and the occurrence of alloimmunization during the first 4 days after a DEA 1-compatible blood transfusion in dogs. Cross-match tests are usually performed in dogs before transfusion when an initial transfusion occurred more than 4 days ago or the transfusion history is unknown.[24,27] In this study, 12/21 dogs (57%) developed alloantibodies within the first 4 days of transfusion. The earliest weakly positive cross-match test was detected as early as 12 hours after transfusion.

Although preexisting alloantibodies could be detected in one study in 17% of the dogs even before an initial transfusion,[17] they are believed to be of no clinical relevance.[21] It might be exclusive nonspecific agglutination of naturally occurring antibodies. However, in some studies, the increase in PCV because of a transfusion could be improved by performing cross-matching.[17] In contrast, for cats, it is recommended to perform a cross-match test before every transfusion, if possible, because red blood cell incompatibility was noted in one study in 15% even before an initial transfusion.[28]

In this study all cross-matches before transfusion were negative, but the formation of alloantibodies could be detected as early as 1 day after a transfusion. There was no correlation between the alloimmunization and the type of anemia, transfusion volume, blood storage duration, or immunosuppressive treatment. Dogs might form alloantibodies earlier as a response to a transfusion than previously thought. A further possible explanation is a low titer of naturally occurring antibodies, below the detectable limit, which increases because of the transfusion. The formation of alloantibodies in dogs within the first few days after a transfusion was described in only one study; thus far, anti-Dal alloantibodies were detected as early as 4 days after transfusion in 1 of 2 dogs in this study.[25] In cats, alloimmunization could be

detected as early as 2 days and in 25% within the first 10 days,[22] in human medicine only in a few patients (0.4%-1.43%) alloantibodies were detected within the first 4 days after transfusion.[29,30]

By examining the formation of alloantibodies over a longer time period after the transfusion, higher rates of alloimmunization are detectable. In our study, overall, 16 of 21 dogs (76%) had signs of alloimmunization within 13 days after a single transfusion based on a microscopically weak positive cross-match test. This agrees with the results of previous studies in dogs. In these studies 44% to 71% were sensitized by the transfused RBCs as shown by incompatible major cross-match results.[11,21] In cats, an alloimmunization rate up to 25%[22,28] and in human medicine of 1.8% to 20% was described.[29-33]

However, it is assumed that based on the frequency and timing of antibody testing in human medicine, only 1/3 of the alloantibodies formed are actually detected.[34] In human medicine the risk of alloimmunization is dependent on various factors; for example, infections, autoimmune diseases, or certain blood diseases, such as sickle cell anemia, can increase the risk of alloimmunization.[32,35,36] Furthermore, the number of transfusions required plays a significant role in alloimmunization.[37] In addition, the risk of alloimmunization can be reduced by extended red blood cell antigen matching as well as treatment with immunosuppressants.[31,38-40] In this study, there was no correlation between the alloimmunization and the type of anemia, transfusion volume, blood storage duration, or immunosuppressive treatment.

While antibody screening and cross-match testing is routinely performed before transfusion in human medicine,[41,42] it is rarely an integral part of routine compatibility testing before an initial transfusion in dogs.[43] Although the clinical relevance of naturally occurring alloantibodies is questionable, the integration of cross-matching into compatibility testing even before an initial transfusion has been discussed by several authors.[16-18] To date, no correlation has been demonstrated between the performance of cross-matching before initial transfusion and the occurrence of acute transfusion reactions.[24,44] However, several studies have suggested that there might be an increase in antibody titer as a result of transfusion of cross-match-incompatible blood.[16,17] This could possibly lead to delayed transfusion reactions or even complications in further transfusions. In the present study, only initial cross-match compatible transfusions were administered, and in the further course, agglutination was only microscopic and weak. No acute transfusion reactions, but a discrepancy of the actual PCV increase compared to the calculated 1, was frequently observed. However, a significantly lower PCV after transfusion in comparison to the calculated value could indicate continuing blood loss/hemolysis or a hemolytic transfusion reaction, whereas a higher increase than expected could be explained by resorptive mechanisms, splenic contraction, dehydration, or regenerative bone marrow response.[45] Thus, the clinical impact of alloimmunization in this study is unclear.

In 4 dogs, in addition to a microscopically weak positive major cross-match test (AD 1+ to 2+), 1 or 2 microscopically weak positive recipient-controls (AD 1+) were detected. Agglutination in the recipient-control could indicate an immune reaction as a result of the transfusion, but also a secondary immune-mediated hemolysis.

During the study, a decrease of agglutination in the major cross-match test results was detected in 9 dogs and 6 of those dogs had complete resolution of agglutination. This could be an indication of the formation of unstable antibodies, which were degraded early after formation. The decline or disappearance of agglutination has also been described in several studies in humans.[19,46] Here, antibodies have evanesced or fallen below the level of detection a few months to years after initial occurrence. Upon renewed contact with the respective antigen, a rapid anamnestic RBC alloantibody response is described. Therefore, the decrease of agglutination below a detectable level poses a high risk to miss a previous alloimmunization and to provoke a transfusion reaction in case of a further transfusion.[19] A decrease in agglutination in dogs was also described in a study in 3 of 10 recipient-donor pairs.[11] Neither the mentioned study nor our study investigated against which antigens the described antibodies were formed. In previous studies antibodies against blood group Dal were detected up to a period of 2 years and against blood group DEA 1 even up to 4.5 years after transfusion.[25,47]

A part of the study group was treated with immunosuppressants during the study period. Because the influence of treatment with immunosuppressants on the occurrence of transfusion reactions could not be proven in a previous study in dogs,[48] premedication with glucocorticoids before transfusion is not recommended in veterinary medicine.[49] However, it is questionable whether treatment could have an effect on alloimmunization. In human medicine, several studies have already described a lower rate of alloimmunization by treatment with immunosuppressants.[31,50] In this study, 9 dogs were treated with immunosuppressants, and treatment was started at different time points. Thus, the influence of immunosuppressants at the time of alloimmunization was variable. In this study, no significant correlation with formation of alloantibodies within the first 4 days was demonstrated. In 2 dogs treated with prednisolone, a decrease in agglutination was evident immediately after initiation of therapy. However, a decrease in agglutination within the study period was also detected in both 4/7 treated and 5/9 untreated recipients. Thus, a correlation between treatment with immunosuppressants and the decrease of alloantibodies was not evident.

Based on an equine study, an influence of the storage of blood samples on the cross-match test result was assumed.[51] In this study, the cross-match test results were not reproducible after 1 week of sample storage. A more recent study, which examined canine cross-match tests, did not demonstrate any association between duration of pRBC storage and development of major cross-match incompatibilities.[52] In this study, donor blood samples were drawn from the preserved blood units and stored at 4°C until cross-matching was performed. During the first 4 days after transfusion, the storage period of the donor samples was nearly equivalent (≤4 days longer) to that of the blood units and the effects of the storage duration of the blood unit on alloimmunization up to day 4 were not demonstrated. However, during the rest of the study period, the storage duration of donor blood samples varied from 6 to 39 days (median 19 days). Even during this study period, there was no significant correlation between the storage duration of the donor blood samples and the outcome of the cross-match tests.

Cross-match tests in this study were performed using the conventional tube method. This is often referred to as the gold standard, but it has also been suggested that this method is too sensitive and identifies also clinically irrelevant incompatibilities.[53] The addition of antiglobulins might even has enhanced the agglutination reaction.[53,54] Therefore, it is uncertain whether this increases the sensitivity in detecting clinically relevant or irrelevant antibodies. However, in comparative studies, the tube method, even without enhancement, was more sensitive than different commercial test kits, which were enhanced with antiglobulins.[14,55]

In this study, only microscopically weak positive cross-match tests were detected. Although the clinical relevance is not clear, it is speculated that transfusion of a blood unit with only a weak cross-match incompatibility might lead to a secondary or anamnestic response.[16] The exclusion of blood products with a low level of cross-match incompatibility can limit blood product availability, whereas the transfusion of incompatible blood products can result in hemolytic transfusion reactions. For this reason, it is recommended that only cross-match-compatible blood units be transfused whenever possible.[24]

This study has several limitations: Evaluation of the clinical impact of alloimmunization was difficult because of the small study group and the diverse underlying diseases in addition to a persistent blood loss or hemolysis. Another limitation of this study is the short study period of 5 to 24 days, as some follow-up examinations were missing because of, for example, lack of compliance of the owners or euthanasia of the dogs.

5 | CONCLUSION

Cross-matching is usually performed only when an initial transfusion had occurred more than 4 days earlier or the transfusion history is unknown. In this study, alloantibody formation was detected in 12/21 transfused dogs within the first 4 days after an initial transfusion. Therefore the 4-day rule should be reconsidered in dogs. Because alloimmunization was detected as early as 12 hours after transfusion, cross-matching should be performed before every subsequent transfusion.

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CONFLICT OF INTEREST DECLARATION

Lisa Herter declares that she is supported by a grant from the Ernst Reuter Foundation and that she has ongoing research collaborations with companies that offer commercial cross-matching test kits. Christiane Weingart declares that she has held lectures for veterinary pharmaceutical and diagnostic companies. Roswitha Merle, Nina Merten, and Nicole Bock declare that they have no conflict of interest. Barbara Kohn declares that she repeatedly has lectured for and acted as consultant for veterinary pharmaceutical, nutritional and diagnostic companies and has previous and ongoing research collaborations with various veterinary pharmaceutical, nutritional and diagnostic companies.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

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

Approved by the Freie Universität Brlin, Protocol Number: O 0246/19.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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