Prevalence of naturally occurring antibodies against dog erythrocyte antigen 7 in a population of dog erythrocyte antigen 7–negative dogs from Spain and Italy

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OBJECTIVE
To determine the prevalence of naturally occurring anti-dog erythrocyte antigen (DEA) 7 antibodies in DEA 7–negative dogs from Spain and Italy.

ANIMALS
252 DEA 7–negative dogs from a population of 312 dogs that were previously tested for DEA 1, DEA 4, and DEA 7.

PROCEDURES
A plasma sample was obtained from each dog and evaluated for anti–DEA 7 antibodies by the use of gel column agglutination. Each plasma sample underwent major crossmatching with RBCs from DEA 7–positive dogs. Samples that resulted in agglutination were then crossmatched with RBCs from DEA 1–negative, DEA 4–positive, and DEA 7–negative dogs to confirm the presence of anti–DEA 7 antibodies. Results were then used to calculate the risk for a delayed transfusion reaction in a DEA 7–negative dog with anti–DEA 7 antibodies after a transfusion with blood that was not crossmatched or typed for DEA 7.

RESULTS
96 of 252 (38.1%) plasma samples contained anti–DEA 7 antibodies. A DEA 7–negative dog with anti–DEA 7 antibodies had a 5.9% chance of developing a delayed hemolytic reaction after transfusion with blood not crossmatched or typed for DEA 7.

CONCLUSIONS AND CLINICAL RELEVANCE
Results indicated that canine blood used for transfusion should be crossmatched with the blood or plasma of the intended recipient prior to transfusion to minimize the likelihood that the recipient will develop a hemolytic reaction associated with anti–DEA 7 antibodies. Ideal canine blood donors should be negative for both DEA 1 and DEA 7. (*Am J Vet Res* 2016;77:877–881)

Detection of antibodies against RBC antigens is critical during pretransfusion compatibility testing, and it is one of the principle tools for investigating potential hemolytic transfusion reactions and immune-mediated hemolytic anemias. Although blood transfusions can be lifesaving, they are also associated with adverse events that can be life threatening.[1] The purpose of pretransfusion testing is to minimize immediate or delayed adverse reactions after an RBC transfusion.

Two types of antibodies (naturally occurring and immune) are of concern in blood banking. Both types of antibodies are produced in reaction to encountered antigens. Naturally occurring anti-RBC antibodies are found in the serum of individuals that have never been exposed to RBC antigens by transfusion, injection, or pregnancy and are likely produced in response to environmental substances that resemble RBC antigens such as pollen grains and bacterial membranes.[2] Conversely, immune anti-RBC antibodies are found in the serum of individuals that have received transfusions.[2] Either type of anti-RBC antibodies can become clinically relevant if they induce destruction of transfused RBCs that express the target antigen, resulting in anemia and transfusion reactions of varying severity. Those antibodies are typically IgG antibodies that react at 37°C.[2]

Dog erythrocyte antigen 1 is considered the most immunogenic RBC antigen. Naturally occurring anti–DEA 1 antibody has not been described. However, exposure of a DEA 1–negative dog that was previously sensitized with DEA 1–positive RBCs induces the production of anti–DEA 1 antibodies, and subsequent administration of an incompatible RBC transfusion might cause a severe acute hemolytic reaction.[3] Unlike DEA 1, naturally occurring antibodies against DEA 3, DEA 5, and DEA 7 are present in some dogs, and occasionally, dogs may have antibodies against other, less common RBC antigens.[4–6,a] The DEA 7 antigen is expressed in approximately 8% to 54% of dogs

ABBREVIATIONS
DEA Dog erythrocyte antigen
LISS Low ionic-strength solution

and is moderately antigenic to DEA 7–negative transfusion recipients.[6–11,b] The prevalence of low titers of weak, nonhemolytic anti–DEA 7 antibodies in DEA 7–negative dogs varies from 0%[3] to 50%.[12,a] When DEA 7–negative dogs receive DEA 7–positive RBCs during a transfusion, there is rapid clearance of transfused RBCs, with sequestration and loss of RBCs from the circulation within 72 hours.[13–15]

Major and minor crossmatching are performed to determine whether there is incompatibility between a donor and recipient and should be done prior to a blood transfusion to identify antibodies and avoid transfusion reactions. The primary purpose of major crossmatching is to determine whether the recipient has antibodies against the donor's RBCs. Minor crossmatching determines whether the donor plasma contains antibodies against the recipient's RBCs and should be considered for recipients that are receiving plasma or whole blood transfusions. Major and minor crossmatching to identify antigen-antibody agglutination reactions are useful for assessing the possibility of and preventing hemolytic reactions, which are characterized by intra- or extravascular hemolysis and unexpected decreases in PCV.[1] If an agglutination reaction occurs during crossmatching, an incompatibility exists between the donor and recipient, and the donor RBCs or plasma should not be administered to the recipient. However, a compatible crossmatch does not guarantee normal RBC survival and does not completely eliminate the risk of a transfusion reaction because low titers of anti-RBC antibodies do not always cause agglutination during crossmatching,[1,16] and reactions to donor leukocytes or plasma proteins are not identified during crossmatching.[1]

In human blood-banking laboratories, antibody-screening tests are used to detect anti-RBC antibodies in the sera of recipients. A recipient's serum is tested against RBCs of known phenotypes to identify problematic interactions and prevent hemolytic reactions during or after blood transfusion. Additionally, that screening process can reveal the frequency with which an unexpected antibody is found within a potential recipient population, and that frequency can be used to calculate the likelihood that such an antibody will cause an adverse transfusion reaction.[17] In small animal veterinary transfusion medicine, a commercial antibody-screening test is currently unavailable, and antibody screening is not a part of routine testing. However, antibody screening has been used in specialized veterinary research settings to determine antibody specificity.[3,5,10,a] The objectives of the study reported here were to assess the prevalence of anti–DEA 7 antibodies in a population of DEA 7–negative dogs and to determine the risk for transfusion reactions associated with the presence of those antibodies in that population.

Materials and Methods

Animals

The study population consisted of 252 DEA 7–negative dogs and included 138 Spanish Greyhounds from a shelter in South Madrid, Spain; 68 Ibizan Hounds from the Island of Ibiza, Spain; and 46 privately owned active blood donors for the Veterinary Transfusion Unit at the University of Milan, Milan, Italy. Those blood donors included 12 Bullmastiffs, 12 Cane Corsos, 6 German Shepherd Dogs, 4 mixed-breed dogs, 3 Bernese Mountain Dogs, 2 Rhodesian Ridgebacks, 2 Dogo Argentinos, 2 Labrador Retrievers, 1 Rottweiler, 1 Doberman Pinscher, and 1 Greyhound. The study population was part of a larger population of 312 dogs that had been previously tested for the presence of DEA 1, DEA 4, and DEA 7. Dog erythrocyte antigen 1 was measured by use of card agglutination[c] in the Spanish Greyhounds[11] and Ibizan Hounds and by immunochromatography[d] in all other dogs. Gel column agglutination was used to detect both DEA 4 and DEA 7 in all dogs.

All study procedures were performed in accordance with European legislation for animal research (2010/63/EU), and owner consent was obtained for all privately owned dogs prior to study enrollment. Blood-typing and crossmatching procedures were performed at the Veterinary Transfusion Unit, University of Milan, Milan, Italy. The polyclonal anti–DEA antibodies[e] used in the study were obtained from a US company and imported to the University of Milan with the authorization of the Italian Health Minister (protocol authorization No. 0021278- 15/10/2014-DGSAF-DGSAF-P).

Identification of anti–DEA 7 antibodies by gel column agglutination

A blood sample (2.5 to 5 mL) was obtained from each dog via jugular or cephalic venipuncture into blood collection tubes that contained EDTA as an anticoagulant. The blood samples were centrifuged, and the plasma was harvested from each sample. A major crossmatch test was performed with gel column agglutination to detect anti–DEA 7 antibodies in each plasma sample. The gel column agglutination test was performed in accordance with the manufacturer's instructions as described[6] with standard gel test cards[f] developed for human plasma. Each test card included 6 microtubes that contained a neutral gel. Donor RBCs were isolated from blood samples collected from DEA 1–negative (as determined by results of an immunochromatographic test), DEA 4–positive (4+ agglutination results on gel column agglutination), and DEA 7–positive (3+ results on gel column agglutination) donor dogs. A 0.8% RBC suspension was generated by the addition of 10 µL of packed donor RBCs to 1 mL of modified LISS.[g] A 50-µL aliquot of the 0.8% RBC–LISS suspension was then added to the test gel column and mixed with 25 µL of plasma from each DEA 7–negative dog in the column reaction chamber. For each sample, a recipient autocontrol test column was prepared by mixing 25 µL of plasma with 50 µL of 0.8% RBC-LISS suspension that was generated with RBCs from the same dog.

Column gel cards were incubated at 37°C for 15 minutes. The cards were then centrifuged for 10 minutes at 80 × *g* in a special gel card centrifuge[h] and evaluated for the presence and extent of agglutination in accordance with the manufacturer's instructions. The cards were visually interpreted and scored on a 5-point scale as follows: 0 = negative (no agglutination; all RBCs located at the bottom of the column), 1+ = very few RBC agglutinates dispersed in the lower part of the gel with most RBCs located at the bottom of the column, 2+ = all RBCs agglutinated and dispersed in the gel, 3+ = some RBC agglutinates dispersed in the upper portion of the gel with most of the RBCs forming a red line on the surface of the gel, and 4+ = all RBCs form a red line at the top of the gel. Results were considered negative for samples with no agglutination and positive for samples with agglutination scores ≥ 1+. Only samples for which the autocontrol column yielded negative results were considered valid.

In transfusion medicine, the specificity, or identity, of an anti-RBC antibody can be determined by testing a recipient's serum or plasma with a panel of RBC suspensions with a known antigenic composition. As a rule, serum or plasma that reacts with an RBC suspension that is positive for a given antigen, but not with an RBC suspension that is negative for that antigen, is suspected to contain antibodies against the given antigen.[17] To confirm that the plasma samples that tested positive for anti–DEA 7 antibodies when crossmatched with DEA 7–positive RBCs contained anti–DEA 7 antibodies, those samples were subsequently tested by use of the same gel column agglutination method in which the 0.8% RBC–LISS suspension was generated from a DEA 7–negative donor dog.

Data analysis

The prevalence of dogs in the study population that had anti–DEA 7 antibodies was calculated as the number of dogs with positive anti–DEA 7 antibody results divided by the number of dogs tested. The probability that 2 independent variables will occur simultaneously can be calculated by multiplying the individual probabilities of occurrence for each variable. To calculate the risk for a hemolytic transfusion reaction in a DEA 7–negative dog with antibodies against DEA 7 after 1 random transfusion of blood that was not crossmatched or typed for DEA 7, we used a previously described formula.[18,19] Specifically, the percentage of DEA 7–negative dogs with anti–DEA 7 antibodies in the original population of 312 dogs was multiplied by the percentage of DEA 7–positive dogs in the original population of 312 dogs.

Results

Of the 252 DEA 7–negative dogs in the study population, 96 (38.1%) had anti–DEA 7 antibodies (ie, positive results on the gel column agglutination when crossmatched with DEA 7–positive RBCs). Those 96 dogs included 66 (68.8%) Spanish Greyhounds, 17 (17.7%) Ibizan Hounds, 9 (9.4%) Cane Corsos, 2 (2.1%) Rhodesian Ridgebacks, 1 (1%) Dogo Argentino, and 1 (1%) mix. Of the 96 samples with positive results, 24 (25%) had 1+ agglutination, 44 (45.8%) had 2+ agglutination, and 28 (29.2%) had 3+ agglutination; none of the positive samples had 4+ agglutination. For all samples that were positive for anti–DEA 7 antibodies, the autocontrol columns yielded negative results and no evidence of agglutination was detected when those samples were crossmatched with RBCs from DEA 1–negative, DEA 4–positive, and DEA 7–negative donor dogs. The potential risk for a hemolytic transfusion reaction in a DEA 7–negative dog with anti–DEA 7 antibodies after 1 random transfusion with blood that had not been crossmatched or typed for DEA 7 was 5.9% (96/312 × 60/312).

Discussion

Results of the present study indicated that 96 of 252 (38.1%) DEA 7–negative dogs had plasma antibodies against DEA 7. Dog erythrocyte antigen 7 is a soluble antigen that is not produced by RBCs; instead it is produced by other tissues and absorbed from the plasma onto the RBC membrane. It is believed that the structure of DEA 7 is similar to that of an antigen found in common bacteria.[7,20] Results of 1 study[a] indicate that up to 9.8% of dogs have naturally occurring antibodies against DEA 7. In DEA 7–negative dogs with anti–DEA 7 antibodies that receive blood transfusions, those antibodies might cause accelerated removal of transfused DEA 7–positive RBCs. In dogs with anti–DEA 7 antibodies, infusion of chromium-tagged DEA 7–positive RBCs results in the removal of all transfused RBCs from the circulation within 4 to 5 days.[15]

The prevalence of DEA 7–negative dogs with anti–DEA 7 antibodies (38.1%) in the present study was higher than that (9.8% [245/2,500]) in 1 study[a] but lower than that (50%) in another study.[12] Those discrepancies might be the result of differences in the breed composition of the populations screened. The prevalence of DEA 1 is associated with breed and geographic location,[6,10,11,18,19,b] and it is likely that the same is true for other DEAs. The population of the present study was primarily composed of Spanish sighthounds, in particular Spanish Greyhounds. In the present study, 66 of 138 (47.8%) Spanish Greyhounds had anti–DEA 7 antibodies, and those dogs represented the majority (66/96 [68.8%]) of all dogs with anti–DEA 7 antibodies. Thus, breed bias may have influenced the overall prevalence of dogs with antibodies against DEA 7 in this study. Except for the 46 blood donors from the Veterinary Transfusion Unit at the University of Milan, which were selected because they were considered optimum candidates for blood donation, the health status for most of the dogs evaluated in the present study was unknown, and those dogs could have previously received blood transfusions or been exposed to diseases or parasites that might

have induced production of antibodies against DEA 7.

Transfusion reactions caused by naturally occurring antibodies are infrequent in dogs, perhaps because of the low prevalence of dogs with clinically relevant naturally occurring antibodies, the low prevalence of dogs that require > 1 blood transfusion, or, more likely, underreporting. In dogs, the rate of adverse effects associated with blood transfusions ranges between 3% and 13%.[21,22] In human patients, delayed hemolytic transfusion reactions occur in 1 of every 524 to 954 blood transfusion recipients (ie, for 1/4,000 to 10,000 U of blood transfused) and account for 2 to 4 deaths/y.[17] On the basis of the results of the present study, approximately 1 in 17 (5.9%) DEA 7–negative dogs that receive a blood transfusion that is not crossmatched or typed for DEA 7 can be expected to develop a delayed hemolytic transfusion.

Implications of the presence of antibodies against DEA 7 vary depending on whether a dog is a blood donor or blood recipient. For blood donors, detection and identification of anti–DEA 7 antibodies are important for the banking of plasma and in areas of the world where only whole blood can be transfused. Dogs positive for DEA 7 that receive plasma or whole blood transfusions that contain anti–DEA 7 antibodies are at risk of developing a hemolytic reaction. In that instance, minor crossmatching could be used to identify potential transfusion problems. For blood recipients, dogs with anti–DEA 7 antibodies should only receive DEA 7–negative RBCs; otherwise, they may develop a hemolytic reaction. In that instance, major crossmatching between the recipient's plasma and the donor's RBCs should be performed to prevent such reactions.

A compatible crossmatch does not guarantee normal survival of transfused RBCs or completely eliminate the risks associated with plasma or blood transfusions. Delayed transfusion reactions are caused by the production of antibodies against transfused antigens (eg, RBCs) shortly after transfusion. Unfortunately, some individuals may have antibodies against transfused antigens at such low titers prior to transfusion that they are not detected during crossmatching.[1,16] The use of LISS enhances the reaction between an RBC antigen and its corresponding antibody and thus enhances the sensitivity of the gel column agglutination technique.[23] In 1 study,[5] there was substantial agreement between the results of a gel column assay and a standard tube assay for measurement of *Dal* canine erythrocyte antigen. In human medicine, the gel column assay is comparable to the tube assay for both indirect and direct antiglobulin tests.[24] In the present study, use of the gel column assay allowed us to clearly identify weak (1+) agglutination reactions that were present in the plasma for 24 of the 96 (25%) dogs with anti–DEA 7 antibodies.

Other factors can influence the sensitivity of antibody screening. In pretransfusion compatibility testing, the focus is on clinically relevant antibodies, which generally react at 37°C, and technicians can omit the immediate spin and room-temperature phases to limit the detection of clinically irrelevant cold antibodies.[2,17] The anti–DEA 7 antibody screening protocol used in the present study was the same as that used in a previous study,[a] and the antibody-antigen reaction was examined at only 37°C; thus, only physiologically active antibodies that are able to agglutinate at body temperature were identified.

A limitation of the present study was that we did not evaluate dogs for DEA 3 and DEA 5. The prevalence of dogs positive for DEA 3 and DEA 5 is low; however, antibodies against either DEA 3 or DEA 5 can cause delayed transfusion reactions.[13,25] Unfortunately, DEA 3, DEA 5, and *Dal* antisera were not commercially available at the time this study was conducted; therefore, screening blood samples for those antigens could not be performed. Additionally, the fact that the majority (66/96 [68.8%]) of dogs with anti–DEA 7 antibodies were Spanish Greyhounds suggested that breed bias might have influenced our results, which precludes extrapolation of the risk assessment results to the general dog population. Finally, although the gel column technique is a sensitive and specific method for antibody detection in both veterinary and human transfusion medicine,[5,24] its accuracy for the detection of low titers of anti–DEA 7 antibodies that could result in delayed transfusion reactions is unknown.

The findings of the present study suggested that canine blood used for transfusion should be crossmatched with the blood or plasma of the intended recipient prior to transfusion (even for the first transfusion) to minimize the likelihood that the recipient will develop a hemolytic reaction associated with anti–DEA 7 antibodies. We are in agreement with other investigators[3,25]; however, until further studies of RBC survival after transfusion of mismatched DEA 7 blood have been conducted, only dogs negative for both DEA 1 and DEA 7 should be considered ideal blood donors.

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Footnotes

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c. DEA 1, Agrolabo SpA, Torino, Italy.
d. Canine laboratory test DEA 1, Alvedia, Lyon, France.
e. Animal Blood Resources International, Stockbridge, Mich.
f. ID-CARD NaCl, enzyme test and cold agglutinins, DiaMed, Cressier FR, Switzerland.
g. LISS ID-DILUENT 2, modified LISS solution, DiaMed, Cressier FR, Switzerland.
h. ID-CENTRIFUGE 24 S, DiaMed-ID Micro Typing System, DiaMed, Cressier sur Morat, Switzerland.

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