

Lack of Evidence of Pregnancy-Induced Alloantibodies in Dogs

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Background: It is controversial whether or not pregnant bitches become sensitized to red blood cell (RBC) antigens.

Hypothesis: Bitches do not develop alloantibodies to RBC antigens during gestation and can be used safely as blood donors.

Animals: The study group included 35 healthy female dogs with a prior history of 1 (n = 12), 2 (n = 14), or ≥ 3 (n = 9) pregnancies. The control group consisted of 15 healthy female dogs without any history of pregnancy.

Methods: All dogs were blood typed for dog erythrocyte antigens (DEA) 1.1, 1.2, 3, 4, 5, and 7 using ethylenediaminetetraacetic acid blood samples and polyclonal antisera. Antibody screening was performed with serum and canine RBC panels of known blood type. An autocontrol and direct antiglobulin test were performed to rule out the presence of autoantibodies.

Results: The only alloantibodies identified were those against DEA 7 and the prevalence of anti-DEA 7 alloantibodies was similar in dogs with known history of pregnancy (11.4%) and in the control group (13.3%).

Conclusions and Clinical Importance: These results confirm previous studies and clinical transfusion medicine experience. Naturally occurring anti-DEA 7 alloantibodies have been reported but their clinical relevance has not been shown. Pregnancy does not appear to sensitize dogs to RBC antigens. Consequently, dogs with prior history of pregnancy can be used safely as blood donors. Conversely, no additional pretransfusion compatibility studies would be required should these dogs themselves need to be transfused.

Key words: Alloantibody screening; Blood compatibility; Blood donor program; Dog erythrocyte antigen; Gestation.

In 1946, Abelson reported natural immunization by pregnancy of female dogs leading to hemolytic disease in their newborns,^a but the characteristics of the alloantibodies were never published.¹ Although dog erythrocyte antigens (DEA) 1, 3, 4, and 5 appear to be fully developed at birth,² and thus could cause immunization, transplacental sensitization of the bitch during pregnancy has not been documented. Similarly, neonatal isoerythrolysis does not appear to occur in puppies unless the bitch has been previously sensitized by a transfusion.¹⁻³ Despite this lack of scientific evidence, current veterinary reviews and some animal blood banks suggest that pregnancy sensitizes dogs to red blood cell (RBC) antigens, and recommend that dogs with prior history of pregnancy not be used as blood donors.⁴⁻⁸ These recommendations likely were extrapolated from human medicine because transplacental sensitization of women, most notably to RhD antigens, is well established. Many veterinary blood banks encourage the use of dogs with a prior history of pregnancy as blood donors, and such recommendations are based on clinical experience and previously published experimental data.^{1,2} The purpose of this study was to investigate whether pregnancy causes dogs to become sensitized to RBC antigens

as reflected by the presence of alloantibodies in bitches that previously had been pregnant.

Methods

Animals

Fifty healthy female dogs, 35 with and 15 without prior pregnancy, were entered in the study. All had normal physical examinations, normal CBC, and absence of transfusion history. The number of pregnancies, date of whelping, number of puppies, and complications during delivery were recorded. The last parturition had to have been > 1 and < 24 months before sampling.

Ethylenediaminetetraacetic acid (EDTA)-anticoagulated blood and serum samples were used for serologic testing, including blood typing, alloantibody screening and direct antiglobulin testing (DAT). This study was approved by the Institutional Animal Care and Use Committee (IACUC) of Tufts University and informed consent was obtained from owners. Use of the dogs for the panel of known cell types and the generation of typing polyclonal canine antisera was approved by the IACUC of Midwest Animal Blood Services.

Blood Typing

RBC suspensions from all dogs were tested by tube agglutination assay for blood types for which reagents are commercially available, (ie, DEA 1.1, 1.2, 3, 4, 5, and 7). Briefly, polyclonal antisera recognizing DEA 1.X, 1.1, 3, 4, 5, and 7 are generated by alloimmunization between members of mismatched donor recipient pairs. Immunized dogs are titrated monthly and serum collected when the immunoglobulin G (IgG) titer is $> 1:254$. After collection over an appropriate period of time, 1000 mL of serum is pooled. Evaluation against 100 dogs of known type is performed to minimize batch variance. On confirmation of the reaction pattern, antisera are assigned lot numbers and aliquoted for use. Routine evaluation of activity in standard tube agglutination is performed quarterly by blinded randomized testing. Blood typing was done within 14 days of blood collection according to the manufacturer's protocol.^b The addition of canine polyvalent anti-IgG reagent (canine Coombs reagent)^c was required to facilitate the agglutination reactions with the DEA 1.X and 1.1 reagents.⁹ For blood samples

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≥ 2 days old, 0.2 mL of RBC preservation solution (Adsol)^d was added per 1 mL of EDTA blood.

DAT

To assess the presence of RBC autoantibodies, DAT was performed with a commercially available polyvalent Coombs' reagent^c for IgG, IgM, and complement as previously described.¹⁰

Alloantibody Screening and Identification

Alloantibody screening refers to testing for agglutinating antibodies in an animal's serum using different RBC suspensions of known blood type (known as panel of known type cells).^e Initially, sera from all dogs were screened against 4 reference panel cells: DEA 1.2 and 4 positive; DEA 1.1, 3, and 4 positive; DEA 1.1, 3, 4, and 5 positive; DEA 1.1, 4, and 7 positive. Briefly, 50 µL of heat-inactivated serum from each bitch and 25 µL of washed 3–5% RBC suspensions of known type are mixed and incubated at 37 °C for 15 minutes. This process is repeated for each reference panel cell. After centrifugation (1000 × *g* for 30 seconds), the tubes are examined for macroscopic and microscopic agglutination. The degree of agglutination is scored from 1+ to 4+. Any agglutination ≥2+ was considered positive for the presence of antibodies. Auto-controls (ie, a dog's serum incubated with its own RBC suspension) also were performed to exclude the presence of autoantibodies. All positive agglutination reactions were verified in duplicate, and the given serum was retested against a second panel of known type RBC to identify the specific antigen associated with alloantibody production. The second panel contained 6 different RBC suspensions: DEA 1.2, 4, and 7 positive; DEA 1.1, 3, and 4 positive; DEA 4 and 7 positive (from 2 different dogs); DEA 1.1, 4, and 7 positive (from 2 different dogs).

Statistical Analysis

A Fischer's exact test was used to investigate whether the prevalence of alloantibody differed between bitches that had or had not been pregnant. A *P* value < .05 was considered significant.

Results

Of 50 female dogs evaluated for blood type and alloantibodies, the study group included 35 dogs with a history of 1 (*n* = 12), 2 (*n* = 14) or ≥3 (*n* = 9) pregnancies. The control group included 15 dogs (spayed or intact) with no history of pregnancy. All dogs but 1 were purebred: 21 Labrador Retrievers from 7 different New England breeders and 2 private owners, 5 pointers, and 18 different breeds represented by 1 or 2 dogs. The blood typing results between the 2 groups and between Labrador Retrievers and other breeds were not significantly different, except there were more DEA 1.1-negative bitches that had been pregnant (Table 1). Only anti-DEA 7 alloantibodies were detected and there was no difference in the prevalence of alloantibodies between groups (*P* = .75). Alloantibodies were identified in 2 control dogs, and in 4 dogs with a history of 1 (*n* = 2), 2 (*n* = 1) or 3 (*n* = 1) pregnancies. The 4 dogs in the study group with positive alloantibody screens had 5, 6, 12, and 20 puppies, respectively, compared with a mean of 13 puppies per study dog. Anti-DEA 7 alloantibody reactions were 2+, but titers were not specifically determined. All DAT and auto-control results were negative. Nine dogs

Table 1. Blood typing results of bitches with and without prior pregnancy.

| DEA Blood Typing | Nulliparous Bitches | Bitches with Previous Pregnancies |
|------------------|----------------------|-----------------------------------|
| | N = 15 (7 Labradors) | N = 35 (14 Labradors) |
| | Percent Positive | Percent Positive |
| 1.1 | 80 (71) | 40 (43) |
| 1.2 | 6.7 (14) | 22.8 (14) |
| 3 | 13.3 (0) | 0 (0) |
| 4 | 100 (100) | 100 (100) |
| 5 | 13.3 (14) | 0 (0) |
| 7 | 6.7 (14) | 5.7 (0) |

Currently commercially available polyvalent canine antisera were used for typing and a ≥2+ agglutination reaction was considered a positive result. Beside 21 Labrador Retrievers (results in parentheses) there were 19 other breeds represented among the 50 bitches typed. Note only anti-DEA 7 alloantibodies were found in 12% of all dogs screened independent of prior pregnancy.

DEA, dog erythrocyte antigens.

had 1 or more complications during delivery including stillborn puppies (*n* = 7), uterine inertia (*n* = 3), metritis (*n* = 1), and requirement for a caesarean section (*n* = 2). However, no complications were reported in any dog with anti-DEA 7 alloantibodies.

Discussion

The blood type distribution of dogs in this study was similar to previously published results (Table 1).^{8,11,12} Because an individual must be negative for a given blood type in order to be sensitized to it, a higher proportion of DEA 1.1-negative dogs that were previously pregnant simply extended the potential for sensitization by pregnancy.

Based on this limited survey, pregnancy did not appear to sensitize dogs to RBC antigens, in contrast to what can occur in humans and horses. The prevalence of naturally occurring alloantibodies (all of which were anti-DEA 7) was 12% and was similar in nulliparous dogs compared with dogs with a prior history of pregnancy. Swisher and Young² recognized naturally occurring alloantibodies in low titer in 15% of 145 dogs with no history of transfusion, but the specificity of the alloantibodies was not determined. In a typing and alloantibody survey of 2500 dogs, predominantly selected as blood donors, the prevalence of naturally occurring serum alloantibodies was 13.5%, and the following alloantibodies were found: 0.3% anti-DEA 1.1; 1.2% anti-DEA 3; 0.8% anti-DEA 5; 9.8% anti-DEA 7; and 2.0% nonspecified antibodies.^e

The lack of any evidence of transplacental immunization of bitches against fetal RBC antigens agrees with previous experimental studies and extensive clinical experience.^{1,2} In studies by Young et al,¹ 6 DEA 1-negative bitches were immunized with IV injections of DEA 1-positive RBC and mated with DEA 1-positive sires. All DEA 1-positive pups permitted to nurse during the first day of life developed hemolysis because of intestinal absorption of colostral alloantibodies. There was no

evidence of transplacental isomunization in the dams' sera. Indeed the isoagglutinin titer induced by blood transfusion decreased during pregnancy, and did not increase after delivery. Similarly, no transfer of antibody occurred transplacentally from mother to puppy. Anti-DEA 1 could not be demonstrated in the blood of puppies at birth, and was not acquired by puppies that did not receive colostrum within 24 hours of birth. In addition, 12 DEA 4-positive puppies born to DEA 4-negative bitches that were immunized against DEA 4 by blood transfusions showed no evidence of hemolytic anemia.^{1,2} There is no experimental or clinical evidence that anti-DEA 7 alloantibodies cause neonatal isoerythrolysis. Weak naturally occurring alloantibodies against DEA 3, DEA 5, and DEA 7 have been identified in dogs that have never received transfusions, but they do not seem to have any clinical relevance because no hemolytic reactions have ever been reported associated with these blood types.^{2,13} Weak anti-DEA 7 alloantibodies have been described in 0–50% of all DEA 7-negative dogs.^{a, 11,14} It is believed that DEA 7 is a soluble antigen, not produced by RBC but instead adsorbed onto the RBC membrane, and structurally related to a common bacterial antigen.¹⁵ Although anti-DEA 7 alloantibodies may result in increased clearance of DEA 7-positive transfused RBC,² in vitro hemolysis, hemolytic transfusion reaction, and hemolysis of the newborn have never been reported.^{3,11}

Basic anatomic differences among species may explain the likelihood of sensitization during gestation. For instance, the placenta of higher primates is classified as hemochorial (ie, the fetal chorionic epithelium is directly bathed in maternal blood). In contrast, the endotheliochorial placenta of dogs and cats provides an additional layer of maternal uterine endothelium, which separates the fetal chorionic epithelium from maternal blood. This basic structural difference may explain in part why bitches are not sensitized during pregnancy. However, this structural variation may not account completely for the lack of pregnancy-related sensitization in dogs. Indeed, mares possess an epitheliochorial placenta, which means that the fetal chorionic epithelium remains separated from the maternal blood by 3 layers of tissue.¹⁶ Despite this even thicker placenta, sensitization of the mare during gestation has been documented and may lead to neonatal isoerythrolysis. Several RBC antigens have been implicated in equine neonatal isoerythrolysis with the most common antigens being Qa and Aa.¹⁷ The actual mechanism of sensitization of the mare has not yet been demonstrated, but it is believed that they are sensitized either because of placental disease, or as a consequence of uterine trauma during delivery.^{18–20} In addition to the number of layers, the shape and area of contact (ie, diffuse in the horse versus zony in dogs) between fetal and maternal tissue determine the nature of molecular transport across the placenta. Antibodies are not transferred in utero in horses, rendering colostrum essential to foals.¹⁶ In dogs, only 5–10% of maternal antibody is obtained in utero across the placenta with the majority being obtained from colostrum during the first 24 hours after birth.^{21,22}

Although neonatal isoerythrolysis is well documented in cats, the pathophysiology differs. Cats possess clini-

cally relevant naturally occurring alloantibodies against the blood type antigen they lack. Type B cats especially have very strong anti-A antibodies.^{23–25} Consequently, blood type A and AB kittens that receive colostral anti-A alloantibodies during their first day of life from type B queens (including primiparous queens) are at risk of severe neonatal isoerythrolysis. In contrast, blood type A queens have much weaker anti-B alloantibodies that have not been associated with neonatal isoerythrolysis.²⁶

In human medicine, some fetal, pregnancy, and delivery complications can influence a woman's chance of hemorrhage and development of Rh incompatibility. Although few complications associated with pregnancy and delivery were noted in the study reported here, no induced alloantibodies were found in any of these bitches.

In addition to the small size of the survey, there are several limitations to this study. The dogs were not typed for the *Dal* antigen. However, to date only *Dal* negative Dalmatians have been reported and there were no Dalmatians among the typed dogs of this study.²⁷ The blood types of puppies and sires were not available and thus the number of occasions the bitches could have been sensitized remains undocumented. Because we included bitches with several pregnancies (a known risk factor for transplacental sensitization in humans^{28,29} and mares^{19,20,30}), this approach rendered access to puppies and sires from previous pregnancies impossible. Despite the large proportion of Labrador Retrievers, the blood typing and alloantibody prevalence was not different from what was observed in the remainder of the screened bitches. Indeed more breed-specific information may be desirable because there may be breed differences in blood types and generation of alloantibodies. There were no DEA 4-negative dogs and thus no statement regarding the presence and induction of anti-DEA 4 can be made. However, >98% of all dogs are DEA 4-positive and anti-DEA 4 alloantibodies have only been reported after transfusion of 1 dog.³¹ Finally, the strength (generally believed to be weak) and type (IgG or IgM) of the anti-DEA 7 alloantibodies were not determined in this and previous studies.

In conclusion, the lack of pregnancy-induced alloantibodies in dogs shown here confirms previous experimental data and extensive clinical experience by large canine blood banks and transfusion centers. Consequently, one need not exclude previously pregnant dogs from blood donor programs. Including them will increase the supply of donors, especially in a time of increasing demand for canine blood products.^{8,15,32,33} Finally, a history of pregnancy in a dog in need of a transfusion is not a reason for additional pretransfusion compatibility studies (eg, crossmatching) beyond DEA 1.1 typing.

Footnotes

^a Abelson, NM: Paper read before Interuban Club, Philadelphia, April 5, 1946

^b Midwest Animal Blood Services Inc, Stockbridge, MI

^c Canine Coombs Reagent, VMRD Inc, Pullman, WA

^d Adsol Red Cell Preservation Solution, Baxter Healthcare, Deerfield, IL

^e Hale AS and Werfelmann J. Incidence of canine serum antibody to known Dog Erythrocyte Antigens in potential donor population. Abstract presented at ACVIM meeting, 2006

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