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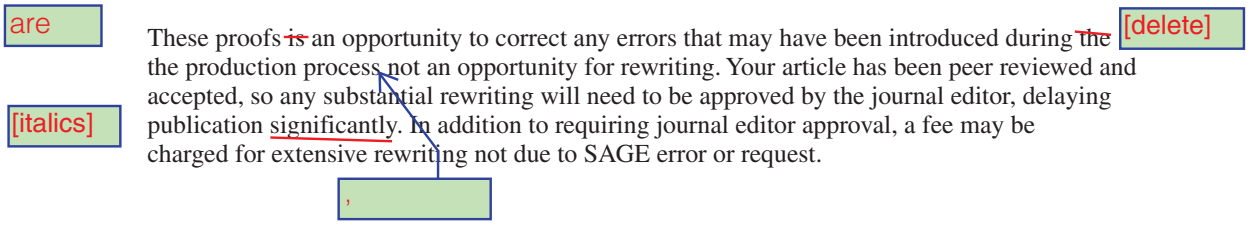

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Prevalence of dog erythrocyte antigens 1, 4, and 7 in galgos (Spanish Greyhounds)

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Abstract. Galgos (Spanish Greyhounds), in common with other sighthounds, have higher hematocrits, hemoglobin concentrations, and red blood cell counts than other breeds. In addition to these hematological characteristics, the physical characteristics of these dogs (medium to large dogs with an easily accessible jugular vein and a good temperament) make galgos ideal blood donors. However, to date, there are only published reports concerning dog erythrocyte antigen (DEA) 1 in this breed. Information on DEAs 4 and 7 would be useful when recruiting blood donors to donation programs, as DEA 1 and 7–negative and DEA 4–positive dogs can be considered universal donors. Ethylenediamine tetra-acetic acid–anticoagulated jugular blood samples were collected from 205 galgos. Dogs were aged between 1 and 10 years, 102 were female (49.8%) and 103 male (50.2%), and all were living in South Madrid, Spain. All 205 blood samples were tested for DEA 1 by card agglutination, and 150 of these samples were tested for DEA 4 and DEA 7 by gel column agglutination using polyclonal anti-DEA antibodies. Of the 205 galgos blood samples typed, 112 out of 205 (54.6%) were positive for DEA 1. Of the 150 blood samples tested, all (150/150, 100%) were positive for DEA 4, and 12 out of 150 (8%) samples tested positive for DEA 7. Of these samples, 70 out of 150 (46.7%) were positive only for DEA 4. There was no relationship between blood types and sex. In addition to the hematological characteristics previously reported and the physical characteristics of these dogs, the relative prevalence of blood types DEA 1, 4, and 7 make galgos good candidates for blood donation in blood donor programs.

Key words: Blood type; canine transfusion medicine; dog erythrocyte antigen; galgos.

Dogs have a number of blood group antigens, which are termed dog erythrocyte antigens (DEAs 1, 3, 4, 5, 7).[9,21,22] Other antigen systems, not yet fully characterized, have been reported as the *Dal* blood group.[3] The DEA 1 blood group is the most significant in terms of transfusion reactions. Spontaneously arising alloantibodies to DEA 1 occur at a very low prevalence in the canine population (0.3%),[9,12] but dogs can be sensitized following an incompatible first transfusion and can experience potentially fatal acute hemolytic reactions with subsequent DEA 1–mismatched transfusions.[7,21] The distribution of DEAs 3, 4, 5, and 7 has been poorly studied in comparison to DEA 1 mainly due to the limited availability of blood-typing reagents. Spontaneously arising alloantibodies to DEAs 3, 5, and 7 are also documented[9,10] and, in dogs that have previously received a transfusion, serious hemolytic transfusion reactions have been described in response to DEA 4 and an unidentified common red blood cell (RBC) membrane antigen.[4,14] Weak anti–DEA 7 antibodies have been described in some dogs, and such antibodies may result in shortened erythrocyte survival.[9,10,21]

The galgos is a Spanish sighthound that is very popular in Spain. These dogs are used for sport, hunting, and as companion animals, and their importance worldwide, especially in Europe, has increased due to adoption programs. In common with other sighthounds, galgos have higher hematocrits, hemoglobin (Hb) concentrations, and RBC counts than other breeds.[16] Studies have shown that blood gas values in the galgos are also commonly outside the reference limits described for dogs; galgos have higher bicarbonate concentration, pCO_2, total carbon dioxide, total Hb content and oxygen content, and lower pH, chloride concentration, and P50 than mixed-breed dogs.[23] In addition to these hematological and biochemical characteristics, their physical characteristics (medium to large dogs with an easily accessible jugular vein and a good temperament) make galgos ideal blood donors.

In galgos, the assessment of blood types is of special interest because of the increasing use of this breed as a blood donor.[15,16] The prevalence of DEA 1 has already been shown

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to be higher in galgos (51.7%)[15] than in Greyhounds (13.1%).[12] In addition, Greyhounds showed 100% positivity for DEA 4 and 29.1% positivity for DEA 7.[12] However, no data is available on the prevalence of DEA 4 and DEA 7 in galgos, and this information would be useful when recruiting blood donors to donation programs. The aims of our study were to confirm the prevalence of DEA1 in galgos as reported in a previous study,[15] and to evaluate the prevalence of DEA 4 and DEA 7 in galgos.

Ethylenediamine tetra-acetic acid–anticoagulated jugular blood samples were collected from 205 healthy galgos in December 2014. Dogs were aged between 1 and 10 years, 102 were female (49.8%) and 103 male (50.2%), and all were living in South Madrid, Spain. All blood samples were collected as part of a health program evaluation of galgos available for adoption. Surplus blood from the health program samples was utilized for our study, which was conducted according to the European legislation (2010/63/EU).

All samples were tested within 24 hr of collection for autoagglutination, and packed cell volume (PCV) and total protein (TP) were measured. All 205 blood samples were tested for DEA 1, and 150 of these samples were tested for DEA 4 and DEA 7. Anti–DEA 4 and 7 antibodies were imported and used in our study with the authorization of Italian Health Minister (authorization n. 0021278-15/10/2014-DGSAF-DGSAF-P). Blood typing was performed at the Veterinary Transfusion Unit (REV), Department of Health, Animal Science and Food Safety (VESPA) of the University of Milan, Milan, Italy.

DEA 1 was identified using card agglutination[a] performed according to the manufacturer's instructions as previously described.[8,19] The principle of this card-based agglutination test is a visible hemagglutination reaction resulting from the binding of the DEA 1 RBC surface antigen to a murine monoclonal antibody lyophilized on the card test.

Analysis for DEAs 4 and 7 was by gel column card agglutination within microtubes[b] as previously described,[13] using polyclonal anti-DEA antibodies raised in dogs by Animal Blood Resources International.[c] Briefly, 25 µl of a 0.8% RBC suspension (made by suspending 10 µl of the RBC pellet in 1 ml of low ionic strength solution[d]) were mixed with 25 µl of DEA 7 antisera or with 15 µl of DEA 4 antisera in the reaction chamber of saline gel columns. For all samples, a negative control column with saline was included. The gel columns were incubated at 4°C for 30 min and were than centrifuged in a special gel column card centrifuge[e] at 80 × *g* for 10 min. Finally, the gel column cards were evaluated for presence and strength of agglutination. The cards were visually interpreted as follows: (0) negative, all RBCs were at the bottom of the column; 1+, very few RBC agglutinates were dispersed in the lower part of the gel, with most RBCs at the bottom of the tube; 2+, all RBCs were agglutinated and dispersed in the gel; 3+, some RBC agglutinates were dispersed in the upper part of the gel and most of the RBCs formed a red line on the surface of the gel; and 4+, all RBCs formed a red line on top of the gel. Results were interpreted as negative if no agglutination or 1+ agglutination was present, whereas 2+ agglutination reactions were considered positive. Results were analyzed by descriptive statistical analysis, and Fisher exact test or Pearson chi-square test were used for comparing results in males and females using a statistical software[f] with significance set at $P < 0.05$.

No sample showed autoagglutination and anemia (PCV mean: 56%, median: 57%, min–max: 40–76%, SD: ±6.9%; TP mean: 7.3 g/dl, median: 7.2 g/dl, min–max: 5.4–9.7 g/dl, SD ±0.8 g/dl). Of the 205 galgos blood typed for DEA 1, 112 out of 205 (54.6%) were positive. All (150/150, 100%) blood samples tested positive for DEA 4 (4+: $n = 150$), 12 out of 150 (8%) samples tested positive for DEA 7 (2+: $n = 7$; 3+: $n = 5$), and 138 out of 150 (92%) tested negative (1+: $n = 3$; 0: $n = 135$). Of the tested dogs, 70 out of 150 (46.7%) were positive only for DEA 4 ("universal donors"). There was no significant relationship between blood types DEA 1, 4, and 7 and sex at $P < 0.05$.

It is important to perform blood typing and cross-matching prior to a blood transfusion to determine the compatibility between the blood donor and the recipient. This approach minimizes the frequency of reactions and their severity.[5–7] The prevalence of different blood group antigens shows breed and geographical differences.[9,11]

In our study, the prevalence of 54.6% for DEA 1 expression in galgos was similar to the prevalence (51.7%) previously reported in this breed,[15] and to the prevalence found in other breeds[2,9,11,12,20,21] and in mixed-breed dogs,[18] with approximatively half of dogs testing positive for this antigen. DEA 1 prevalence in galgos is much higher than in Greyhounds (shown in a previous study to be 13.1%).[12]

A previous study[1] using flow cytometry has demonstrated that DEAs 1.1, 1.2, and 1.3 are nothing more than variable expressions of the same red cell antigen DEA 1, and the named variables are nothing more than a subjective determination of agglutination reaction, not separate blood types. The blood group system DEA 1 is a continuum from negative to strongly positive antigen expression. These findings suggest that all alleles within the DEA 1 system have a similarly based epitope recognized by the monoclonal antibody. For this reason, in our study we have used the term DEA 1 rather than DEA 1.1 as used in previous studies.

Naturally occurring, weak, low titered, nonhemolytic anti–DEA 7 is present in 9.8–50% of DEA 7–negative dogs,[9,10] and sensitized DEA 7–negative dogs, when transfused with DEA 7–positive RBCs show increased clearance of red blood cells after transfusion,[21] with sequestration and loss of RBCs within 72 hr.[9] For this reason, this blood group should be identified in the donor and recipient before a transfusion. The prevalence of DEA 7 in the galgo population in our study was lower than previously reported in other canine breeds, such as Greyhounds (29.1%),[12] Turkish kangal dogs (71.1%),[2] and Golden Retrievers (25–27%),[11,20] and similar to prevalence in mixed breeds (11%) and German Shepherd

Dogs (8%) in Brazil.[18] Galgos negative for DEA 4 were not found among our study population, which was not surprising as up to 98–100% of the general dog population expresses this antigen.[2,11–13,18,20–22]

The definition of a canine universal donor is not unanimously agreed on among veterinary transfusion experts. The most restrictive definition of the universal donor would be a dog negative for DEAs 1, 3, 5, 7, and positive for DEA 4. Even though a hemolytic transfusion reaction due to DEA 4 alloantibodies has been reported in a dog,[14] because 98–100% of all dogs are positive for DEA 4,[11,22] it is rare to find DEA 4–negative dogs and this antigen is not likely to influence donor selection. Some experts do not exclude DEA 7–positive dogs from the donor pool.[5] However, when possible, it is useful to test donors and recipients for DEA 7, bearing in mind that extending donor DEA testing does not eliminate the need for cross-matching after the first transfusion. The prevalence of universal donors (i.e., dogs positive only for DEA 4), in our population of galgos, was 46.7%, which was slightly lower than the prevalence of 57.3% found in Greyhounds.[12]

As in a previous study,[13] all of the positive DEA 7 samples in our study showed an agglutination reaction of 2+ or 3+, but no 4+ reactions, and all of the DEA 4–positive samples in our study showed a 4+ agglutination. As previously discussed,[13] this different strength of agglutination is most likely a result of the titer and affinity of polyclonal antibodies to the different RBC antigens.

Blood types can be related to some diseases. A study reported that the absence of DEA 7 was associated with an increased risk of immune-mediated hemolytic anemia (IMHA) in Cocker Spaniels. The mechanisms proposed to account for an increased risk of IHMA in dogs that lack DEA 7 are manifold, but lack of a specific RBC surface antigen, such as DEA 7, could result in substantial instability in the cell membrane structure, with a functional defect at the cellular level that could result in cell lysis or abnormal cell shape and survival.[17] Galgos in this survey showed a low frequency of DEA 7; however, to the authors' knowledge, no report has been published suggesting a higher predisposition to IMHA in this breed. Further studies are needed to determine whether lack of DEA 7 could be related to these mechanisms that could predispose to IHMA.

The card agglutination technique has been used for decades and is sensitive for detecting DEA 1 and is suited for screening blood donors in a blood bank program.[8,19] Our study uses column gel agglutination with polyclonal antibodies for DEAs 4 and 7.[13] The first study that used this technique with polyclonal antibodies for DEAs 4 and 7 demonstrated that this technique was not 100% sensitive for identification of DEA 7.[13] When agglutination on gel was compared with agglutination in tube (considered to be the gold standard), there were 12 discordant results for DEA 7 with a concordance of 84%. In particular, the gel method had a specificity of 100% and a sensitivity of 53% for identification of DEA 7–positive samples when compared with agglutination in tube.[13] However, the gel technique has many advantages over tube agglutination testing: The procedure is standardized (there is no tube shaking or resuspension of an RBC button that may introduce subjectivity into the interpretation of the test), it is simple to perform, does not require RBC washing, and the results are reliable (there are well-defined endpoints of the reactions), clear to read, and stable for observation from hours to days after completion of the test. In addition, gel agglutination requires a smaller sample volume and this is undoubtedly an advantage in epidemiological studies with mass testing, given the difficulties in obtaining DEA 7 and DEA 4 antisera. The major disadvantage of the gel technique is the need to purchase special equipment (i.e., a special centrifuge to accommodate the microtube cards used for testing).

Another limitation of our study is that we tested only 3 blood types, because at the time of the study, DEA 3, DEA 5, and *Dal* antisera were not commercially available. However, we did include the blood types with greater antigenicity in canine transfusion medicine.

In conclusion, the frequency of positivity for DEA 1 and DEA 4 in galgos in our study was similar to that previously reported in the general canine population, but the frequency of DEA 7 was lower. In addition to the hematological characteristics previously reported and the physical characteristics of galgos, the prevalence of blood types DEA 1, 4, and 7 as reported in our study (with nearly half of the galgo population being identified as universal blood donors), make galgos good candidates for blood donation in blood donor programs. However, blood typing of blood donor and recipient should always be performed before a blood transfusion.

Authors' Note

This study was presented as a poster at the XXXII Annual Congress of the Asociación Madrileña de Veterinarios de Animales de Compañia (AMVAC), Vetmadrid 2015, March 5–7, 2015.

Sources and manufacturers

a. RapidVet-H (canine DEA 1.1), Agrolabo SpA, Scarmagno, Turin, Italy.
b. ID-Card "NaCl enzyme test and cold agglutinins", DiaMed GmbH, Cressier FR, Switzerland.
c. Animal Blood Resources International (ABRINT), Stockbridge, MI.
d. ID-Diluent 2 (modified LISS solution), DiaMed GmbH, Cressier FR, Switzerland.
e. ID-Centrifuge 24 S, DiaMed-ID micro typing system, DiaMed GmbH, Cressier FR, Switzerland.
f. MedCalc v. 14.10.2, MedCalc Software bvba, Ostend, Belgium.

Declaration of conflicting interests

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