Prevalence of dog erythrocyte antigen 1.1 in galgos (Spanish greyhounds)

I. Mesa-Sanchez, R. Ruiz de Gopegui-Fernández, M. M. Granados-Machuca, A. Galan-Rodriguez

Dog erythrocyte antigen (DEA) 1.1 is the most clinically important blood group in dogs, as negative recipients for this group may develop a life-threatening acute haemolytic transfusion reaction if they receive several DEA 1.1 positive blood transfusions. Due to their physical features, galgos are frequently used as blood donors in clinical practice, however, there are no published data regarding the prevalence of DEA 1.1 in this breed. Expression of DEA 1.1 was determined in 118 galgos and 88 dogs of other breeds being screened as potential blood donors, using an immunochromatographic cartridge typing kit (Quick Test DEA 1.1, Alvedia, Lyon, France). Of the total dogs, 53.4 per cent (110/206) were positive for DEA 1.1. The prevalence of DEA 1.1 positive blood among our population of galgos and other-breed dogs were 51.7 per cent (61/118) and 55.7 per cent (49/88), respectively. Potential risk of sensitisation in a recipient of other breed following non-typed blood transfusion using blood from galgos was 22.9 per cent. Due to the clinical significance of DEA 1.1 and the high prevalence of this blood group in galgos of Spain, we strongly recommend blood-typing for this group before administering any blood transfusion using galgos as donors, as with transfusions from other commonly used breeds.

Introduction

Transfusion medicine is a growing area in veterinary medicine. Nowadays, red blood cell (RBC) transfusion is a common clinical practice for treatment of anaemia, and it is not uncommon to have patients requiring several transfusions throughout their lives. Canine blood groups are determined by glycolipids and glycoproteins on the erythrocyte surface and they are designated as dog erythrocyte antigen (DEA) 1, 3, 4, 5 and 7, and a new group called Dal. A dog can be positive or negative for each blood type, except for the DEA 1, which contains multiple alleles determining two subgroups (DEA 1.1 and DEA 1.2) and a null type (Hale 1995, Hohenhaus 2004, Blais and others 2007). Of all the canine blood groups, DEA 1.1 is the most clinically important; although dogs do not have naturally occurring antibodies against this group, it is extremely antigenic, and antibodies will appear in a DEA 1.1 negative recipient dog exposed to positive RBCs within 4–14 days (Haldane and others 2004). If a second DEA 1.1 positive RBC transfusion is administered to the same DEA 1.1 negative patient, acute intravascular haemolysis (classified as type 2 hypersensitivity), haemoglobinemia, haemoglobinuria, renal ischaemia, acute renal failure, disseminated intravascular coagulation, and even death may occur (Giger and others 1995).

Veterinary Record (2014) doi: 10.1136/vr.102087

I. Mesa-Sanchez, M. M. Granados-Machuca, A. Galan-Rodriguez
Veterinary Faculty, Department of Animal Medicine and Surgery, University of Cordoba, Cordoba, Spain
E-mail for correspondence: i.mesa84@gmail.com
Provenance: not commissioned; externally peer reviewed
Accepted February 3, 2014

However, this reaction is rare in clinical practice. The clinical consequences of blood types other than DEA 1.1 are controversial, and determination of these blood groups is limited to specialised laboratories (Hale 1995, Hohenhaus 2004, Giger and others 2005). Although there is no consensus as regards universal canine donors (Hale 1995, Andrews 2006, Tocci and Ewing 2009, Kessler and others 2010), blood typing for the presence of DEA 1.1 should be performed in all donor and recipient dogs prior to transfusion to minimise the occurrence of adverse effects and to optimise survival of transfused RBCs (Giger and others 1995, Hale 1995, Tocci and Ewing 2009). Currently, several commercial kits for typing DEA 1.1 antigen have been developed, such as typing cards (DMS RapidVet-H, DMS Laboratories, Flemington, New Jersey, USA), cartridge kits (Quick Test DEA 1.1, Alvedia, Lyon, France), QuickVet/RapidVet DEA 1.1 Blood Typing Cartridge (Scandinavian Micro Biodevices ApS, Farum, Denmark), or gel column agglutination within microtubes (ID-Gel Test Canine DEA 1.1, Dia-Med Vet) (Giger and others 2005, Kessler and others 2010, Kohn and others 2012, Seth and others 2012, Blois and others 2013). DEA 1.1 is expressed in approximately 40–60 per cent of the general canine population. However, marked differences in DEA 1.1 frequencies among breeds and geographic locations have been reported (Novais and others 1999, van der Merwe and others 2002, Nottidge and others 2006, Graemer and others 2007, Hale and others 2008, Zubic and others 2008, Jazbik and others 2010, Ergul Ekviz and others 2011, Ferreira and others 2011).

The galgo (Spanish greyhound) is a popular breed in Spain. Galgos are mainly used in sports and hunting, but their importance as companion pets has increased in recent years. Due to their physical features: large breed, readily accessible jugular vein, and good temperament, allowing the blood collection without sedation, galgos are frequently used as blood donors in clinical practice. However, despite the clinical importance of the DEA 1.1 and the increased selection of galgos as blood donors, to the authors’ knowledge, there is no published data of the prevalence of DEA 1.1 in this breed. Interestingly, a recent study

10.1136/vr.102087 | Veterinary Record | 1 of 3

Accepted February 3, 2014

doi: 10.1136/vr.102087
in greyhounds, which are closely related to the galgo (they are both sightshound in group 10 and section 3 according to the Fédération Cynologique Internationale) reported that only 15.1 per cent of them expressed DEA 1.1 and 52.2 per cent were considered universal donors (negative for DEA 1.1, 1.2, DEA 3, DEA 5, DEA 7, and positive for DEA 4), whereas 39.7 per cent and 37.5 per cent of all other breeds contained were DEA 1.1 positive and universal donors, respectively (Iazbik and others 2010). Genetic studies have demonstrated that sightshound breeds cluster together (Parker and others 2007), therefore, we hypothesised that galgos, similar to greyhounds, would also have lower prevalence of DEA 1.1 when compared with other breeds that are not sightshounds. Therefore, the aims of this study were (1) to identify the prevalence of DEA 1.1 expression in galgos in Spain, (2) to compare this prevalence of DEA 1.1 with a control group of dogs of other breeds commonly used as blood donors in Spain and (3) to estimate the risk of sensitisation in a population of recipients that are not typed or cross-matched, when galgos are used as blood donors.

**Material and methods**

**Animals**

Expression of DEA 1.1 was determined in a total of 206 client-owned dogs (118 galgos and 88 dogs of a wide variety of breeds) being screened as potential blood donors in the animal blood bank of the Veterinary Teaching Hospital at the University of Córdoba (Spain). The following breeds were represented in the control group: golden retriever (18), mixed breed (13), beagle (10), German shepherd dog (9), boxer (7), Alaskan Malamute (5), labrador retriever (4), bernese mountain dog (3), doberman (3), bobtail (3), great Dane (2), and one each of dogo Argentino, German shorthaired pointer, American Staffordshire terrier, pit bull, bull mastiff, shar pei, Belgian shepherd, Spanish mastiff, Newfoundland, English setter and Weimaraner. All dogs included in the study were at least one year of age, weighed over 25 kg (or donated less than 20 ml/kg if they weighed less than 25 kg), were current on vaccinations, and had no prior history of blood-borne infections or received a blood transfusion. All dogs were considered to be healthy on the basis of their clinical histories, physical examination, complete blood count (CBC), and serum biochemistry profile at the time of sample collection. All dogs were negative for five vector-borne diseases (Leishmania infantum, Ehrlichia canis, Babesia burgdorferi, Dirofilaria immitis and Anaplasma phagocytophilum) tested using a commercial ELISA SNAP test (Leishmania Snap and SNAP 4Dx, Idexx Laboratories, Barcelona, Spain). The animals were assessed to have a good temperament and to be cooperative, and no sedation was required for any donor. All dogs were presented for screening as potential blood donors between February 2010 and May 2013. Signed informed owner consent was obtained from the owners of the dogs. This study was conducted according to European legislation (2005/85/EU).

**Blood typing and calculation of risk of sensitisation**

Blood samples for blood-typing were obtained from the jugular or saphenous vein. For each animal, 1 ml of blood was collected into EDTA vacutainer tubes (K3E/EDTA 1 ml tubes, Aquisel SL, Abdera, Spain), and processed immediately. DEA 1.1 expression was determined using an immunochromatographic cartridge typing kit (Quick Test DEA 1.1, Alvedia, Lyon, France) according to the manufacturer’s instructions.

Potential risk of sensitisation after a non-typed blood transfusion using blood from a galgo in a recipient of other breed was calculated by multiplying the frequency of DEA 1.1 negative blood in the control group and the frequency of DEA 1.1 positive blood in galgos (Novais and others 1999, Kessler and others 2010, Ferreira and others 2011). The data were analysed using the statistical software SPSS V.15.0. The Pearson’s χ² test was used for statistical comparison of DEA 1.1 frequencies among groups. Statistical significance was accepted at P<0.05.

**Results**

Two hundred and six dogs were screened as potential donors over the enrolment period. Overall, 53.4 per cent (110/206) were positive for DEA 1.1, and 46.6 per cent (96/206) were negative for DEA 1.1. The prevalence of DEA 1.1 positive blood among our population of galgos and other-breed dogs was 51.7 per cent (61/118) and 55.7 per cent (49/88), respectively. The prevalence of DEA 1.1 negative blood among this population of galgos and other breeds was 48.3 per cent (57/118) and 44.3 per cent (39/88), respectively. There was no significant difference between the groups with respect to DEA 1.1 (P=0.57). Potential risk of sensitisation in a recipient of other breed after a non-typed blood transfusion using blood from a galgo was 22.9 per cent.

**Discussion**

Although transfusion reactions are rare in clinical practice, the potential for life threatening acute haemolytic reactions in DEA 1.1 negative dogs previously sensitised by being given DEA 1.2 positive donor blood means it is prudent to use DEA 1.1 negative blood for donations to recipients of unknown blood type or which are DEA 1.1 negative (Giger and others 1995, Tocci and Ewing 2009). As discussed in the introduction, the prevalence of the DEA 1.1 blood group varies with breed and this study tested the prevalence in galgos. We found there was no significant difference between the prevalence of the DEA 1.1 blood group the control group composed of dogs of other breeds commonly used as blood donors in Spain (55.7 per cent) and galgos (51.7 per cent). There is a potential risk of 22.9 per cent of sensitisation following the first transfusion if we use galgos as blood donors and blood that is not typed or cross-matched. These probabilities were similar to that reported previously by Novais and others (1999) and Ferreira and others (2011) and to that which might be predicted giving blood from other dogs likely to be used as donors in Spain.

We had hypothesised that galgos might share the low DEA 1.1 prevalence recently reported in greyhounds (Iazbik and others 2010) since the breeds are closely related in the sightshound group (group 10 and section 3 according to the Fédération Cynologique Internationale), but that was not the case. Diverse genetic pools of ancestors for sightshounds and different levels of inbreeding among different geographic locations may explain the difference in the prevalence of DEA 1.1 between galgos and greyhounds. That the DEA 1.1 prevalence is not similar in the two breeds is important because, due to the phenotypic resemblance, there is a risk that the use of non-typed galgos as donors might be perceived as relatively safe practice.

The relatively high proportion of galgos that are DEA 1.1 means blood typing is still prudent before any transfusion, but does not detract from the breed otherwise making ideal blood donors due to their large size, easily accessible jugular vein, and good temperament. Moreover, previous studies have reported that galgos, similar to other sightshounds, have higher haemoglobin, haemoglobin (Hb) concentration, RBC count, oxygen content, and high oxygen-affinity Hb (lower P50), and lower platelet count when compared with dogs of other breeds (Zaldivar-López and others 2011, Mesa-Sánchez and others 2012). A limitation of this study was that only one blood typing method was used, however, there is no currently accepted gold standard for canine blood typing (Giger and others 2005) and previous studies have demonstrated that the cartridge kit (QuicK Test DEA 1.1, Alvedia, Lyon, France) is accurate (Seth and others 2012, Blois and others 2013) and performs well so long as dogs have haemoglobin >40 and do not have immune mediated haemolytic anaemia (Seth and others 2012, Blois and others 2013). Another limitation of this study was that extensive blood typing for multiple DEAs was not performed, however, the blood type of the ideal canine blood donor is not uniformly agreed on among transfusion experts (Hale 1995, Andrews 2006, Tocci and Ewing 2009, Kessler and others 2010). The most restrictive definition of the universal donor would be a dog negative for DEA 1.1, 1.2, DEA 3, DEA 5, DEA 7, and positive for DEA 4 (Hale 1995), whereas some authors consider that the importance of antigens other than DEA 1.1 is unclear, and thus cross-matching and testing for DEA 1.1 is more realistic and practical (Andrews 2006, Tocci and Ewing 2009, Kessler and others 2010). Given that debate, which has been well reviewed (Hale 1995, Hohenhaus 2004, Tocci and Ewing 2009) this study focussed on DEA 1.1 status only since it is widely recognised as the most clinically relevant.

In conclusion, the frequency of DEA 1.1 in galgos is similar to that of other breeds commonly used as donors in Spain, and similar to that reported in the literature for the general canine population, but is considerably higher than the prevalence of DEA 1.1 reported
in greyhounds, despite of they are both shorthair sighthounds. Due to the clinical significance of DEA 1.1 and the high prevalence of this blood group in galgos of Spain, we strongly recommend blood-typing for this group, as with other donors, before their use as blood donors to limit the probability of sensitisation and subsequent life-threatening haemolytic reactions.

References


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Veterinary Record published online February 26, 2014
doi: 10.1136/vr.102087