Original Research

Frequency of dog erythrocyte antigen 1.1 in 4 breeds native to different areas in Turkey

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Key Words
Blood type, DEA, hemolytic reaction, sensitization, transfusion medicine

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Background: Dog erythrocyte antigen (DEA) 1.1 is the most important RBC antigen clinically, as it is highly immunogenic and causes acute hemolytic transfusion reactions (HTR) in sensitized dogs.

Objectives: The aims of this study were to determine the frequency of DEA 1.1 expression in 4 Turkish dog breeds, and to estimate the potential risk of HTR when blood from a DEA 1.1-positive donor is administered to a DEA 1.1-negative recipient following sensitization by a prior mismatched transfusion.

Methods: EDTA blood samples (n = 178) were typed for DEA 1.1 using a commercial gel-column agglutination test (ID-Gel-Test Canine DEA 1.1). Probabilities of sensitization and risk of an HTR were calculated.

Results: The frequency of positivity for DEA 1.1 among Kars (n = 59), Kangal (n = 53), Akbash (n = 50), and Catalburun (n = 16) breeds was 71.2%, 67.9%, 60.0%, and 50.0%, respectively. Potential risk for occurrence of an HTR after administration of blood from a dog of the same breed ranged from 12.5% to 14.8%, whereas HTR induced by blood of a dog from a different breed ranged from 7.2% to 25.3%.

Conclusions: The frequency of DEA 1.1-positive dogs among 4 Turkish breeds is high compared with that of most other breeds previously surveyed. The predicted risk of both sensitization and occurrence of DEA 1.1-related HTR following transfusion between dogs of either the same or different Turkish breeds was considerable. Although few dogs are transfused ≥ 4 days after the first transfusion, we recommend that (1) all donors and recipients be typed for DEA 1.1, (2) DEA 1.1-negative recipients receive only DEA 1.1-negative blood, and (3) blood be cross-matched prior to transfusing any dog ≥ 4 days after the first transfusion. These guidelines are also applicable to other breeds and countries.

Introduction

Blood transfusions are frequently required to support anemic and bleeding animals.¹ ² Although they can be potentially life-saving, transfusions are not without risks due to blood type incompatibility, transmission of infectious diseases, and inadequate quality of blood products.³ ⁴ ⁵ Although dogs lack naturally occurring alloantibodies to disparate blood groups of clinical importance, induced alloantibodies can lead to a life-threatening acute hemolytic transfusion reaction (HTR) in previously sensitized recipients. Dogs may remain permanently sensitized to a blood group,⁶ and the risk of sensitization can be reduced by performing blood-typing and cross-matching procedures, prior to the first transfusion. Similar procedures performed before the second transfusion (≥ 4 days after the first transfusion) would significantly reduce the risk of an HTR, provided that units of compatible blood are administered.⁵ ⁹
Many canine blood groups are classified into dog erythrocyte antigen (DEA) systems. Although more than 20 different canine blood groups have been described thus far, only 7 groups (DEA 1 [1.1 and 1.2], 3, 4, 5, 6, 7, 8) have received international standardization.\(^{10,11}\) Although all canine blood group antigens can stimulate formation of alloantibodies, DEA 1.1 seems to be the most immunogenic and is considered the primary “lytic factor” in canine transfusion medicine.\(^{7,11,12}\) Hence, DEA 1.1-matched transfusions are generally recommended.\(^9\) The absence of naturally occurring alloantibodies to other blood groups\(^5,11\) may preclude an acute incompatibility reaction following the first transfusion of DEA 1.1-positive RBCs to a DEA 1.1-negative recipient\(^5,6\), however, alloantibody production and sensitization of the recipient will occur, potentially resulting in a serious acute HTR and even death of the recipient if subsequent transfusions of DEA 1.1-positive RBCs are administered to the same DEA 1.1-negative recipient.\(^3,6,12\)

The risk for transfusion reactions against antigens other than DEA 1.1 are less well defined, but common antigens like DEA 4 and Dal can elicit an immune reaction in the rarely encountered RBC antigen-negative dog.\(^9,13,15\) An acute HTR against these other RBC antigens, including DEA 4,\(^14\) Dal,\(^15\) and an unknown common RBC antigen,\(^9,13\) has been reported only in dogs previously sensitized by a mismatched transfusion. Furthermore, there is no clinical evidence for an HTR caused by DEA 1.2, 3, 5, and 7 (antibody for DEA 8-typing is no longer available), although it has been suggested that DEA 1.2 and 7 alloantibodies may reduce the lifespan of transfused RBCs.\(^3,11\) Moreover, typing of many canine RBC antigens is based on polyclonal alloantibodies and tube agglutination assays that may require antiglobulin (Coombs’) reagents, and the results are difficult to interpret.\(^9\)

The frequency of DEA 1.1 expression in dogs has been investigated in small studies in which the focus was on determining the frequency in geographically restricted canine populations composed of various breeds.\(^16,17\) However, there may be marked differences in DEA 1.1 frequencies among breeds,\(^18\) and knowledge of breed differences may be useful for efficient recruitment of typed blood donors.\(^19\) Currently, information on the frequency of DEA 1.1 expression among breeds native to Turkey is unavailable, except for that reported for a colony of Kangal dogs.\(^20\) The objectives of the present study were (1) to determine the frequency of DEA 1.1 positivity in Kangal, Akbash, Catalburun, and Kars breeds in Turkey and (2) to estimate the probability of a previously sensitized DEA 1.1-negative dog receiving a second transfusion of DEA 1.1-positive RBCs.

### Materials and Methods

Four canine breeds native to Turkey were evaluated as approved by the Institutional Animal Care and Use Committee at the Veterinary Medicine Faculty of Istanbul University. Blood samples from privately owned dogs were obtained with consent of the owners from 4 different regions of Turkey: (1) Kangal dogs from the Sivas region of central Anatolia, (2) Akbash dogs from western Anatolia, (3) Catalburun dogs from the Tarsus-Mersin region of southeastern Anatolia, and (4) Kars dogs from the Kars region of northeast Anatolia.

Blood was collected from the cephalic or jugular vein into 2-mL EDTA vacutainer tubes (Becton, Dickinson and Company, Plymouth, UK) and shipped to the Animal Blood Bank, Istanbul, Turkey. Blood tubes were stored at 4°C for <1 day before analysis. The DEA 1.1 expression was determined using a commercial gel-column agglutination test (ID-Gel-Test Canine DEA 1.1, DiaMed Microtyping System, Cressier-sur-Morat, Switzerland). The test is based on detecting RBC agglutination in microcolumns containing anti-DEA 1.1 monoclonal antibodies within the gel matrix. Agglutination reactions were graded on a scale of 0 to 4+ with 0 indicating absence of agglutination and 4+ representing the strongest positive agglutination reaction. The typing procedure and interpretation of results were performed, according to the manufacturer’s instructions.\(^9,21,22\)

Based on the observed DEA 1.1 frequencies, the probability of occurrence of an acute HTR after a transfusion between dogs of either the same or different breeds was calculated as described previously.\(^17\) Briefly, to calculate the probability of a DEA 1.1-negative dog receiving DEA 1.1-positive blood during the first transfusion, signifying the potential risk of sensitization, the DEA 1.1-positive and DEA 1.1-negative frequencies were multiplied. To estimate the potential risk of an acute HTR, this product was then multiplied by the frequency of DEA 1.1-positive dogs to estimate the probability of a previously sensitized DEA 1.1-negative dog receiving DEA 1.1-positive blood, during the second transfusion.

### Statistical analysis

The Pearson’s chi-square test was used for statistical comparison of DEA 1.1-positive and DEA 1.1-negative frequencies among breeds (SPSS for Windows Advanced Statistics Release 10.0, SPSS Inc., Chicago, IL, USA). Statistical significance was set at \( P < .05.\)
Results

A total of 178 dogs from 4 native breeds and regions in Turkey were typed for DEA 1.1 expression, by the gel-column technique (Table 1). Overall 65.2% of dogs were DEA 1.1-positive, and the frequency of DEA 1.1 positivity among the 4 breeds varied between 50.0% and 71.2% with no significant differences ($P = .34$). For samples positive for DEA 1.1, only 3+ and 4+ agglutination reactions were observed in the DEA 1.1 gel columns, and all DEA 1.1-negative dogs showed no (0) agglutination reactions. Autoagglutination reactions were not detected in the control columns of any of the 178 samples tested, and all results were considered valid.

Assuming that blood-typing prior to transfusion is performed very rarely in Turkey and that any DEA-1.1 mismatch results in sensitization of DEA 1.1-negative recipients, the potential risk of sensitization of a DEA 1.1-negative recipient administered blood from a DEA 1.1-positive donor of the same breed during the first transfusion varied between 20.5% and 25.0%. The probability of occurrence of an acute HTR following a second transfusion of blood from a dog of the same breed administered at least 4 days after the first transfusion ranged from 12.5% to 14.8% (Table 2). The probability of sensitizing a DEA 1.1-negative recipient at the time of the first transfusion with blood from a DEA 1.1-positive donor of any of the 4 Turkish breeds evaluated in this study varied between 14.4% and 35.6%. The probability of the same DEA 1.1-negative dog having an HTR following a second transfusion with blood from a DEA 1.1-positive donor ranged from 7.2% to 25.3%.

Discussion

Kangal, Akbash, and Kars dogs are typical Turkish livestock-guarding breeds that together comprise the Turkish Shepherd dogs, whereas the Catalburun dog is the only native pointing breed of Turkey.23 These 4 breeds originated from different regions of Turkey. The Kangal is the dog of central Anatolia, and the Akbash is the pure white dog of western Anatolia. Kars dogs are bred in the northeast part of Turkey, especially in the Kars region,24 and the Catalburun is a breed raised in the Tarsus-Mersin region of Turkey.25 In this study of DEA 1.1 expression, a limited survey of these 4 native Turkish breeds revealed a higher frequency of DEA 1.1 positivity than has been reported for other canine populations, except for dogs in Croatia where the frequency is 84%.6,7,16,17,19,26 The high frequency of DEA 1.1 expression in all 4 Turkish breeds is interesting as these dogs live in different regions in Turkey, near where they originated, and their owners and breeders carefully avoided mating these dogs with other breeds or mixed breed dogs. Furthermore, these unique regional breeds are currently preserved by government-controlled breeding centers and some private farms. Although the number of dogs surveyed from each breed was relatively small, the high frequency is substantiated by a recent report demonstrating similar high frequency (61%) of DEA 1.1-positive Kangal dogs.20 All 4 breeds, which are common to Turkey, fulfill common requirements for blood donors as they are of large size (≥ 23 kg body weight), good-tempered, and easy to handle during blood collection.

Based on a large survey of dogs in the US, it is estimated that 42% of dogs express the DEA 1.1 antigen on their RBCs.10,18 In addition, frequencies lower than those found in our study were reported for 15 breeds in Japan (44%),16 16 different breeds in Brazil (51%),17 33% of the dogs in Pennsylvania,6 and 47% in South Africa.19 It has been suggested that the frequency of DEA 1.1 expression might differ depending on geographic variation and breed,6,16,17,20 as has been shown for domestic shorthair cats.27 However, most studies have surveyed small populations and have included a variety of breeds in different proportions; these factors hamper direct comparisons.6,17,19,26,28

Table 1. Frequency of DEA 1.1 positivity and negativity in Kangal, Akbash, Catalburun, and Kars dogs reared in Turkey and intensity of the agglutination reaction to the DEA 1.1 alloantibody in a gel-column assay.

<table>
<thead>
<tr>
<th>DEA 1.1 Type</th>
<th>All Dogs ($n = 178$)</th>
<th>Kangal ($n = 53$)</th>
<th>Akbash ($n = 50$)</th>
<th>Catalburun ($n = 16$)</th>
<th>Kars ($n = 59$)</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>n = 62</td>
<td>62</td>
<td>20</td>
<td>8</td>
<td>17</td>
<td>28.8</td>
</tr>
<tr>
<td></td>
<td>% 34.8</td>
<td>34.8</td>
<td>40.0</td>
<td>50.0</td>
<td>28.8</td>
<td>3.329*NS</td>
</tr>
<tr>
<td>Positive</td>
<td>n = 116</td>
<td>116</td>
<td>30</td>
<td>8</td>
<td>42</td>
<td>71.2</td>
</tr>
<tr>
<td></td>
<td>% 65.2</td>
<td>65.2</td>
<td>60.0</td>
<td>50.0</td>
<td>71.2</td>
<td>3.329*NS</td>
</tr>
<tr>
<td>3+ Agglutination*</td>
<td>n = 21</td>
<td>21</td>
<td>6</td>
<td>–</td>
<td>10</td>
<td>23.8</td>
</tr>
<tr>
<td></td>
<td>% 18.1</td>
<td>18.1</td>
<td>20.0</td>
<td>–</td>
<td>23.8</td>
<td></td>
</tr>
<tr>
<td>4+ Agglutination*</td>
<td>n = 95</td>
<td>95</td>
<td>24</td>
<td>8</td>
<td>32</td>
<td>76.2</td>
</tr>
<tr>
<td></td>
<td>% 81.9</td>
<td>81.9</td>
<td>80.0</td>
<td>100.0</td>
<td>76.2</td>
<td></td>
</tr>
</tbody>
</table>

*NS indicates percentage between breeds did not differ significantly ($P = .34$).

*1+ or 2+ reactions were not observed.
In limited surveys of specific breeds, DEA 1.1 positivity ranges from 0% to 97% in Collies and Dalmatians, respectively.\(^{16,26}\) Interestingly, with the exception of Collies all breeds examined have both DEA 1.1-positive and negative individuals, despite the relatively narrow gene pool of most breeds. The 4 breeds we evaluated had relatively high frequencies of DEA 1.1 expression similar to Labrador Retrievers (55%), German Shepherds (64%), Istrian Hounds (66%), and Cocker Spaniels (71%).\(^{17,19,26}\) Even higher frequencies have been reported for Rottweilers (78%), Shibas (79%), Croatian Sheepdogs (90%), and Dalmatians (97%).\(^{16,19,26,29}\) In contrast, frequencies of DEA 1.1 positivity reported for Collies (0%), Boxers (13%), Nigerian indigenous dogs (32%), Beagles (39%), and English Setters (43%) were lower than those of the Turkish breeds.\(^{7,16,19,28}\) The number and relationship of animals tested for each breed might affect the frequency of DEA 1.1 expression, but the current surveys at least provide a starting point when considering donor/recipient pairs for transfusion.\(^{16,17}\)

The standard tube-typing assay used for earlier typing surveys was difficult to interpret and resulted in relatively weak agglutination reactions.\(^{21}\) The newer gel column-typing method used in the current study was easy to perform, resulted in strong agglutination reactions, and clearly differentiated between DEA 1.1-positive and negative RBCs.\(^{9,21,22}\) Unfortunately, the column has been discontinued; however, adequate DEA 1.1-typing methods, such as card-typing (DMS RapidVet-H, DMS Laboratories Inc., Flemington, NJ, USA) and cartridge (Quick Test DEA 1.1, Alvedia, Lyon, France) kits, have been established for laboratory and clinical use.\(^{21,22}\) Extended typing for other DEAs and common RBC antigens is complicated and less reliable. As alloantibodies and HTRs are rarely, if ever, associated with these antigens,\(^{5,9}\) it is generally considered unnecessary to test for them.

In practice, DEA 1.1-negative dogs are considered the preferred donors,\(^{6,11}\) and breeds of a large size and low frequency of DEA 1.1 expression are desirable for blood donor selection.\(^{7,26}\) Although Turkish breeds are of appropriate size, the relatively low proportion that are DEA 1.1-negative makes the search for donors difficult to accomplish in Turkey. On the other hand, there is also a smaller proportion of DEA 1.1-negative dogs that will require blood transfusions. Whereas, blood from DEA 1.1-negative dogs can be used for either DEA 1.1-negative or positive recipients, blood from DEA 1.1-positive donors is suitable for use only in DEA 1.1-positive recipients.\(^{5,11}\)

Owing to the similar frequency of DEA 1.1-positive and negative dogs among breeds native to Turkey, the risk for sensitization with the first transfusion and an acute HTR reaction with a second transfusion is similar among Kars, Kangal, Akbash, and Catalburun breeds. As Kars dogs had a higher frequency of DEA 1.1 positivity and Catalburun dogs had a higher frequency of DEA 1.1 negativity, the probability of accidental transfusion of DEA 1.1-positive blood being administered to a DEA 1.1-negative recipient for both the first and second transfusions is lowest when the donor is a Catalburun and the recipient is a Kars dog, and highest when the donor is a Kars dog and the recipient is a Catalburun dog.

Most veterinarians in practice in Turkey lack the typing kits and the expertise to type dogs or do not have access to laboratories that offer blood-typing on an emergency basis. Thus, although they rarely administer blood transfusions, in an emergency situation some veterinarians may need to transfuse a dog that has not been typed.\(^{1,17}\) Therefore, previous identification of potential blood donors that have been appropriately screened and typed in advance as DEA 1.1-negative should be selected as donors for untyped recipients to reduce the potential for a life-threatening HTR following subsequent transfusions.

### Table 2

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Donor</th>
<th>Kangal Transfusion</th>
<th>Akbash Transfusion</th>
<th>Catalburun Transfusion</th>
<th>Kars Transfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First</td>
<td>Second</td>
<td>First</td>
<td>Second</td>
<td>First</td>
</tr>
<tr>
<td>Kangal</td>
<td>21.8</td>
<td>14.8</td>
<td>19.3</td>
<td>11.6</td>
<td>16.0</td>
</tr>
<tr>
<td>Akbash</td>
<td>27.2</td>
<td>18.5</td>
<td>24.0</td>
<td>14.4</td>
<td>20.0</td>
</tr>
<tr>
<td>Catalburun</td>
<td>34.0</td>
<td>23.1</td>
<td>30.0</td>
<td>18.0</td>
<td>25.0</td>
</tr>
<tr>
<td>Kars</td>
<td>19.6</td>
<td>13.3</td>
<td>17.3</td>
<td>10.4</td>
<td>14.4</td>
</tr>
</tbody>
</table>

Numbers are % probability.
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