Case-control study of blood type, breed, sex, and bacteremia in dogs with immune-mediated hemolytic anemia

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Objective—To determine whether blood type, breed, or sex were risk factors for immune-mediated hemolytic anemia (IMHA) in dogs and whether bacteremia was common in dogs with IMHA.

Design—Case-control study.

Animals—33 dogs with IMHA, 1,014 dogs without IMHA for which blood type (dog erythrocyte antigen 7) was known, 15,668 dogs without IMHA for which breed was known, and 15,589 dogs without IMHA for which sex was known.

Procedure—Blood type, breed, and sex distribution of dogs with IMHA were compared with data for control dogs with Fisher exact tests and by calculating odds ratios (ORs). Results of bacterial culture of blood samples were documented for dogs with IMHA, when available.

Results—Dog erythrocyte antigen 7 was associated with a significant protective effect (OR, 0.1) in Cocker Spaniels with IMHA (n = 10), compared with control dogs. Cocker Spaniels, Bichon Frise, Miniature Pinschers, Rough-coated Collies, and Finnish Spitz had a significantly increased risk of IMHA, as did female dogs (OR, 2.1). Blood samples from 12 dogs with IMHA were submitted for bacterial culture, and none had bacteremia.

Conclusions and Clinical Relevance—Results suggest that blood type, breed, and sex may play a role in IMHA in dogs. (J Am Vet Med Assoc 2004;224:232–235)
1997, and July 1, 1998, that did not have IMHA. No dogs were excluded from analyses of breed distributions, but 79 control dogs were excluded from analyses of sex distributions because sex was not recorded.

**Blood-typing**—To prevent interference by transfused cells, results of blood-typing were recorded only if blood samples submitted for typing had been collected prior to administration of a transfusion or ≥3 weeks after the most recent transfusion. For blood-typing, 6 mL of blood was collected and combined with 1 mL of citric acid-trisodium citrate-dextrose solution. All blood samples were tested by a single laboratory for dog erythrocyte antigens (DEA) 1.1, 1.2, 3, 4, 5, and 7 by use of a tube agglutination method that incorporated canine-derived polyclonal antisera. Briefly, RBCs for typing were washed according to standard techniques and resuspended in a 4% solution with phosphate-buffered saline solution. Three polyclonal antisera were used to identify DEA 1.1 and 1.2. Tubes were incubated at 37°C for 15 minutes, and 2+ or greater agglutination was considered a positive reaction. Negative reactions were verified through Coomb’s enhancement. Individual polyclonal antisera recognizing DEA 3, 4, 5, and 7 were used to identify minor RBC antigens. Tubes for these reactions were incubated at 4°C for 30 minutes, and 2+ or greater agglutination was considered a positive reaction.

**Bacterial culture of blood samples**—Blood samples for bacterial culture were collected according to a standard protocol prior to administration of antimicrobials and within 2 hours of initial examination of the dog at the Animal Medical Center.6,7 Three samples were collected within a 3-hour period and submitted for aerobic and anaerobic culture. Blood was collected aseptically from a peripheral vein or from a recently (within 30 minutes) placed indwelling central venous catheter. Indwelling catheter insertion and collection of blood from peripheral veins were preceded by skin disinfection with chlorhexidine and alcohol, and indwelling catheters were placed with sterile technique.

**Statistical analyses**—Proportions of case dogs and the 1,014 control dogs tested by Midwest Animal Blood Services with each blood type were calculated and compared. In addition, proportions of Cocker Spaniels with IMHA with each blood type were compared with proportions of all 1,014 control dogs with each blood type and with proportions of the 15 control Cocker Spaniels with IMHA with each particular blood type were not significantly different from the proportion of control dogs that had that blood type (ie, for all individual blood types, P values were not less than 0.05). Similarly, when results of blood-typing for the 10 Cocker Spaniels with IMHA were compared with results of blood-typing for the 15 control Cocker Spaniels, proportions of Cocker Spaniels with IMHA with each particular blood type were not significantly different from proportions of control Cocker Spaniels. However, when results of blood-typing for the 10 Cocker Spaniels with IMHA were compared with results for the 1,014 control dogs of all breeds, proportions of dogs with DEA 7 were significantly (P = 0.039) different between groups. The OR was 0.1 (95% CI, 0 to 0.9).

Statistical analyses were performed with standard software; P values were calculated by means of Fisher exact tests, with values of P ≤ 0.05 considered significant.

**Results**

Thirty-three dogs with IMHA met the criteria for inclusion in the study (case dogs), of which 10 were Cocker Spaniels. The most common blood type among dogs with IMHA was DEA 4 (Table 1).

When results of blood-typing for all 33 dogs with IMHA were compared with results for the 1,014 control dogs (Table 2), the proportion of dogs with IMHA that had each particular blood type was not significantly different from the proportion of control dogs that had that blood type (ie, for all individual blood types, P values were not less than 0.05). Similarly, when results of blood-typing for the 10 Cocker Spaniels with IMHA were compared with results of blood-typing for the 15 control Cocker Spaniels, proportions of Cocker Spaniels with IMHA with each particular blood type were not significantly different from proportions of control Cocker Spaniels. However, when results of blood-typing for the 10 Cocker Spaniels with IMHA were compared with results for the 1,014 control dogs of all breeds, proportions of dogs with DEA 7 were significantly (P = 0.039) different between groups. The OR was 0.1 (95% CI, 0 to 0.9).

**Table 1—Results of blood group typing for 33 dogs with immune-mediated hemolytic anemia (IMHA)**

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**Table 2—Comparison of proportions of dogs with each individual blood type between groups of dogs with and without IMHA**

<table>
<thead>
<tr>
<th>Groups</th>
<th>1.1</th>
<th>1.2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>7</th>
</tr>
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<tbody>
<tr>
<td>Dogs with IMHA (any breed) vs dogs without IMHA (any breed)</td>
<td>0.270</td>
<td>0.195</td>
<td>&gt;0.999</td>
<td>&gt;0.999</td>
<td>0.519</td>
<td>0.176</td>
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<tr>
<td>Cocker Spaniels with IMHA vs dogs without IMHA</td>
<td>0.508</td>
<td>0.631</td>
<td>&gt;0.999</td>
<td>&gt;0.999</td>
<td>&gt;0.999</td>
<td>0.039*</td>
</tr>
<tr>
<td>Cocker Spaniels with IMHA vs Cocker Spaniels without IMHA</td>
<td>0.099</td>
<td>&gt;0.999</td>
<td>&gt;0.999</td>
<td>&gt;0.999</td>
<td>&gt;0.999</td>
<td>0.250</td>
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Values represent P values obtained by use of Fisher exact tests.
*Odds ratio, 0.1; 95% confidence interval, 0 to 0.9.
Discussion

Results of the present study suggest that blood type, breed, and sex may be associated with development of IMHA in dogs. The absence of DEA 7 was associated with an increased risk of IMHA in Cocker Spaniels, compared with control dogs, and Cocker Spaniels, Bichon Frise, Miniature Pinschers, Rough-coated Collies, and Finnish Spitz had an increased risk of IMHA, as did female dogs. Although DEA 4 was the most common blood type identified in dogs with IMHA, DEA 4 is the most common blood type reported in dogs, with 98% of all dogs being positive for this blood type.

Multiple mechanisms can be proposed to account for an increased risk of IMHA among dogs that lack blood group DEA 7. In particular, the absence of a functional cell membrane protein can result in a functional defect at the cellular level that could result in cell lysis. For example, in humans, the glycoprotein that determines the Kidd blood group is also an important urea transport protein. Humans with the rare homozygous absence of the Kidd glycoprotein are susceptible to urea-induced hemolysis because the cells lack the ability to transport urea across the cell membrane. Alternatively, lack of a specific RBC surface antigen, such as DEA 7, could result in substantial instability in the cell membrane structure. In humans with the RH null syndrome, for instance, an absence of the RH antigens and cell membrane proteins is associated with abnormal cell shape and survival and can result in severe hemolytic anemia, although some affected individuals may only have mild, compensated anemia.

Finally, a lack of DEA 7 may allow expression of a unique autoantigen or modification of the cell membrane such that autoantibody production is stimulated or autoreactive T cells are activated. Antibodies to DEA 7 are present in 20% to 50% of DEA 7-negative dogs, and 1 of these antibodies could be autoreactive. However, until the structure and function of DEA 7 is known, what role, if any, it plays in hemolysis in dogs with IMHA is unknown. Further study is needed to determine whether any of these mechanisms are important in dogs with IMHA.

Breed has long been suspected of being linked to IMHA, and a previous study reported that IMHA occurred more frequently in Cocker Spaniels. The English Springer Spaniel, Poodle, Old English Sheepdog, and Collie breeds have also been associated with increased risk of IMHA in previous studies. Our data show that Cocker Spaniels were 12.2 times as likely to have IMHA as were dogs of other breeds. Bichon Frise, Miniature Pinschers, Rough-coated Collies, and Finnish Spitz also had an increased risk of IMHA in our study; however, because of the low number of dogs of each breed, the 95% CIs were wide, indicating that the study could not provide very precise estimates of the actual increase in risk for these particular breeds.

The present study was designed only to identify breeds that had an increased risk of developing IMHA, as dogs of a breed that have no risk would not have...
been included in the case group. The study population was too small to determine whether the other 139 breeds not represented in the case group had a decreased risk of IMHA or simply did not appear in the study because the population sampled was too small. A larger study population is needed to draw further conclusions regarding breed and risk of IMHA for breeds other than the Cocker Spaniel.

A female sex predilection for IMHA in human and veterinary medicine has long been postulated, even though in most case series of human patients with IMHA, the incidence is approximately equal for males and females. Any increased incidence of IMHA in human females may be attributable to a higher incidence of systemic lupus erythematosus and concurrent associated IMHA. In contrast to the situation in adults, there was a male preponderance in a case series involving children. This may reflect a genetic predisposition in children, whereas IMHA in adults may be acquired or a result of long-term environmental influences. In veterinary medicine, 1 study reported equal male and female representation, and another reported that there were more females than males. Our data strongly support an association between female sex and IMHA. Of the 33 dogs with IMHA, 22 (67%) were female, whereas only 49% of the control dogs were female. Further studies are needed to determine the reason for the increased risk of IMHA among female dogs.

Infectious diseases are known to cause hemolytic anemia by many mechanisms and may be direct or indirect causes of IMHA. Bacteria or 1 of their components may nonspecifically adsorb to the cell membrane and act as a hapten, inducing formation of antibodies against the hapten-membrane complex and leading to hemolysis upon recognition of the altered cell membrane by the immune system. This is similar to the mechanism by which cephalosporin and penicillin are believed to cause IMHA. Other agents that have been reported in association with IMHA in dogs are vaccines and bee sting venom.

The clinical signs of IMHA in dogs may be similar to those expected with bacteremia or septicemia, including neutrophilic leukocytosis with a left shift, monocytosis, and fever. Consequently, bacteremia or septicemia should be considered before treatment with glucocorticoids is instituted for IMHA. However, immediate glucocorticoid treatment may be necessary if the hemolysis is fulminant and results of bacterial culture of blood samples are not available. Also, some systemic infections may resolve more quickly when antimicrobials and glucocorticoids are given concurrently as can occur with ehrlichiosis. In the present study, blood samples were submitted for bacterial culture only from the 12 dogs identified prospectively, and results were negative for all 12. However, bacterial culture of blood samples is a technically challenging procedure with a low yield, so false-negative results are possible. It is also possible that IMHA could have been induced by bacterial infection that had resolved by the time blood samples were collected. Samples would have to be collected from a larger number of dogs before conclusions can be drawn regarding the usefulness of bacterial culture of blood samples from dogs with IMHA.

References