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

Sybille A. Miller, DVM, DACVIM; Ann E. Hohenhaus, DVM, DACVIM; Anne S. Hale, DVM

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 antigens 1.1, 1.2, 3, 4, 5, and 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)

Immune-mediated hemolytic anemia (IMHA) is a common and important cause of hemolytic anemia in dogs. Hemolysis and anemia usually occur rapidly, and the outcome can be catastrophic even with early recognition and treatment.[1,2] However, little is known about the causes of IMHA in dogs.[3] Consequently, measures to promote early recognition and prevention and improve treatment are needed.

The purpose of the study reported here was to determine whether blood type, breed, or sex were risk factors for IMHA in dogs. Once identified, risk factors could be used as screening criteria to modify the prevalence of IMHA or increase the probability of diagnosis in affected dogs. For example, a breeder may attempt to avoid breeding dogs with a specific blood type if it was associated with an increased risk for IMHA. Or, an increased awareness of breed or sex predisposition may lead to a higher index of suspicion for IMHA by clinicians. In humans, bacteremia has been shown to modify RBC surface antigens, leading to antibody binding and ultimately hemolysis.[4] To our knowledge, however, bacteremia in association with IMHA in dogs has not been investigated. Therefore, an additional objective of our study was to determine whether dogs with IMHA had concurrent bacteremia.

From The Department of Medicine, The Bobst Hospital, The Animal Medical Center, 510 E 62nd St, New York, NY 10021-8302 (Miller, Hohenhaus); and Midwest Animal Blood Services Inc, PO Box 626, 120 E Main St, Ste 1, Stockbridge, MI 49285 (Hale). Dr. Miller's present address is The Marlboro Animal Hospital, 441 Lakeside Ave, Marlboro, MA 01752.

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Address correspondence to Dr. Hohenhaus.

Materials and Methods

Case selection—All dogs examined at the Animal Medical Center between July 1, 1995, and July 1, 1999, in which a diagnosis of IMHA had been made were considered for inclusion in the study. Case dogs were identified retrospectively by examining the case logs of the Internal Medicine Service. Owners of dogs identified retrospectively were contacted by letter or telephone to schedule a physical examination and blood-typing. Dogs were identified prospectively as they were admitted to the hospital with clinical signs consistent with IMHA and included in the study if the diagnosis was confirmed. Dogs were considered to have IMHA if they were anemic (PCV < 30%), had laboratory evidence of hemolysis (ie, hemoglobinemia, hemoglobinuria, and spherocytosis), and had evidence of antibody directed against RBCs (ie, persistent RBC autoagglutination or positive Coomb's test results). Dogs in which a diagnosis of IMHA had been made were included in the study only if results of a concurrent CBC, serum biochemistry panel, and test of RBCs for autoagglutination or a Coomb's test were available.

Dogs were excluded from the study if blood-typing was not performed prior to transfusion or the dog was unavailable for blood-typing ≥ 3 weeks after the most recent transfusion, if an underlying disease process was identified during recheck examinations, if the medical record was incomplete, or if an underlying cause for the hemolytic anemia was identified (eg, neoplasia, uremia, toxicosis, ehrlichiosis, babesiosis, or other infection). Information on results of bacterial culture of blood samples was obtained for dogs included in the study, but dogs were not excluded from the study if blood samples had not been submitted for bacterial culture.

Control selection—For analysis of blood type as a risk factor for IMHA, the control population consisted of 1,014 dogs without IMHA for which samples had been submitted to Midwest Animal Blood Services for blood-typing. In addition, 15 overtly healthy Cocker Spaniels that underwent routine physical examination and blood-typing at the Animal Medical Center between January 1, 1998 and January 1, 1999, were included as control dogs for comparison with Cocker Spaniels with IMHA.

For analysis of breed and sex as risk factors for IMHA, the control population consisted of all dogs (n = 15,668) evaluated at the Animal Medical Center between July 1,

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,[5] 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 overtly healthy Cocker Spaniels with each blood type. *P* values, **odds ratios (ORs)** and their 95% **confidence intervals (CIs)** were calculated for each comparison made.

Sex and breed distributions of dogs with IMHA were compared with sex and breed distributions of the control dogs examined at the Animal Medical Center. For analysis of sex distributions, dogs were classified as sexually intact females, sexually intact males, spayed females, or castrated males. For each comparison, the *P* value, OR, and 95% CI of the OR were calculated.

Statistical analyses were performed with standard software[a]; *P* values were calculated by means of Fisher exact tests, with values of $P \leq 0.05$ 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

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

Breed	No. of dogs	Dog erythrocyte antigen type					
		1.1	1.2	3	4	5	7
Bichon Frise	3	2	0	0	3	1	3
Cairn Terrier	1	0	0	0	1	0	0
Cocker Spaniel	10	2	2	1	10	1	0
Finnish Spitz	1	0	1	0	1	1	1
Golden Retriever	2	1	0	0	2	0	0
Greyhound	1	0	0	0	1	0	0
Jack Russell Terrier	1	1	0	0	1	0	0
Labrador Retriever	1	1	0	0	1	0	1
Miniature Pinscher	2	2	0	0	2	0	0
Mixed	3	2	0	0	3	0	0
Pomeranian	1	0	1	0	1	0	0
Pug	1	1	0	0	1	0	0
Rottweiler	2	1	1	0	2	0	0
Rough-coated Collie	1	1	0	0	1	0	1
Shetland Sheepdog	1	0	1	0	1	0	0
Shih Tzu	1	0	1	0	0	0	0
Toy Poodle	1	1	0	0	1	1	0
Total	**33**	**15**	**7**	**1**	**32**	**4**	**6**

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

Groups	Dog erythrocyte antigen type					
	1.1	1.2	3	4	5	7
Dogs with IMHA (any breed) vs dogs without IMHA (any breed)	0.270	0.195	0.722	> 0.999	0.519	0.176
Cocker Spaniels with IMHA vs dogs without IMHA	0.508	0.631	> 0.999	> 0.999	> 0.999	0.039*
Cocker Spaniels with IMHA vs Cocker Spaniels without IMHA	0.099	> 0.999	> 0.999	> 0.999	>0.999	0.250

Values represent *P* values obtained by use of Fisher exact tests.
*Odds ratio, 0.1; 95% confidence interval, 0 to 0.9.

Table 3—Breed distribution of 33 dogs with (case dogs) and 15,668 without IMHA (control dogs)

Breed	No. (%) of case dogs	No. (%) of control dogs	*P* value	OR	95% CI
Bichon Frise	3 (9)	292 (2)	0.024	5.3*	1.2–22.5
Cairn Terrier	1 (3)	92 (1)	0.178	5.3	NC
Cocker Spaniel	10 (30)	541 (3)	< 0.001	12.2	4.5–33.1
Finnish Spitz	1 (3)	7 (< 1)	0.017	69.9*	2.1–2,287
Golden Retriever	2 (6)	572 (4)	0.341	1.7	NC
Greyhound	1 (3)	44 (< 1)	0.091	11.1	NC
Jack Russell Terrier	1 (3)	174 (1)	0.309	2.8	NC
Labrador Retriever	1 (3)	811 (5)	> 0.999	0.6	NC
Miniature Pinscher	2 (6)	136 (1)	0.034	7.4*	1.2–47.1
Mixed	3 (9)	3,487 (22)	0.091	0.3	NC
Pomeranian	1 (3)	198 (1)	0.344	2.4	NC
Pug	1 (3)	193 (1)	0.337	2.5	NC
Rottweiler	2 (6)	532 (3)	0.310	1.8	NC
Rough-coated Collie	1 (3)	22 (< 1)	0.047	22.2*	1.0–473
Shetland Sheepdog	1 (3)	101 (1)	0.194	4.8	NC
Shih Tzu	1 (3)	500 (3)	> 0.999	0.9	NC
Toy Poodle	1 (3)	191 (1)	0.334	2.5	NC
Total	**33 (100)**	**15,668 (100)**	**NA**	**NA**	**NA**

*Number of dogs of this breed with IMHA may be too low to accurately assess the risk of IMHA.
NC = Not calculated because OR was not significantly different from 1. NA = Not applicable.

Table 4—Sex distribution of 33 dogs with (case dogs) and 15,589 dogs without IMHA (control dogs)

Sex	No. (%) of case dogs	No. (%) of control dogs	*P* value	OR
All females	22 (67%)	7,652 (49%)	0.032	2.1*
Sexually intact females	8 (24%)	3,020 (19%)	0.302	1.3
Spayed females	14 (42%)	4,632 (30%)	0.083	1.7
All males	11 (33%)	7,937 (51%)	0.987	0.5
Sexually intact males	3 (9%)	4,368 (28%)	0.998	0.3
Castrated males	8 (24%)	3,569 (23%)	0.494	1.1
Total	**33 (100%)**	**15,589 (100%)**	**NA**	**NA**

*95% confidence interval, 1.1 to 4.1.
NA = Not applicable.

0.9), suggesting a decreased risk for IMHA in Cocker Spaniels that were positive for DEA 7 (ie, suggesting that DEA 7 may have had a protective effect on risk of IMHA in Cocker Spaniels).

The 33 dogs with IMHA consisted of 30 purebred dogs representing 16 breeds and 3 mixed-breed dogs, and the 15,668 control dogs examined at the Animal Medical Center represented 176 breeds. Proportions of Cocker Spaniels, Bichon Frise, Miniature Pinschers, Rough-coated Collies, and Finnish Spitz were significantly different between the case and control groups (**Table 3**), with dogs of these breeds having a significantly increased risk of IMHA.

Proportions of sexually intact females, spayed females, sexually intact males, and castrated males were not significantly different between the case group of 33 dogs with IMHA and the control group of 15,589 dogs examined at the Animal Medical Center (**Table 4**). However, the risk of IMHA was significantly (P = 0.032) increased for female dogs (sexually intact and spayed; OR, 2.1; 95% CI, 1.1 to 4.1).

Blood samples from 12 of the dogs with IMHA were submitted for aerobic and anaerobic bacterial culture. Results of bacterial culture indicated that none of these dogs had bacteremia.

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.[8]

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.[9] 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.[9] 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,[8] 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[3] 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.[3,10,11] 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 159 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.[12] 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.[12] 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[3] reported equal male and female representation, and another[13] 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[14] and penicillin[15] are believed to cause IMHA. Other agents that have been reported in association with IMHA in dogs are vaccines[16] and bee sting venom.[17]

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.[18] 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[19] with a low yield,[20] 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.

[a]StatView for Windows, version 5, SAS Institute Inc, Cary, NC.

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