Evaluation of prognostic factors, survival rates, and treatment protocols for immune-mediated hemolytic anemia in dogs: 151 cases (1993–2002)

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Objective—To evaluate prognostic factors, survival, and treatment protocols for immune-mediated hemolytic anemia (IMHA) in dogs.

Design—Retrospective study.

Animals—151 dogs with IMHA not associated with underlying infectious or neoplastic disease.

Procedure—Information recorded from review of medical records included signalment at the time of initial evaluation; vaccination history; 30-, 60-, and 365-day follow-up outcomes; laboratory data; results of imaging studies; and necropsy findings. Dogs were grouped according to the presence of spherocytes, autoagglutination, a regenerative erythrocyte response, and treatments received (azathioprine, azathioprine plus ultralow-dose aspirin, azathioprine plus mixed–molecular-weight heparin [mHEP], or azathioprine plus ultralow-dose aspirin plus mHEP) for comparisons. All dogs received glucocorticoids.

Results—Cocker Spaniels, Miniature Schnauzers, neutered dogs, and female dogs were overrepresented. Alterations in certain clinicopathologic variables were associated with increased mortality rate. Rates of survival following treatment with azathioprine, azathioprine plus ultralow-dose aspirin, azathioprine plus mHEP, and azathioprine plus ultralow-dose aspirin plus mHEP were 74%, 88%, 23%, and 70%, respectively, at hospital discharge; 57%, 82%, 17%, and 67%, respectively, at 30 days; and 45%, 69%, 17%, and 64%, respectively, at 1 year. In comparison, mean survival rates at discharge and at 30 days and 1 year after evaluation collated from 7 published reviews of canine IMHA were 57%, 58%, and 34%, respectively.

Conclusions and Clinical Relevance—Treatment with a combination of glucocorticoids, azathioprine, and ultralow-dose aspirin significantly improved short- and long-term survival in dogs with IMHA. (*J Am Vet Med Assoc* 2005;226:1869–1880)

Immune-mediated hemolytic anemia (IMHA) in dogs is a frustrating clinical problem because of high complication and mortality rates, expense, and the paucity of standardized immunosuppressive and anticoagulant treatment protocols. In some reports,[1-4] it is suggested that severe anemia, absence of erythrocyte regeneration, autoagglutination, thrombocytopenia, high serum activity of **alkaline phosphatase [ALP]**, high serum concentration of **total bilirubin (TB)**, and low serum concentration of albumin are associated with poor prognosis for survival. Certain treatments, such as administration of bovine **hemoglobin-based oxygen carriers (HBOCs)** and cyclophosphamide, may adversely affect survival.[5] Although data are available regarding short-term complication and mortality rates,[1-8] published information[3,9] pertaining to long-term survival in dogs with IMHA is limited.

A variety of immunosuppressive drugs have been used to treat IMHA in dogs. Although treatment with glucocorticoid drugs remains the mainstay of management of this disease, augmentation of glucocorticoid monotherapy with other immunosuppressive agents has been reported[3,9-14] to improve acute and long-term control of the disease.[3,9-14] Pharmaceutical agents used in combination with glucocorticoids include azathioprine, cyclophosphamide, cyclosporine, mycophenolate mofetil, leflunomide, danazol, and high-dose human immunoglobulin. Although results of other retrospective studies[3,9] have suggested that addition of azathioprine to a treatment protocol is associated with improved patient response and survival, the long-term outcome has been determined in only a small number of dogs.

Development of thromboembolism is an important complication in dogs with IMHA.[4,15,16] Alterations in coagulability, endothelial integrity, and blood flow are likely contributing factors. Anticoagulation treatments specifically devised for dogs with IMHA have not been developed.[17] Although long-term administration of variable doses of aspirin has been used in veterinary patients to reduce the risk of embolic complications in cardiac and pulmonary disease,[18,19] the therapeutic value of aspirin in dogs with IMHA has not been considered. Results of many clinical studies[20-24] in humans suggest that treatment with aspirin, alone or in combination with other anticoagulants, reduces the risk of acute thromboembolism in several disease conditions.

We hypothesized that ultralow-dose aspirin (0.5 mg/kg/d [0.23 mg/lb/d]) provides effective thromboprophylaxis in dogs with IMHA on the basis of results of a pilot study[a] of whole blood platelet impedance aggregometry in clinically normal dogs that was conducted in our laboratory 15 years ago. In 10 adult dogs with platelet counts within reference range, aspirin was administered at a dosage of 0.5 mg/kg orally once daily for 2 days. A significant reduction in platelet aggregation was confirmed 24 hours after the last aspirin dose.

The study protocol was repeated at the conclusion of the retrospective study reported here in 11 healthy adult dogs with whole blood platelet counts within reference range. Once again, a significant reduction in platelet aggregation was confirmed 24 hours after the last aspirin dose.

The objectives of the study reported here were to determine historical, physical, and clinicopathologic features in dogs with this disease and evaluate differences in outcome among treatment groups.

Criteria for Selection of Cases

Medical records of the Cornell University Hospital for Animals from 1993 to 2002 were searched to identify dogs for which a diagnosis of IMHA had been made. The search yielded 186 cases. Study inclusion criteria included PCV ≤ 35% associated with either positive results of a slide agglutination test, positive results of a direct Coombs' test or anti-erythrocyte antibody test, or evidence of hemolysis (ie, hyperbilirubinemia, hemoglobinemia, or hemoglobinuria) or spherocytosis. Records were excluded (n = 35) if an underlying disease process was identified or if results were positive for serologic tests for ehrlichiosis, borreliosis, leptospirosis, or dirofilariasis.

Procedures

Data collected from medical records at the time of initial evaluation included body weight; age; sex; breed; rectal temperature; heart rate; respiratory rate; date of examination; vaccination history and date relative to onset of IMHA; treatments; 30-, 60-, and 365-day follow-up outcomes; necropsy findings; and laboratory data (ie, hematology, coagulation tests, serum biochemical analyses, urinalysis, and immunologic tests). Imaging reports (radiographic and ultrasonographic examinations of the thorax and abdomen) were reviewed for abnormalities consistent with thromboembolism.

A control hospital population was derived by reviewing medical records from 3 representative years (1993, 1997, and 2001) to determine whether the IMHA-affected dog population was unique in age, sex, or breed association. The season of disease occurrence was defined as winter (January to March), spring (April to June), summer (July to September), or fall (October to December). Treatment information included number and type of transfusions (ie, whole blood, packed RBCs, fresh frozen plasma, or HBOCs[b]), number of IV catheters placed, immunosuppressive drugs used (ie, glucocorticoids, azathioprine, cyclosporine, cyclophosphamide, vincristine, leflunomide, IV human immunoglobulin, or danazol), and anticoagulant treatments (aspirin or **mixed–molecular-weight heparin [mHEP]**). Follow-up information was recorded by review of medical records or contact with the referring veterinarian or pet owner and included length of time of survival from initial examination, length of time to disease relapse, and cause of death. Euthanasia or death was considered related to IMHA if it occurred during the initial period of hospitalization (failure to respond to treatment), if it was associated with complications of IMHA (development of thromboembolism before or after discharge from the hospital), or if it occurred as a complication of treatment (medication adverse effects). Necropsy reports were reviewed for evidence of a primary disease process and thromboembolism.

Hematologic variables evaluated included PCV; mean corpuscular volume; spherocytosis; autoagglutination; erythrocyte regeneration (ie, a corrected reticulocyte percentage > 1); counts of mature neutrophils, band neutrophils, and platelets; and plasma color. All blood counts had been determined by use of automated techniques,[c] and blood smears had been manually examined to detect spherocytes. An in-saline autoagglutination test was performed by diluting blood with saline (0.9% NaCl) solution to a concentration meeting or exceeding a 1:4 dilution; cells were not repeatedly washed before this assessment. Reticulocyte percentage was determined from new methylene blue-stained blood smears examined by use of oil-immersion light microscopy with the aid of a Miller disk ocular.[d] Bone marrow aspirates were performed in dogs with nonregenerative anemia and in some dogs to exclude lymphoma as an underlying diagnosis, and these results were recorded. Coagulation variables recorded included **activated partial thromboplastin time** (**APTT**); **prothrombin time** (**PT**); and plasma concentrations of fibrinogen, fibrinogen degradation products, and D-dimers. The coagulation tests were performed by use of commercial kits[e-g] and end point determination with a fibrometer.[h] Pertinent serum biochemical analyses included concentrations of total protein, albumin, potassium, TB, bicarbonate, and creatinine and activities of **alanine aminotransferase** (**ALT**), ALP, and **creatine kinase** (**CK**). Serum biochemical analyses were performed via automated systems.[i] Urinalysis included determination of specific gravity and routine dipstick and sediment evaluations. Recorded immunologic information included the results of direct Coombs', anti-erythrocyte antibody, and **antinuclear antibody** (**ANA**) tests. Direct Coombs' testing was performed by use of polyvalent antisera (IgG, IgM, and C3)[j] with a rabbit-origin, anti-dog labeling antibody in microtiter plates at 37°C. Direct immunofluorescent flow cytometry for detection of RBC surface immunoglobulins was performed in 3 dogs by use of goat anti-canine fluorescein isothiocyanate-conjugated antibodies against IgG (heavy- and light-chain specific), IgM (μ-chain specific), and IgA (α-chain specific), as previously described.[25] Antinuclear antibody tests were completed with sections of rat liver that reacted with serial dilutions of the patient's sera; after incubation and duplicate washing with phosphate-buffered saline solution, fluorescein-labeled rabbit-origin anti-dog IgG[k] was applied and incubated. A positive control, phosphate-buffered saline solution negative control, and negative serum control were simultaneously evaluated. Results of an ANA test were considered positive if the serum titer was ≥ 1:80.

Statistical analyses—Histograms and box-and-whisker plots were used to evaluate data distribution. For most physical and clinicopathologic variables, nongaussian distributions required application of nonparametric statistical analyses; these values were

expressed as median value and range. χ^2 Goodness-of-fit tests or 2 × 2 tables were used to determine differences between dogs with IMHA and control dogs for age, sex, and breed distributions; seasonal occurrence of IMHA; seasonal association with autoagglutination; and the relationship between autoagglutination and survival. Descriptive statistics for these relationships were expressed as mean ± SD.

Dogs were grouped for evaluation of diagnostic and treatment variables (spherocytes, agglutination, regenerative erythroid response, and treatments) versus disease outcome (survival vs nonsurvival). Treatments were recorded as the following: azathioprine, azathioprine plus ultralow-dose aspirin, azathioprine plus mHEP, and azathioprine plus ultralow-dose aspirin plus mHEP. Survival intervals were expressed as days after arrival at the hospital. Dogs lost to follow-up within the discrete intervals of 30 days (n = 11), 60 days (13), and during the full course of the study (17) were excluded from that and subsequent survival analyses. Because only 8 dogs had not received azathioprine and only 4 of these survived beyond hospital discharge, response of these dogs was not considered beyond their contribution to the overall survival of all study dogs with IMHA.

Significant differences between groups (survival vs nonsurvival, spherocytes vs no spherocytes, autoagglutination vs no autoagglutination, regenerative anemia vs nonregenerative anemia, azathioprine plus ultralow-dose aspirin vs azathioprine, azathioprine plus mHEP vs azathioprine, azathioprine plus ultralow-dose aspirin plus mHEP vs azathioprine, azathioprine plus ultralow-dose aspirin vs azathioprine plus mHEP, azathioprine plus ultralow-dose aspirin vs azathioprine plus ultralow-dose aspirin plus mHEP, and azathioprine plus ultralow-dose aspirin plus mHEP vs azathioprine plus mHEP) in clinicopathologic features or survival at each interval were tested by use of the Wilcoxon signed rank test. An association between death and the number of IV catheter placements was evaluated by use of the Wilcoxon rank sum test. Kaplan-Meier survival curves were used to depict differences in survival of dogs grouped according to certain clinicopathologic variables and treatments. Cutoff values for the hematologic and biochemical variables examined (band neutrophil count ≥ 3,000 cells/µL; platelet count < 150,000 platelets/µL; serum concentrations of TB > 1.5 mg/dL; albumin < 3.0 g/dL; potassium < 3.5 mEq/L; and serum activities of CK > 250 U/L, and ALT twice the upper limit of the reference range) were determined after review of descriptive statistics for survival category at the time of hospital discharge. Significant differences were determined by use of the Gehan-Wilcoxon test (weighting early survival more heavily than later survival) and the log-rank test (weighting all deaths equally irrespective of time of death). Comparison of survival rates with survival data reported in other retrospective studies[1-3,5-7,9] of IMHA in dogs was accomplished by use of goodness-of-fit tests (2 × 2 tables). Retrospective studies selected for comparison met the following criteria: the report was published in a peer-reviewed journal, the study dogs were derived from a single clinical facility, case number

exceeded 10, and there were clearly defined survival outcome analyses (eg, days of follow-up, death loss, or loss to follow-up unambiguously defined) and selection criteria for inclusion in the study. Survival outcomes from those studies were divided into 3 categories (survival to time of hospital discharge [n = 177 dogs],[3,5,6] survival at 30 days [201],[1-3,7,9] and survival at 365 days [83][3,9]) for comparison with outcomes in the present study. For calculation of survival rates in the present study, dogs lost to follow-up at each time interval were excluded from that and subsequent survival analyses; similar adjustments could not consistently be determined for the other studies. A 2-tailed P test was used for nonparametric analyses; a value of $P < 0.05$ was considered significant for all comparisons. All statistical analyses were performed by use of data-analysis software.[1]

Results

Although the age of dogs with IMHA (mean ± SD, 6.6 ± 2.9 years) was similar to that (6.4 ± 3.9 years) of the control dogs, the distribution of age within the IMHA population was significantly ($P = 0.008$) different. Compared with control dogs, significantly fewer dogs with IMHA were younger than 2 years of age and more dogs were 6 years of age. A significantly greater proportion of neutered male and female dogs (129/151 [85%]; $P = 0.006$), neutered and sexually intact female dogs (96/151 [64%]; $P = 0.02$), and neutered female dogs (86/151 [57%]; $P = 0.001$) had IMHA, compared with corresponding control dogs (10,038/13,266 [76%], 6,814/13,266 [51%], and 5,492/13,266 [41%], respectively). A significantly lower proportion of sexually intact male dogs (12/151 dogs [8%]; $P = 0.03$) was in the affected group, compared with the control group (1,907/13,266 [14%]). The Cocker Spaniel breed was overrepresented among affected dogs, compared with control dogs (32/151 [21%] vs 534/13,266 [4%]; $P < 0.001$). Although the Cocker Spaniel breed had a predilection for IMHA in this study, the Kaplan-Meier survival curves for those dogs versus all affected dogs were identical up to 1,200 days. Among other breeds with ≥ 4 dogs represented (Labrador Retriever [n = 7], Rottweiler [6], Miniature Schnauzer [6], and Shih Tzu [4]), only the Miniature Schnauzer breed was significantly overrepresented, compared with the control group (6/151 [4%] vs 127/13,266 dogs [1%], respectively; $P < 0.001$). Significantly ($P = 0.04$) fewer cases were detected during the fall (29/151), compared with the spring (44/151), and significantly ($P = 0.02$) more cases were detected during the warm months (April through September; 86/151 [57%]), compared with the cold months (October through March; 65/151 [43%]). The frequency of a positive autoagglutination test was not significantly ($P = 0.1$) different between dogs detected with IMHA during warm versus cold months. A vaccination history was available for 76 of 151 (50%) dogs with IMHA. Twenty-two percent (17/76) of those dogs had been vaccinated in the 30 days preceding evaluation, whereas 30% (23/76) had been vaccinated in the preceding 60 days. The vaccination history could not be determined for all of the control dogs, and type of immunization given to some dogs with IMHA

could not be obtained from the medical record, via querying the client, or via communication with the referring veterinarian. Most dogs with IMHA received treatment with doxycycline during the initial period of hospitalization, while results of serologic examinations for agents of tick-borne diseases were pending.

Transfusions (ie, whole blood, packed RBCs, fresh frozen plasma, or HBOCs) were provided to 106 of 151 (70%) dogs, 80% of which received 1 or 2 transfusions (range, 1 to 8). Twenty-one percent of transfused dogs received HBOCs. Glucocorticoids were given to all dogs within hours of evaluation; orally administered prednisone was the most commonly prescribed treatment (> 98% of dogs). Ninety-five percent (143/151) of study dogs also received orally administered azathioprine within 24 hours of the diagnosis of IMHA; a loading dose of 1.4 to 2.2 mg/kg/d (0.6 to 1.0 mg/lb/d) was given for 4 to 7 days, followed by long-term administration every other day. Other immunosuppressive agents administered to dogs included cyclosporine A (7/151 dogs), cyclophosphamide (7/151), vincristine (3/151), danazol (2/151), leflunomide (1/151), and IV human immunoglobulin (1/151). Ultralow-dose aspirin (0.5 mg/kg/d) was given orally to 106 of 151 (70%) dogs within the first 24 hours of diagnosis of IMHA. All dogs that received aspirin were administered this ultralow-dose. Mixed–molecular-weight heparin (75 to 125 U/kg [34 to 57 U/lb], SC, q 6 to 8 h) was given to 41 of 151 (27%) dogs during the initial week of hospitalization and only while hospitalized; treatment was tapered and discontinued before discharge from the hospital. Treatment groups included dogs that received azathioprine (n = 27), azathioprine plus ultralow-dose aspirin (76), azathioprine plus mHEP (13), and azathioprine plus ultralow-dose aspirin plus mHEP (27).

Median length of hospitalization for all dogs with IMHA was 6 days (range, 1 to 19). Dogs that survived to discharge had a significantly ($P < 0.001$) longer period of hospitalization (median, 6 days), compared with dogs that were euthanatized or died (median, 4 days). The number of IV catheter placements per hospital stay was not significantly associated with death ($P = 0.67$). There were no significant differences in median pulse rate, rectal temperature, or body weight between dogs with IMHA that survived to the time of hospital discharge and those that died or were euthanatized. However, dogs with IMHA that survived to be discharged from the hospital were significantly ($P = 0.05$) younger (median age, 6 years) and less tachypneic (median respiratory rate, 40 breaths/min) than dogs that died or were euthanatized (median age, 8 years; median respiratory rate, 60 breaths/min).

Median PCV of dogs with IMHA at the time of initial examination was 15% (range, 4% to 35%). There was no association between degree of anemia and mortality rate. However, dogs that survived to hospital discharge had significantly lower mature and band neutrophil counts; higher platelet count; shorter APTT and PT; higher serum concentrations of total protein, albumin, potassium, and bicarbonate; lower serum concentrations of TB; and lower serum activities of ALT and CK, compared with dogs that died or were euthanatized (**Tables 1 and 2**).

Spherocytosis was detected in 134 of 151 (89%), autoagglutination in 118 of 151 (78%), and a regenerative erythroid response in 107 of 151 (71%) dogs. The result of a direct Coombs' test was positive in 58 of 75 (77%) dogs tested (dogs with overt autoagglutination were not tested); a small number of dogs (n = 10) had nonspherocytic, nonagglutinating, Coombs'-positive IMHA. The ANA titer was ≥ 1:80 in 39 of 101 (39%) tested dogs. Cytologic analysis of a bone marrow aspirate revealed nonregenerative marrow or ineffective erythropoiesis in 23 of 45 dogs that underwent the procedure. There were significant differences in median values of physical features and certain laboratory test results between dogs with spherocytes, autoagglutination, or a regenerative erythroid response and dogs that lacked those hematologic changes at the time of initial examination (**Table 3**). Dogs with spherocytes and autoagglutination had significantly lower PCV, higher band neutrophil count, longer APTT, lower serum potassium concentration, higher serum TB concentration, and higher serum activity of CK, compared with dogs without these changes ($P ≤ 0.05$).

Differences among treatment groups in median values of physical features and clinicopathologic vari-

Table 1—Laboratory variables in dogs with immune-mediated hemolytic anemia (IMHA) that died (or were euthanatized) or survived to the time of discharge from the hospital.

Survived or died	PCV (%)	MCV (fL)	Neut (×10³/μL)	Band (×10³/μL)	Platelet (×10³/μL)	APTT (s)	PT (s)	Fibrinogen (mg/dL)	D-dimer (ng/mL)
Survived (n =114)									
No. tested	114	114	114	114	114	85	85	75	23
Median	16	74	19.8	1.1	200	15	7	577	500–1,000
(range)	(7–35)	(31–126)	(3.4–83.9)	(0–23.5)	(4–1,200)	(10–25)	(5–21)	(160–1,938)	(< 250 – > 1,000)
Died (37)									
No. tested	37	37	37	37	37	30	30	25	11
Median	15	78	24.6	3.5	110	19	12	612	500–1,000
(range)	(4–32)	(63–105)	(4.5–71.7)	(0.1–13)	(4–338)	(10–41)	(6–26)	(52–1,399)	(< 250–1,000)
P values	0.46	0.08	0.02	< 0.001	< 0.001	< 0.001	0.02	0.81	0.54
Reference interval*	42–57	63–74	3.4–9.7	0–0.3	179–483	12–21	5–8	105–510	< 250

*Reference interval established at the College of Veterinary Medicine, Cornell University, Ithaca, NY.
MCV = Mean corpuscular volume. Neut = Neutrophil concentration. Band = Band neutrophil concentration. APTT = Activated partial thromboplastin time. PT = Prothrombin time.

ables (Tables 1 and 2) at the time of initial hospitalization that might indicate a selection bias favoring survival were determined (**Table 4**). Significant differences in serum concentrations of potassium, TB, and bicarbonate between dogs that received azathioprine and those that received azathioprine plus ultralow-dose aspirin should have predicted reduced survival in the latter group. However, the only feature associated with improved survival in dogs that received azathioprine plus ultralow-dose aspirin versus dogs that received azathioprine was younger age. Thus, 1 variable was associated with survival (younger age), and 3 variables were associated with nonsurvival (serum concentrations of potassium, TB, and bicarbonate), a finding that suggests there was no selection bias favoring survival for dogs that received ultralow-dose aspirin. However, dogs that received azathioprine plus mHEP had significantly different laboratory results (higher band neutrophil count, lower platelet count, longer APTT and PT, and higher serum TB concentration), compared with dogs that received azathioprine plus ultralow-dose aspirin. Those laboratory results were consistent with failure to survive (Tables 1 and 2). Similarly, dogs that received azathioprine plus ultralow-dose aspirin plus mHEP also had laboratory results (higher band neutrophil count, longer APTT, and higher TB concen-

tration) consistent with nonsurvival, compared with dogs that received azathioprine plus ultralow-dose aspirin. Collectively, these observations suggest that dogs that received mHEP had a consumptive coagulopathy that could have confounded their survival and that also may have contributed to an mHEP treatment selection bias favoring nonsurvival.

Overall, 114 of 151 (76%) dogs with IMHA were discharged, 23 of 151 (15%) were euthanatized, and 14 of 151 (9%) died during the initial period of hospitalization. Within 60 days of initial hospitalization and diagnosis of IMHA, an additional 15 dogs died (12 from IMHA-related causes and 3 from unrelated causes) and 13 dogs were lost to follow-up. If the best-case scenario was assumed (ie, that all dogs lost to follow-up or that died from unrelated causes would have lived to 60 days), the 60-day survival rate would have been 68% (102/151). However, a more reasonable 60-day survival rate (86/138 [62%]) was determined by eliminating the 13 dogs lost to follow-up from the study population and considering that dogs dying (regardless of cause) succumbed to IMHA. Necropsy evaluations performed on 9 of the 37 dogs that died or were euthanatized during initial hospitalization revealed evidence of thromboembolism in each; lesions were detected in the spleen (6/9 dogs), lungs (5/9), kidneys (4/9), heart (2/9) and lymph nodes (2/9).

Table 2—Laboratory variables in dogs with IMHA that died or survived to the time of discharge from the hospital.

Survived or died	TP (g/dL)	Albumin (g/dL)	K (mEq/L)	TB (mg/dL)	ALT (U/L)	ALP (U/L)	CK (U/L)	HCO$_3^-$ (mEq/L)	Creatinine (mg/dL)
Survived (n = 114)									
No. tested	113	113	113	113	113	113	101	108	113
Median	6.3	3.3	3.8	0.9	42	212	183	18	0.5
(range)	(4.5–8.2)	(1.6–5.3)	(2.4–5.9)	(0–50.2)	(6–1,863)	(34–7,910)	(36–4,574)	(8–26)	(0.1–2.5)
Died (37)									
No. tested	37	37	37	37	37	37	37	35	37
Median	5.8	2.9	3.2	3.6	104	234	352	15	0.5
(range)	(3.5–7.8)	(1.5–5.3)	(2.2–6.3)	(0.2–79.2)	(8–2,625)	(28–1,242)	(77–13,728)	(7–29)	(0.1–2.1)
P values	< 0.001	0.02	< 0.001	< 0.001	0.002	0.97	0.008	0.05	0.52
Reference interval*	5.6–7.1	3.1–4.1	3.9–5.3	0.1–0.2	25–106	12–122	58–241	15–25	0.5–1.3

TP = Total protein. K = Potassium. TB = Total bilirubin. ALT = Alanine aminotransferase. ALP = Alkaline phosphatase. CK = Creatine kinase. HCO$_3^-$ = Bicarbonate.
See Table 1 for remainder of key.

Table 3—Differences in median values of physical features and laboratory test results at the time of initial diagnosis in dogs with IMHA grouped according to certain hematologic findings.

Variable group	RR (/min)	n	PCV (%)	n	MCV (fL)	n	Neut (×10³/μL)	n	Band (×10³/μL)	n	Platelet (×10³/μL)	n	APTT (s)	n	Fibrinogen (mg/dL)	n	K (mEq/L)	n	TB (mg/dL)	n	CK (U/L)	n	HCO$_3^-$ (mEq/L)	n
Spherocytes (n = 134)	—		15†	134	76†	134	—		1.85†	134	—		16*	104	617*	90	3.5*	133	1.1†	133	216*	122	—	
No spherocytes (17)	—		24	17	70	17	—		0.2	17	—		14	11	298	10	4	17	0.4	17	128	17	—	
Autoagglutination (118)	44*	118	15*	118	—		23.4†	118	1.95†	118	200*	118	16*	89	—		3.5*	117	1.3*	117	221*	107	16*	111
No autoagglutination (33)	36	33	19	33	—		15.8	33	0.3	33	240	33	15	26	—		4.1	33	0.4	33	144	31	18	32
Regenerative anemia (107)	—		—		78†	107	23.5*	107	1.9*	107	—		—		—		—		—		—		—	
Nonregenerative anemia (44)	—		—		70	44	17.3	44	0.7	44	—		—		—		—		—		—		—	

— = No significant (P < 0.05) difference. *P ≤ 0.05. †P ≤ 0.001.
n = Number of dogs tested. RR = Respiratory rate.
See Tables 1 and 2 for remainder of key.

Table 4—Differences in median values of physical features and laboratory test results at the time of initial diagnosis in dogs with IMHA grouped according to treatments.

Treatment group	Age (y)	n	PCV (%)	n	MCV (fL)	n	Band (X 10³/µL)	n	Platelet (X 10³/µL)	n	APTT (s)	n	PT (s)	n	Fibrinogen (mg/dL)	n	K (mEq/L)	n	TB (mg/dL)	n	HCO₃⁻ (mEq/L)	n
AZA + aspirin (76)	6*	76	15*	76	—		—		—		—		—		579*	43	3.5*	75	0.9*	76	17*	73
AZA (27)	7	27	7	27	—		—		—		—		—		358	18	4.1	27	0.5	27	19	25
AZA + mHEP (13)	—		13*	13	79*	13	—		—		17*	13	15.5†	13	—		3.2*	13	4.3*	13	—	
AZA (27)	—		20	27	71	27	—		—		15	21	7	21	—		4.1	27	0.5	27	—	
AZA + aspirin + mHEP (27)	—		—		—		—		200†	27	—		—		—		—		—		—	
AZA (27)	—		—		—		—		108	27	—		—		—		—		—		—	
AZA + aspirin (76)	—		—		—		1.2*	76	200†	76	15*	76	7†	76	—		—		0.9*	75	—	
AZA + mHEP (13)	—		—		—		3.5	13	66	13	17	13	15.5	13	—		—		4.3	13	—	
AZA + aspirin (76)	—		—		—		1.2*	76	—		15*	76	—		—		—		0.9†	75	—	
AZA + aspirin + mHEP (27)	—		—		—		2.3	27	—		16	27	—		—		—		2.9	27	—	
AZA + aspirin + mHEP (27)	—		—		—		—		200†	27	—		—		—		—		—		—	
AZA + mHEP (13)	—		—		—		—		66	13	—		—		—		—		—		—	

AZA = Azathioprine. mHEP = Mixed–molecular-weight heparin.
See Tables 1–3 for remainder of key.

Significant differences were detected in median values of physical features and laboratory test results in dogs with IMHA grouped by certain hematologic features or treatments between survivors and nonsurvivors at 60 days after initial hospitalization and diagnosis of IMHA (**Table 5**). Factors significantly associated with survival included younger age; slower respiratory rate; lower mature and band neutrophil counts; higher platelet count; shorter APTT; higher serum concentrations of total protein, albumin, and potassium; lower serum concentration of TB; and lower serum activities of ALT and CK. These significant differences were also identified in dogs that survived to initial discharge from the hospital (Tables 1 and 2). Thus, features distinguishing acute and 60-day survival were similar. However, the prognostic importance of finding significant differences in APTT, total protein, and ALT between groups was questionable because median values for those variables remained within reference ranges.

Autoagglutination was significantly (P = 0.001) more common in dogs that died or were euthanatized during initial hospitalization, compared with dogs that survived to discharge (36/37 [97%] vs 82/114 [72%]). However, in dogs that survived to 60 days, autoagglutination was not significantly associated with mortality rate; 45 of 52 (87%) dogs that died had positive results of an autoagglutination test, compared with 63 of 86 (73%) dogs that survived (P = 0.07). Dogs with serum TB concentration > 1.5 mg/dL had reduced survival (Kaplan-Meier survival analysis), compared with those with lower concentrations (P < 0.001). Other clinicopathologic variables significantly associated with reduced survival included band neutrophil count ≥ 3,000 cells/µL (P ≤ 0.001), platelet count < 150,000 platelets/µL (P < 0.001), serum albumin concentration < 3.0 g/dL (P = 0.02), serum potassium concentration < 3.5 mEq/L (P = 0.003), and serum activity of CK
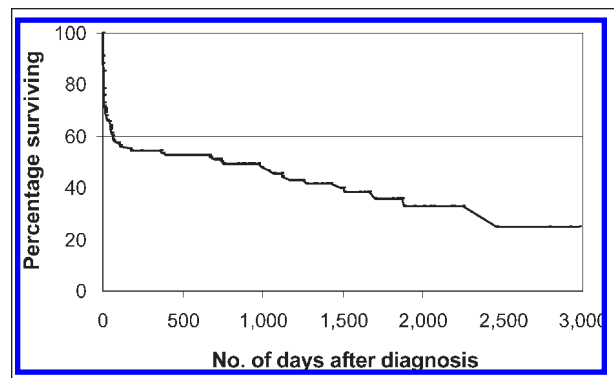

Figure 1—Kaplan-Meier survival curve for 151 dogs with immune-mediated hemolytic anemia (IMHA).
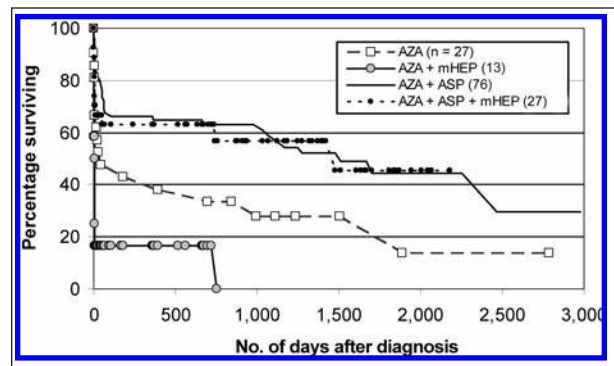

Figure 2—Kaplan-Meier survival curves for dogs with IMHA that were treated with glucocorticoids (all dogs) and azathioprine (AZA), aspirin (ASP), or mixed–molecular-weight heparin (mHEP) in various combinations.

> 250 U/L (P = 0.03). A relationship between reduced short-term survival and transfusion was detected by use of the Gehan-Wilcoxon test (P = 0.05; early events weighted more heavily), but the log-rank assessment

(P = 0.08) did not detect a difference. Transfusion with HBOCs was not negatively associated with survival (P = 0.08). Survival rates in dogs with and without a regenerative erythroid response at the time of initial examination were not significantly different at the time of initial discharge from the hospital or at 30 and 60 days after discharge (76% vs 75%, 70% vs 62%, and 64% vs 60%, respectively; P > 0.2). There was 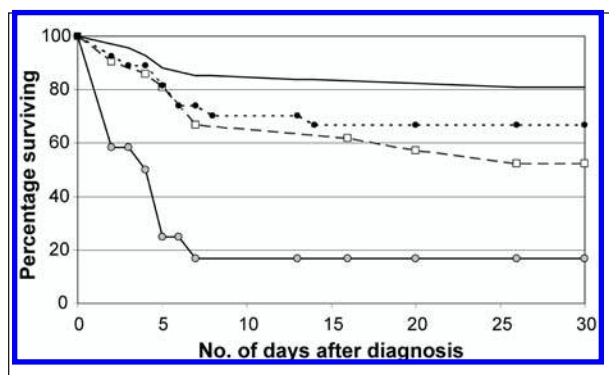no difference in overall survival rate between dogs with and without autoagglutination (P = 0.09); between dogs with nonspherocytic nonagglutinating IMHA and dogs with spherocytic agglutinating IMHA (P = 0.65); between dogs with relapsing IMHA during treatment titration and dogs with acute IMHA (P = 0.28); between dogs with regenerative anemia and those without regeneration (P = 0.31); between dogs with serum ALT activity twice the upper limit of the reference range and dogs with ALT activity within the reference range (P = 0.06); or between Cocker Spaniels and other breeds (P = 0.52).

Median survival time was 455 days (range, 2 to 2,980 days) for 134 dogs that remained after exclusion of 17 dogs for which long-term (> 60 days) follow-up information was unavailable. The Kaplan-Meier survival plot indicated a death event because of IMHA or any cause (**Figure 1**). Dogs that survived > 60 days (long-term survivors; n = 82) had a median survival time of 984 days (range, 63 to 2,980 days), whereas dogs that died within 60 days had a median survival of 26 days (range, 2 to 52 days). Among long-term survivors, medications were tapered and then discontinued for 46 of 82 (56%) dogs; drug dosages were reduced but not discontinued for 36 of 82 (44%). Relapse of IMHA developed in 12 of 82 (15%) dogs

Figure 3—Kaplan-Meier survival curves for dogs with IMHA during the initial 30 days after diagnosis. *See* Figure 2 for key.

Table 5—Differences in median values of physical features and laboratory test results in dogs with IMHA grouped according to certain hematologic findings or treatments that survived or did not survive to 60 days after initial diagnosis.

Variable or treatment group	Age (y)	Temp (°F)	RR (/min)	Neut (X 10³/µL)	Band (X 10³/µL)	Platelet (X 10³/µL)	APTT (s)	n	TP (g/dL)	Alb (g/dL)	K (mEq/L)	n	TB (mg/dL)	n	ALT (U/L)	CK (U/L)	n
Overall																	
Survival (n = 86)	6*	—	40*	19.8*	1.1*	200*	15†	63	6.3†	3.3*	3.7†		0.8†		37†	164*	74
Nonsurvival (52)	7	—	49	23.8	3.2	130	17	42	6.0	3.1	3.4		2.7		103	270	52
Spherocytes																	
Survival (74)	6*	—	40*	20.2*	1.3*	200*	15*	55	6.3†	3.3*	3.6†		0.9†		36*	183*	63
Nonsurvival (49)	8	—	48	24.0	3.3	128	17	39	6.0	3.0	3.3		3.2		97	280	49
No spherocytes																	
Survival (12)	—	—	—	—	—	—	—		—	—	—		—		54*	—	
Nonsurvival (3)	—	—	—	—	—	—	—		—	—	—		—		396	—	
Autoagglutination																	
Survival (63)	6*	—	40*	20.2*	1.6*	200*	15*	45	6.3†	3.3*	3.6*		0.9†		35†	193*	53
Nonsurvival (45)	7	—	55	24.6	3.3	127	18	36	6.0	3.0	3.3		3.2		101	352	45
No autoagglutination																	
Survival (23)	—	—	—	—	—	—	—		—	—	—		—		—	—	
Nonsurvival (7)	—	—	—	—	—	—	—		—	—	—		—		—	—	
Regenerative anemia																	
Survival (61)	6*	—	—	20.5*	1.5*	—	15*	47	6.5*	—	3.6*		0.8†		38*	183*	52
Nonsurvival (35)	8	—	—	28.5	3.5	—	17	27	6.0	—	3.4		2.3		90	259	35
Nonregenerative anemia																	
Survival (25)	—	101.8*	—	—	0.5*	215*	16*	16	6.0*	—	3.8*		0.6*		—	118*	22
Nonsurvival (17)	—	103	—	—	2.0	100	19	15	5.9	—	3.3		4.3		—	339	17
AZA																	
Survival (11)	—	—	—	—	0.2*	237*	—		—	3.7*	—		—		—	—	
Nonsurvival (11)	—	—	—	—	2	17	—		—	2.9	—		—		—	—	
AZA + aspirin																	
Survival (50)	6*	—	—	—	—	—	—		—	—	3.6†	49	0.7†	49	—	160*	41
Nonsurvival (18)	7.5	—	—	—	—	—	—		—	—	3.1		2.6		—	317	
AZA + mHEP																	
Survival (2)	—	—	—	—	—	—	—		—	—	4.6*		0.4*		—	—	
Nonsurvival (10)	—	—	—	—	—	—	—		—	—	3.7		9.8		—	—	
AZA + aspirin + mHEP																	
Survival (17)	—	—	—	—	—	—	—		—	—	—		—		—	—	
Nonsurvival (10)	—	—	—	—	—	—	—		—	—	—		—		—	—	

Unless otherwise specified, dogs tested = group n.
See Tables 1-4 for key.

subsequent to reducing or discontinuing immunosuppressive treatment.

Compared with dogs treated with azathioprine, dogs treated with azathioprine plus ultralow-dose aspirin had significantly ($P < 0.001$) greater survival rates despite having clinicopathologic features that suggested nonsurvival, whereas dogs treated with azathioprine plus mHEP had a significantly ($P = 0.001$) lower survival rate (**Figures 2 and 3; Table 5**). A reduced survival rate in dogs treated with azathioprine plus mHEP could be explained by the severity of disease. However, dogs treated with azathioprine plus ultralow-dose aspirin plus mHEP (a group not distinguishable from the azathioprine plus mHEP treatment group by physical features or results of laboratory tests, except for lower median platelet count in the latter group) had significantly ($P = 0.001$) better survival rates than dogs that received azathioprine plus mHEP. Thus, our observations support that a survival advantage was bestowed by aspirin.

In this study, survival rates for all dogs and treatments combined (**Figure 4**) equaled or exceeded those reported in previous reviews of IMHA in which treatment protocols that did not include aspirin were administered. The only treatment group with a survival rate at time of discharge from the hospital that significantly ($P = 0.007$) exceeded rates reported in past studies[3,5,6] was the group of dogs that received azathioprine plus ultralow-dose aspirin. Dogs treated with azathioprine plus ultralow-dose aspirin also had a significantly ($P = 0.007$) greater 30-day survival rate than was reported in previous studies.[1-3,7,9] Dogs treated with azathioprine and mHEP had significantly (each $P = 0.02$) lower survival rates at both the time of hospital discharge and at 30 days, compared with survival rates in other studies. Survival at 365 days was significantly ($P < 0.007$) better in all dogs treated with azathioprine
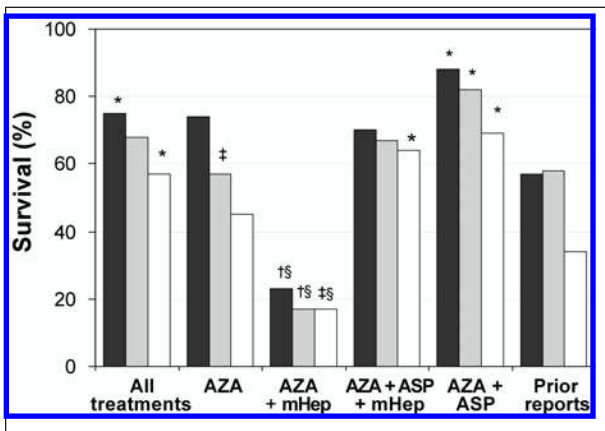

Figure 4—Percentage survival in dogs of this report with IMHA, compared with percentage survival in prior reports.[1-3,5-7,9] Dogs were grouped according to survival at time of hospital discharge (solid bars), at 30 days after admission (shaded bars), or at 1 year after admission (open bars); dogs were also grouped according to treatment with all combinations of drugs (all treatments), AZA, AZA plus mHep, AZA plus mHep plus ASP, or AZA plus ASP. *Significant ($P = 0.007$) difference from dogs in prior reports. †Significant ($P = 0.02$) difference from dogs in the AZA group, the AZA plus ASP group, and prior reports. ‡Significant ($P < 0.02$) difference from dogs in the AZA plus ASP group. §Significant ($P = 0.02$) difference from dogs in the AZA plus ASP plus mHEP group. *See* Figure 2 for key.

plus ultralow-dose aspirin (including dogs that also received mHEP), compared with previous reports.[3,9] Treatment with azathioprine without ultralow-dose aspirin had no apparent survival advantage at the time of hospital discharge, 30 days, or 365 days, compared with collated results for similar time intervals from prior reports ($P > 0.05$). The finding that azathioprine combined with ultralow-dose aspirin, with or without mHEP, significantly improves chronic survival further suggests that aspirin imparts a beneficial therapeutic effect in dogs with IMHA.

Discussion

The ages of dogs with IMHA in this study were similar to those reported in previous studies,[2,8,9] indicating a predisposition for the disease in adults. Sexually intact female dogs and all neutered dogs were overrepresented in the affected group, compared with the control hospital population. Although previous studies[3,7] have also revealed a predisposition for IMHA in female dogs, the association between sex and risk for the disease remains unclear. The overrepresentation of female dogs and neutered dogs of either sex may indicate that androgens are protective. Similar to findings in previous reports,[1,3,4,7,9] the Cocker Spaniel breed was overrepresented in the present study. Although Miniature Schnauzer dogs were also overrepresented in the present study, other breed predilections for IMHA were not substantiated. Our study did not reveal a significant difference in mortality rate or survival time between Cocker Spaniels and other breeds with IMHA, corroborating the findings in a previous study.[9]

Prior to data analysis, we had a clinical impression that IMHA in dogs was more common during cold months, possibly because of changes in microvascular autoagglutination and appendicular hypothermia. However, we found a higher frequency of IMHA during warm months (April through September) in this northeastern region of the United States, an observation that may reflect an increase in the number of owners scheduling visits for routine health care, including vaccinations, during warmer months. However, this speculated relationship could not be adequately investigated because of incomplete vaccination records in affected dogs. Although results of a previous study[2] suggest a causal relationship between vaccination and development of IMHA, other investigations have not detected this association.[4,9] In our study, 30% of dogs with IMHA had been vaccinated within 60 days of initial diagnosis. The incomplete vaccination data for the control group prevented a thorough investigation of an association between vaccination and IMHA.

Results of previous studies[1-4] indicate that certain clinicopathologic variables (ie, high serum TB concentration, high serum ALP activity, low platelet count, low PCV, autoagglutination, and lack of a regenerative erythroid response) were associated with a poor prognosis in dogs with IMHA. The present study revealed that dogs that died had more severe thrombocytopenia and hyperbilirubinemia than dogs that survived, but did not detect an association between severity of anemia, serum ALP activity, or absence of a regenerative erythroid response and mortality rate. Thrombocytopenia can

develop in dogs with IMHA because of consumptive coagulopathy resulting from disseminated intravascular coagulation, concurrent immune-mediated platelet destruction, splenic sequestration of thrombocytes, or failure of platelet production (immune-mediated or chemotherapeutic toxicosis). Although severe hyperbilirubinemia (serum concentration > 5 mg/dL) was associated with a poor prognosis in previous studies,[1,4] results of the present study indicated an association between hyperbilirubinemia and mortality rate at lower serum TB concentrations (ie, TB > 1.5 mg/dL; reference range, < 0.3 mg/dL). Most dogs that died had jaundice, whereas 50% of surviving dogs did not. This serum concentration is the concentration above which hyperbilirubinemia is manifested clinically as jaundice. Hyperbilirubinemia associated with IMHA may result from hemolytic or hepatobiliary causes (eg, impairment of bilirubin uptake by hepatocytes, cytosolic transport, storage, conjugation, or canalicular egress). Alterations in hepatobiliary mechanisms causing hyperbilirubinemia in dogs that succumb to IMHA include hepatocellular necrosis secondary to hypoxia, thromboembolism, ischemia, or hepatic hemoglobinemic endothelial damage.[16] An inconsistent association between histologic liver lesions at necropsy and magnitude of hyperbilirubinemia has been reported.[16] Increased concentrations of serum TB and free hemoglobin may complicate systemic responses in IMHA via proinflammatory effects.[26]

There was a wide overlap of clinicopathologic values between survivors and nonsurvivors. Dogs that died had a higher median neutrophil count, lower median concentrations of albumin and potassium, and a higher median serum CK activity. However, none of these tests provided a cutoff value for predicting outcome. An association between hypoalbuminemia and thromboembolism has been reported previously.[4] Because a wide variety of circumstances can lead to hypoalbuminemia (eg, a negative acute-phase response with downregulation of albumin synthesis in coordination with upregulation of inflammatory cytokines, failure of liver synthesis, proteinuria, enteric protein loss, vascular leakage, or hemorrhagic loss), multiple factors have the potential to reduce serum albumin concentrations in dogs with IMHA.[27-30] Although an association between hypokalemia (serum potassium concentration < 3.5 mEq/L) and an increased rate of mortality has not been reported, the association between critical illness and hypokalemia is not novel.[31] Hypokalemia promotes vascular constriction and may increase the risk for vascular and thromboembolic complications.[32,33] The higher median serum CK activity in dogs with IMHA that died may reflect the combined influences of hypoxia, recumbency, altered cell membrane permeability, and muscle injury secondary to impaired perfusion; thromboembolic complications (including embolic myopathy); repeated IV catheter placement (thrombosed catheters); and SC or IM administered medications.[34] Although IMHA is typically characterized by a regenerative marrow response that is accompanied by mature and band neutrophilia, an association between mortality and the severity of leukocytosis and left shift has recently been reported.[16] This finding may reflect ischemic or hypoxic tissue necrosis accompanying severe anemia. The results of the study reported here also indicated an association between mortality rate and band neutrophilia (≥ 3,000 cells/μL) in dogs with IMHA.

Erythroid regenerative response is an unreliable predictor of survival in canine IMHA. One retrospective study[1] that evaluated IMHA with peripheral erythrocyte destruction reported a lower survival rate in dogs that lacked a vigorous regenerative response during initial hospitalization, but this association was not substantiated in a study of dogs with marrow-mediated IMHA.[35] In the present study, although 29% of dogs had nonregenerative anemia at the time of evaluation, neither short- nor long-term survival rates differed from the survival rate in dogs with a regenerative erythroid response. Therefore, grouping patients on the basis of erythroid regeneration at the time of initial evaluation is inappropriate because the lack of erythroid regeneration could be attributable to insufficient time to mount a reticulocyte response, bone marrow-targeted erythroid destruction, or an undetected infectious disorder (eg, *Ehrlichia canis* infection). Whether dogs with IMHA were referred to our hospital earlier in the course of disease than were dogs in other studies cannot be determined. We believe it is unlikely that an underlying infectious disorder caused impaired erythroid regeneration in dogs in our study because medical records were reviewed for evidence of concurrent disease. However, because most dogs were treated with doxycycline while results of serodiagnostic tests were pending, an underlying doxycycline-responsive infectious disorder cannot be ruled out in some dogs. Dogs in the present study lived in the northeastern section of the United States, where anemia-causing infectious disorders are uncommon.

Autoagglutination has been variably associated with reduced survival rate in dogs with IMHA.[2,5] In the study reported here, a significant association between autoagglutination and acute, but not long-term, mortality rate was detected. This may have resulted from resolution of autoagglutination over time as immunotargeting of erythrocytes abated in response to treatment with azathioprine.

Spherocytosis was detected on a blood smear in 89% of the affected dogs in this study. Compared with dogs that lacked this finding, dogs with spherocytosis had clinicopathologic results consistent with severe disease, but these dogs did not have significantly reduced survival rates. Our data also suggest that nonspherocytic, nonagglutinating IMHA is rare in dogs as it is in humans.[36] Dogs (n = 10) with this form of disease had positive results of direct Coombs' tests and did not differ in survival, compared with dogs that had autoagglutination or spherocytosis.

Seventy percent (106/151) of dogs were treated via transfusion; 21% of these received HBOCs. A positive association between transfusion with blood, plasma, or HBOCs and a higher acute mortality rate (from the time of initial treatment to hospital discharge) may reflect the severity of disease in dogs that received these treatments, compared with dogs that did not receive transfusion therapy. However, transfusion with blood or HBOCs did not have a negative long-term survival

effect. The positive long-term outcome in dogs that received HBOCs contradicts findings reported in a previous study[5] in which HBOC administration was associated with a poor prognosis. Our findings indicate that successful clinical management of dogs with IMHA commonly involves transfusion of blood components or HBOCs. Although most clinicians preferentially used blood (fresh whole blood or packed RBCs) to provide oxygen-carrying capacity for dogs with clinical signs of anemia, HBOCs were used regularly and repeatedly in patients for which compatible blood products were unavailable or that had severe hemolysis.

A variety of immunosuppressive protocols are used in the management of IMHA in veterinary medicine. Unfortunately, small data sets, lack of uniform treatment strategies, and limited long-term follow-up information have restricted the ability of veterinarians to make conclusions regarding treatment efficacy. Considering all treatments and all dogs in the present study, survival rates matched or exceeded those reported in previous studies.[1-3,5-7,9] Although some dogs (18/151 [12%]) received additional immunomodulatory drugs, treatment with glucocorticoids, azathioprine, and ultralow-dose aspirin was the most common management protocol (76/151 [50%]). In our study, administration of azathioprine alone did not increase survival, compared with results of previous studies that reported management of the disease with immunosuppressive agents (but not aspirin). However, the combination of ultralow-dose aspirin with azathioprine significantly increased survival, whether mHEP was included in the treatment protocol (27/151 [18%]) or not (76/151 [50%]).

Azathioprine, a purine-analogue antimetabolite, preferentially affects T cells and has minimal short-term effects on immunoglobulin production.[37,38] Although the drug's purported onset of action is controversial, evidence suggests that it influences lymphocyte blastogenesis in as early as 7 days.[39,40] Azathioprine is commonly combined with glucocorticoids to take advantage of the rapid steroid-mediated immunomodulation during the acute stages of an immune-mediated disorder. Administering the drugs in combination is thought to achieve a rapid clinical response and allow for lower drug dosages and longer administration intervals with fewer adverse effects. Reported adverse effects of azathioprine, including pancreatitis, cholestatic hepatopathy, myelosuppression, vomiting, anorexia, and diarrhea, are encountered infrequently and were not reported in the dogs of this study.[11,41]

In the present study, there was a relapse in clinical disease in 15% of dogs that survived beyond 60 days. During this time interval, treatments had either been discontinued or were on a tapering course. Relapse was as likely to occur while on a tapering course of medication as after treatment was discontinued. Our results indicate that most dogs treated with glucocorticoids, azathioprine, and ultralow-dose aspirin that survived > 60 days did not have disease relapse.

All necropsy examinations in dogs that died or were euthanatized while hospitalized revealed thromboembolic lesions, similar to observations in previous studies.[15,16,42] Because the spleen was the most common site of embolization, ultrasonographic assessment of splenic vasculature may prove useful in assessing patients for this complication. Predisposing factors for thromboembolism include venous stasis, endothelial injury, and hypercoagulability, all of which may be exacerbated by cage confinement, recumbency, and the process of placing and securing (by bandaging) IV catheters. Vascular injury in dogs with IMHA occurs secondary to the release of inflammatory mediators as a result of RBC destruction, hyperbilirubinemia, and hypoxia and can potentiate the tendency for clot formation initiated by autoagglutinating erythrocytes and the release of RBC stromal elements upon hemolysis. These factors mandate thromboprophylaxis in dogs with IMHA.

Consistent with our findings, a poor response to prophylactic administration of heparin as an antithrombotic strategy in the treatment of canine IMHA was recently reported.[9] Optimal titration to effect of mHEP administration necessitates serial monitoring with coagulation tests (APTT or anti-Xa activity) to verify patient response and optimize treatment safety. The dose of mHEP used in this study (75 to 125 U/kg, SC, q 6 h) was conservative and may not have achieved an optimal anticoagulant response. Conservative dosing was used because adverse effects of mHEP impose unacceptable complications, including hemorrhage, thrombocytopenia, and thrombotic purpura, in humans.[43,44] Because the dogs that received mHEP had more markers of severe clinical disease, it is possible that treatment bias influenced their survival outcome. However, the finding that survival rate improved when aspirin was combined with mHEP in a group of dogs that were not significantly different in physical features or laboratory test results (except for higher platelet count) from those given azathioprine plus mHEP suggests that aspirin imparted a survival benefit even in the most severely affected patients.

Because an ideal prophylactic anticoagulant for dogs with IMHA has yet to be identified, we initiated a long-term treatment protocol that included ultralow-dose aspirin on the basis of our pilot study investigations. Furthermore, in humans, there is clear indication that low-dose aspirin administration reduces morbidity and mortality rates associated with thromboembolic disease.[20, 23,24] Depending on dose, aspirin can substantially influence multiple organs by inhibiting cyclooxygenase-dependent systems, which reduces eicosanoid production. High aspirin doses should be avoided because these can lead to detrimental enteric, renal, and hepatic effects, along with impaired platelet aggregation and hemorrhagic diathesis. Titrated low-dose aspirin administration can minimize iatrogenic complications while retaining a modulatory influence on platelet aggregation that inhibits clot formation. Because aspirin has a dose-dependent inhibitory effect on prostacyclin activity in various cells, careful dose titration is necessary to preserve beneficial vasodilatory and thromboprotective effects.[22,45] Applying these dosing principles, ultralow-dose aspirin offers greater potential for reducing risk for thrombosis, compared with higher dose aspirin administration.[45,46]

Although the data reported here circumstantially suggest that ultralow-dose aspirin has an antithrom-

botic effect in dogs with IMHA, we did not specifically test platelets in these patients. Aspirin is a useful antithrombotic agent because a single dose can impair platelet response for approximately 1 week (the life span of the platelet). Even very low doses of aspirin cause irreversible acetylation of the platelet cyclooxygenase active site.[45] In humans, the influence of ultralow-dose aspirin on platelet aggregation varies among individuals.[45] Nevertheless, it has been reported[47] that even dosages at the low end of the ultralow-dose regimen for aspirin in humans inhibit arachidonate-induced coronary vasoconstriction in canine myocardium (an effect unmeasurable by use of conventional techniques).

Adverse effects of ultralow-dose aspirin administration were not evident in our study despite concurrent treatment with immunosuppressive doses of glucocorticosteroids. The absence of adverse effects suggested similarity to the dose-dependent enteric toxicity of aspirin in humans. Avoidance of adverse effects is easily accomplished in humans with doses as low as 0.5 mg/kg/d used for antithrombotic effects.[22,45]

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a. Pilot study of platelet impedance aggregometry; data available from authors on request.
b. Oxyglobin, Biopure Corp, Cambridge, Mass.
c. Coulter S+ IV electronic counter, Coulter Electronics, Hialeah, Fla.
d. Spectra Services, Rochester, NY.
e. Simplastin Excel, Organon Teknika Corp, Durham, NC.
f. General Diagnostic Automated apt, Organon Teknika Corp, Durham, NC.
g. Dade fibrinogen determination reagents for mechanical instruments, Dade International Inc, Miami, Fla.
h. Fibrometer, Becton, Dickinson & Co, Franklin Lakes, NJ.
i. Hitachi 911, Boehringer, Mannheim, Germany (Indianapolis, Ind).
j. Canine anti-globulin (Coombs') reagent, ICN Biomedicals Inc, Aurora, Ohio.
k. Rabbit anti-dog IgG (H & L) FITC conjugated, ICN Biomedicals Inc, Aurora, Ohio.
l. Statistix 7, version 7.0, Analytical Software, Tallahassee, Fla.

References

1. Klag AR, Giger U, Shofer FS. Idiopathic immune-mediated hemolytic anemia in dogs: 42 cases (1986–1990). *J Am Vet Med Assoc* 1993;202:783–788.
2. Duval D, Giger U. Vaccine-associated immune-mediated hemolytic anemia in the dog. *J Vet Intern Med* 1996;10:290–295.
3. Reimer ME, Troy GC, Warnick LD. Immune-mediated hemolytic anemia: 70 cases (1988–1996). *J Am Anim Hosp Assoc* 1999;35:384–391.
4. Carr AP, Panciera DL, Kidd L. Prognostic factors for mortality and thromboembolism in canine immune-mediated hemolytic anemia: a retrospective study of 72 dogs. *J Vet Intern Med* 2002;16: 504–509.
5. Grundy SA, Barton C. Influence of drug treatment on survival of dogs with immune-mediated hemolytic anemia: 88 cases (1989–1999). *J Am Vet Med Assoc* 2001;218:543–546.
6. Bennett D, Finnett SL, Nash AS, et al. Primary autoimmune haemolytic anaemia in the dog. *Vet Rec* 1981;109:150–153.
7. Mason N, Duval D, Shofer FS, et al. Cyclophosphamide exerts no beneficial effect over prednisone alone in the initial treatment of acute immune-mediated hemolytic anemia in dogs: a randomized controlled clinical trial. *J Vet Intern Med* 2003;17:206–212.
8. Jackson ML, Kruth SA. Immune-mediated hemolytic anemia and thrombocytopenia in the dog: a retrospective study of 55 cases diagnosed from 1969 though 1983 at the Western College of Veterinary Medicine. *Can Vet J* 1985;26:245–250.
9. Burgess K, Moore A, Rand W, et al. Treatment of immune-mediated hemolytic anemia in dogs with cyclophosphamide. *J Vet Intern Med* 2000;14:456–462.
10. Kellerman DL, Bruyette DS. Intravenous human immunoglobulin for the treatment of immune-mediated hemolytic anemia in 13 dogs. *J Vet Intern Med* 1997;11:327–332.
11. Miller E. The use of cytotoxic agents in the treatment of immune-mediated diseases of dogs and cats. *Semin Vet Med Surg (Small Anim)* 1997;12:157–160.
12. Miller E. The use of danazol in the therapy of immune-mediated disease of dogs. *Semin Vet Med Surg (Small Anim)* 1997;12:167–169.
13. Vaden SL. Cyclosporine and tacrolimus. *Semin Vet Med Surg (Small Anim)* 1997;12:161–166.
14. Gregory CR, Stewart A, Sturges B, et al. Leflunomide effectively treats naturally occurring immune-mediated and inflammatory diseases of the dog that are unresponsive to conventional therapy. *Transplant Proc* 1998;30:4143–4148.
15. Klein MK, Dow SW, Rosychuck RAW. Pulmonary thromboembolism associated with immune-mediated hemolytic anemia in dogs: ten cases (1982–1987). *J Am Vet Med Assoc* 1989;195:246–250.
16. McManus PM, Craig LE. Correlation between leukocytosis and necropsy findings in dogs with immune-mediated hemolytic anemia: 34 cases (1994–1999). *J Am Vet Med Assoc* 2001;218:1308–1313.
17. Mischke RH, Schüttert C, Grebe SI. Anticoagulant effects of repeated subcutaneous injections of high doses of unfractionated heparin in healthy dogs. *Am J Vet Res* 2001;62:1887–1891.
18. Keith JC, Rawlings CA, Schaub RG. Pulmonary thromboembolism during therapy of dirofilariasis with thiacetarsamide: modification with aspirin or prednisone. *Am J Vet Res* 1983;44:1278–1283.
19. Smith SA, Tobias AH, Jacob KA, et al. Arterial thomboembolism in cats: acute crisis in 127 cases (1992–2001) and long term management with low-dose aspirin in 24 cases. *J Vet Intern Med* 2003;17:73–83.
20. Pulmonary embolism prevention trial collaborative group. Prevention of pulmonary embolism and deep vein thrombosis with low dose aspirin: pulmonary embolism prevention (PEP) trial. *Lancet* 2000;35:1295–1302.
21. Robertson AJ, Nargund V, Gray DWR, et al. Low dose aspirin as prophylaxis against renal vein thrombosis in renal transplant recipients. *Nephrol Dial Transplant* 2000;15:1865–1868.
22. Patrono C. Aspirin: new cardiovascular uses for an old drug. *Am J Med* 2001;110:62S–65S.
23. Lauer MS. Aspirin for primary prevention of coronary events. *N Engl J Med* 2002;346:1468–1474.
24. Spencer FA, Santopinto JJ, Gore JM, et al. Impact of aspirin on presentation and hospital outcomes in patients with acute coronary syndromes (the global registry of acute coronary events [GRACE]). *Am J Cardiol* 2002;90:1056–1061.
25. Wilkerson MJ, Davis E, Shuman W, et al. Isotype-specific antibodies in horses and dogs with immune-mediated hemolytic anemia. *J Vet Intern Med* 2000;14:190–196.
26. Graça-Souza AV, Arruda MAB, de Freitas MS, et al. Neutrophil activation by heme: implications for inflammatory processes. *Blood* 2002;99:4160–4165.
27. Mazzaferro EM, Rudloff E, Kirby R. The role of albumin replacement in the critically ill veterinary patient. *J Vet Emerg Crit Care* 2002;12:113–124.
28. Margarson MP, Soni N. Serum albumin: touchstone or totem? *Anaesthesia* 1998;53:789–803.
29. Nicholson JP, Wolmarans MR, Park GR. The role of albumin in critical illness. *Br J Anaesth* 2000;85:599–610.
30. Franch-Arcas G. The meaning of hypoalbuminemia in clinical practice. *Clin Nutr* 2001;20:265–269.
31. Gennari JF. Hypokalemia. *N Engl J Med* 1998;339:451–458.
32. Cohn JN, Kowey PR, Whelton PK, et al. New guidelines for potassium replacement in clinical practice. *Arch Intern Med* 2000; 160:2429–2436.
33. Whang R, Sims G. Magnesium and potassium supplementation in the prevention of diabetic vascular disease. *Med Hypotheses* 2000;55:263–265.
34. Wyss M, Kaddurah-Daouk R. Creatine and creatine metabolism. *Physiol Rev* 2000;80:1107–1213.
35. Weiss DJ. Primary pure red cell aplasia in dogs: 13 cases (1996–2000). *J Am Vet Med Assoc* 2002;221:93–95.

36. Kerr R, Rawlinson PSM, Cachia PG. Direct antiglobulin test negative, non-spherocytic autoimmune haemolytic anaemia. *Clin Lab Haem* 2000;22:365–367.

37. Beale KM. Azathioprine for treatment of immune-mediated disease of dogs and cats. *J Am Vet Med Assoc* 1988;192:1316–1318.

38. Miller E. Immunosuppression—an overview. *Semin Vet Med Surg (Small Anim)* 1997;12:144–149.

39. Ogilvie GK, Felsburg PJ, Harris CW. Short-term effect of cyclophosphamide and azathioprine on selected aspects of canine blastogenic response. *Vet Immunol Immunopathol* 1988;18:119–127.

40. Rinkardt NE, Kruth SA, Kaushik A. The effects of prednisone and azathioprine on circulating immunoglobulin levels and lymphocyte subpopulations in normal dogs. *Can Vet J* 1999;63:18–24.

41. Rinkardt NE, Kruth SA. Azathioprine-induced bone marrow toxicity in four dogs. *Can Vet J* 1996;37:612–613.

42. Scott-Moncrieff JC, Treadwell NG, McCullough SM, et al. Hemostatic abnormalities in dogs with primary immune-mediated hemolytic anemia. *J Am Anim Hosp Assoc* 2001;37:220–227.

43. Cines DB, Tomaski A, Tannenbaum S. Immune endothelial-cell injury in heparin-associated thrombocytopenia. *N Engl J Med* 1987;316:581–589.

44. Warkentin TE, Kelton JG. Temporal aspects of heparin-induced thrombocytopenia. *N Engl J Med* 2001;344:1286–1292.

45. Schrör K. Aspirin and platelets: the antiplatelet action of aspirin and its role in thrombosis treatment and prophylaxis. *Semin Thromb Hemost* 1997;23:349–356.

46. Hofman WF, Fravel J, Ehrhart IC. Effect of aspirin on vascular tone and reactivity to vasoactive amines in the dog lung. *Res Commun Chem Pathol Pharmacol* 1992;78:47–56.

47. Capurro NL, Lipson LC, Bonow RO, et al. Relative effects of aspirin on platelet aggregation and prostaglandin-mediated coronary vasodilation in the dog. *Circulation* 1980;62:1221–1227.

Selected abstract for *JAVMA* readers from the *American Journal of Veterinary Research*

Epidemiologic evaluation of diarrhea in dogs in an animal shelter
### Susanne H. Sokolow et al

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Objectives—To determine associations among infectious pathogens and diarrheal disease in dogs in an animal shelter and demonstrate the use of geographic information systems (GISs) for tracking spatial distributions of diarrheal disease within shelters.

Sample Population—Feces from 120 dogs.

Procedure—Fresh fecal specimens were screened for bacteria and bacterial toxins via bacteriologic culture and ELISA, parvovirus via ELISA, canine coronavirus via nested polymerase chain reaction assay, protozoal cysts and oocysts via a direct fluorescent antibody technique, and parasite ova and larvae via microscopic examination of direct wet mounts and zinc sulfate centrifugation flotation.

Results—*Salmonella enterica* and *Brachyspira* spp were not common, whereas other pathogens such as canine coronavirus and *Helicobacter* spp were common among the dogs that were surveyed. Only intestinal parasites and *Campylobacter jejuni* infection were significant risk factors for diarrhea by univariate odds ratio analysis. *Giardia lamblia* was significantly underestimated by fecal flotation, compared with a direct fluorescent antibody technique. Spatial analysis of case specimens by use of GIS indicated that diarrhea was widespread throughout the entire shelter, and spatial statistical analysis revealed no evidence of spatial clustering of case specimens.

Conclusions and Clinical Relevance—This study provided an epidemiologic overview of diarrhea and interacting diarrhea-associated pathogens in a densely housed, highly predisposed shelter population of dogs. Several of the approaches used in this study, such as use of a spatial representation of case specimens and considering multiple etiologies simultaneously, were novel and illustrate an integrated approach to epidemiologic investigations in shelter populations. (*Am J Vet Res* 2005;66:1018–1024)
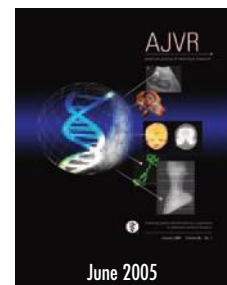
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SMALL ANIMALS