Vaccine-Associated Immune-Mediated Hemolytic Anemia in the Dog

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Vaccination has been incriminated as a trigger of immune-mediated hemolytic anemia (IMHA) in dogs and in people, but evidence to support this association is lacking. In a controlled retrospective study, idiopathic IMHA was identified in 58 dogs over a 27-month period. When compared with a randomly selected control group of 70 dogs (presented for reasons other than IMHA) over the same period, the distribution of cases versus time since vaccination was different ($P < .05$). Fifteen of the dogs (26%) had been vaccinated within 1 month (mean, 13 days; median, 14 days; range, 1 to 27 days) of developing IMHA ($P < .0001$), whereas in the control group no marked increase in frequency of presentation was seen in the first month after vaccination. The dogs with IMHA were divided into 2 groups based on time since vaccination: the vaccine IMHA group included dogs vaccinated within 1 month of developing IMHA; the nonvaccine IMHA group included dogs that developed IMHA more than 1 month after vaccination. The recently vaccinated dogs with IMHA (vaccine IMHA group) had significantly lower platelet counts ($P < .05$) and a trend towards increased prevalence of intravascular hemolysis and autoagglutination when compared with the nonvaccine IMHA group. Similar mortality rates were seen in the vaccine IMHA group (60%) and the nonvaccine IMHA group (44%), with the majority of fatalities (>75%) occurring in the first 3 weeks after presentation. Persistent autoagglutination was a negative prognostic indicator for survival in both groups ($P < .05$). Presence of icterus and hyperbilirubinemia were negative prognostic indicators for survival in the nonvaccine IMHA group ($P < .0001$ and $P < .01$, respectively) but not in the vaccine IMHA group. In the recently vaccinated dogs, combination vaccines from various manufacturers against canine distemper, adenovirus type 2, leptospirosis, parainfluenza, and parvovirus (DHLPP) were involved in each case. Vaccines against rabies virus, *Bordetella* spp, coronavirus, and Lyme *Borrelia* were administered concomitantly to some dogs. This study provides the first clinical evidence for a temporal relationship of vaccine-associated IMHA in the dog.

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Immune-mediated hemolytic anemia (IMHA) is characterized by immune destruction of red blood cells resulting in extravascular, and occasionally, intravascular hemolysis. IMHA is a common hematologic disorder in dogs,[1-3] and may be primary, known as autoimmune hemolytic anemia or idiopathic IMHA, or secondary to a number of causes. Autoagglutination persisting after saline washing, a positive direct Coombs' test, and/or spherocytosis are diagnostic findings in IMHA, but do not differentiate primary from secondary forms. The anemia is typically regenerative, but can also present without reticulocytosis.[1,3-6] Signs of hemolysis include bilirubinuria and icterus, and rarely, hemoglobinemia and hemoglobinuria. IMHA may be secondary to toxin or drug exposure, neoplasia, or concurrent infection.

Based on clinical impression, anecdotal and other reports, recent vaccination (within 1 month) has been implicated as a trigger for immune-mediated disease in dogs and humans, but evidence showing a causal relationship is lacking. Previous studies of dogs with IMHA have not investigated an association between IMHA and recent vaccination in depth.[1,4,6-10] In this report, the temporal relationship between vaccination and onset of IMHA in dogs was studied and compared with a control population without IMHA.

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Materials and Methods

Dogs with IMHA were selected from medical records at the Veterinary Hospital of the University of Pennsylvania (VHUP) from December 1992 to March 1995. IMHA was diagnosed when there was anemia and evidence of hemolysis (as evidenced by hyperbilirubinemia, bilirubinuria, and/or hemoglobinuria) with a positive direct Coombs' test or persistent autoagglutination after saline washing of red blood cells (which precluded performing a Coombs' test). Dates, types, and brands of most recent vaccines were recorded. Seventy adult dogs with complete vaccination records that did not have IMHA, anemia, or other immune diseases were randomly selected throughout the same time period to act as a control group. To investigate the association between recent vaccination and IMHA, the time from last vaccination to presentation (control group) or onset of IMHA were compared for the dogs with idiopathic IMHA and the control dogs. Dogs with IMHA were then assigned to 1 of 2 groups depending on the time from last vaccination to the appearance of clinical signs of IMHA. The vaccine IMHA group included dogs that had been vaccinated within 1 month of developing IMHA. The nonvaccine IMHA group included dogs that had been vaccinated more that 1 month before developing IMHA. Dogs were excluded if another potential cause of IMHA such as drug or toxin exposure, infectious disease, or neoplasia was identified from clinical findings or the patient's history. Two dogs were excluded because vaccine histories were unavailable.

Blood samples from all patients were evaluated for spherocytosis and microscopic autoagglutination. Autoagglutination was considered positive if it persisted through 3 washes with phosphate-buffered saline solution. The Coombs' test was performed within 24 hours of collection. EDTA-anticoagulated blood cells were washed 4 times and incubated as a 2% cell suspension at 37°C with immunoglobulin (Ig) G, IgM, or C_{3b} (Organon Teknika Corp, West Chester, PA) at different doubling concentrations. Depending on the degree of erythrocyte agglutination, Coombs' test results were scored +1 to +4.

Time from vaccination to onset of IMHA was compared with the time from vaccination to presentation in the control group with a Kolmogorov-Smirnov test (SAS Institute, Cary, NC). To assess the association between clinical findings and mortality, categorical data

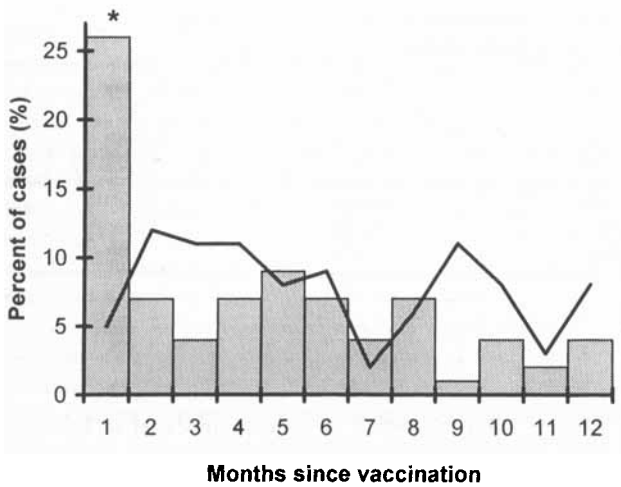

Fig 1. Time of presentation of dogs with IMHA versus control dogs in months since last vaccination. Presentation of 13 dogs with IMHA (22%) and 10 control dogs (14%) occurred more than 1 year after last vaccination, with 0 to 2 cases per month, and are not shown. (□) IMHA dogs. The solid line represents the control group of 70 dogs. *$P < .0001$.

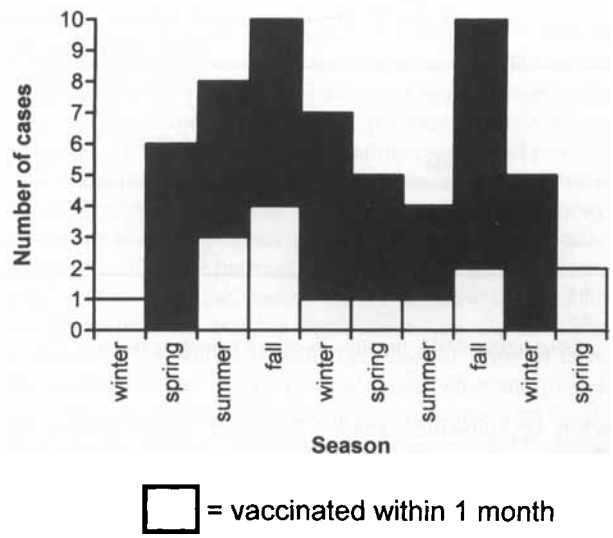

Fig 2. Season of presentation of IMHA in 58 dogs from winter 1992 to spring 1995. Winter, December–February; spring, March–May; summer, June–August; fall, September–November. (□) vaccine IMHA group, (■) nonvaccine IMHA group, (▬) combined IMHA groups.

were analyzed using the chi-squared test or Fisher's exact test when comparing 2 dichotomous variables. The vaccine IMHA group and nonvaccine IMHA group were compared using Student's *t*-test. The time from vaccination to onset of IMHA was analyzed with the binomial solution to the classical occupancy problem.[11] A probability of $P < .05$ was considered statistically significant. Data were analyzed using the SAS software package (SAS Institute). Epistat software was used for the binomial test (True Epistat; Epistat Services).

Results

Fifty-eight dogs with IMHA fitting the inclusion criteria were identified during the 27-month study period. When time since last vaccination was compared for the control group and the dogs with IMHA, the distributions were stochastically different ($P < .05$). A disparately large number (15 of 58 [26%], $P < .0001$) of cases of IMHA had been vaccinated within 1 month or less from onset of illness (Fig 1), whereas in the control group no significant increase in frequency of presentation was seen in the first month after vaccination. Within the vaccine IMHA group, mean time from vaccination to onset of IMHA was 13 days (standard deviation 8.7 days), with a median of 14 days and a range of 1 to 27 days. The dogs vaccinated more than 1 month before the onset of IMHA (nonvaccine IMHA group) were fairly evenly distributed, with 0 to 5 presentations per month throughout the next 11 months (Fig 1). The 13 dogs that received their last vaccination more than 1 year (13 to 55 months) before the onset of IMHA had 0 to 2 cases per month. There was an increased frequency of dogs with IMHA (ie, when the vaccine IMHA group and nonvaccine IMHA group were combined) in the fall (September through November, Fig 2). The small number of dogs in individual groups did not allow substantiation of a seasonal pattern in these subsets (Fig 2).

The signalment, history, and clinicopathologic features were similar between the 2 groups with the exception of a significantly lower platelet count in the vaccine IMHA group ($P < .05$) (Table 1). The frequency of intravascular hemolysis and autoagglutination were also slightly increased in the vaccine IMHA group, although these differences were not statistically significant (Table 2). Autoagglutination was found in 9 of the vaccine IMHA group dogs (60%) at presentation to VHUP. In 3 of these 9 dogs, autoagglutination prevented performing a Coombs' test, and in the other 6 dogs Coombs' test results were positive within 1 to 3 days,

Table 1. Selected Clinical and Pathological Findings of 58 Dogs With IMHA by Recent Vaccination Status

Variable	Vaccine IMHA group (n = 15) Mean ± SD	Nonvaccine IMHA group (n = 43) Mean ± SD
Age (yr)	6.5 ± 2.5	6.7 ± 2.9
Temp (°C)	39.4 ± 0.7	39.2 ± 0.8
Weight (kg)	20.4 ± 11.6	19.6 ± 11.6
WBC ($\times 10^3$)	29.6 ± 11.5	28.1 ± 11.5
RBC ($\times 10^6$)	2.2 ± 1	2.5 ± 1.3
Hb (g/dL)	6.1 ± 0.6	6.9 ± 3
Hct (%)	19.9 ± 7.9	20.9 ± 8.8
MCV (fL)	86.6 ± 7.1	91.1 ± 23.4
MCHC (g/dL)	31.0 ± 3	32.5 ± 4.4
Reticulocytes (%)	8.6 ± 11.6	7.5 ± 5.9
Platelets/mm^3	93.7 ± 49.8	157.2 ± 132.9*
Total bilirubin (mg/dL)	3.7 ± 4.5	4.0 ± 6
Mortality (%)	60.0	44.4

* $P < .05$.

Abbreviations: IMHA, immune-mediated hemolytic anemia; SD, standard deviation.

Table 2. Relationship of Selected Clinicopathologic Findings and Mortality in 58 Dogs With IMHA by Recent Vaccination Status

	Vaccine IMHA Group (n = 15)		Nonvaccine IMHA Group (n = 43)	
Variable	Total Cases: n (%)	Fatalities: n (%)	Total cases: n (%)	Fatalities: n (%)
Icterus	11 (73)	7 (64)	28 (65)	18 (64)*
Autoagglutination	9 (60)	6 (67)†	21 (49)	13 (62)†
IV hemolysis	5 (33)	3 (60)	7 (17)	3 (43)

* $P < .001$.

† $P < .05$.

Abbreviation: IMHA, immune-mediated hemolytic anemia.

when agglutination subsided. Likewise, in the nonvaccine IMHA group, autoagglutination precluded the performance of a Coombs' test in 5 of 21 dogs. In the 16 remaining dogs with autoagglutination, a Coombs' test was performed in 1 to 3 days, when agglutination subsided. Thus, Coombs' test results were positive in all dogs tested (n = 12) in the vaccine IMHA group for IgG and 2 of the dogs were also positive for C_{3b}. In 34 of 39 dogs tested in the nonvaccine IMHA group, the Coombs' test results were positive for IgG; the 5 remaining dogs tested in this group had negative Coombs' test results, but presented with persistent autoagglutination. None of the dogs in this study had a positive Coombs' test for IgM.

In the nonvaccine IMHA group, 5 of 31 dogs tested had a positive antinuclear antibody (ANA) titer, although no other signs compatible with systemic lupus erythematosus were observed. In contrast, the ANA titers in all the dogs tested in the vaccine IMHA group were negative (n = 11). Forty-four dogs evaluated had no detectable serum antibody titers against *Ehrlichia canis*, Rocky Mountain spotted fever, and *Babesia canis*.

Follow-up time for survivors ranged from 92 to 750 days (mean, 290 days; median, 268 days). The mortality rate was similar in the vaccine IMHA group (60%) and the nonvaccine IMHA group (44%) ($P = .3$). In both IMHA groups most of the fatalities (>75%) were seen in the first 3 weeks after presentation (Fig 3). A higher mortality rate was associated with the presence of icterus ($P < .0001$). In the nonvaccine IMHA group, but not in the vaccine IMHA group, hyperbilirubinemia was associated with a higher mortality. The mean total bilirubin concentration of survivors and nonsurvivors in the nonvaccine IMHA group were 1.37 mg/dL and 7.32 mg/dL, respectively ($P < .01$). Autoagglutination was associated with a higher mortality in both IMHA groups ($p < .05$) (Table 2). The presence of intravascular hemolysis did not affect mortality in either IMHA group (Table 2).

All 58 dogs in the study had been previously vaccinated against canine distemper, adenovirus type 2, leptospirosis, parainfluenza, and parvovirus (DHLPP) and rabies virus. Dogs in the vaccine IMHA group had been given a lyophilized modified-live virus vaccine (DHPP), and in 14 of 15 cases, killed leptospira bacterin in the diluent, within the last month. Vaccine manufacturers included Fort Dodge Laboratories (Ft. Dodge, IA), Rhone Merieux (Athens, GA), SmithKline Beecham (Exton, PA), and Solvay (Mendota Heights, MN). No brand of vaccine appeared to be overrepresented. In addition, killed rabies virus vaccine (manufactured by Rhone Merieux or Solvay) was used simultaneously in 8 of the 15 dogs that were recently vaccinated. Killed borrelia bacterin, *Bordetella* bacterin, and coronavirus vaccine manufactured by Fort Dodge Laboratories were also administered to 3, 2, and 1 dogs, respectively.

Discussion

Vaccines have been implicated as a cause of IMHA in dogs, but little supportive evidence exists.[1,4,6-10,12,13] In this controlled retrospective study one fourth (26%) of dogs that had been diagnosed with idiopathic IMHA had been vaccinated within 1 month of onset of illness. Two thirds of the remaining dogs were seen within the next year and 13 dogs were seen 13 to 55 months later. There was a marked difference in the frequency of IMHA between the first month after vaccination and subsequent months ($P < .0001$), which was not seen in the control group. This temporal relationship strongly supports that vaccination can trigger IMHA in dogs.

A variety of adverse vaccine reactions have been reported but there are few surveys of these reactions in the literature.

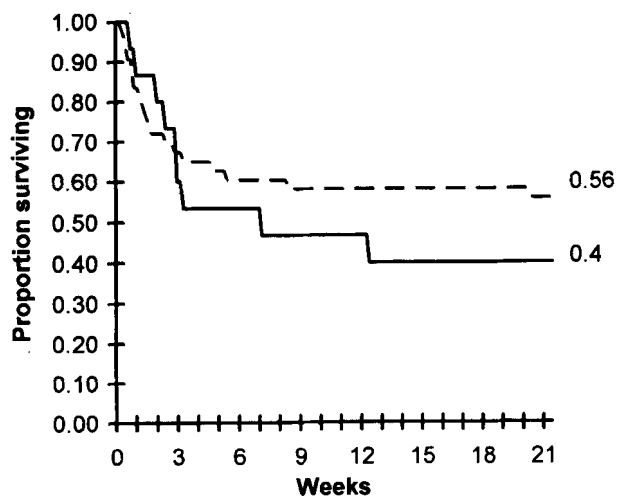

Fig 3. Actuarial survival curves in vaccine IMHA group (solid line) and nonvaccine IMHA group (broken line) of dogs over the first 21 weeks, $P = .3$.

Although reactions are frequently reported to vaccine manufacturers, these companies consider this to be proprietary information and are hesitant to release such data. Reactions can be caused by the chemical constituents of the vaccine or the immune reaction that they induce.[14] Vaccination can produce local swelling and inflammation acutely,[14] and with time, granulomatous inflammation.[14-16] In cats, vaccination has been associated with the development of fibrosarcomas.[17-19] The association between these reactions and vaccination is based on the location of the reaction (ie, at the vaccine site), as well as their temporal relationship.[15,17-19] Because vaccine components can remain in the body for extended periods of time,[14,16,20] chemical reactions caused by these vaccine components may continue to occur later than with other drugs that are excreted or metabolized more quickly. Immune reactions may occur immediately, such as urticaria or anaphylaxis, or be delayed over days to weeks.[14] Markers that define the relationship between immune reactions and vaccination have not been identified; therefore, these associations are based on their temporal relationship.[21]

Vaccines have been incriminated in triggering immune-mediated hematologic disease in people. Immune-mediated thrombocytopenia (IMTP) has been reported in people after vaccination against poliomyelitis, morbilli, rubella, typhus, smallpox, influenza, and pneumococcal infection.[22-25] Thrombocytopenia has developed after distemper vaccination in dogs.[26-28] Platelet counts decreased by approximately 100,000 cells/mm^3 within 3 to 7 days; the lowest platelet counts were seen at 7 days and returned to normal by 3 weeks.[26-28] Thrombocytopenia can also be seen within a similar time frame in naturally occurring distemper virus infection[26,27,29] and in the closely related human measles (morbilli) virus infection.[25,30]

Four mechanisms by which vaccines can induce IMTP have been proposed, but direct evidence is lacking. First, the vaccine virus may inhibit thrombocytopoesis at the megakaryocyte stage.[23,27,28] However, in people, bone marrow aspirates showed increased megakaryocyte numbers, and blood smears revealed enlarged platelets suggestive of accelerated thrombopoiesis in these cases.[22-25] Second, vaccine components may attach to or alter the platelet membrane and elicit an immune response.[2,6] Surface antigens or cross-reactivity with platelets and vaccine components have not yet been demonstrated. In a similar fashion, vaccine components may combine with proteins to act as haptens and invoke immune complex formation.[29,31] This appears to be the case in distemper-induced thrombocytopenia in experimentally infected dogs, although the specificity of the platelet-bound antibodies was not determined.[29] Fourth, vaccination may alter the immune response so that normal platelets are removed by an overactive immune system.[2,6,23] Injection with pneumococcal vaccine antigens in mice triggers a polyclonal B-lymphocyte stimulation that can result in IMTP similar to that seen in sepsis-induced IMTP.[32] Experimentally, immune modulatory drugs such as levamisole may reduce the prevalence of the thrombocytopenia induced after vaccination for distemper virus in dogs.[26,27] Further studies are needed to elucidate the cause of vaccine-induced IMTP.

Vaccine-associated IMHA has been reported after diphtheria-pertussis-tetanus vaccination in children.[12] Similar mechanisms are proposed for vaccine-induced IMHA as for IMTP. Surface erythrocyte binding and agglutination were seen when diphtheria and tetanus portions of the vaccine were added to erythrocytes from healthy people and patients with vaccine-associated IMHA.[12] Immunoglobulins eluted off erythrocytes from these patients had antigen specificity for the various components of the diphtheria-pertussis-tetanus vaccine.[12] These findings may support an immune-mediated model, with antibodies formed against erythrocyte membrane-attached vaccine components. In a guinea pig model of IMHA, antibody-coated erythrocyte removal from the circulation was markedly accelerated when the guinea pig's immune system was previously stimulated with bacillus Calmette-Guerin.[33] This evidence suggests that immune stimulation and macrophage activation, such as might be induced by vaccination, could exacerbate pre-existing IMHA.

It is not known whether the vaccine components induced autoantibody production in the dogs in this study, or whether the macrophages or the immune system were activated by vaccination to destroy red blood cells with pre-existing antibodies on the erythrocyte surface. In drug-induced IMHA, drugs can induce autoantibodies, or can induce antibody production against red blood cells that may require the involvement of the drug in the antibody-erythrocyte binding.[31,34] Although vaccine components are given in small quantities, they contain relatively large amounts of immune reactive antigens and other components that may remain in the body for extended periods of time. Therefore, vaccination can elicit an immune response days to weeks after inoculation. Drug-induced IMHA resolves with drug withdrawal and has a good prognosis.[31,34] Vaccine-associated IMHA, in contrast, has a mortality rate and clinical course more closely resembling idiopathic IMHA.[4]

The vaccines reported in this study are commonly used canine vaccines (DHLPP, rabies, *Borrelia*, *Bordetella*, and coronavirus). They included modified-live viruses, killed viruses, and killed bacterins. Any of these components, as well as the adjuvants used in these vaccines, may stimulate or disrupt the function of the immune system or elicit increased antibody production that could lead to IMHA. Each year hundreds of thousands of dogs are vaccinated with these products. The overall adverse reaction rate reported by manufacturers for canine vaccines is approximately 0.002%. Estimates from the vaccine manufacturers suggest that less than 5% of these reactions involve immune-mediated disease such as IMHA (personal communications: Hall J, Fort Dodge Animal Health Corp, 1995; Klink B, Rhone Merieux Inc, 1995). This would place the reported prevalence of vaccine-induced IMHA at less than 0.0001% or 1 per 1 million vaccinated dogs. However, because not all cases are reported to the manufacturers (none of the cases in this retrospective study had been reported) and the association between IMHA and recent vaccination may not have been considered in clinical practice, the prevalence of vaccine-associated IMHA is likely to be underestimated. The results of the present study should alert clinicians to this association and prompt notification of such cases to vaccine manufacturers.

Effective and safe vaccination is an important part of preventative health care for dogs and people, since the risks of contracting infectious diseases are much higher than those of vaccine-associated problems, including IMHA. However, the currently available vaccines for dogs and cats may be capable of providing long-lasting immunity, and the need for yearly vaccination in healthy adult animals has been recently questioned.[16,35] Reducing the frequency of vaccination or the number of vaccine components may decrease the incidence of vaccine-induced IMHA in susceptible dogs. Dogs that have had idiopathic IMHA or vaccine-induced IMHA may be predisposed to relapse if subsequent vaccinations are given.[2,4] In humans with IMTP in remission, vaccination with pneumococcal or influenza vaccines has triggered relapses of their disease.[23,24] Careful monitoring of platelet counts is recommended if follow-up vaccination is attempted in people with vaccine-induced IMTP.[24,25] More studies will be required to determine the role of vaccination in inducing relapse of IMTP or IMHA in dogs. Recommendations for vaccinating dogs with IMHA or IMTP should consider the relative risk of vaccine reactions versus developing an infectious disease. Individual circumstances may vary, and breed, age, gender, immune status, and risk of disease exposure must be considered. Pertinent legal issues, such as exist with rabies virus vaccination, must also be considered in formulating a vaccination plan. A genetic predisposition to develop immune-mediated disease has been reported within related groups of dogs.[2,4,6,8,28] In the present study, the number of dogs in the vaccine IMHA group is too small to document or discount any genetic predisposition, but it may be prudent to evaluate related dogs for immune-mediated diseases and consider this factor when vaccinating healthy relatives of affected dogs. The American Cocker Spaniel has been over-represented in previous studies of idiopathic IMHA in dogs.[1,2,6,10]

The clinical course of disease in the vaccine IMHA group was similar to the dogs in the nonvaccine IMHA group. Indeed, only retrospectively were these dogs identified as being recently vaccinated and the temporal association made with IMHA. A seasonal prevalence was observed, with the peak incidence of cases seen in the fall. The seasonal pattern in this report could not be accounted for by variations in hospital admissions or month of vaccination (data not shown). A previous report of a canine population at VHUP (42 cases over a 60-month period) reported May and June to be the most common presentation months for idiopathic IMHA, with 60% of cases seen in those 2 months.[1] The significance of the difference in seasonal distribution of these 2 studies is unknown. The cause of the apparent increase in the prevalence of IMHA seen since this earlier report is also unclear. Improved record-keeping or reporting, increased awareness and referral, or an actual increased incidence of IMHA may all have contributed to the greater number of cases in the present study. Because vaccination histories were not available or not obtained in the previous study, only 1 of the 42 cases could be related to recent vaccination.[1]

The nonvaccine IMHA group was similar to other reported groups of idiopathic IMHA in dogs. As previously reported,[1] the presence of icterus and hyperbilirubinemia indicated a poorer prognosis in the present study. Autoagglutination at presentation was correlated with higher mortality in both IMHA groups, which is in agreement with previous studies on idiopathic IMHA.[1,5] Icterus, intravascular hemolysis, and autoagglutination were common in the vaccine IMHA group (see Table 1). The vaccine IMHA group had slightly higher mortality (60%) than the nonvaccine IMHA group (44%), although this difference was not statistically significant ($P = .3$).

This report is the first to document and define the temporal association between vaccination and IMHA in dogs. Whether pre-existing IMHA was accelerated or IMHA was indeed caused by the vaccination in these dogs is unknown. It is important that clinicians obtain an accurate vaccine history on all dogs presenting for IMHA or IMTP. Careful reconsideration of vaccine protocols in patients with IMHA or IMTP, as well as in healthy adult animals, may also be warranted.

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