Serum antibodies against human albumin in critically ill and healthy dogs

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Objective—To characterize the magnitude and duration of the antibody response against human albumin (HA) in critically ill and healthy dogs.

Design—Cohort and cross-sectional study.

Animals—Fourteen critically ill dogs that received 25% HA as part of their treatment protocol, 2 healthy dogs with no known previous exposure to HA that received 2 infusions of 25% HA (positive control dogs), and 47 healthy dogs and 21 critically ill dogs with no known exposure to HA (negative control dogs).

Procedures—An ELISA to detect IgG against HA was developed. Serum samples were obtained from the critically ill dogs prior to infusion of HA, at the time of hospital discharge, and 4 to 6 weeks and 6 months after HA administration. Serum samples were obtained at 2- to 4-week intervals from both positive control dogs for 101 weeks. A single serum sample was obtained from each of the negative control dogs.

Results—All 14 critically ill dogs developed serum IgG against HA. Peak antibody response was detected 4 to 6 weeks after HA administration. In both positive control dogs, IgG against HA was detected 10 days after HA administration and continued past 97 weeks. The peak antibody response was detected at 3 weeks in 1 dog and at 9 weeks in the other. Five of the 68 (7%) negative control dogs had a positive antibody response.

Conclusions and Clinical Relevance—Results suggested that dogs developed a pronounced IgG response following exposure to HA and that some dogs with no history of HA administration were positive for anti-HA IgG. (*J Am Vet Med Assoc* 2008;232:1004–1009)

Albumin is one of the most important proteins in the body because of its role in maintenance of colloid oncotic pressure, substrate transport, buffering capacity, and free-radical scavenging and as a mediator of coagulation and wound healing.[1–3] Hypoalbuminemia is a common consequence of many critical illnesses, including sepsis, systemic inflammatory response syndrome, severe thermal injury, end-stage hepatic failure, protein-losing enteropathy, and nephrotic syndrome.[1–3] The ensuing hypoalbuminemia can result in life-threatening complications such as systemic organ dysfunction, pulmonary edema, poor wound healing, and hypercoaguability.[1–8] Furthermore, hypoalbuminemia has been associated with high morbidity and mortality rates in human and veterinary patients.[9–13]

Because of the real and potential clinical problems associated with hypoalbuminemia, albumin supplementation has become a frequently used component of the treatment regimen for critically ill dogs. However, concentrated canine albumin solutions are not commercially available at this time. As a result, plasma transfusion is currently the only available method for providing species-specific albumin in dogs.[1,2] Unfortunately, use of plasma transfusions alone to increase albumin concentration is expensive and frequently not feasible because of inadequate supplies. For these reasons, the use of commercially available pharmaceutical-grade concentrated HA solutions in veterinary patients has been investigated. Small case series[14,a–d] have reported the successful use of 1-time and repeated administration of HA in dogs, cats, and horses. However, acute hypersensitivity reactions, typically facial edema, vomiting, and fever, have been observed in clinically ill veterinary patients,[14,b] and acute hypersensitivity reactions, type III hypersensitivity reactions, and anaphylactic reactions have been observed in healthy dogs given HA or BA.[15,16,e]

Comparison of the amino acid sequences of canine and human albumin reveals only 79.3% homology,[17] raising concerns about the antigenicity of HA when administered to dogs. The differences in amino acid sequence result in differences in molecular weight, relative charge, and isoelectric point.[f] Thus, it seems likely that at least some dogs will develop antibodies against unique epitopes on HA. However, information is lacking on the time frame over

Abbreviations

HA	Human albumin
BA	Bovine albumin
OD	Optical density

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which dogs would develop circulating antibodies against HA and the clinical importance of those antibodies. Therefore, the purpose of the study reported here was to characterize the magnitude and duration of the antibody response to HA in critically ill and healthy dogs. To assess the antibody response, we developed an ELISA to detect anti-HA IgG in canine serum.

Materials and Methods

Dogs—All aspects of the study were approved by the institutional animal care and use committees at Washington State University and at the Animal Emergency Hospital and Referral Center in Leesburg, Va. Fourteen critically ill, client-owned dogs admitted to the intensive care unit at Washington State University or the Animal Emergency Hospital and Referral Center that received 25% HA as part of their treatment protocol were enrolled in the study; all dogs were > 1 year old.

Two healthy purpose-bred dogs (a 26-month-old 25-kg [55-lb] spayed female hound mix and a 23-month-old 20-kg [44-lb] sexually intact female hound mix) with no known exposure to HA that received 2 infusions of 25% HA were enrolled in the study as positive control dogs. Positive control dogs were considered to be healthy on the basis of results of a physical examination, CBC, serum biochemical analysis, and urinalysis and were housed in a routine manner in animal facilities at Washington State University, which was accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Finally, 47 healthy dogs and 21 critically ill dogs with no known exposure to HA were enrolled in the study as negative control dogs. The 47 healthy dogs were owned by faculty, students, and staff members at Washington State University and were considered to be healthy on the basis of history and results of a physical examination. The 21 critically ill dogs were privately owned and had been brought to Washington State University for treatment of a variety of illnesses. Owners of all dogs enrolled in the study provided their consent.

Experimental design and data collection—The 14 critically ill dogs had all been given 25% HA[g] at the discretion of the attending veterinarian. Dosage of HA and administration rate varied on the basis of each dog's perceived need for supplemental albumin and underlying disease process. In all instances, HA was administered IV via a peripheral or central venous catheter. Serum samples were obtained from these 14 dogs prior to infusion of HA, at the time of hospital discharge, and 4 to 6 weeks and 6 months after HA administration. All serum samples were frozen at –80°C until analyzed.

The 2 positive control dogs were given 25% HA solution[g] at a dosage of 2 mL/kg (0.91 mL/lb; 0.5 g/kg [0.23 g/lb]), IV, over 2 hours. Ten days later, they were given a second infusion at a dosage of 1 mL/kg (0.45 mL/lb; 0.25 g/kg [0.11 g/lb]), IV, over 1 hour. Both infusions were administered via a peripheral venous catheter. Prior to the second infusion, each dog was pretreated with diphenhydramine (1 mg/kg, IM). During each infusion, dogs were monitored every 15 minutes for any signs of adverse reactions. Body temperature, heart rate, and respiratory rate were monitored; pulmonary auscultation was performed; and dogs were examined for signs of pruritus, urticaria, muscle tremors, vomiting, and facial or peripheral edema. Serum samples were obtained before each of the 2 infusions, then every 2 to 4 weeks for 101 weeks. All serum samples were frozen at –80°C until analyzed.

A single serum sample was obtained from each of the negative control dogs. All serum samples were frozen at –80°C until analyzed.

Detection of serum anti-HA IgG—An ELISA was developed to detect anti-HA IgG in serum samples from dogs. In brief, HA[g] diluted in sodium bicarbonate buffer to a concentration of 5 ng/µL was added to wells on 96-well microtitration plates[h] (50 µL/well), and plates were incubated at room temperature for 30 minutes. Plates were then washed 4 times in phosphate-buffered saline solution containing 0.05% Tween, 5% nonfat dried milk was added to each well (150 µL/well) to block nonspecific binding, and plates were incubated overnight at 4°C. Serum samples were diluted 1:500 in phosphate-buffered saline solution containing 0.05% Tween and added in triplicate to wells on the plate (50 µL/well). Each plate also contained 5 dilutions of a positive control standard, 4 dilutions of a negative control standard, and control wells with serum only, HA only, and neither HA nor serum, each in duplicate. Plates were incubated for 30 minutes at room temperature and washed 4 times. Horseradish peroxidase–conjugated goat anti-dog IgG antibody[i] diluted 1:2,000 in 5% nonfat dried milk was added to the wells (50 µL/well), and plates were incubated for 30 minutes at room temperature, then washed 4 times. Bound antibodies were detected by adding a peroxidase substrate[j] to each well (100 µL/well) and incubating plates for 10 minutes. Color development was stopped by adding stop solution[k] (100 µL/well), and OD of each well was read with a photometer[l] at a wavelength of 450 nm. Checkerboard titrations were performed to optimize the signal-to-noise ratio. Assay results were validated by comparison with results of western blotting performed on select serum samples. On the basis of results for the negative control dogs, the threshold value for a positive ELISA result was determined to be an OD value of 0.385. This value was calculated as mean OD plus 3 times the SD for the negative control dogs, after exclusion of outlier values, defined as those OD values outside the 90th percentile.

Intradermal testing—Intradermal testing was conducted 97 weeks after the initial HA infusion in one of the positive control dogs. The other positive control dog was no longer available for this procedure. A third, healthy, purpose-bred dog with no known history of exposure to HA was used as a negative control dog for the intradermal testing. Physiologic saline solution was used as the negative control solution, and histamine[m] diluted in saline solution to a concentration of 5 µg/mL was used as the positive control solution. Three dilutions of 25% HA (50, 5, and 0.5 mg/mL) were used. Dogs were placed in lateral recumbency, the lateral aspect of the thorax was clipped of hair, and 0.05 mL of each solution was injected ID, resulting in HA doses of 2.5 mg, 250 µg, and 25 µg, respectively. Diameter of the wheal reactions was measured 15 and 30 minutes after ID injections.

Statistical analysis—Data were summarized as median and range. The Kruskal-Wallis comparison of mean ranks test was used to compare baseline OD values (ie, values obtained prior to HA administration) for the 14 critically ill dogs that received HA to values for the negative control dogs with no known exposure to HA. For the critically ill dogs, paired *t* tests were used to compare baseline OD values (n = 14) with values obtained at the time of hospital discharge (12), 4 to 6 weeks after HA administration (10), and 6 months after HA administration (8). Because of the multiple comparisons, a critical α value of 0.017 was used. For each positive control dog, the 2-sample *t* test was used to compare OD values from various time points. Except as noted, values of $P \leq 0.05$ were considered significant. All statistical analyses were performed with standard software.[n]

Results

Critically ill dogs—Of the 14 critically ill dogs that received HA as part of their treatment protocol, 7 were admitted because of primary gastrointestinal tract disorders (pancreatitis, gastric dilatation-volvulus, protein-losing enteropathy, sustained diarrhea, hemorrhagic diarrhea, ruptured cecal mass, and metallic foreign body), 4 were admitted because of traumatic injuries (2 were hit by motor vehicles, 1 fell from a moving motor vehicle, and 1 was shot with a pellet gun), and 3 were admitted because of other disorders (dehiscence of a thoracotomy incision; presumed vasculitis; and acute hypersensitivity characterized by fever, generalized erythema, and facial and peripheral edema). All 14 dogs were given HA because of hypoalbuminemia. Additional reasons for HA administration included pleural or abdominal effusion (3 dogs), facial or peripheral edema (3 dogs), and hypotension (1 dog). Twelve of the 14 dogs survived to hospital discharge, 10 survived at least 6 weeks after HA administration, and 8 survived at least 6 months after HA administration.

Three of the 14 dogs were Labrador Retrievers, and 3 were German Shepherd Dogs; the remaining 8 dogs represented a variety of breeds. Median age was 7.0 years (range, 1.4 to 10.6 years), and median weight was 30.4 kg (66.9 lb; range, 1.3 to 55 kg [2.9 to 121 lb]). There was 1 sexually intact female, 6 neutered females, 1 sexually intact male, and 6 neutered male dogs. Median dose of 25% HA was 5.2 mL/kg (2.36 mL/lb; range, 1.8 to 47.1 mL/kg [0.82 to 21.41 mL/lb]); median administration rate was 0.8 mL/kg/h (0.36 mL/lb/h; range, 0.2 to 1.8 mL/kg/h [0.09 to 0.82 mL/lb/h]). Median hospitalization time was 7.5 days (range, 2 to 20 days). Two of the 14 dogs received multiple HA infusions (median time between infusions, 20 hours; range, 16 to 24 hours), and 4 received a continuous infusion over multiple days (median duration of continuous HA infusion, 5.5 days; range, 2 to 12 days). A transient fever was identified during the initial infusion in 1 dog but not during the second infusion. Otherwise, no acute hypersensitivity reactions were observed. No type III hypersensitivity reactions were reported in the 12 dogs that survived > 7 days after HA administration.

Median OD value for the 14 critically ill dogs prior to HA administration was 0.053 (range, 0.042 to 0.149), and median OD values at the time of hospital discharge, 4 to 6 weeks after HA administration, and 6 months after HA administration were 0.107 (range, 0.043 to 2.605), 2.227 (range, 0.166 to 3.210), and 0.589 (range, 0.150 to 0.955), respectively (**Figure 1**). Values obtained at the time of hospital discharge were not significantly ($P = 0.093$) different from baseline values; however, values obtained 4 to 6 weeks after HA administration ($P < 0.001$) and 6 months after HA administration ($P = 0.001$) were significantly higher than baseline values.

Positive control dogs—No acute hypersensitivity reactions were seen during the first administration of HA in either of the positive control dogs. However, 1 dog developed facial edema and had a poor appetite 8 days later. The edema resolved after administration of diphenhydramine (1 mg/kg, SC, once), and the dog's appetite subsequently improved. Despite pretreatment with diphenhydramine, both dogs developed erythema, vomiting, and diarrhea during the second infusion of HA; however, body temperature, heart rate, and respiration rate were within reference limits. The adverse reactions resolved after the infusion was completed. Both dogs were given a second dose of diphenhydramine (1 mg/kg, IM) and fluids SC after the second HA infusion. No additional adverse events were identified in either dog.

For the 2 positive control dogs, median OD values were 0.083 and 0.111 prior to HA administration and were 0.975 and 1.308 ten days after administration of the first dose of HA (**Figure 2**). Values peaked 3 weeks after administration of the first dose of HA in 1 dog and 9 weeks after administration of the first dose of HA in the other. For one of the dogs, OD value at the end of the study period (97 weeks) was significantly ($P = 0.004$) higher than the value obtained prior to HA administration (0.266 and 0.111, respectively). Similarly, for the other dog, OD value at the end of the study period

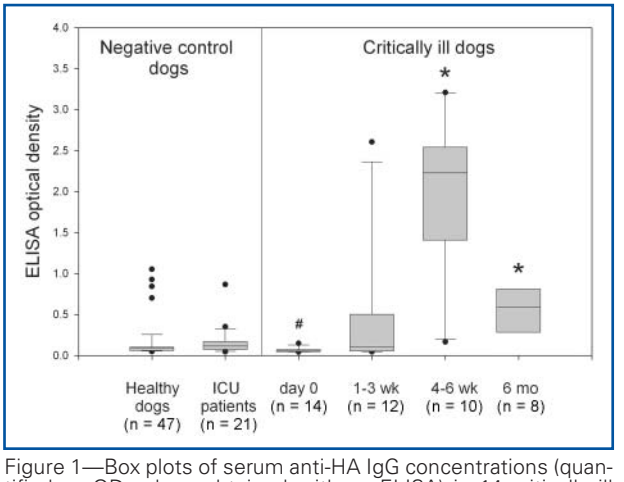


Figure 1—Box plots of serum anti-HA IgG concentrations (quantified as OD values obtained with an ELISA) in 14 critically ill dogs before (day 0) and after IV infusion of 25% HA and in 2 populations of negative control dogs with no known exposure to HA. For each plot, the box represents the interquartile (ie, 25th to 75th percentile) range, the horizontal line within each box represents the median, the bars represent the 10th and 90th percentiles, and the black circles represent outlying values. *Significantly ($P < 0.017$) different from the baseline (day 0) value. #Significantly ($P < 0.05$) different from values for both groups of negative control dogs.

(101 weeks) was significantly (P = 0.006) higher than the value obtained prior to HA administration (0.305 and 0.083, respectively). One of the dogs had a brief increase in OD values beginning 77 weeks after the first HA infusion; the dog had received a rabies vaccine and distemper combination vaccine 2 weeks earlier.

The positive control dog had positive responses following ID injection of all 3 dilutions of HA (50, 5, and 0.5 mg/mL), with wheal diameters of 10, 7, and 6 mm, respectively, 15 minutes after HA injection and wheal diameters of 11, 6, and 5 mm, respectively, 30 minutes after HA injection. By contrast, the wheal diameters were 11 mm 15 minutes after injection of histamine and 10 mm 30 minutes after injection of histamine. The negative control dog had a positive response to injection of histamine but did not have any response to injection of saline solution or any of the 3 dilutions of HA. In both dogs, OD values for serum anti-HA IgG were substantially increased for several weeks after intradermal testing (**Figure 3**).

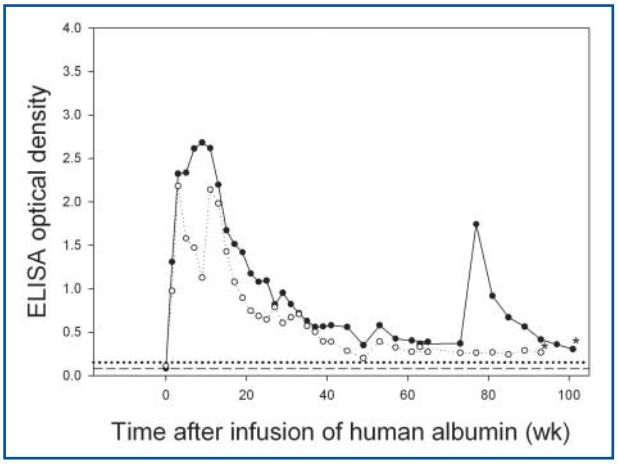


Figure 2—Serum anti-HA IgG concentrations in 2 healthy dogs given IV infusions of 25% HA on days 0 and 10. The dashed line represents mean serum anti-HA IgG concentration in a reference population of dogs without any exposure to HA; the dotted line represents the mean concentration plus 3 SD. *Significantly (P < 0.05) different from the baseline (week 0) value.

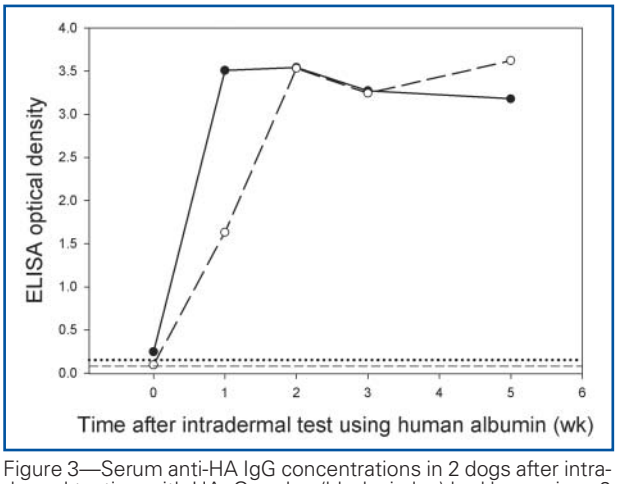


Figure 3—Serum anti-HA IgG concentrations in 2 dogs after intradermal testing with HA. One dog (black circles) had been given 2 infusions of HA 97 weeks earlier; the other (white circles) had no history of exposure to HA. *See* Figure 2 for key.

Negative control dogs—Median age of the negative control dogs was 6.3 years (range, 1.2 to 15.6 years), and median body weight was 28.6 kg (62.9 lb; range, 3.6 to 45 kg [7.9 to 99 lb]). There were 5 Golden Retrievers, 4 Boxers, 4 Greyhounds, 4 German Shepherd Dogs, and 3 Australian Cattle Dogs; the remaining dogs represented a variety of breeds. There was 1 sexually intact female, 34 neutered females, 3 sexually intact males, and 30 neutered males. The critically ill negative control dogs had been admitted to the intensive care unit for a variety of reasons, including gastric dilatation-volvulus, coagulopathy, trauma, and seizures.

Median OD value for the 68 negative control dogs was 0.087 (range, 0.046 to 1.048; Figure 1). Four of the 47 healthy dogs and 1 of the 21 critically ill dogs had outlier values (ie, values > the 90th percentile). Median OD value for the 5 dogs with outlier values was 0.865 (range, 0.699 to 1.048). Median OD value for the 47 healthy negative control dogs was not significantly different from median OD value for the 21 critically ill negative control dogs; however, median OD value prior to HA administration in the 14 critically ill dogs that received HA was significantly lower than median OD value for either of the negative control dog groups.

Discussion

Results of the present study indicated that both critically ill and healthy dogs developed an IgG immune response following administration of HA. We were able to verify development of anti-HA antibodies in all 14 critically ill dogs enrolled in the present study. Antibody concentrations, determined on the basis of OD values obtained with an ELISA for anti-HA IgG, were highest 4 to 6 weeks after HA administration in these dogs and were still high 6 months after HA administration. We also found high IgG concentrations in the 2 healthy positive control dogs beginning 10 days after and persisting up to 101 weeks after the initial infusion of HA. Antibody concentrations peaked 3 weeks after HA administration in one of these dogs and 9 weeks after HA administration in the other. These results were similar to results of a previous study[16] of the clinical and immunologic responses of 9 healthy dogs to HA infusion. In that study, anti-HA antibodies could be detected 7 days after HA administration and appeared to peak 14 days after HA administration. Development of anti-HA antibodies was also documented in a study[15] in which 6 healthy dogs were given HA to examine the effects of various fluid therapy solutions on coagulation parameters, hemodilution, and colloid oncotic pressure. All 6 dogs had high concentrations of anti-HA antibodies shortly after administration of HA, with antibody concentrations peaking 2 to 4 weeks after HA administration and 4 dogs still having substantial concentrations 8 to 13 weeks after HA administration.

In the present study, the critically ill patients that received HA had lower antibody concentrations prior to administration of HA than did the healthy or critically ill negative control dogs. The reason for this difference was difficult to explain but may have been attributable to the disease processes that caused hypoalbuminemia in the critically ill patients. Case reports[18–21] have doc-

umented concurrent hypoalbuminemia and hypogammaglobulinemia in human patients with lymphangiectasia, reflux esophagitis, and histiocytic sarcoma of the spleen. The exact pathophysiologic mechanisms of this phenomenon are unknown, but for patients with gastrointestinal tract disease, it has been speculated that albumin and immunoglobulins are lost through the gastrointestinal tract.[18,19] None of the critically ill dogs in the present study had lymphangiectasia, reflux esophagitis, or histiocytic sarcoma of the spleen; however, 7 of the 14 critically ill dogs were admitted to the intensive care unit for treatment of primary gastrointestinal tract disorders, including 1 dog with protein-losing enteropathy and 2 dogs with diarrhea.

Results of intradermal testing in the present study also suggested that HA induces a prolonged IgE immune response. Even though we did not specifically test serum samples for anti-HA IgE concentrations, the positive intradermal test results in 1 dog 97 weeks after HA administration were suggestive of specific anti-HA IgE antibody production. In a previous study,[16] similar intradermal responses were seen 5 to 10 weeks after HA administration in 8 of 9 healthy dogs. An IgE immune response to HA could explain some of the adverse reactions (edema, urticaria, circulatory collapse, vomiting, and diarrhea) to HA that have been described previously.[14–16,b]

The substantial increases in serum IgG antibody concentrations following intradermal testing in the 2 dogs in the present study suggested that intradermal administration of a very small volume of HA could stimulate a strong serum IgG response in a dog that had not been previously exposed to HA and could stimulate an anamnestic response in a dog that had been previously exposed.

Five of the 68 negative control dogs in the present study were classified as positive for anti-HA IgG on the basis of OD values obtained with the ELISA for anti-HA IgG. It seems unlikely that the high IgG concentrations in these dogs were a result of laboratory error, in that all serum samples were tested in triplicate and testing of additional serum samples from these dogs again resulted in high measured concentrations. Possibly, the high anti-HA antibody concentrations in these dogs represented a cross-reaction with anti-BA antibodies that developed when dogs were exposed to BA through vaccination or ingestion. Fetal bovine serum is used as a nutrient source for cell cultures used in the manufacture of canine vaccines, and large amounts of BA and bovine IgG have been shown to be included in monovalent and multivalent vaccines for dogs.[22] In another study,[23] inoculation of dogs with rabies vaccine was found to induce transient increases in total IgE and specific anti-BA IgE concentrations, whereas inoculation of dogs with a multivalent vaccine induced only a slight increase in total IgE concentration. A possible link between vaccination and anti-HA antibodies was also suggested by the abrupt increase in anti-HA IgG concentration 2 weeks after vaccination of one of the healthy positive control dogs in the present study.

It is also possible that dogs with defective mucosal barrier function may become sensitized to BA following ingestion. In a study[24] of serum IgE and IgG responses to food antigens in healthy dogs, atopic dogs, and dogs with gastrointestinal tract disease, it was found that dogs with gastrointestinal tract disease had significantly higher concentrations of IgG against various food antigens, including beef, than did dogs in the other 2 groups. The authors theorized that this may reflect increased antigen exposure secondary to increased mucosal permeability, a commonly recognized feature of gastrointestinal tract disease in dogs. It is also possible that some dogs may have become sensitized to HA as a result of ingestion of human blood secondary to biting a person. However, none of the negative control dogs with outlier values in the present study had clinical signs consistent with gastrointestinal tract disease or had any history of biting a person.

Adverse reactions are an important concern with any treatment. The therapeutic use of HA in dogs is a relatively recent development, and the efficacy of and adverse effects associated with HA have not yet been fully elucidated or quantified. There appears to be a dichotomy in the type and severity of adverse reactions to HA between healthy and critically ill dogs,[14–16,a–c] and a similar dichotomy was seen in the present study. The reasons for this dichotomy are unclear but may involve immunocompetence and the fact that healthy dogs have normal serum albumin concentrations.[15,16] Results of the present study suggest that HA is highly antigenic in dogs and poses a risk if given more than once. Even if HA has never been administered previously, it may still pose a risk if given to dogs previously sensitized to albumin. Thus, when considering administering HA to dogs, the potential benefits of HA administration should be carefully weighed against the risks of adverse reactions.

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j. SureBlue TMB, Kirkegaard & Perry Labs Inc, Gaithersburg, Md.
k. TMB Stop Solution, Kirkegaard & Perry Labs Inc, Gaithersburg, Md.
l. Multiskan EX, Labsystems, Helsinki, Finland.
m. Histatrol, Center Laboratories, Port Washington, NY.
n. Statistix, version 7.0, Analytical Software, Tallahassee, Fla.

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