The ARs are some of the most common toxicants that are ingested by dogs. They result in life-threatening coagulopathy by antagonizing vitamin K epoxide reductase in the liver. This enzyme is required to reduce vitamin K epoxide back to active vitamin K. Without appropriate recycling of vitamin K, factors II, VII, IX, and X and the anticoagulant proteins C and S are unable to be carboxylated, a necessary step for them to become active factors. As plasma levels of these factors are depleted, coagulopathy will develop unless vitamin K is supplemented.

Anticoagulant rodenticide screening is a valuable tool for confirming the diagnosis of AR intoxication in cases where ingestion was not witnessed. It is exceptionally valuable in cases in which owners are adamant that there has been no possible exposure to these toxicants. The clinical signs of AR are vague and usually consist of lethargy, anorexia, dyspnea, hemoptysis, tachycardia, poor pulses, pale mucous membranes, and collapse secondary to bleeding. Thoracic auscultation may reveal dull lung sounds if there is a pleural effusion and dull heart sounds if pericardial effusion is present. These signs are consistent with bleeding from any cause of coagulopathy. When patients are initially examined with severe coagulopathies, the differential diagnoses that should be considered include AR intoxication, DIC secondary to neoplasia or other disease processes including angiostrongylosis, heat stroke, sepsis or systemic inflammatory response syndrome.
hemophilia or other hereditary coagulation disorders, liver failure, or severe autoimmune disease such as immune-mediated thrombocytopenia. Anticoagulant rodenticide intoxication is generally regarded as having the best prognosis of these disease processes, making correct diagnosis and treatment essential.

The purpose of the study reported here was to evaluate the use of AR screening in dogs, determine the frequency of positive results, identify any correlation between prolongation of PT and aPTT and concentration and type of AR, and evaluate the prognostic value of both positive and negative results for AR screening. The final diagnoses and prognosis for dogs with negative results for AR screenings were also evaluated.

Materials and Methods

Criteria for selection of cases—Medical records of all dogs that were examined at the Matthew J. Ryan Veterinary Hospital of the University of Pennsylvania that had serum or whole blood samples submitted antemortem for AR screening from January 1996 through January 2003 were evaluated.

Medical records review—Data extracted from the medical records included signalment, historical information, possible or known exposure to AR, initial clinical signs, physical examination findings, PCV and TS, PT, aPTT, platelet count, toxicology screening results, number of days hospitalized, type and amount of blood products administered, final diagnosis, and outcome.

Sample analysis for AR screening—Whole blood or serum samples were analyzed for brodifacoum, bromadiolone, chlorophacinone, coumachryl, dicoumarol, difenacoum, diphacinone, pindone, valone, and warfarin by means of high-performance liquid chromatography. Briefly, 4 mL of acetonitrile was added to 2 mL of serum in a 16 X 100-mm glass disposable culture tube. The tube was vortexed to mix the sample and solvent and then centrifuged for 5 minutes at 700 X g (approx 1,200 rpm). The supernatant was collected from the column, followed by a 4-mL acetonitrile wash. The initial effluent and subsequent wash were combined and evaporated under a stream of nitrogen at 55°C. The residue was redissolved in 500 µL of ion pair reagent (19.2 mM tetrabutylammonium hydroxide [pH, 7] in 80:20 methanol/water), filtered through a 45-µm filter, and injected into the high-performance liquid chromatography system equipped with UV (wave length, 280 nm) and fluorescent detectors (excitation, 280 nm; emission, 410 nm). Stock solutions for each anticoagulant standard were prepared in acetonitrile at 1,000 ppm concentrations, stored at −5°C, and protected from light. Working standard mixtures were prepared in the range of 0.010 to 1.0 µg/mL by dilution of stock solutions in an ion pair reagent. The LOQ were 0.01 ppm for brodifacoum, 0.02 ppm for bromadiolone and difenacoum, and 0.10 ppm for the others. A test was considered positive if an AR anticoagulant was detected below the LOQ but could not be quantified (defined as a trace concentration); all other samples were considered negative.

Statistical analysis—All statistical analyses were performed with a commercially available software program. For descriptive purposes, continuous variables are expressed as mean ± SD or median and range, depending on whether the data were normally or not normally distributed, respectively. The Shapiro-Wilk test was used to determine whether the variables had a normal distribution. Categorical variables are expressed as a percentage of the total.

Comparisons between groups with normally distributed continuous or ordinal variables were made via the Wilcoxon rank sum test, whereas comparisons between groups with normally distributed continuous variables were made via the unpaired t test. Proportions between groups for dichotomous variables were compared with the Pearson χ² test if the expected count was > 5 in any cell of the table or the Fisher exact test if the expected count was ≤ 5 in any cell of the table. Values of P < 0.05 were considered significant. Finally, correlations between PT and aPTT with rodenticide concentrations were performed by means of the Pearson correlation or Spearman correlation, depending on whether the variables were normally or not normally distributed, respectively.

Results

One hundred twenty-three dogs that had results from AR screenings submitted antemortem were identified in the medical records. All were included in this study. Seventy-five (61%) dogs were positive (positive group) and 48 (39%) dogs were negative (negative group) for AR.

There were 24 (50%) males (15 sexually intact) and 24 (50%) females (9 sexually intact) in the negative group, and 29 (39%) males (13 sexually intact) and 46 (61%) females (13 sexually intact) in the positive group. The median age of the negative group was 4 years (range, 0.08 to 14 years) and 3.5 years (range, 0.3 to 11 years) for the positive group. Overall, several breeds were represented, including 22 mixed breeds, 20 Labrador Retrievers, 7 Bichon Frise, 7 Cocker Spaniels, 6 American Pitbull Terriers, 5 Lhasa Apsos, 5 Jack Russell Terriers, 4 Golden Retrievers, 3 Dachshunds, 3 German Shepherds, 2 Miniature Pinchers, 2 Dobermans, 2 Shih Tzus, 2 Yorkshire Terriers, 2 Chihuahuas, 2 English Bulldogs, and 1 each of 24 other breeds. There was no significant difference between groups for sex, age, or breed.

A known ingestion or possible exposure to AR was noted in 60 of 123 (48.8%) dogs. For the negative group, 33 of 48 (72.9%) had no known exposure to AR, whereas 12 of 48 (25%) had a possible exposure. No exposure history was recorded in the medical records for 1 of 48. For dogs in the positive group, 21 of 75 (28%) had no known exposure, whereas 49 of 75 (65.3%) had a known or possible exposure. No exposure history was recorded in 5 of 75 (6.7%). Dogs in the positive group were more likely to have a known or possible exposure.

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than dogs in the negative group ($P < 0.001$). Four of 75 (5.3%) dogs in the positive group received vitamin K$_\text{a}$ at their referring veterinary hospitals prior to initial examination because of the high suspicion of AR exposure and clinical evidence of hemorrhage.

Initial clinical signs were recorded in 121 dogs. The most frequently reported conditions included lethargy or collapse (73/121 [60.3%]), anorexia or decreased appetite (49/121 [40.5%]), vomiting (31/121 [25.6%]), dyspnea (25/121 [20.7%]), and cough (22/121 [18.2%]). There was no difference between positive and negative groups for any initial clinical sign.

Median rectal temperature, heart rate, and respiratory rate were not significantly different between the 2 groups (Table 1). Median weight was significantly ($P = 0.008$) different between the 2 groups, with the positive group weighing less (13.9 kg [30.6 lb]; $n = 73$; range, 2.7 to 99) than the negative group (27.4 kg [60.3 lb]; $n = 47$; range, 1.8 to 76).

The median PCV, TS, platelet count, and aPTT were not significantly different between the 2 groups (Table 2). Five dogs in the positive group had severely decreased platelet counts ($\leq 20,000/\mu$L) and were anemic (PCV range, 12% to 27%). These dogs had physical evidence of severe hemorrhaging, including extensive bruising, pale mucous membranes, tachycardia, and hypothermia. The median PT of the positive group (52.3 seconds; $n = 49$; range, 7.1 to >100 seconds) was significantly ($P < 0.001$) more prolonged, compared with that of the negative group (12.8 seconds; $n = 36$; range, 6.3 to >100 seconds).

A significantly ($P = 0.023$) greater volume of FFP (18.8 mL/kg [8.5 mL/lb]; $n = 73$; range, 0 to 68.5 mL/kg [0 to 31.1 mL/lb]) was administered to the positive group, compared with that given to the negative group (15.0 mL/kg [6.8 mL/lb]; $n = 46$; range, 0 to 88.7 mL/kg [0 to 40.3 mL/lb]); conversely, there was no significant ($P = 0.739$) difference in the amount of packed RBCs administered to the positive group (10.9 mL/kg [5.0 mL/lb]; $n = 73$; SD, 13.5 mL/kg [6.1 mL/lb]), compared with that given to the negative group (12.7 mL/kg [5.8 mL/lb]; $n = 46$; SD, 17.1 mL/kg [7.8 mL/lb]).

Seventy-five of the 123 (61%) dogs were positive for 1 or more ARs. Of those with positive screening results, 60 of 75 (80%) were positive for brodifacoum, 14 of 75 (18.7%) were positive for difaphacinone, and 2 of 75 (2.7%) were positive for chlorophacinone. One dog was positive for both brodifacoum and dichophacinone. Concentrations of AR were measured in ppb. Dogs that were positive for brodifacoum had a median concentration of 50 ppb ($n = 58$; range, 10 to 280 ppb), those that were positive for difaphacinone had a median concentration of 310 ppb ($n = 13$; range, 44 to 1,120 ppb), and those that were positive for chlorophacinone had a median concentration of 353 ppb ($n = 2$; range, 86 to 620 ppb). Two dogs were positive for brodifacoum, but the concentrations could not be quantitated (ie, reported as trace concentration). No correlation was found between prolongation of the PT or aPTT and concentration of AR when each of the ARs was evaluated separately.

There was no significant difference ($P = 0.378$) in the median duration of hospitalization between the positive group (2.7 days; $n = 73$; range, 0 to 9 days) and the negative group (4.2 days; $n = 48$; range, 0 to 20 days).

Significantly ($P < 0.001$) more dogs in the positive group (74/75 [98.7%]) survived, compared with dogs in the negative group (30/48 [62.5%]). One dog in the positive group did not survive; this was a young puppy that was initially examined for severe respiratory distress and suffered a cardiopulmonary arrest within several hours of initial examination despite appropriate treatment being administered. All of the dogs in the negative group that did not survive (18/48 [37.5%]) were euthanized.

The most common final diagnoses for dogs with negative screens included neoplasia (15/48 [31.3%]), immune-mediated disease (7/48 [14.6%]), and severe gastrointestinal bleeding (5/48 [10.4%]). Less common diseases included liver disease (3 [6.3%]), factor X deficiency (2 [4.2%]), and renal disease and gastroenteritis (1/48 [2.1%]). A diagnosis was not determined for 14 (29.2%) dogs with negative results from AR screenings.

### Table 1—Physical examination parameters at admission for 123 dogs that had serum or whole blood samples submitted antemortem for AR screening from January 1996 through January 2003.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AR positive group (median [range])</th>
<th>AR negative group (median [range])</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rectal temp (°C)</td>
<td>38.1 (32.8–41.4) $n = 70$</td>
<td>38.5 (37.2–40.8) $n = 46$</td>
<td>0.099</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>140 (80–220) $n = 71$</td>
<td>134 (80–200) $n = 47$</td>
<td>0.252</td>
</tr>
<tr>
<td>Respiratory rate (breaths/min)</td>
<td>40 (20–120) $n = 60$</td>
<td>36 (8–134) $n = 33$</td>
<td>0.223</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>13.9 (2.7–49) $n = 73$</td>
<td>27.4 (1.8–76) $n = 47$</td>
<td>0.008*</td>
</tr>
</tbody>
</table>

To convert kg to lb, multiply by 2.2.

*Significantly different between groups.

### Table 2—Clinical pathological parameters for the dogs in Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AR positive group (median [range])</th>
<th>AR negative group (median [range])</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV (%)</td>
<td>29.5 (10 to 64) $n = 72$</td>
<td>35.5 (10 to 62) $n = 46$</td>
<td>0.105</td>
</tr>
<tr>
<td>TS (g/dL)</td>
<td>5.5 (3.7 to 8.0) $n = 72$</td>
<td>5.7 (2 to 9.7) $n = 46$</td>
<td>0.335</td>
</tr>
<tr>
<td>Platelet count (µL)</td>
<td>112,000 (6,000 to 423,000) $n = 62$</td>
<td>84,200 (2,000 to 398,000) $n = 40$</td>
<td>0.110</td>
</tr>
<tr>
<td>PT (s)</td>
<td>52.3 (7.1 to &gt;100) $n = 49$</td>
<td>12.8 (6.3 to 100) $n = 36$</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>aPTT (s)</td>
<td>34.3 (12.8 to &gt;100) $n = 49$</td>
<td>22.4 (11 to &gt;100) $n = 36$</td>
<td>0.322</td>
</tr>
</tbody>
</table>

*Significantly different between groups.
Discussion

In the present study that evaluated the clinical use of toxicology screening for ARs in dogs over a 7-year period, AR concentrations were not correlated with clinical signs or the degree of prolongation of coagulation times. Coagulation abnormalities that were severe enough to prompt screening for AR were associated with a poor prognosis in dogs when results were negative; neoplasia was the most common disease process identified. Anticoagulant rodenticide intoxication had the best prognosis, with a survival rate of 98.7% in this study.

In the present study, the concentrations of AR varied widely and did not correspond to the severity of changes in the PT. We suggest that this was most likely due to the fact that the samples were drawn from some dogs when they had no clinical signs but had a history of exposure or possible ingestion, whereas others were submitted when the dogs had severe hemorrhage. Other studies have described the clinical signs, radiographic findings, and laboratory abnormalities associated with AR intoxication. Evaluating AR concentrations and prolongation of PT can be complicated by treatment with vitamin K, before coagulation times are measured as well as variability in the time from ingestion to when PT is measured; however, this will not affect serum or blood concentrations of the detected AR. Four of our dogs received vitamin K, treatment before initial coagulation times were obtained because of clinical evidence of hemorrhage and high suspicion of AR toxicity, which may have reduced PT times. Other cases had blood obtained for AR screenings immediately following possible ingestion of AR. Finally, individual dogs may be more susceptible to the effects of AR because of predisposing factors that may enhance their toxic effects, such as drugs (sulfonamides, phenylbutazone, aspirin, and chloramphenicol), hypoalbuminemia, and liver and renal disease.

There were several other disease processes that led to coagulation abnormalities severe enough that AR intoxication was considered as a differential, leading to submission of samples for AR screening. Severe coagulopathies that were not associated with AR toxicity generally had a poor prognosis in this study; with neoplasia being the most common disease process. Other differentials that should be considered in patients initially examined with coagulation abnormalities include DIC associated with a variety of diseases including neoplasia; liver failure; angiostrongylosis; autoimmune thrombocytopenia; and hereditary coagulation disorders, many of which are associated with a poor prognosis. The AR screening provides definitive information as to whether the coagulopathy is a result of AR toxicity. This allows clinicians to better guide owners when making decisions about treatment versus euthanasia because of the high cost of blood products and intensive care that some of these patients require. Anticoagulant rodenticide screenings are available at many veterinary diagnostic laboratories. Either serum or whole blood can be collected for antemortem analysis; ARs are stable, and samples typically require only refrigeration prior to testing. Which sample (ie, serum or whole blood) is preferred is laboratory dependent. Serum concentrations will be higher than whole blood concentrations, but both samples are appropriate. Proteins induced by vitamin K antagonism were previously thought to offer a quick and reliable way to distinguish between AR intoxication and other causes of severe coagulopathy, but have since been shown to be elevated with other disease processes, particularly severe liver disease. The AR screening does not allow for misinterpretation, since there are no acceptable concentrations of AR in dogs unless they are receiving coumadin treatment. A positive result means that the animal has had exposure to and absorption of some quantity of AR. Quantification is typically not necessary, since any detectable serum or whole blood AR concentration is clinically significant in dogs. In our study, 28% of the dogs that were positive had no known possible exposure to AR.

Since the completion of this study, we have continued to use the AR screen to confirm the diagnosis of AR intoxication in dogs that the owners were convinced had no possible exposure to AR. The AR screen has also been used to confirm AR intoxication in a cat at our hospital since the study period. A negative AR screen has also been useful in providing owners with an idea of prognosis in dogs examined for coagulopathies due to other causes, as the results of the present study indicated that these patients typically have a disease process that is less treatable and have a poorer prognosis than those patients with positive results of AR screens.

Dogs that ingested AR weighed less than dogs that did not. This might be because the smaller dogs were able to gain access to rodenticide easier than the larger dogs. Owners often think it is not possible for the dog to gain access to areas where AR has been placed, although smaller dogs are more likely to fit into these areas. Additionally, owners will often place standardized packets of bait containing AR and dogs will often ingest the entire contents irrespective of size. This could result in a much higher dosage of AR in smaller dogs, resulting in clinical evidence of hemorrhage and therefore submission of the AR screen.

Age, sex, and breed were not significantly different between the 2 groups in this study. It might be expected that younger dogs would be more likely to ingest AR, but the age range of the dogs in our positive group was 0.3 to 11 years, very similar to that of the negative group (age range, 0.08 to 14 years).

Initial clinical signs were remarkably similar between the 2 groups, with lethargy, collapse, anorexia, decreased appetite, vomiting, dyspnea, and cough the most common in each group. This reflects the severity of disease in each group, with cardiovascular and respiratory compromise frequently occurring in dogs of both groups. Both groups had median heart rates that were tachycardic (134 beats/min in the negative group and 140 beats/min in the positive group) and median respiratory rates that were tachypneic (36 beats/min in the negative group and 40 beats/min in the positive group). Median body temperatures were similar between the 2 groups, although each group included dogs that were hyperthermic or febrile, and those that were hypothermic.

Initial median PCV and TS were similar and just below reference range in both groups in this study. How-
ever, there were dogs in both groups that had severely decreased PCV and TS as well as dogs that had evidence of hemoconcentration. Median platelet count was not significantly different between the groups, although severe thrombocytopenia was noted in dogs from both the positive and negative groups. Immune-mediated thrombocytopenia was the most common cause in the latter, but several dogs in the positive group had platelet counts that were just as low. This has been attributed to consumption of platelets from severe hemorrhage. It was reflected in the 3 dogs of the positive group with platelet counts ≤ 20,000/µL, which all were anemic (PCV range, 12% to 27%) and had physical evidence of severe hemorrhage such as extensive bruising, pale mucous membranes, tachycardia, and hypothermia.

The median PT was significantly more prolonged in the positive group, but aPTT was not significantly different. This is consistent with the fact that ARs cause antagonism of vitamin K epoxide reductase, which depletes the body's stores of active vitamin K–dependent factors: factors II, VII, IX, and X. Of these, factor VII has the shortest half-life (6.2 hours). The PT, which tests the tissue factor pathway of the clotting cascade, is dependent on factor VII, and this test is affected first in animals that ingest AR. The aPTT also becomes prolonged as factors II, IX, and X are depleted, and all coagulation factors begin to become consumed once prolonged beyond measurement. A recent study evaluated the usefulness of PT in dogs 48 to 72 hours after known or highly suspected ingestion of AR rodenticides and gastrointestinal decontamination. Only 8% had prolongation of their PT by 72 hours and required treatment with vitamin K. None of these dogs showed clinical signs of hemorrhage, indicating that delaying vitamin K treatment until after measurement of PT 48 to 72 hours after ingestion is safe. In this situation, submission of an AR screening would not be beneficial unless the exact AR needed to be identified for some reason. The AR screening is most helpful in diagnosing the patient with clinical evidence of hemorrhage with no or uncertain exposure history to ARs and when other differential diagnoses with a poorer prognosis are being considered for the coagulopathy.

In the present study, the dogs in the positive group received more FFP than did the dogs in the negative group. A few possible theories as to why the positive group received more FFP are that the dogs in the AR positive group had more prolonged bleeding times on the coagulation screening or had more severe hemorrhage at initial examination or that clinicians were treating with FFP until the coagulation screening returned to normal in dogs with AR toxicity. A combination of the first and last theories seems most likely because the positive group had longer PT values than the negative group and it was common for the treating clinician to repeat coagulation panel testing until the PT and aPTT normalized. This may not have been a similar goal in the negative group, depending on the diagnosis that was ultimately made. An interesting finding is that the 2 groups did not receive significantly different amounts of packed RBCs. This seems to lend credence to the theory that these patients received more FFP on the basis of coagulation testing results rather than severity of bleeding, otherwise, it would be expected that the positive AR dogs would have also received more packed RBCs.

The number of days in the hospital was not significantly different between the 2 groups. It might be expected that the AR-intoxicated dogs would have shorter hospital stays, considering the course of their disease is usually sudden in onset and has a rapid recovery. However, dogs with AR toxicity can have severe respiratory embarrassment on initial examination and require several days of supplemental oxygen treatment. Some of the dogs in the AR negative group were euthanized relatively rapidly once AR was ruled out and other diagnoses with poorer prognoses were definitively made. These variations in both groups caused a wide range of days in the hospital, with a high degree of overlap between the 2 groups.

Overall, the prognosis was much better for AR-intoxicated dogs of this study. Only 1 of the 75 positive dogs did not survive. The 1 nonsurvivor was a young puppy that was initially examined in severe respiratory distress and underwent cardiopulmonary arrest within several hours despite appropriate treatment being administered. This was a case that was initially examined too late for treatment to be successful. All of the other cases that were positive were discharged and fully recovered. Of the dogs with negative screens, only 30 survived 18 were euthanized. Neoplasia, immune-mediated disease, liver disease, severe gastrointestinal bleeding, factor X deficiency, renal disease, and severe gastroenteritis were the final diagnoses made in 34 dogs, with an open diagnosis in 14 dogs.

The AR toxicology screening is able to detect the presence of multiple ARs, but only 3 were detected in the 75 dogs with positive AR screenings. Of these, 80% were positive for brodifacoum, 18.7% were positive for diphacinone, and 2.7% were positive for chlorophacinone. One dog was positive for both brodifacoum and diphacinone. This would imply that brodifacoum is the most commonly used AR. All of the ARs that were detected were longer-acting or second-generation rodenticides, which are used because of rodent resistance to the short-acting AR warfarin. This is important in that even without identifying the AR involved, veterinarians should treat these patients with vitamin K, for 28 days at a minimum.

Blood AR concentrations were not correlated with prolongation of the PT or aPTT even when each of the ARs was evaluated separately. This is probably due to several factors. There is a large variation in the duration of time after ingestion that these dogs are evaluated, from immediately after ingestion (which may be associated with high concentrations and no changes in coagulation) to dogs that are bleeding from the coagulopathy (typically 4 to 5 days after ingestion and may
now have reduced blood concentrations). Also, there is a large variation in the amount that dogs ingest and over what time period they have ingested it. They may have barely eaten the toxic dose or may have consumed several times the toxic dose if a large amount of bait was available to them. Other dogs are examined after eating smaller doses on multiple different days. Additionally, some dogs received vitamin K1 SC before coagulation times are obtained. This can result in decreased or even normalized PT values despite high blood concentrations of AR. Dogs may also have had some predisposing factors that would have increased the toxic effects of the AR, such as underlying liver disease or concurrent drug treatment. These factors were not evaluated in this study. Also, there could be variability in the rate of consumption of and demand for coagulation factors, such as would occur if the patient sustained some minor trauma or started bleeding for any other reason such as a gastrointestinal ulcer. All of these factors can contribute to the variable concentrations seen with both normal coagulation and severe prolongation of the PT and aPTT. Additional studies would be needed with standardization of time that blood was drawn after ingestion to determine whether blood concentrations of AR could be used to predict which patients ingested a toxic dose. However, this information would probably be of limited use in most clinical cases, as the exact time of ingestion is infrequently known.

References