Clinical and clinicopathologic features of dogs that consumed foodborne hepatotoxic aflatoxins: 72 cases (2005–2006)

Diane M. Dereszynski, DVM; Sharon A. Center, DVM, DACVIM; John F. Randolph, DVM, DACVIM; Marjory B. Brooks, DVM, DACVIM; Alice G. Hadden, DVM; Kiran S. Palyada, BVSc, MVSc, PhD; Sean P. McDonough, DVM, DACVP, PhD; Joanne Messick, DVM, DACVP, PhD; Tracy Stokol, BVSc, DACVP, PhD; Karyn L. Bischoff, DVM, MS, DABVT; Stuart Gluckman, DVM; Sara Y. Sanders, DVM

Objective—To characterize clinical signs, clinicopathologic features, treatments, and survival in dogs with naturally acquired foodborne aflatoxicosis.

Design—Retrospective case series.

Animals—72 dogs that consumed aflatoxin-contaminated commercial dog food.

Procedures—Medical records of affected dogs were reviewed. Between December 2005 and March 2006, dogs were identified as having foodborne aflatoxin hepatotoxicosis on the basis of the history of consumption of contaminated food or characteristic histopathologic lesions (subject dog or a recently deceased dog in the same household or kennel). Recorded information included signalment, clinical features, clinicopathologic test results, treatments, and survival. Data were analyzed by survival status.

Results—Most dogs were of large breeds from breeding kennels. No significant differences were found in age or weight between 26 (36%) survivor dogs and 46 (64%) nonsurvivor dogs. Severity of clinical signs varied widely; 7 dogs died abruptly. In order of onset, clinical features included anorexia, lethargy, vomiting, jaundice, diarrhea (melena, hematochezia), abdominal effusion, peripheral edema, and terminal encephalopathy and hemorrhagic diathesis. Common clinicopathologic features included coagulopathic and electrolyte disturbances, hypoproteinemia, increased serum liver enzyme activities, hyperbilirubinemia, and hypocholesterolemia. Cytologic hepatocellular lipid vacuolation was confirmed in 11 dogs examined. In comparisons of clinicopathologic test results between survivor and nonsurvivor dogs, only granular cylindruria (7/21 dogs) consistently predicted death. Best early markers of aflatoxicosis were low plasma activities of anticoagulant proteins (protein C, antithrombin) and hypocholesterolemia. Despite aggressive treatment, many but not all severely affected dogs died.

Conclusions and Clinical Relevance—Serum liver enzyme activities and bilirubin concentration were unreliable early markers of aflatoxin hepatotoxicosis in dogs. Hypocholesterolemia and decreased plasma protein C and antithrombin activities may function as exposure biomarkers. (*J Am Vet Med Assoc* 2008;232:1329–1337)

Aflatoxins are potent hepatotoxic and hepatocarcinogenic agents produced primarily by strains of the fungus *Aspergillus*.[1–4] Involved fungi are widely distributed in nature, making aflatoxicosis an ever-present danger. Food contamination with aflatoxins can occur in the field or after harvest during storage and processing.[5] Since the early 1960s, when aflatoxicosis caused a mysterious epidemic of acute hepatic necrosis in 100,000 dying turkey poults, multiple other species have been similarly affected.[3,6–14] Indeed, aflatoxin hepatotoxicosis has been characterized in many species, including dogs.[3,15-19,a]

Abbreviations

AFB_1	Aflatoxin B_1
CYP450	P450 cytochromes
GSH	Glutathione
CUHA	Cornell University Hospital for Animals
aPTT	Activated partial thromboplastin time
PT	Prothrombin time
FVII:C	Coagulation factor VII

Although many different aflatoxins exist, AFB_1 is the most common, potent, and oncogenic moiety implicated

From the Departments of Clinical Sciences (Dereszynski, Center, Randolph, Hadden), Population Medicine and Diagnostic Sciences (Brooks, Messick, Stokol, Bischoff), and Biomedical Sciences (Palyada, McDonough), College of Veterinary Medicine, Cornell University, Ithaca, NY 14853; and Mendon Village Animal Hospital, 1380B Pittsford-Mendon Rd, Mendon, NY 14506 (Gluckman, Sanders). Dr. Dereszynski's present address is VCA West Los Angeles Animal Hospital, 1818 S Sepulveda Blvd, Los Angeles, CA 90025. Dr. Hadden's present address is VCA Roberts Animal Hospital, 516 Washington St, Hanover, MA 02339. Dr. Messick's present address is Department of Comparative Pathobiology, School of Veterinary Medicine, Purdue University, West Lafayette, IN 47907.

Presented in part at the 24th Annual Forum of the American College of Veterinary Internal Medicine, Louisville, June 2006.

The authors thank Dr. Hollis N. Erb for statistical assistance.

Address correspondence to Dr. Center.

in food-related aflatoxicosis.[3] Hepatotoxicosis is dose-related and is caused by rapidly formed metabolites. The conversion (largely under the influence of CYP450) of AFB_1 to active electrophilic and arylating metabolites accounts for cellular toxicity.[3,20,21] Specifically, rapid hepatocellular formation of a noxious 8,9-epoxide of AFB_1[22] overwhelms detoxification pathways by depleting intracellular GSH. Loss of hepatocellular GSH not only compromises the detoxifying conjugation of AFB_1, but also results in secondary oxidative injury that augments the direct epoxide-mediated damage. Accumulated AFB_1 adducts irreversibly bind to cell enzymes, proteins, and DNA causing impaired metabolism, gene transcription, and protein synthesis.[5] Inhibition of RNA polymerase and interaction of AFB_1 epoxide with polyribosomes impair secretory protein synthesis.[23] Additionally, some evidence indicates that the AFB_1 epoxide binds to mitochondrial DNA where it impairs cell energy production.[3] Furthermore, hepatocarcinogenesis has been confirmed in several species; in humans, development of hepatocellular carcinoma has been linked to a mutation in the p53 tumor-suppressor gene.[3,24]

Different species have varying susceptibility to aflatoxins, with dogs being highly susceptible.[25,26] The vulnerability of dogs to acute and subacute liver disease in response to aflatoxins might reflect their inherently lower hepatocellular concentration of GSH, compared with other species, and perhaps their interindividual differences in activity of GSH-S-transferase.[3,27–30] Sporadic instances of individually affected dogs and outbreaks of dogs exposed to hepatotoxic aflatoxins have been reported since the 1970s.[16–19,31]

During late 2005, a serious foodborne aflatoxin contamination that was derived from moldy corn in manufactured dog food was recognized through collaborative efforts of regional New York State veterinarians and the College of Veterinary Medicine at Cornell University. Contaminated food was distributed widely in the eastern and southeastern United States and was exported to other countries, including members of the European Union. Although product recall was rapidly announced, high rates of morbidity and mortality of dogs nevertheless was encountered in the United States. The purpose of the study reported here was to characterize clinical signs, clinicopathologic features, treatments, and survival of dogs with naturally acquired foodborne aflatoxicosis in December 2005 through March 2006. We retrospectively report the spectrum of clinical and clinicopathologic features of dogs following consumption of foodborne hepatotoxic aflatoxins in the largest patient series yet described and relate findings to survival.

Materials and Methods

Case selection—Dogs considered affected with aflatoxicosis were identified during December 2005 through March 2006 on the basis of ≥ 1 of the following criteria: a several week or month history of consumption of contaminated dog food identified by product-date and plant-facility codes; or confirmation of aflatoxin concentration in consumed food ≥ 60 ppb[32]; or characteristic histopathologic hepatic lesions of aflatoxicosis in the subject dog or a recently deceased dog in the same household or kennel consuming the same batch of contaminated food. Dogs included in this study were either patients of the CUHA (n = 22) or were identified during discussions with consulting veterinarians in the eastern and southeastern United States. Inclusion of affected dogs (n = 50) from consulting practitioners required access to medical records, clinicopathologic test results (hematologic, biochemical, urinalysis, and coagulation tests), information regarding treatments and outcome, and (if available) hepatic histologic evaluations on the subject dog or deceased dog in the same household that substantiated aflatoxicosis.[33]

Medical records review—Recorded information included signalment (age, sex, body weight, and breed), clinical signs and physical examination findings, routine clinicopathologic tests (including CBC, serum biochemical profile, and urinalysis) and coagulation assessments from samples collected during initial evaluation, imaging studies (routine abdominal radiography and abdominal ultrasonography), hepatic cytologic findings, treatments, and outcome. Dogs were categorized as survivor or nonsurvivor dogs. When available, the aflatoxin concentration of food was recorded.

Clinicopathologic variables—Hematologic variables included determination of PCV, mean corpuscular volume, total and differential WBC counts, and platelet count. Coagulation assays included coagulation screening tests (aPTT and PT); fibrinogen concentration as determined via the Clauss method[34]; and activities of antithrombin, protein C, and FVII:C. Serum biochemical tests included concentrations of sodium; potassium; chloride; bicarbonate; SUN; creatinine; calcium; phosphate; magnesium; glucose; total protein; albumin; globulin; cholesterol; and total, direct, and indirect bilirubin and activities of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, γ-glutamyltranspeptidase, amylase, and creatine kinase. Urinalyses included determination of specific gravity and routine dipstick and microscopic sediment evaluations. Multiple laboratories were involved with sample analyses with the exception of coagulation assessments, which were all determined at the Comparative Coagulation Section, Animal Health Diagnostic Center, Cornell University College of Veterinary Medicine. As guidelines for routine laboratory data interpretation, reference ranges established by use of automated procedures[b,c] at the Cornell University College of Veterinary Medicine are provided.

Blood samples for coagulation testing were collected into sodium citrate vacuum tubes (1 part sodium citrate:9 parts whole blood) and centrifuged, and the supernatant plasma was stored at 4°C for same-day assay (CUHA patients) or shipped overnight on cold packs (non-CUHA patients). Coagulation screening tests and fibrinogen were performed by use of commercial reagents,[d–f] as previously described.[35] Plasma antithrombin and protein C activities were measured by use of synthetic chromogenic substrate kits[g,h] configured with a canine (rather than human) calibration standard. The FVII:C activity was performed by use of a modified 1-stage PT technique,[36] rabbit brain thromboplastin reagent,[e] and canine substrate-deficient plasma.

Results of antithrombin, protein C, and FVII:C analyses were reported as a percentage of the canine standard (prepared from 20 healthy adult dogs), with the assigned value of 100% activity.

Statistical analysis—Box-and-whisker plots and histograms were used to evaluate data distributions to select the most appropriate analyses. Because most data were non-Gaussian, nonparametric methods were used and data expressed as median (range). The Wilcoxon rank sum test was used to determine differences in signalment and clinicopathologic variables between survivor and nonsurvivor dogs. A 2-tailed value of $P \leq 0.05$ was used to detect significance with an approximate Bonferroni-like correction applied within sets of interrelated tests to account for multiple comparisons (signalment, $P \leq 0.025$; hematologic tests, $P \leq 0.01$; coagulation tests, $P \leq 0.008$; and biochemical tests, $P \leq 0.002$). Two-by-two tables were used to detect differences in the number of dogs in survival categories having abnormal clinicopathologic test values; a 2-tailed value of $P \leq 0.05$ was applied. Analyses were performed by use of data-analysis software.[i]

Results

Median (range) age of dogs with aflatoxicosis was 4 years old (0.8 to 13 years old). The study included 13 sexually intact males, 17 neutered males, 23 sexually intact females, and 19 neutered females. Thirty-six dogs were sexually intact (most living in purebred dog kennels). Median weight was 29.6 kg (65.1 lb) with a range of 2.3 to 57.8 kg (5.1 to 127.2 lb). Most (51/72; 71%) dogs were of large breeds (**Table 1**) with 34 of 51 dogs from 5 kennels. No significant differences were found in age or weight between the 26 (36%) dogs that lived and 46 (64%) that died (**Table 2**).

Severity of clinical signs varied among dogs. Seven dogs died acutely with no recognized antecedent illness. Often, these dogs served as sentinels for aflatoxicosis within affected kennels. Because of the complexity of deriving consistent historic information from records of dogs managed outside of our teaching hospital, we enumerated frequency of clinical signs and physical examination findings only in the 22 dogs treated within our hospital. In order of onset, features in these dogs included anorexia (20/22; 91%), lethargy (19/22; 86%), vomiting (20/22; 91%), jaundice (16/22; 73%), diarrhea (18/22; 81%; frequently associated with melena progressing to hematochezia), abdominal effusion (14/22; 64%; usually a modified transudate, rarely hemorrhagic), peripheral edema subsequent to critical supportive fluid therapy (15/22; 67%), and terminal encephalopathy associated with hemorrhagic diathesis (9/22; 41%). Some dogs were reluctant to eat the contaminated food yet readily consumed alternative feed. Of CUHA patients that developed an abdominal effusion, most died (12/14). However, we are aware of 5 surviving dogs in this retrospective study that resolved large-volume abdominal effusion within 60 days of first recognition. We confirmed the presence of acquired portosystemic shunts in chronically affected dogs that died and in 2 surviving dogs that resolved abdominal effusion. Failure to survive in most dogs was linked with severe enteric hemorrhage, as witnessed clinically. In 3 dogs, polyuria and polydipsia were initial prominent clinical signs. Three surviving dogs had a preexistent disease process (hypoadrenocorticism, diffuse vacuolar hepatopathy, or intestinal malabsorption syndrome).

Table 1—Breeds of dogs with naturally acquired foodborne aflatoxicosis

Dog breed	No. of dogs
Labrador Retriever	19
Malamute	7
Staffordshire Terrier	6
Cocker Spaniel (American)	5
Plott Hound	5
Golden Retriever	4
Miniature Pinscher	4
Mixed-breed	4
Border Collie	2
Doberman Pinscher	2
Rottweiler	2
Scottish Terrier	2
Pembroke Welsh Corgie	1
Dachshund	1
Great Dane	1
Irish Setter	1
Lakeland Terrier	1
Pomeranian	1
Shetland Sheepdog	1
Siberian Husky	1
Skye Terrier	1
Standard Poodle	1

Clinicopathologic data were stratified by survival category (Table 2). Common features in survivor and nonsurvivor dogs included evidence of coagulopathic disturbances, electrolyte disturbances (reflecting dehydration, vomiting, diarrhea, or abnormal body fluid retention or distribution), hypoalbuminemia, increased liver enzyme activities, hyperbilirubinemia, and hypocholesterolemia. Surprisingly, 32 of 42 (76%) dogs with a quantitative differential leukocyte count failed to have a stress leukogram (defined as coexistent mature neutrophilia, lymphopenia, and eosinopenia). Two of these dogs had ACTH response tests pursued to determine whether atypical hypoadrenocorticism was a covert complication of systemic aflatoxicosis; normal cortisol responses were found for each dog. Nonsurvivor dogs had significantly prolonged aPTT and PT, lower fibrinogen concentrations, and lower plasma activities of antithrombin, protein C, and FVII:C than survivor dogs. Nonsurvivor dogs also had significantly lower serum concentrations of sodium, calcium, total protein, albumin, and cholesterol than survivor dogs. Urinalysis was completed in 21 dogs (10 survivor dogs and 11 nonsurvivor dogs). Median specific gravity was 1.020 (1.008 to 1.045) with no significant difference between survival categories. Granular casts were identified in 7 of 21 (33%) dogs; each dog with cylindruria succumbed to aflatoxicosis, yet only 1 dog was mildly azotemic.

Evaluating the frequency of abnormal test results in survival categories identified markers of aflatoxin exposure, some with prognostic value. Variables reflecting aflatoxin exposure (ie, abnormalities detected in both survival categories) included reduced plasma antithrombin and protein C activities, hypocholesterolemia, and increased liver serum enzyme activities (Table 2). In fact, on initial evaluation, 96%

Table 2—Selected signalment, hematologic, plasma coagulation, and serum biochemistry variables at initial evaluation in dogs with naturally acquired foodborne aflatoxicosis.

	Survivor dogs				Nonsurvivor dogs					
Variables	**Median (range)**	**No.**	**Low**	**High**	**Median (range)**	**No.**	**Low**	**High**	***P* value**	**RR**
Age (y)	4 (1–13)	26	NA	NA	3.8 (0.8–12)	46	NA	NA	0.200	NA
Weight (kg)*	28.7 (2.3–57.8)	26	NA	NA	29.8 (5.4–57.3)	46	NA	NA	0.600	NA
PCV (%)	49 (35–56)	22	1	0	49 (19–69)	33	3	3	1.000	39–57
MCV (fL)	68 (61–74)	20	1	1	68 (60–77)	30	3	1	0.600	64–73
WBC ($\times 10^3$ cells/μL)	10.4 (6.0–36.1)	22	1	1	14.7 (4.8–40.0)	31	2	15	0.007	7.5–19.9
Neutrophils ($\times 10^3$ cells/μL)	7.6 (3.9–23.2)	22	0	5	11.4 (4.0–37.2)	31	0	21	0.008	3.9–14.7
Platelets ($\times 10^3$ cells/μL)	266 (69–548)	19	4	1	186 (21–400)	31	14	0	0.050	179–510
aPTT (s)	16.3 (14–120)	10	0	2	31 (18.5–120)	15	0	15	0.002	10–17
PT (s)	19 (15–70)	10	0	5	29.5 (18.6–100)	16	0	16	0.007	13–18
Fibrinogen (mg/dL)	192 (76–323)	9	2	0	37 (11–344)	14	12	0	0.004	100–510
AT (% activity)	48 (26–75)	11	10	NA	17 (6–43)	13	13	NA	< 0.001	> 75
Protein C (% activity)	40 (20–70)	11	10	NA	18 (4–55)	14	14	NA	0.006	> 70
FVII:C (% activity)	81 (13–124)	6	2	NA	32 (1.4–67)	8	6	NA	0.200	> 50
Sodium (mEq/L)	150 (145–163)	21	0	7	145 (126–156)	35	13	3	< 0.001	142–151
Potassium (mEq/L)	4.2 (3.4–5.1)	21	5	0	4.1 (3.2–5.7)	35	13	2	0.080	3.9–5.3
Chloride (mEq/L)	116 (110–122)	20	0	5	108 (95–123)	28	10	6	0.009	107–117
Bicarbonate (mEq/L)	22 (18–25)	9	0	0	23 (12–26)	14	2	3	0.600	15–25
SUN (mg/dL)	10 (4–19)	23	5	0	11 (4–97)	44	7	10	0.100	8–30
Creatinine (mg/dL)	0.8 (0.4–1.6)	22	1	2	0.8 (0.5–3.8)	36	0	7	0.700	0.5–1.3
Calcium (mg/dL)	10.0 (8.7–10.8)	21	4	0	9.3 (6.9–10.4)	36	18	0	< 0.001	9.3–11.6
Phosphate (mg/dL)	3.8 (2.6–4.7)	20	1	0	4.4 (2.8–13.0)	35	0	13	0.030	2.8–5.3
Magnesium (mEq/L)	1.6 (1.2–1.8)	9	1	0	1.5 (1.2–1.9)	15	3	0	0.500	1.4–2.0
Glucose (mg/dL)	110 (74–156)	23	0	6	110 (45–135)	41	1	8	0.800	58–120
Total protein (g/dL)	6.6 (5.5–7.6)	23	1	1	5.1 (2.6–6.5)	43	25	0	< 0.001	5.6–7.1
Albumin (g/dL)	3.3 (2.0–3.9)	22	8	0	2.4 (0.5–3.5)	41	33	0	< 0.001	3.1–4.1
Globulin (g/dL)	3.3 (2.4–4.4)	22	0	3	2.3 (1.0–4.7)	41	10	7	0.006	1.9–3.6
ALT (U/L)	143 (10–695)	26	1	19	284 (52–2,226)	45	0	40	0.030	12–106
AST (U/L)	55 (20–200)	13	0	7	148 (46–657)	20	0	19	0.008	13–56
ALP (U/L)	142 (21–2,247)	26	0	14	189 (31–3,477)	45	0	33	0.100	4–122
GGT (U/L)	4 (3–20)	13	NA	3	12 (3–33)	19	NA	11	0.020	<12
Total bilirubin (mg/dL)	0.7 (0.1–7.5)	24	NA	15	5.2 (0.1–25)	44	NA	43	< 0.001	0–0.3
Direct bilirubin (mg/dL)	0.3 (0.1–1.2)	9	NA	6	5.1 (0.2–11)	16	NA	16	< 0.001	0–0.1
Indirect bilirubin (mg/dL)	0.5 (0.2–0.8)	9	NA	6	2.7 (0.5–6.9)	16	NA	16	< 0.001	0–0.3
Amylase (U/L)	448 (243–764)	16	1	0	304 (153–947)	32	11	0	0.010	286–1124
Cholesterol (mg/dL)	135 (62–400)	21	15	1	63 (0–288)	31	22	0	< 0.001	150–335
CK (U/L)	90 (62–123)	9	0	0	230 (69–1,700)	15	0	6	0.005	58–241

*To convert kilograms to pounds, use a factor of 2.2.

No. = Number of dogs for each measurement. Low = Number of dogs with values below the reference range. High = Number of dogs with values above the reference range. RR = Reference range. NA = Not applicable. MCV = Mean corpuscular volume. AT = Antithromin activity. ALT = Alanine aminotransferase. AST = Aspartate aminotransferase. ALP = Alkaline phosphatase. GGT = γ-Glutamyltransferase. CK = Creatine kinase.

(24/25) of dogs tested had subnormal plasma protein C and antithrombin (23/24 dogs) activities, as many as 83% (eg, 83% [59/71] alanine aminotransferase, 79% [26/33] aspartate aminotransferase, 66% [47/71] alkaline phosphatase, and 44% [14/32] γ-glutamyltranspeptidase) had increased serum liver enzyme activities ranging from mild to marked, and 71% (37/52) were hypocholesterolemic. Abnormalities of protein C, antithrombin, and cholesterol preceded development of clinical signs in 3 of 22 (14%) dogs and were present in an additional 2 dogs having only inappetence. Sequential testing confirmed that abnormalities in protein C, antithrombin, and cholesterol persisted beyond several weeks (**Figure 1**). Sequential testing of serum liver enzyme activities revealed nonspecific mildly increased activity or activity within reference range (data not shown). Variables that might predict death included neutrophilic leukocytosis, prolonged aPTT and PT, hyponatremia, hypochloremia, hypocalcemia, hyperphosphatemia, hypoproteinemia including hypoalbuminemia and hypoglobulinemia, and a high SUN concentration.

In 10 dogs that had abdominal ultrasonography findings at CUHA, the liver appeared hyperechoic in 6 and 3 others had hypoechoic hepatic nodules (diameters, 2 to 20 mm). The gallbladder wall was thick (> 3 mm) in 5 dogs, large mesenteric lymph nodes were imaged in 2 dogs, anechoic abdominal effusion of varying severity was imaged in 4 dogs, hepatic surface contour was judged to be irregular in 2 dogs, and liver size was subjectively normal in 9 dogs. Additionally, 3 dogs had gastric atony on contrast radiographic studies or ultrasonographic imaging that did not respond to prokinetic gastrointestinal motility enhancers.

Cytologic preparations made from tissue imprints and needle aspirates of liver from dogs that died all had diffuse hepatocyte lipid vacuolation. The lipid content of hepatocyte vacuoles was confirmed with Sudan III staining. Degenerating hepatocytes and minor inflammatory cell infiltrates also were observed. Cytologic features corresponded with the histologic confirmation of diffuse hepatocyte lipid vacuolation.[33]

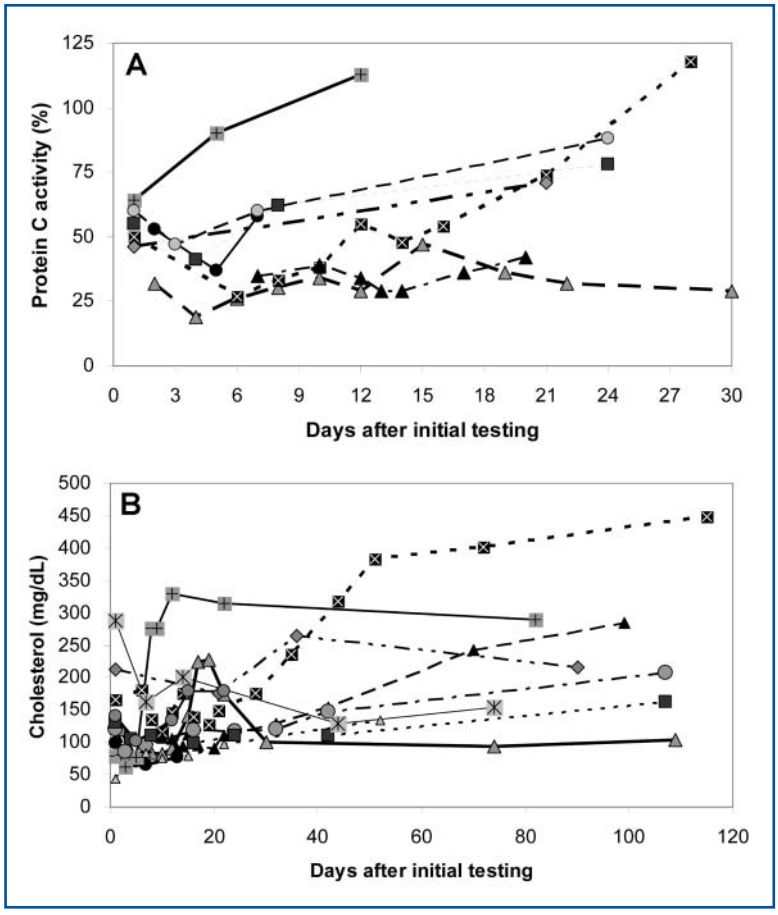

Figure 1—Plasma protein C activity (A) and serum cholesterol concentrations (B) in dogs that survived naturally acquired foodborne aflatoxicosis and that had sequential laboratory monitoring. Individual dogs represented by different symbols and connecting lines. Reference range values are provided in Table 2.

Aflatoxin concentration (median, 300 ppb; range, 48 to 800 ppb) in food was verified for 28 dogs from 9 households. Measurements were completed in multiple toxicologic reference laboratories by use of ELISA, high-pressure liquid chromatography, and liquid chromatography-tandem mass spectrometry methods.

Treatments for all dogs managed at CUHA and vigorously managed in private hospitals included IV administration of crystalloid fluids with judicious electrolyte supplements and blood-component treatment (packed RBCs and fresh frozen plasma), thiol donors (IV administration of n-acetylcysteine or oral administration of s-adenosylmethionine), antiemetics (metoclopramide, dolasetron, or ondansetron), gastroenteric protectants (famotidine and sucralfate), milk thistle derivative hepatic protectants, vitamin K_1, broad-spectrum antimicrobials (ampicillin-sulbactam, enrofloxacin, or both), vitamin E, and L-carnitine. After IV administration of n-acetylcysteine, 3 dogs developed urticaria that resolved with stopping the drug and administering antihistamines.

Discussion

Despite much investigational work on aflatoxicosis, this condition is rarely identified antemortem in small animal practice in part because it masquerades as many other illnesses. Accordingly, this retrospective study reports the breadth of clinical features and clinicopathologic findings in a widely disseminated foodborne outbreak in dogs. Following telecommunication and internet news reports of the food contamination, wide surveillance for intoxicated dogs was conducted by some regional veterinary clinics. However, the actual number of dogs that developed clinical illness from aflatoxicosis might never be known. As predicted by experimental work in dogs, early clinical signs of many affected pets included inappetence, vomiting, lethargy, and diarrhea[a]; however, these features were inconsistent. Notably, not all exposed dogs were clinically ill at the time of initial evaluation. Nevertheless, some of these dogs had laboratory abnormalities and proceeded to develop severe clinical signs (in some instances leading to death within days). In retrospect, waiting until a dog has clinical signs of aflatoxicosis before seeking veterinary care, as had been instructed initially during the food recall, might have deprived affected dogs of acquiring supportive care when it might have been most efficacious. Additionally, 10% (7/72) of dogs died acutely with no recognized antecedent illness. Often, these individuals served as sentinels for the naturally acquired foodborne aflatoxicosis of dogs within affected kennels. Thus, wide interindividual differences in clinical features were seen (likely reflecting pharmacogenetic diversity in toxin metabolism and detoxification mechanisms, the amount and duration of toxin exposure, nutritional status, interactions with other toxic insults, or antecedent disease processes).[4,25,37] Three surviving dogs each had a preexistent disease process (hypoadrenocorticism, diffuse vacuolar hepatopathy, or intestinal malabsorption syndrome) that may have altered aflatoxin absorption or metabolism. Some dogs that survived were reluctant to eat contaminated food, either fasting or stealing food from the kitchen counter or from companion pets fed other rations. This avoidance of contaminated food prior to any signs of illness in some dogs is noteworthy because the odorless and tasteless properties of aflatoxin should not taint food palatability. Thus, it is possible that some additional mycotoxin or food contaminant may have altered food palatability.

Early in the outbreak, we recognized that markedly increased serum liver enzyme activities and overt hyperbilirubinemia (total bilirubin > 2.5 mg/dL, consistent with recognition of jaundice) were inconsistent laboratory features. Markedly increased liver enzyme activity and jaundice were seemingly identified more commonly in the most severely affected dogs, consistent with findings in dogs with experimental aflatoxicosis.[a] In the experimental aflatoxicosis study,[a] dogs developed hyperbilirubinemia only after ≥ 1 month

of aflatoxin ingestion. Rapid development of marked hyperbilirubinemia in the patients described herein, however, often followed administration of blood transfusions, possibly reflecting impaired hepatic heme detoxification or dysfunctional membrane bilirubin transporters. In support of this theory, glucuronides of AFB_1 have been identified as biliary metabolites in some species and might competitively inhibit the processing of heme pigments.[1]

Serum activity of liver enzymes typically reflect cellular changes and usually correspond with the histologic features of liver injury.[38] However, prior studies of dogs with aflatoxicosis confirm differences in biochemical features in acute high-dose poisoning as compared with lower-dose chronic intoxication.[18,a] In the former dogs, death may occur secondary to fulminant hepatic failure before marked abnormalities in liver enzymes or any illness is recognized. In the latter group, chronic intermittent cell necrosis, architectural remodeling, and associated oval cell and biliary epithelial hyperplasia are associated with illness and increases in membrane-associated enzymes (alkaline phosphatase and γ-glutamyltranspeptid ase).[39,a] In general, the magnitude of liver enzyme activity did not reconcile with the severity of hepatocellular injury documented at necropsy. This is not a unique phenomenon to this episode of hepatic damage, or in general to aflatoxicosis, because similar discordance between circulating liver enzyme activities and hepatic histologic features was reported for dogs sequentially tested during chronic experimental aflatoxicosis[a] as well as for rats with microcystin toxicosis.[40] Microcystin impairs hepatic alanine aminotransferase synthesis thereby curtailing development of high circulating liver enzyme activity.[40] Although limited information is found regarding the clinicopathologic features associated with microcystin-induced hepatotoxicosis in dogs, results of 1 study[41] revealed that alanine aminotransferase activity was within the reference range 48 hours after toxin ingestion despite acute fulminant hepatic necrosis. Although previous studies[42,a] of aflatoxicosis in dogs and chickens revealed minimally increased alanine aminotransferase activity, similar to that found in many dogs reported herein, it remains unclear whether impaired enzyme synthesis explains our findings. Nevertheless, early surveillance for dogs at risk for illness and death from aflatoxicosis based only on liver enzyme activities can be misleading. Furthermore, the diverse spectrum of systemic disorders associated with mild increases in liver enzyme activities and the relationship between the dose of aflatoxin ingested and ensuing liver injury impair their utility as a diagnostic marker for clinically important aflatoxin exposure.

Compared with liver enzyme abnormalities, low plasma protein C and antithrombin activities and low serum cholesterol concentrations are derived from a more restricted spectrum of disease processes (including hepatic function or circulation abnormalities). Although not all dogs were tested, subnormal plasma protein C and antithrombin activities and hypocholesterolemia were the most sensitive biomarkers of aflatoxin ingestion in dogs with minimal clinical signs. In fact, on initial evaluation, 96% of dogs tested had subnormal plasma protein C and antithrombin activities and 71% were hypocholesterolemic. Liver disease can be associated with complex hemostatic abnormalities affecting platelet function, procoagulant and anticoagulant factors and fibrinolysis. Because the liver is the predominant site of protein C and antithrombin synthesis, hepatic dysfunction could explain our findings. Alternatively, aflatoxicosis is known to induce disseminated intravascular coagulation that can secondarily deplete procoagulant and anticoagulant factors.[16] Thus, finding low protein C and antithrombin activities along with significantly prolonged aPTT and PT and lower fibrinogen and FVII:C in nonsurvivor dogs suggests that disseminated intravascular coagulation was a contributing pathologic mechanism. However, because low protein C and antithrombin activities were the sole hemostatic abnormalities in many survivor dogs, we contend that these biomarkers might reflect specific aflatoxin-mediated abnormalities. Nevertheless, it is important to clarify that these proteins cannot be considered as a toxin-specific test. Because we coincidentally recognized that low protein C and antithrombin activities and hypocholesterolemia preceded development of clinical signs in 3 of 22 (14%) dogs and an additional 2 dogs only had signs of inappetence, we speculate that aflatoxicosis might simultaneously impair biosynthesis of certain proteins and cholesterol. Results of a study[a] of dogs with experimental aflatoxicosis revealed a mean decrease of 61% to 75% in serum concentrations of total, free, and esterified cholesterol. Although the exact mechanism of impaired cholesterol synthesis has not been clearly identified, results of 1 study[43] in rats revealed impaired acetate incorporation. Even though aflatoxin-mediated inhibition of protein synthesis is recognized,[3] we cannot ascribe the subtle but steady decline in albumin concentrations seen in some dogs (data not shown) simply to impaired hepatic function as crystalloid fluid administration, enteric bleeding, and third-space fluid distribution (edema and ascites) may each have contributed to these findings.

Aflatoxin B_1 is biotransformed within seconds to its toxic epoxide that instantaneously binds to nucleic acids and proteins (including albumin). Biotransformation is rapidly facilitated by CYP450 such that the toxic effects of adducts can be circumvented only if reduced GSH is immediately available in adequate concentrations and GSH-S-transferase activity is high.[20,21,30,44,45] Through noncovalent bonds, albumin is the main protein that binds and transports AFB_1 in rat or human plasma.[46–48] Toxin persists (bound to albumin) in the peripheral circulation of humans from 20 to 60 days.[5] Furthermore, aflatoxin metabolites undergo enterohepatic circulation.[5] The GSH-adduct conjugates are exported into bile by the multidrug resistance–associated protein-2 pump.[3] In the intestine, these can be resorbed and subsequently transported to the kidney.[49] The GSH-conjugates might be "dismantled" by renal γ-glutamyltranspeptidase (as shown for ochratoxin) resulting in bioactivation of AFB_1 adducts and subsequent renal tubular damage.[49] Thus, the appearance of granular casts in the urine of dogs succumbing to aflatoxicosis might reflect adduct-mediated injury, altered redox status, or impaired renal perfusion from the systemic effects of terminal aflatoxicosis. Although we identified some variables that might comparatively predict death be-

tween survivor and nonsurvivor dogs (eg, neutrophilic leukocytosis, prolonged aPTT and PT, hyponatremia, hypochloremia, hypocalcemia, hyperphosphatemia, hyperbilirubinemia, hypoproteinemia including hypoalbuminemia and hypoglobulinemia, high SUN, and cylindruria), application of this information would not be generally useful on an individual basis with the exception of granular casts in urine. All dogs (7/21; 33%) identified with granular casts died. However, the relationship between purported renal metabolism of aflatoxin and the appearance of granular casts only in dogs that died remains speculative.

Of the 72 dogs in this study, 26 (36%) lived and 46 (64%) died; many dogs already had severe clinical signs at initial evaluation. General treatment for aflatoxicosis was directed at providing supportive care while the source of the toxin was eliminated. Treatments devised for intoxicated dogs were based on knowledge of aflatoxin biotransformation, toxic mechanisms, and interventions in the experimental literature in a variety of animal species.[50,51] We also considered the importance of GSH to these detoxification mechanisms; the inherently lower hepatic GSH status in clinically normal dogs, compared with other species; and the pathologic reductions in GSH found in many dogs with spontaneous liver disease.[28] Because aflatoxin metabolites undergo enterohepatic circulation and no information details recirculation of eluted toxin from dying cells or catabolized proteins, it was unclear whether long-term administration of thiol donors to replenish systemic and hepatic GSH was necessary.[50] Thus, we elected to provide hepatic supportive care with thiol donors for 2 months following initial diagnosis. In dogs requiring critical supportive care (eg, hospitalization for IV fluid or blood component treatment), we initially administered n-acetylcysteine IV as the thiol donor but changed to oral administration of s-adenosylmethionine when dogs could tolerate this route of administration.[50] Antiemetics were used to combat persistent vomiting or nausea. The unexpected gastric atony found in 3 dogs did not respond to any form of antiemetic or prokinetic treatment and led to euthanasia in 2 dogs. Because hypomotility of the stomach or intestines has not been previously described in aflatoxicosis of dogs, not all critically ill dogs were evaluated for this problem. It remains unclear whether gastroenteric atony represents a newly discovered clinical manifestation of aflatoxicosis in dogs or is attributable to another cause such as an unidentified concurrently present neurotoxic mycotoxin in the contaminated food. The histamine-2 receptor blocking agent famotidine and the gastric cytoprotective drug sucralfate were incorporated into the treatment of all dogs to treat clinically suspected gastric ulcers and esophagitis secondary to protracted vomiting. Silybin (a flavonoid derived from milk thistle) was administered because it is considered hepatic protective having potential to modify formation of CYP450 AFB_1 adducts, enhance GSH-S-transferase activity, and promote GSH synthesis.[42,51,52] Blood-component treatment and vitamin K_1 were administered to curtail concurrent coagulopathies. Treatment with vitamin K_1 was intended to replenish depleted stores of this important coagulation cofactor, as such supplementation in rats with AFB_1 aflatoxicosis normalized prolonged wholeblood clotting times.[53] It has also been proposed that AFB_1 may impose a coumarin-like anticoagulant effect.[16] Vitamin E was administered because it is not synthesized by mammalian cells, must be supplemented or acquired from the diet, and is the most important fat-soluble chain terminator of membrane lipid peroxidation.[52] Dosing of vitamin E was limited to ≤ 10 U/kg/d (≤ 4.5 U/lb/d) because high-dose α-tocopherol supplementation inhibits γ-carboxylation of vitamin K–dependent coagulants.[54] Broad-spectrum antibiotics were used to protect against systemic infection in consideration of potential aflatoxin-mediated immunosuppression as documented in rats, poultry, and swine.[5,22] L-carnitine was added to the treatment regimen to ameliorate hepatic lipidosis shown cytologically and histologically and in consideration of other benefits shown in experimental aflatoxicosis.[55–58] For example, in 1 rodent study[57] of aflatoxin protein binding, L-carnitine protectively deviated adduct binding from hepatocellular DNA to plasma proteins. Furthermore, L-carnitine administered to quail chronically exposed to AFB_1 conserved hepatic GSH and reduced oxidative injury.[58]

Several other treatments reported in the literature were not used in the dogs of this study. Oltipraz, a drug originally used to treat schistosomiasis, has been investigated in humans and experimental animals exposed to aflatoxin.[4] Through its effects as an enhancer of GSH-S-transferase activity and modulator of certain CYP450 oxidases, oltipraz reduces toxic adduct formation and promotes AFB_1-GSH conjugation (thereby limiting toxic effects in nonhuman primates and rabbits).[59,60] However, the lower GSH concentrations in dogs seemingly would preclude simple benefit from enhanced GSH-S-transferase activity. In addition, the suspected chronic ingestion of aflatoxin in dogs involved with this outbreak precluded rational use of oltipraz as a hepatic protectant. In rabbits, concurrent ingestion of aflatoxin and oxytetracycline minimizes toxic effects owing to unique metabolism of aflatoxin in this species, compared with humans, dogs, and other animals.[61,62] Although multiple CYP450s activate AFB_1 to toxic and mutagenic adducts in humans and rodents,[1] the response to specific CYP450 inhibitors such as cimetidine remains unclear. Furthermore, because CYP450s do not share substrate specificities among species, information derived from rodent studies might not be applicable to dogs. In addition, the chronic aflatoxin exposure status of affected dogs would obviate the utility of an enzyme inhibitor targeting toxic adduct formation.

Treated dogs frequently underwent a progressive clinical deterioration despite outlined treatments. Based on experimental work in the dog[3,a] and clinical observations and clinicopathologic features in the affected dogs described herein, we propose a scenario that might explain the escalation of clinical illness in severe aflatoxicosis of dogs. Initially, AFB_1 adducts rapidly accumulate forming covalent bonds with hepatocyte DNA and proteins and disrupting intracellular metabolism, cell renewal, protein synthesis, and lipid exportation. Simultaneous depletion of hepatocellular GSH permits oxidative membrane damage and loss of cellular enzymes (including GSH-S-transferase). Cells surrounding the he-

patic venule (zone 3) are first affected because this is the predominant location for CYP450s producing the AFB_1 epoxide. Ultimately, toxic adducts cause cytolytic necrosis and parenchymal collapse of zone 3 hepatocytes. Architectural disruption occludes egress of blood from the hepatic sinusoids imposing intrahepatic portal hypertension and increased formation of ultrafiltrate (lymph) in the space of Disse. Increased hepatic lymph subsequently leaks from the hepatic capsule into the abdominal cavity (modified transudate). The acute portal hypertension coupled with impaired synthesis of clotting factors allows for intestinal RBC diapedesis and bleeding. In dogs with experimental aflatoxicosis,[a] point sources of gastrointestinal hemorrhage have not been grossly or histologically identified despite laboratory data supportive of a coagulopathy, as also noted in dogs of this outbreak. The presence of enteric blood promotes onset of hepatic encephalopathy, consistent with the semiconscious status of dogs during the terminal stages of aflatoxicosis. We verified formation of multiple acquired portosystemic shunts in some dogs, corroborating development of portal hypertension.

The findings reported herein provide veterinarians with the spectrum of features likely to be encountered in dogs with spontaneous naturally acquired foodborne aflatoxicosis. Although proven protocols were not available to guide treatment of dogs at onset of this outbreak, critical supportive care and interventional strategies designed to intercept aflatoxin-mediated liver injury seemingly allowed some profoundly ill dogs to survive beyond all reasonable expectations.

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c. Advia 120, Bayer Diagnostics, Norwood, Mass.
d. Actin FS, Dade Behring, Newark, Del.
e. Thromboplastin LI, Helena Diagnostics, Beaumont, Tex.
f. Fibrinogen, Diagnostica Stago, Parsippany, NJ.
g. Stachrom ATIII, Diagnostica Stago, Parsippany, NJ.
h. Stachrom Protein C, Diagnostica Stago, Parsippany, NJ.
i. Statistix 7, version 7.0, Analytical Software, Tallahassee, Fla.

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