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Timely Topics in Nutrition

Aflatoxicosis in dogs and dealing with suspected contaminated commercial foods

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In December 2005, 2 dogs died of acute hepatic failure after consuming a commercially available food formulated for dogs. Food toxicosis was suspected. The product manufacturer was notified by the attending veterinarian, and necropsy of the dogs was performed by a university pathology service. Product date codes for the product consumed by the dogs were not provided to the manufacturer, and the FDA was not notified of potential product contamination. Screening of raw ingredients and finished product to detect aflatoxin by the manufacturer yielded negative results.

However, additional dogs in the eastern United States developed clinical signs of suspected toxicosis. These additional animals, along with the product description and date code, were reported to the product manufacturer. The FDA was notified of the possible toxicosis, and testing confirmed contamination with aflatoxin. The manufacturer issued a recall of 19 products produced at a plant in South Carolina. After the manufacturer conducted additional extensive testing of samples of finished products, the list of products and potentially affected lots was narrowed to specific production and use-by dates. Recall instructions advised pet owners to discontinue feeding the product and to return bags of food to their retailer. Concerned pet owners were instructed to consult a veterinarian if their dog had clinical signs that included loss of appetite; yellow coloration of the whites of the eyes, gums, or skin in the belly or other areas where the hair is thin; severe, persistent vomiting combined with bloody diarrhea; discolored urine; and fever.

As consumers and veterinarians became informed about the contamination, the number of reported dogs in the eastern United States increased rapidly. Internet-based discussions on a general veterinary Web site and list servers for recognized veterinary specialty organizations facilitated communication and sharing of recommendations on monitoring, testing, and treating affected dogs. Despite the timely recognition of the toxicity and dissemination of information, >100 dogs apparently died as a result of the toxic effects of contaminated products. This highlights the need for veterinarians to rapidly recognize adverse events involving consumption of contaminated foods; contact the appropriate groups involved (eg, retailers, product manufacturers, and regulatory agencies); and retain suitable food samples, product labels, and biological samples to aid in the identification of contaminated foods, rapid notification of pet owners, and documentation for clients.

Clinical Signs of Aflatoxicosis

Eight dogs were examined or necropsied (or both) at the University of Tennessee College of Veterinary Medicine and confirmed to have aflatoxicosis linked to consumption of a commercially available food formulated for dogs. Review of the information for these 8 dogs provides a spectrum of the medical history, clinical signs, and progression of disease seen with aflatoxicosis. In addition, 4 other dogs with suspected aflatoxicosis were evaluated or necropsied at the university. However, aflatoxicosis could not be confirmed in those 4 dogs because of atypical clinical signs or pathologic characteristics and lack of evidence for toxicologic exposure (eg, lack of food samples or product date codes or negative results for analysis of food and tissues). Therefore, the information provided here does not include data for those 4 suspect dogs.

The 8 dogs consisted of 6 Basset Hounds, 1 Australian Shepherd, and 1 Airedale Terrier. These dogs had a median age of 2 years. The Australian Shepherd died at home after a 4-day episode of lethargy, anorexia, and vomiting that had progressed to icterus, melena, and hematemesis. Hematologic testing conducted by the referring veterinarian confirmed that the dog had increases in hepatic enzyme activity and total bilirubin concentration. A necropsy was per-
formed on the Australian Shepherd. A seventh Basset Hound from the same household had also died after an episode of an acute onset of lethargy, anorexia, icterus, gingival petechial hemorrhages, and increased ALT activity, as determined by testing conducted by the referring veterinarian. Although a necropsy was not performed on this Basset Hound, subsequent analysis of the food fed to the dogs of that household supported a diagnosis of aflatoxicosis.

Historically, several affected dogs had a decrease in interest in consuming the food prior to the knowledge that it was contaminated, which was suggestive of altered palatability of the diet. Three of the 7 dogs examined at the university were sick at the time of initial examination, with signs of lethargy, anorexia, vomiting, melena, and icterus. Four Basset Hounds, which comprised half of the 8 dogs in which aflatoxicosis was definitively confirmed, did not have clinical signs at the time of our initial evaluation and were examined because of the death of the aforementioned Basset Hound and 2 ill Basset Hounds from the same household. Analysis of results of laboratory testing conducted at the time of initial examination confirmed that all 7 dogs examined (including the 4 dogs that did not have clinical signs of aflatoxicosis) had varied increases in activity of ALT (range, 269 to 2,259 U/L; median, 803 U/L; reference range, 25 to 106 U/L) and AST (range, 75 to 1,090 U/L; median, 148 U/L; reference range, 16 to 30 U/L), hyperbilirubinemia (total bilirubin concentration; range, 0.3 to 8.6 mg/dL; median, 1.4 mg/dL; reference range, 0.1 to 0.2 mg/dL), and a prolonged prothrombin time (32 to > 70 seconds; median, 52 seconds; reference range, 10.0 to 16.8 seconds).

**Treatment and Outcome**

All 7 dogs received supportive treatment by IV administration of an electrolyte solution that contained supplemental potassium chloride. Dogs also were administered S-adenosylmethionine, milk thistle, vitamin K<sub>1</sub>, ampicillin, famotidine, sulfacetamide, nutritional support via total parenteral nutrition, and transfusions of fresh-frozen plasma. The condition of the Airedale Terrier quickly deteriorated, which led us to euthanatize the dog within 12 hours after initial examination, and the 2 ill Basset Hounds died within 3 days after initial examination at our university facility.

Despite the aggressive treatment early during the course of the condition, clinical signs (anorexia, icterus, vomiting, melena, and severe peripheral edema) developed in all 4 remaining Basset Hounds within 8 days of our initial examination during admission. Clinical signs corresponded with progression of increases in ALT and AST activities, hyperbilirubinemia, hypoalbuminemia, hypocholesterolemia, and severe coagulopathy. Three of the 4 dogs died or were euthanized within 2 days after onset of clinical signs. The last of the Basset Hounds that developed clinical signs was a 2-year-old sexually intact male that had been receiving a restricted amount of the contaminated food because of the dog’s inclusion in a weight-loss program. This dog had a slower progression of changes in laboratory variables and was the final dog to develop clinical signs on day 8 after admission. Despite continued aggressive supportive care, the dog’s condition worsened during the subsequent 7 days and this final Basset Hound was euthanatized on day 15 after admission.

**Necropsy Findings**

Necropsies were performed on all 8 dogs with confirmed aflatoxicosis. All had severe icterus of the mucous membranes and sclera. Hepatomegaly and a diffuse yellow friable appearance of the liver were consistently identified. Six of 8 dogs had severe acute edema and hemorrhage in the subcutaneous tissues as well as intestinal hemorrhage. Four dogs had systemic petechiae, 4 had gastric ulcers with melena, and 3 had reactive femoral bone marrow.

Histologic lesions were similar in all dogs but varied in extent and duration. Marked cytoplasmic vacuolar degeneration consistent with accumulation of hepatocellular lipids was evident in all 8 dogs. Portal fibrosis was identified in 7 dogs, and 7 dogs had biliary hyperplasia. Hepatocellular cholestasis was detected in all 8 dogs. Inflammation with primarily lymphocytes was identified in the portal triads of 7 dogs, and scattered hepatocyte necrosis was identified in 4 dogs. Lesions of lesser importance included sinusoidal extramedullary hematopoesis in 4 dogs, nodular hyperplasia in 2 dogs, and arteriolization of central veins in 3 dogs.

**Aflatoxins**

Aflatoxins are a group of related, natural, toxic by-products of the fungi Aspergillus flavus, Aspergillus parasiticus, and a few select Penicillium spp, which are ubiquitous soil contaminants found throughout the world. These fungal organisms grow on most natural products, including corn, peanuts, rice, soybeans, wheat, and oats. Aflatoxin production results when there are specific environmental temperatures and moisture conditions, and risk of contamination is increased when crops are stressed by drought, insect damage, improper field management, or inappropriate handling or storage. Four natural aflatoxins (B1, B2, G1, and G2) are responsible for food contamination, with B1 being the most hepatotoxic. Aflatoxins also can be immunosuppressive, nephrotoxic, and carcinogenic, and they can cause hemolytic anemia and coagulopathies.

Aflatoxins are liposoluble and readily absorbed from the gastrointestinal tract into the portal blood. They are then transported to the liver for metabolism. Toxicosis is a result of binding of essential enzymes, which blocks DNA polymerase and ribosomal translase and causes formation of DNA adducts. Dogs and cats are especially susceptible to the toxic effects of aflatoxin B1 in feed (> 60 μg/kg of feed) with an LD<sub>50</sub> of 0.5 to 1.5 mg/kg of body weight for dogs and 0.3 to 0.6 mg/kg for cats. There is a dose-response correlation with survival time and intensity of histologic changes. Rate of metabolism varies on the basis of genetics, age, hormonal status, nutritional status, and concurrent disease, with young animals and animals that are pregnant being the most susceptible to the toxic effects of aflatoxins.
Aflatoxin Contamination

All corn and corn products are routinely tested for aflatoxin contamination prior to their use in the manufacture of commercial food formulated for dogs. Corn is often screened for fluorescence by use of a 365-nm black light prior to its sale to manufacturers and again by the manufacturer prior to being unloaded from trucks. Fluorescence screening is a presumptive test for aflatoxins and is based on the natural bright greenish-yellow fluorescence of kijic acid produced by the fungi, which signifies fungal growth. However, this test cannot be used to quantify the amount of toxin. Aflatoxins are not evenly distributed throughout each load of contaminated corn, which allows false-negative results during routine screening tests.

A more definitive test uses HPLC to detect and provide a range of aflatoxin contamination. The FDA encourages but does not require HPLC testing; however, the FDA does require that food manufactured for consumption by humans and dogs must contain < 20 µg of aflatoxin/kg of food.

For the situation described here, the FDA concluded on January 19, 2006, that the company did not appropriately adhere to its own stringent guidelines for aflatoxin testing for 12 shipments of corn that arrived at the plant in South Carolina in September and October of 2005. Food manufactured for dogs between October 1 and 15, 2005, was contaminated, and the final food product was shipped from this plant to retailers in 23 states and at least 29 countries. To minimize the risk of aflatoxin contamination in the future, the manufacturer has strengthened premanufacturing monitoring of incoming corn and added additional testing of the final product to their safety protocols.

Prevention of Aflatoxicosis

Because aflatoxin contamination can be found sporadically throughout corn in shipments, and small amounts can potentially escape detection despite regular monitoring, researchers have investigated methods to prevent gastrointestinal absorption of aflatoxins. A promising prospect is the addition of dietary clay to dry diets during the extrusion process or as a coating on the surface of the kibble. Addition of hydrated sodium calcium aluminosilicate, a clay that tightly and selectively adsorbs aflatoxins, is effective in protecting dogs fed aflatoxin-contaminated foods. In the future, the addition of this clay may provide the pet food industry with a means to further ensure the safety of foods formulated for pets.

Diagnosis and Documentation of Aflatoxicosis

Historical evidence of exposure and results of laboratory tests, including an increase in ALT activity, hyperbilirubinemia, hypocholesterolemia, and prolonged clotting times, support a clinical diagnosis of aflatoxicosis. Further supportive diagnostic test results include low concentrations of antithrombin III and low activity of serum protein C. Samples for measurement of concentrations of antithrombin III can be submitted to a specific veterinary diagnostic laboratory and has proved promising for use as a sensitive screening test for aflatoxicosis, although the assay is not specific because activity of protein C may decrease in response to exposure to any hepatotoxin.

Concentrations of the M1 metabolite of aflatoxin B1 in urine samples have been measured by use of HPLC in research settings for documenting aflatoxicosis and monitoring affected animals. However, this metabolite is cleared from the body within 48 hours after ingestion. Therefore, testing of urine samples may be worthwhile for dogs suspected of aflatoxin exposure that are still consuming the diet, but it is not appropriate as a screening test for dogs whose diet has been changed because of suspected food contamination.

Histologic lesions in liver samples obtained during biopsy procedures or necropsy can help confirm aflatoxicosis. Additional confirmation can be obtained by submitting serum or liver samples for aflatoxin testing. In the situation described here, samples of liver from each of the 8 dogs were submitted for analysis of aflatoxin metabolite (M1) and results confirmed high amounts of aflatoxin in 7 of the 8 livers.

Saving a sample of the suspect food as well as packaging information, including the product and date code, is a critical component of documenting food contamination and aiding manufacturers in identifying and isolating the contaminated lots (Figure 1). Pet owners may be encouraged to keep food for their pets in the original packaging, rather than transferring food into a separate storage container. The owner of the Basset Hounds described here saved a sample of food and a package label indicating the manufacturing plant and use-by date involved in the recall. The sample of food had positive results when tested for aflatoxin B1 (579 µg/kg), aflatoxin B2 (19 µg/kg), and total aflatoxin (598 µg/kg). The toxic range for total aflatoxin concentration is > 60 µg/kg of feed. The food fed to the Airedale Terrier also had positive results when tested for total aflatoxin (223 µg/kg).

Providing pet food manufacturers with a clinical diagnosis and detailed description of the suspected product, date codes, and consumption data will help to focus analytic testing to the most likely contaminant and help them to more rapidly identify affected lots of product. Working in conjunction with pet food manufacturers typically results in the rapid identification and resolution of food contamination issues.
Recognizing Food Contamination

Most episodes of contaminated commercially manufactured pet foods are evident as a geographic and temporal cluster of cases within the same household, kennel, or area. The clustered cases are suggestive of an infectious disease or exposure to toxins, and obtaining a thorough medical history may help limit the list of initial differential diagnoses. In the dogs described here, leptospirosis was considered as a primary differential diagnosis for the 1 dead and 2 ill Basset Hounds prior to discovery of aflatoxin contamination. However, PCR assay for leptospirosis yielded negative results. A history of recent feeding from a new bag of food, recent changes in diet, signs of altered food palatability, or decreased appetite are all consistent with a contaminated food. The index of suspicion for a contaminated food is further increased when multiple households with affected dogs feed the same diet, although not all dogs consuming that diet will necessarily have clinical signs or be affected.

Identifying Toxins in Foods

Once a toxin is suspected, an epidemiologic investigation is initiated to identify the unknown toxin or toxins and appropriately deal with the situation. A checklist of components is suggested for use in helping veterinarians identify and document disease caused by contaminated foods (Appendix). A toxin-oriented approach can be used when exposure to a particular toxin is known or suspected and tests can quickly be conducted to verify that toxin. More often, the toxin or toxins are unknown, and investigators must use a disease-oriented approach that involves recognized clinical signs to identify the suspect toxin and then confirm the underlying cause. Steps in this investigation include a descriptive phase, generation and testing of a hypothesis, and confirmatory testing. The descriptive phase involves creating a case definition as well as describing signalment of affected patients, changes in laboratory test results for specific variables, results of histologic examination, and patterns of disease progression. Generation and testing of a hypothesis is conducted on the basis of known toxins that may cause the clinical findings and appropriate documentation from toxicologic testing of the blood or tissues of affected animals. When a toxin can be identified during analysis of a patient’s sample, and exposure to that toxin is consistent with the clinical signs, confirmation testing of the suspect food should be performed to establish a causal relationship.

Contacting the Appropriate Groups about Suspected Food Contamination

As soon as food contamination is suspected, veterinarians should contact the manufacturer of the product. Veterinarians should also contact the FDA when the dietary history and medical evaluation are consistent with food contamination. Samples of food should be obtained from pet owners for testing, and packaging information should be obtained, if possible. Veterinarians should maintain medical records documenting clinical signs and laboratory test results consistent with the suspected contamination. Veterinarians can submit samples to veterinary diagnostic laboratories or toxicology laboratories for testing, but it is not necessary to await laboratory test results prior to contacting the aforementioned groups. Although suspected food toxicoses reported by pet owners are often subsequently found to be incorrect, appropriately documented cases reported by veterinarians are highly credible.

Once contacted, the manufacturer and FDA will perform additional testing to quickly confirm or disprove the suspected contamination and decide whether a product recall is indicated. The manufacturer should then communicate via the media to inform veterinarians and consumers of the potential contamination as well as to provide recommendations for recall and procedures for reporting possible cases. Timely dissemination of knowledge is critical during food contamination episodes to minimize additional exposures and allow for appropriate screening and treatment to begin as soon as possible.

State veterinarians and state, regional, and local veterinary medical associations can help notify veterinarians. Each veterinarian can then inform their clients of the recall and provide medical advice. Retailers of the food products also play a large role in providing information to pet owners and reminding them to save samples of suspected food as well as labels from suspected food products. It is important that all authoritative groups or people involved report only known facts and provide sound and justifiable advice to consumers to avoid unnecessary panic or alarm.

In the aflatoxin-contamination episode that affected >100 dogs, the Internet became an extremely useful modality for communication among veterinarians. Various professional list servers and a general veterinary Web site were excellent sources for up-to-date information as the epidemiologic investigation progressed, which helped further develop the case definition and document the progression of the condition in affected dogs. Use of the general veterinary Web site also allowed open discussion among veterinarians employed by corporations or the pet food industry, veterinarians in general practice, and board-certified veterinary specialists so that timely and appropriate recommendations for diagnostic testing and treatment protocols could be developed. However, despite good intentions by all contributors, it should be remembered that Internet discussions do not represent peer-reviewed publication; thus, caution must be used to avoid dissemination of misinformation.

Submitting Samples for Analysis

A veterinary diagnostic toxicologist can be contacted to help clinicians narrow the list of potential toxins and determine the appropriate samples for submission. Samples of whole blood (collected into tubes containing EDTA as an anticoagulant), serum, urine, fresh or frozen tissues, gastrointestinal contents, and food are all commonly requested in cases of suspected food contamination. As soon as the association between the illness and contaminated food is recognized, every attempt should be made to save a large sample of the food and the entire package label (or at
least the package label with the product code and use-by date). Approximately 1 kg of dry food or 4 cans of food should be saved for testing, but a portion should also be maintained for future reference. Food should be frozen or stored at room temperature in an airtight bag. Unopened cans of food should remain at room temperature. Samples of fresh or frozen tissues for use in toxicologic analysis should be large, whereas samples placed in formalin for histologic examination should be thin to allow for proper penetration by the fixative.3

Samples should be submitted for analysis as soon as possible, although weekend and holiday shipments should be avoided. Testing laboratories can advise veterinarians on shipping conditions, such as whether dry ice is needed. Proper packaging for biohazardous material is required. A full list of accredited veterinary diagnostic laboratories is provided at the American Association of Veterinary Laboratory Diagnosticians Web site.16 For the situation described here, food samples were submitted to 2 separate veterinary diagnostic laboratories15 and liver samples were submitted on dry ice to another veterinary diagnostic laboratory.1

Determining Appropriate Treatment

Initial treatment for sick dogs should be based on documented problems and tentative diagnoses. Often, this includes nonspecific supportive care, such as in the case of aflatoxicosis. To address hepatic damage caused by aflatoxins, treatments include S-adenosylmethionine, milk thistle, vitamin K1, vitamin E, nutritional support, and fresh-frozen plasma. As more information becomes available about the contaminating toxin or toxins, specific treatments may become an option. It can be challenging to determine which dogs require treatment and when to begin treatment, and such decisions should be made on the basis of knowledge about toxin behavior and discussions between veterinarians and clients. In the dogs with aflatoxicosis described here, many had progressive changes in laboratory test results for several variables while still without clinical signs of toxicity and prognosis was guarded once clinical signs developed. This suggests that early treatment before development of clinical signs was indicated to minimize morbidity and fatalities in dogs known to have ingested aflatoxin-contaminated food. Early prophylactic or therapeutic treatments are not necessarily indicated for every patient with potential toxin exposure; thus, each animal must be assessed separately.

Commercial Food Contaminations

Although rare, contaminations of commercial foods formulated for dogs that result in substantial morbidity and fatalities are seen sporadically. To our knowledge, since 1975, there have been 11 other episodes of aflatoxin contamination in foods manufactured for dogs. The most recent episode was in 1998 in Texas, with 55 confirmed deaths of dogs.13 In 2003, 48 dogs (all of which were consuming the same natural dog food) became ill with various clinical problems, including hepatic failure, immune-mediated hemolytic anemia, and sudden death.16 However, despite thorough testing, the final FDA report did not identify the inciting cause, although deviation in product formula by the addition of a synthetic antioxidant to the natural diet was discovered. In early 2006, a manufacturing error in another commercial diet was identified, with excessive amounts of vitamin D3 cited as the cause for hypercalcemia in dogs and cats.25 These episodes of food contamination reinforce the need for quality-control monitoring by food manufacturers as well as quick recognition and action by veterinarians suspecting such a problem.

Conclusions

Despite protective measures and protocols to ensure safety of food components and accuracy of recipe preparation, food contamination and misformulation accidents have resulted in morbidity and fatalities of animals. Veterinarians are instrumental in the timely recognition of adverse health effects associated with adulterated foods. Appropriate notification of manufacturers and regulatory agencies, combined with detailed documentation, sample collection, and analytic testing, will aid in the confirmation or rejection of a suspected toxic exposure.

An outbreak of aflatoxicosis was reported in at least 100 dogs consuming a commercial food manufactured in the southeastern United States. Of the dogs examined at our university facility, 8 were confirmed with aflatoxicosis and served to illustrate the variability in clinical signs of acute aflatoxicosis as well as to highlight the appropriate steps for appropriate notification of the manufacturer and regulatory agencies, documentation for each animal, and confirmation of the involved toxin. Timely and appropriate handling of situations that involve suspected contaminated commercial foods will help minimize exposure, morbidity, and fatalities of animals.

References

5. Bingham AK, Phillips TD, Bauer JE. Potential for dietary pro-

Appendix
Suggested checklist for dealing with suspected contaminated food.

- Retain food samples for analysis.
  - Retain 4 cans or 1 kg of dry food, when possible.
  - Freeze when possible or store at room temperature in airtight bags.
- Document product name, type of product and manufacturing information.
  - Retain all packaging.
  - Identify date codes or production lot numbers.
  - Retain purchase receipts.
- Document product consumption.
  - Dates product or products were fed.
  - Consumption and palatability history.
  - Detailed dietary history (ie, all products fed and feeding methods).
- Contact the manufacturer.
- Contact the FDA Consumer Complaints Coordinator for your state.
  - A list of telephone numbers for each state is available at: www.fda.gov/opacom/backrounders/complain.html.
- Document communication with the FDA, manufacturer, and clients.
  - Record date, time, and contact person.
  - Maintain a unique identification number for each patient.
- Submit samples to a diagnostic laboratory, the FDA, or the manufacturer for analysis.
- Submit all deceased animals for necropsy or collect appropriate samples.
- Store tissue samples in formalin as well as in a freezer.
- Consult with personnel at diagnostic laboratory for required quantity of tissues, tissue preparation, storage conditions, and submission of samples.
- Maintain detailed medical records of affected animals.
  - Clearly record clinical diagnosis, course, specific treatments, and outcome.
  - When possible, retain serum and tissue samples for further testing.
  - Notify clients of potential exposure to contaminated foods or product recalls.
  - Recommend that clients discontinue feeding potentially contaminated food.
  - Suggest that clients always save original food packaging.
- Examine all pets with known or suspected exposure to contaminated foods.
- Submit appropriate samples for diagnostic testing.
- Initiate prophylactic or therapeutic treatment as indicated.
- Obtain written client authorization prior to release of medical information.