Subacute aflatoxicosis due to moldy bread consumption in a dog

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SUMMARY

A 2-year-old male Anatolian shepherd dog was delivered to Firat University veterinary emergency service in a comatose state with a history of anorexia and was in poor general condition with icterus, epistaxis and melena. Marked elevation of alanine aminotransferase (474 IU/L), alkaline phosphatase (463 IU/L), hyperbilirubinemia (9.15 mg/dl) and blood urea nitrogen (192 mg/dl) were highly suggestive of hepato-renal failure. Despite intravascular supportive fluid administration, the dog died two hours after arrival. This patient was the fourth dog to die from the same farm within an eight day period. The necropsy revealed severe diffuse icterus, severe and diffuse intestinal intraluminal hemorrhage, unclotted hemorrhagic fluid in the thoracic and abdominal cavities, enhanced lobular pattern in the liver, mesenteric arterial thrombosis, diffuse splenic, pancreatic and gastrointestinal edema and plant material in the stomach. The main histological findings in the liver included centrilobular hemorrhagic necrosis, degenerative changes, moderate cytomegaly, cholestasis and regenerative nodule formation. Aflatoxin exposure was confirmed by determining the levels of aflatoxins (B1, B2, G1, G2) or their metabolite (M1) in the bread included in the dog’s diet (AFB1: 326 µg/kg, AFB2: 142 µg/kg, AFG1: 1542 µg/kg, AFG2:1151 µg/kg, AFM1:72 µg/kg), in the liver (AFB1: 0.35 µg/kg, AFG1: 0.32 µg/kg, AFG2: 0.75 µg/kg, AFM1: 1.70 µg/kg ) and in the urine (AFG2: 2.55 µg/kg, AFM1: 8.60 µg/kg) by HPLC. Aspergillus flavus and Aspergillus parasiticus were isolated from bread samples. Tests for leptospirosis, adenosivirus infection and mushroom intoxication (alpha, beta and gamma amanitins) were all negative. Unique morphological changes including mesenteric arterial thrombosis and renal tubular megalocytosis have not been reported before in association with canine aflatoxicosis.

Keywords: Aflatoxicosis, Aspergillus, contaminated bread, dog, hepatotoxicity.

Introduction

Aflatoxins represent a major group of harmful by-products of different species of the fungal genus Aspergillus and are common contaminants of food. Fungal growth and toxin production occur between 24°C to 35°C [9]. There are 4 main aflatoxins including: Aflatoxin B1 (AFB1), AFB2, AFG1, AFG2 [23]. AFB1 has the most notable hepatotoxic activity resulting from the interaction between the aflatoxin, its activated metabolites and various cell proteins. These interactions result in the disruption of basic metabolic processes and protein synthesis causing cell death. The carcinogenic, mutagenic and immunosuppressive effects are due to the binding of metabolites to macromolecules and DNA, resulting in disruption of the DNA and carcinogenesis [5, 9, 23]. This report is to document the toxicological and clinicopathological findings of fatal subacute aflatoxicosis in an Anatolian shepherd dog.

Case Report

A 2-year-old, male Anatolian shepherd dog was brought to the Firat University Veterinary Teaching Hospital in a comatose state. Anamnesis indicated that the dog was housed with 3 other dogs that had all died within 8 days with no detectable signs except for anorexia and lethargy. The dogs

RÉSUMÉ

Aflatoxicose subaiguë par ingestion de pain moisi chez un chien

Un chien mâle Berger d’Anatolie de 2 ans a été présenté au service d’urgence vétérinaire de l’université de Firat dans un état comateux accompagné d’ictère, epistaxis et melena avec antécédents d’anorexie. L’analyse biochimique a révélé une augmentation des taux d’alanine aminotransférase (474 UI / L), alcaline phosphatase (463 UI / L), une hyperbilirubinémie (9.15 mg / dl) et une hyperuricémie (192 mg / dl) très suggestifs d’une insuffisance hépatorénale. Malgré une perfusion de soutien, le chien est mort deux heures après son arrivée. Ce patient était le quatrième chien à mourir dans les mêmes circonstances sur une période de huit jours. L’autopsie a révélé un ictère diffus, une hémorragie intestinale intra-luminaire, la présence de sang coagulé dans les cavités thoracique et abdominale, un élargissement de la taille des lobules hépatiques, une thrombose artérielle mésentérique, un œdème gastro-intestinal diffus et la présence de matériel végétal dans l’estomac. Les principales observations histologiques étaient la présence d’une nécrose hémorragique centrolobulaire, avec cytomégalie modérée, cholestase et nodules de régénération. L’exposition à l’aflatoxine a été confirmée par dosage HPLC des aflatoxines et leurs métabolites (B1, B2, G1, G2, M1) dans le pain inclus dans le régime alimentaire du chien (AFB1: 326 ug / kg, AFB2:142 ug / kg, AFG1:1542 µg/kg, AFG2:1151 µg/kg, AFM1:72 µg/kg), dans le foie (AFB1: 0.35 µg/kg, AFG1: 0.32 µg/kg, AFG2: 0.75 µg/kg, AFM1: 1.70 µg/kg ) et dans l’urine (AFG2: 2.55 µg/kg, AFM1: 8.60 µg/kg) par HPLC. Aspergillus flavus et Aspergillus parasiticus ont été isolés à partir de pain contaminy. Les tests pour le leptospirose, l’infection par adénovirus et la recherche d’amanitines (intoxication par champignons) se sont révélés négatifs.

Mots-clés: aflatoxicose, Aspergillus, pain, chien, hépatotoxicité.
were 5, 6 and 8 months old and of the same breed and lived mostly outdoors with a herd of sheep. The owner found the bodies of the other three dogs in the surrounding area and did not submit them for necropsy. The dog’s illness began and progressed suddenly within the past 48 hours for the present case. The typical daily diet for the dogs consisted of bread and boiled meat. Bread was the main component of the diet and was supplied every 7 to 14 days from a local restaurant with an average of 700-800 g consumed per animal per day. Samples of the bread were submitted by the owner and found not to have distinctly linear green discolorations. According to the history, the dogs were vaccinated with a modified live virus vaccine against canine parvovirus (CPV), canine distemper virus (CDV), canine adenovirus-1 and 2 (CAV-1 and CAV-2) and canine parainfluenza virus (CPI) and a bactrin-containing vaccine against Leptospira canicola and Leptospira icterohaemorrhagiae (Vanguard Plus 5/L-Pfizer/Turkey) at 12 weeks of age and received a booster 3 weeks later.

A physical exam was performed and blood was collected into sterile blood containers with EDTA for a complete blood count (Mindray BC-5800, China) and without EDTA for isolating serum for biochemical analysis (Olympus AU2700 Chemical Analyzer, Japan). Despite the initiation of supportive care including intravascular fluid administration by serum physiologic, the dog died two hours after admission and a complete necropsy was performed with subsequent histologic evaluation.

As intoxication was suspected by agents known to cause hepatic necrosis such as chemicals (carbon tetrachloride and xylitol toxicity), drugs (carprofen, sulfonamides), toxins (Amanita mushrooms, blue-green algae) and bacterial infections (Clostridium piliformis, Leptospira spp), the samples of the liver, kidney, urine and bread were collected and stored at -20°C until toxicological analysis could be performed.

HISTOPATHOLOGIC ANALYSIS

For pathological examinations, tissue samples from the liver, lungs, adrenals, pancreas, brain, small and large intestines and kidneys were fixed in 10% formalin, processed routinely, embedded in paraffin and sectioned at 5 μm. The sections were stained with hematoxylin and eosin (H&E) and selected liver sections were also stained with Masson’s trichrome and Gordon and Sweet’s reticulin stains. Frozen sections of formalin-fixed tissues were stained with oil red O. In addition, selected brain sections were examined immunohistochemically with a streptavidin-biotin kit by using rabbit anti-human glial fibrillary acid protein (GFAP, NeoMarker, USA) and S100 protein (NeoMarker, USA) at 100 fold dilution performed on automated immunohistochemistry machine (Benchmark XT, USA). From the bread sample Aspergillus flavus and Aspergillus parasiticus were isolated on Sabouraud dextrose agar.

TOXICOLOGICAL ANALYSIS

Aflatoxin analysis

Bread, liver and urine samples were analyzed for aflatoxins using reverse phase high performance liquid chromatography (RP-HPLC) as previously described [13]. Aflatoxin mix kit (Supelco, Bellefonte, PA, USA) was used as an aflatoxin standard. The mobile phase adopted for the aflatoxin determination was a mixture of water/methanol/acetonitrile (6:3:2; v/v/v). Separations were carried at a flow rate of 1 mL/min at 40°C. Analyses were conducted by using a UV detector for aminotins (303 nm) and a fluorescent detector for aflatoxins (365 nm excitation and 435 nm emission). After homogenization, supernatant was filtered through a 0.22 μm filter and a chloroform extraction was performed. Extracted material was injected in 20 μL. Standard calibration graphics were prepared for aflatoxins B1, B2, G1, G2 and M1. The R² values were 0.9987, 0.9979, 0.9995, 0.9951 and 0.9988 respectively. The retention times for the aflatoxins were AFB1 17.55, AFB2 24.04, AFG1 27.46, AFG2 31.49 and AFM1 12.89. The limits of detection (LOD) for the toxins were 0.2 ng/mL for AFM1 and 0.1 ng/mL for the others. After obtaining the calibration curve equation for each toxin, the amounts were calculated.

Amanitin

Liver and urine samples were also analyzed for α, β and γ aminotins by performing RP-HPLC separation [6] and by using a commercial HPLC system (Shimadzu, Japan) equipped with 250 x 4.6mm C18 column (Agilent, Palo Alto, California) with 5 μm particles at 40°C. The mobile phase adopted for the determination of all of the aminotins was a mixture of 50 mM ammonium phosphate aqueous solution (pH 5.5 adjusted with acetic acid) and acetonitrile (90:10; v/v). Aflatoxin analysis

Fungal Culture

The samples from the bread were passaged on Sabouraud dextrose agar (Oxoid, Hampshire United Kingdom) and incubated at 26°C for 48 hours.

Leptospira and canine adenovirus (CAV1 and 2) status determination

IgG antibody concentrations for Leptospira sp, CAV-1 and CAV-2 were determined by using commercial ELISA kits (Cusabio CSB- Cat. No: E17928 and Demeditec Cat. No: E2480). Polymerase chain reaction (PCR) was used to detect DNA of the CAV.

Results

The results of routine hematologic examination and blood chemistry revealed severe anemia, thrombocytopenia, leukopenia and hepato-renal failure by markedly increased.
enzymatic activity of Aspartate aminotransferase (463 U/L), Alanine aminotransferase (474 U/L), Gamma glutamyl transferase (13 U/L), Creatine kinase (644 U/L), elevated blood urea nitrogen (192 mg/dL), bilirubin (9.15 mg/dL) and creatinine (3.09 mg/dL), and hypocholesterolemia (Table-I).

At the time of necropsy, the dog weighed 43 kg with moderate body condition. The sclera, mucous membranes, renal pelvis, subcutaneous tissues and body fat were markedly icteric. The venipuncture site had a subcutaneous hematoma measuring 3x4x2 cm. The thoracic and abdominal cavities contained 150 and 600 ml of unclotted hemorrhagic fluid, respectively. The gastric mucosa was markedly congested and edematous and the stomach contained undigested bread that was dark yellow. Additionally, the stomach contained two pieces of mushroom-like material. The liver was swollen and friable and mottled red and yellow with a reticulated/ enhanced lobular pattern on external and cut surfaces (Fig.1). The wall of gall bladder was edematous and contained highly viscous, light brown material. The cranial and caudal mesenteric arteries contained thrombi, mesenterium was congested and the associated intestinal segments were pale tan (Fig. 2). The mesenteric lymph nodes were enlarged, uniformly congested and wet on cut surfaces. From the duodenum to the rectum, all intestinal segments showed severe, diffuse, intramural, dark red hemorrhages and some of the large intestinal segments contained mucoid material. There was moderate splenomegaly (85 g, 0.20% of body weight) and severe diffuse pancreatic edema (115 g, 0.267% of body weight). The lungs were moderately atelectatic and the kidneys were dark red and bilaterally had multifocal 1-2 mm diameter subcapsular depressions.

<table>
<thead>
<tr>
<th>Hematological and Biochemical Parameters</th>
<th>Value</th>
<th>Ref. Intervals (10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cells (WBC, 10^3/µL)</td>
<td>1.76</td>
<td>5.0 – 14.1</td>
</tr>
<tr>
<td>Red blood cells (RBC, 10^6/µL)</td>
<td>1.14</td>
<td>5.0 – 7.9</td>
</tr>
<tr>
<td>Hematocrit (HCT, %)</td>
<td>6.3</td>
<td>35 – 57</td>
</tr>
<tr>
<td>Hemoglobin (HB, g/dL)</td>
<td>1.9</td>
<td>12 – 19</td>
</tr>
<tr>
<td>Platelets (10^3/µL)</td>
<td>6</td>
<td>211 – 621</td>
</tr>
<tr>
<td>Mean corpuscular volume (fL)</td>
<td>55.3</td>
<td>66 – 77</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin (MCH, pg)</td>
<td>16.7</td>
<td>21.0 – 26.2</td>
</tr>
<tr>
<td>Mean corpusc hemog. concentration (MCHC g/dL)</td>
<td>30.2</td>
<td>32.0 – 36.3</td>
</tr>
<tr>
<td>Glucose (GLU mg/dL)</td>
<td>66.6</td>
<td>65 – 118</td>
</tr>
<tr>
<td>Blood urea nitrogen (BUN mg/dL)</td>
<td>192</td>
<td>10 – 28</td>
</tr>
<tr>
<td>Creatinine (CR, mg/dL)</td>
<td>3.09</td>
<td>0.5 – 1.5</td>
</tr>
<tr>
<td>Aspartate aminotransferase (AST, U/L)</td>
<td>463</td>
<td>23 – 66</td>
</tr>
<tr>
<td>Alanine aminotransferase (ALT, U/L)</td>
<td>474</td>
<td>21 – 102</td>
</tr>
<tr>
<td>Gamma glutamyl transferase (GGT U/L)</td>
<td>13</td>
<td>1.2 – 6.4</td>
</tr>
<tr>
<td>Total Bilirubin (TB, mg/dL)</td>
<td>9.15</td>
<td>0.1 – 0.5</td>
</tr>
<tr>
<td>Direct Bilirubin (DB mg/dL)</td>
<td>5.98</td>
<td>0.06 – 0.12</td>
</tr>
<tr>
<td>Creatine kinase (CK, U/L)</td>
<td>644</td>
<td>1.15 – 28.40</td>
</tr>
<tr>
<td>Cholesterol (CH, mg/dL)</td>
<td>29.4</td>
<td>135 – 270</td>
</tr>
<tr>
<td>Triglyceride (TG mg/dL)</td>
<td>83.5</td>
<td>20 – 112</td>
</tr>
</tbody>
</table>

Table I: Hematological and biochemical parameters of the dog.

Figure 1: Enhanced lobular pattern of liver characterized by mottled red and yellow color in a reticulated pattern, diaphragmatic surface.

Figure 2: Mesenteric arterial thrombus, mesenterial congestion (arrow) and pale tan appearance of associated intestinal segment.
Histologically, the most common hepatic lesion encountered was severe centri-lobular hemorrhage and necrosis (Fig-3). Hepatocytes in affected areas were largely absent and when present, were dissociated and admixed with accumulations of yellow bile pigments, hemosiderin-laden macrophages and hemorrhage (Fig-4). Centrilobular hepatic parenchyma was replaced by oval cells, mononuclear cells and fibroblasts, which surrounded patent central veins. Granulation tissue surrounded and compressed many central veins, producing concentric narrowing (Fig-5 and 6). There was also reticulin and collagen fibers accumulation in centrilobular areas (fibrosis). Remaining hepatocytes were frequently bi- or multinucleated and enlarged (cytomegaly) (Fig-7). Portal areas also exhibited loss of hepatocytes with granulation tissue formation and variable numbers of lymphocytes, plasma cells and neutrophils and hemosiderin-laden macrophages. Midzonal hepatocytes exhibited moderate to severe microvesicular fatty change. Large bile ducts were dilated and contained blue-gray, inspissated mucus. Randomly located throughout the lobules were sharply circumscribed regenerative nodules composed of hepatocytes exhibiting meglocytosis. There was patchy, mild to moderate subcapsular edema and hyperplasia of the bile ductular epithelium.

![Figure 3: Multifocal areas of centrilobular coagulative necrosis (N). Hema-toxyline and eosin (H&E).](image)

![Figure 4: Centrilobular necrosis and hemorrhage, bile pigment deposits (arrows) and disassociated hepatic cells, (H&E).](image)

![Figure 5: Centrilobular collagen fibers and patent central vein (arrow), Masson's Trichrome.](image)

![Figure 6: Centrilobular fibrosis with central vein, Gordon and Sweet's reticulin stain.](image)

![Figure 7: Early regenerative nodule with megalocytes, (H&E).](image)

Pancreatic and splenic changes included severe subcapsular and interlobular edema in the pancreas and severe and diffuse perivascular edema in the spleen with hemorrhage and erythrophagocytosis in the red pulp.

Nephrosis was characterized by multifocal, tubular coagulative necrosis (Fig-8), severe tubular epithelial
vacuolar degeneration and tubular ectasia with proteinosis. There was a varying degree of glomerulopathy characterized by proteinaceous fluid accumulation in Bowman’s space and capsular thickening. There was moderate, multifocal vascular congestion in the interstitial tissue of the medulla, pelvis and some glomeruli. Rarely hemosiderin deposits were present in tubular epithelial cells and tubular lumens. Multifocally, cortical tubular epithelial cells exhibited megacystis.

There was diffuse superficial mucosal necrosis with mild to moderate mononuclear and less prominent eosinophilic and neutrophilic infiltration of the lamina propria and submucosa of intestinal tissue. The lungs had patchy atelectasis and moderate, diffuse intra-alveolar hemorrhage with moderate perivascular edema.

Sections of the brain stem and midbrain cortex had groups of Alzheimer type II (AAII) astrocytes (Fig-9), characterized by indistinct cytoplasm, chromatin margination, nuclear pallor and swelling. AAII astrocytes had negative or weakly focal positive immunoreaction to glial fibrillary acidic protein (GFAP) and multifocal and moderate positivity to S100. Additionally, there was vacuolation in the neuropil and perineurial edema.

RP-HPLC analysis revealed the aflatoxin content of bread, urine and liver (Table-II). Microscopically, Aspergillus flavus and Aspergillus parasiticus were identified in preparations from the cultured material, which were stained with lactophenol.

<table>
<thead>
<tr>
<th>Sample</th>
<th>AFB1 (µg/kg)</th>
<th>AFB2 (µg/kg)</th>
<th>AFG1 (µg/kg)</th>
<th>AFG2 (µg/kg)</th>
<th>AFM1 (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bread</td>
<td>326</td>
<td>142</td>
<td>1542</td>
<td>1151</td>
<td>72</td>
</tr>
<tr>
<td>Liver (µg/kg)</td>
<td>0.35</td>
<td>-</td>
<td>0.32</td>
<td>0.75</td>
<td>1.70</td>
</tr>
<tr>
<td>Urine (µg/kg)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.55</td>
<td>8.60</td>
</tr>
</tbody>
</table>

Table II: The results of aflatoxin analysis of moldy bread, liver and urine by HPLC.

No amanitin toxins were detected in the liver or urine samples. Nor was CAV virus detected via PCR.

Discussion

Based on the history of acute death, appropriate clinicopathological findings and the detection of aflatoxins and their metabolite in the bread, urine and liver samples by RP-HPLC, a diagnosis of aflatoxicosis was made in the present case. Historically, aflatoxicosis had been called Hepatitis-X because of the difficulty in establishing a relationship between the lesions and the toxic agent until aflatoxin was isolated and the lesions were experimentally induced in dogs in the 1960s [15].

Aflatoxicosis has been reported in dogs [1, 2, 4, 5, 7, 11, 15-19, 21], farm animals [12, 20], poultry [9, 23] and laboratory animals [9]. Differences in susceptibility in various species to acute and chronic aflatoxicosis are explained by the ruminal degradation in the case of ruminants and differences in biotransformation, detoxification pathways and hepatic glutathione levels among species [4, 5, 7, 9, 15, 16, 21]. As dogs are highly sensitive to aflatoxins [4, 9, 15, 16], it is generally believed that the occurrence of aflatoxicosis in dogs is much more prevalent than in other species.

Most aflatoxin-related fatalities in dogs are associated with contaminated commercial feed [5, 11, 16, 18], corn product [21] or moldy bread consumption [19]. The legal limit for AFB1 is 5 µg/kg and all kinds of aflatoxins in the foodstuffs is 10 µg/kg in Turkey.

In this report, similar to previous ones in which contaminated feed contained 300-500 ppb³, the quantity of
Aflatoxin B1 level in the moldy bread was 312 ppb; however, much higher AFBI levels (1640-5791 ppb) have been reported [15, 21], in food in naturally occurring canine cases. However, no constant correlation between the nature and severity of the lesions and aflatoxin content of the feed could be expected. And total dose or amount of food consumption is to determine the toxic effects and outcome.

One of the most obvious microscopic changes in this study was centrilobular hepatic hemorrhagic necrosis and fibrosis, which is consistent with previous reports in the veterinary literature[19]. The differentiation between centrilobular fibrosis and veno-occlusion is highly important, because alkylating agents including aflatoxins are potentially hepatic veno-occlusion inducers. Because of the marked accumulation of collagen and reticulin fibers and the absence of central endophlebitis or completely occluded central veins, the present lesion was identified as centrilobular fibrosis rather than veno-occlusion. In addition to centrilobular changes, there was also periportal fibrosis and necrosis. It is highly probable that the necrotic changes are attributable to disseminated intravascular coagulation (DIC). Alternatively, a high concentration of toxic metabolites in the central vein might be responsible for the necrosis at this zone.

As previously reported in swine [21] and dogs [19], megalocytosis reflecting the antimitotic effects of aflatoxins was detected in this study. Hepatic megalocytosis in addition to regenerative nodule formation indicate a relatively long exposure to aflatoxins in the present case.

The dog’s environment did not contain toxic substances including poisonous mushrooms, blue-green algae, drugs or chemicals. In spite of the mushroom like plant fragments in the gastric contents, mushroom poisoning could not be confirmed in either liver or urine samples toxicologically, nor were the pathological findings consistent with mushroom poisoning. While profound hypoglycemia, vesicular hepatic nuclei, loss of nucleoli in hepatocytes and intestinal crypt necrosis have been reported as the hallmark findings in canine mushroom poisoning [17, 22], these findings have never been reported in canine aflatoxicosis to date and were not observed in this case. In contrast to mushroom poisoning [17, 20], icterus, which was observed in the present case, has always been reported as classic sign in canine aflatoxicosis. In this report, similar to previous reports, melena [7, 16], diffuse hemorrhage [6, 11, 19], enhanced lobular pattern of the liver [21], and coma [16] were typical findings in canine aflatoxicosis. These signs reflect hepato-renal deficiency and coagulopathy [1, 2, 16]. However, to our knowledge, mesenteric arterial thrombosis, which might be indicative of DIC, has not been reported before in cases of aflatoxicosis. The combination of decreased production of clotting factors and DIC was proposed as the mechanism of a coagulation defect in experimentally induced aflatoxicosis in rabbits [3]. We attribute the coagulopathy, at least in part, to the DIC in the present case based on severe thrombocytopenia, mesenteric arterial thrombosis, severe internal hemorrhages and bilateral tubular necrosis. However, neither glomerulor nor renal arteriolar thrombosis was detected in this case. Renal changes including tubular degeneration and necrosis have been documented in previous studies [2, 4, 5, 7, 8, 11, 15, 19] and in the present case. However, glomerulopathy, which was observed in the present case, has only been reported in dogs [8]. The tubular necrosis seen in the kidneys in this case may be a result of re-absorption of aflatoxins and their metabolites, or alternatively DIC might play a role in the tubular necrosis.

Hepatic encephalopathy, defining a complex and reversible syndrome due to hyperammonemia, results from the disturbance of ammonia metabolism arising from hepatic failure [17]. Ammonia in the central nervous system is metabolized by astrocytes to glutamine, and evidence for a direct toxic effect of ammonia on astrocytes has been reported [14, 17]. Hepatic encephalopathy was diagnosed in 21 of 50 canine cases of aflatoxicosis in which high plasma ammonia levels were found in conjunction with neurologic signs [5]. From the evidence presented here, it is clear that hepatic encephalopathy in dogs might cause structural changes in the brain as evidenced by AAII astrocytosis, perineural edema and hydropic changes in the neuropil. AAII cells are regarded as an early activation stage of quiescent to reactive astrocytes with accumulation of intermediate filaments, principally GFAP [14]. The observation that the AAII cells in this report lacked immunoreaction to GFAP is in agreement with earlier reports[16,20] and it might be due to not to an incomplete transformation to reactive astrocytes [14].

In conclusion, the data showed that aflatoxicosis was the most likely cause of the dog’s death in this case and is a reminder that aflatoxins are an important canine health hazard. The occurrence of histological changes including renal megalocytosis and mesenteric arterial thrombosis in association with aflatoxicosis were demonstrated for the first time in this report.

Declaration of conflicting interests

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