Aflatoxicosis in rottweilers after eating moldy bread: clinicopathological features and effective tetrasulphate therapy

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ABSTRACT

The purpose of this study was to describe the clinical, hematologic, gross, histopathologic and toxicologic findings and to report effective therapy application for aflatoxicosis in dogs, ascribed to the ingestion of moldy wet bread contaminated with aflatoxin. A prospective case series of 10 client-owned dogs from the same household developed toxicological signs after eating moldy bread treated with water that had been stored for an undetermined period, fully covered with a grey-green mold. All dogs exhibited vomiting followed by excessive salivation and hyperaesthesia. Among the surviving dogs (n = 7), three of them presented with diarrhea, depression, abdominal pain and two others showed icterus. One of the dead dogs was found on the initial referral. Two others were dead following initial diagnosis and prior to therapy application. The most common gross findings in the dogs were generalized icterus, mucosal or submucosal edema and petechial, to ecchymotic hemorrhages in the organs. Histopathological findings included focal necrotic areas extending from the periacinar to centroacinar regions in the liver, biliary hyperplasia, cholestasis and multifocal hemorrhages in the kidneys and lungs. Due to the sudden onset of clinical signs, the lack of exposure to other toxins, and the confirmed evidence of ingestion of moldy bread, the results of analysis of liver samples and the histopathological signs, the definitive diagnosis was mycotoxicosis. Therapeutic applications included tetrasulphate (an antidote) given orally, and supportive treatment with balanced electrolyte solutions, antiemetics and H_2 receptor antagonist. All treated dogs (7/7) made a full recovery over 18-24 hours. The results of the present study reported here describe the clinicopathological features of aflatoxicosis in Rottweilers, suggesting that the use of tetrasulphate solution as an inexpensive and available therapy may have helped the survival of the dogs and might reverse the adverse health effects of mycotoxins.

Key words: aflatoxicosis, rottweiler, moldy bread, mycotoxicosis, tetrasulphate, therapy

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Introduction

Toxic metabolites produced by fungus may result in mycotoxicosis. In small animal practices, exposure to aflatoxins, trichothecenes, tremorgens, ochratoxin, or penicillic acid may cause disease. Aflatoxins could cause natural outbreaks of mycotoxicoses (PUSCHNER, 2002). Aflatoxicosis has been reported in humans, dogs, cats, cattle, birds, rodents and poultry following exposure to mycotoxin contaminated food (NEWBURNE, 1973; BASTIANELLO et al., 1987). Poisoning with aflatoxin after eating moldy bread was reported previously in dogs (KETTERER et al., 1975, BAILLY et al., 1997). In a case series of 5 pigs, 1 peracute case of aflatoxicosis was diagnosed due to consumption of moldy bread (KETTERER et al., 1982). Apart from reports involving moldy bread, another case series was reported in nine dogs with aflatoxicosis after exposure to contaminated commercial dog food (NEWMAN et al., 2007). Similarly 72 dogs with aflatoxicosis, ascribed to ingestion of dog food, were detected in 2008 (DERESZYNSKI et al., 2008).

There have been a limited number of clinical studies regarding natural aflatoxicosis in dogs, especially in Turkey. To the authors' knowledge no definitive and completely effective treatment protocol has been established to date. In the present study the aim was to report the clinical, histopathological and toxicological features of mycotoxicosis in dogs, ascribed to the ingestion of wet bread contaminated with aflatoxin B2 and G2, and consequently describe the use of tetrasulphate solution as a possible inexpensive and available therapy.

Materials and methods

Animals. Ten Rottweiler dogs (including 6 puppies, 3 females and 1 male) ranging in age from 4 months to 3 years, were presented to the Department of Internal Medicine, Veterinary Faculty, University of Adnan Menderes, over the course of an outbreak. For several weeks prior to presentation, all the dogs had eaten excessive amounts of moldy bread treated with water that had been stored for an undetermined period. During referral, the bread was inspected and found to be completely covered with a grey-green mold.

Blood panels. Hematologic variables included determination of differential white blood cell counts (WBC), red blood cell (RBC), packed cell volume (PCV), mean corpuscular volume (MCV), and platelet (PLT) counts. Serum biochemical tests included urea, creatinine, total protein, total bilirubin and activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkalen phosphatase (ALP) and gamma glutamil transferase (GGT).

Pathology. A standard necropsy was performed in the three cases of mortality. Following necropsy, tissue samples were collected from the liver, gall bladder, kidneys, spleen, trachea, lungs, heart, thymus, oesophagus, stomach, small and large intestines and

brain. Then, the tissue samples were fixed in 10% buffered formalin solution, embedded in parafin, sectioned at 5 μm, and stained with hematoxylin and eosin.

Toxicology. Total aflatoxin levels were investigated by high performance liquid chromatography (HPLC) with a fluorescence detector following the extraction procedure. For this purpose, two samples consisting of gastric content and liver were examined. An Aflatoxin Standard (aflatoxin mix kit) was used from Supelco (Bellefonte, PA, USA) (Cat. No: 46300-U). Aflatoxin from gastric content and liver were assessed by the method of Newman et al. (2007). All solvents used were reagent or HPLC grade.

Therapy application. Therapeutic applications included tetrasulphate (an antidote involving ferrous sulphate 16.6 g, copper sulphate 2.4 g, zinc sulphate 7.5 g, magnesium sulphate 10 g) at the rate of 0.6 g orally for the first day, and then followed by 0.3 g daily for 5 days given orally. Supportive treatment included i.v. 0.9% saline at 90 mL/kg, antiemetic (metoclopramide 0.5 mg/kg i.v. q 8h) and H_2 receptor antagonist (ranitidine 1 mg/kg q 8h) for 2 days.

Statistical analyses. Clinical parameters involving haematological and serum biochemical values in diseased dogs (n = 7) before (day 0) and after treatment (21 days post-treatment) and apparently healthy dogs (n = 7) were compared with analysis of variance (one way Annova). Significance was set as P<0.01.

Results

Clinical and haematological findings. Two days before initial referral, all dogs (n = 10) exhibited vomiting followed by excessive salivation and hyperaesthesia. Among the surviving dogs (n = 7), three of them showed diarrhea, depression, abdominal pain, and two other showed icterus (Table 1). One of them was found dead one day before referral. After that all the animals were brought to our clinic. One adult female dog (case II) that was found dead was forwarded to the Pathology Department for necropsy and routine histopathologic examination. After referral, 2 other dogs (cases I and III) died 2 hours after initial presentation to our clinic, prior to certain diagnosis and antidote therapy, and were also submitted for a necropsy.

Due to the sudden onset of clinical signs, lack of exposure to other toxins, the confirmed ingestion of moldy bread, toxicological analysis of gastric content and liver and the histopathological lesions, the definitive diagnosis was mycotoxicosis. All treated dogs, mainly puppies, made a full recovery over 12-24 hours. The clinical signs disappeared, yet the dogs were monitored for an additional 5 weeks, with no recurrence of clinical symptoms.

Historical data and physical examination findings are summarized in Table 1. Significant clinical laboratory data (involving complete blood counts and clinical biochemistry) are summarized in Table 2. A statistically significant difference was found

among clinicopathological variables, including mean white blood cell counts (P<0.001), serum total protein (P<0.05), ALT (P<0.01) and AST (P<0.001) concentrations in dogs with aflatoxicosis. Values before treatment were significantly higher in contrast to mean values after treatment.

Table 1. Historical data and clinical features of dogs with aflatoxicosis

Case No.	Age (mo)	Sex	Clinical signs	Death
1	36	♂	V, M, I, Ap	Yes
2	14	♀	V, I, D,	Yes
3	21	♀	V, M, I, Ap	Yes
4	24	♀	V, S, H, Ap, D	No
5	4	♀	V, S, H, I, D	No
6	4	♀	V, S, H, Ap, Di	No
7	4	♂	V, S, H, Ap, Di	No
8	4	♂	V, S, H, I, D	No
9	4	♂	V, S, H, Di	No
10	4	♂	V, S, H	No

V: vomiting, M: melena, I: icterus, Ap: abdominal pain, D: depression, Di: diarrhea, S: salivation, H: hyperaesthesia

Table 2. Clinical parameters including haematological and selected serum biochemical values in dogs with aflatoxicosis (before and after treatment) and healthy (control) dogs

Parameters	Clinical cases		Control	P
	Before treatment	After treatment		
WBC	32.4 ± 7.28^b	13.7 ± 1.04^a	9.5 ± 1.12^a	P<0.001
RBC	5.9 ± 0.22	5.8 ± 0.20	6.0 ± 0.19	P>0.05
PCV	41.9 ± 6.76	47.7 ± 1.10	41.9 ± 1.89	P>0.05
TP	7.2 ± 0.28^b	6.4 ± 0.15^{ab}	6.7 ± 0.17^a	P<0.05
ALT	116.4 ± 23.95^b	53.2 ± 4.62^a	51.4 ± 5.28^a	P<0.01
AST	98.7 ± 14.58^b	52.2 ± 4.02^a	48.4 ± 5.09^a	P<0.001

a-b: Mean values with different superscripts in each row, differ significantly (P<0,01). WBC: white blood cells, RBC: red blood cells, PCV: packed cell volume, TP: total protein, ALT: alanine aminotransferase, AST: aspartate aminotransferase

Toxicological results. The levels of aflatoxin were determined for all samples. Mean results of total aflatoxin analysis were 0.230 ppb and 0.051 ppb for liver and gastric content, respectively. Results of mean aflatoxin G_2 and B_2 concentrations of dog livers were 0.198 and 0.032 ppb, gastric content were 0.016 and 0.034 ppb respectively (Fig. 1).
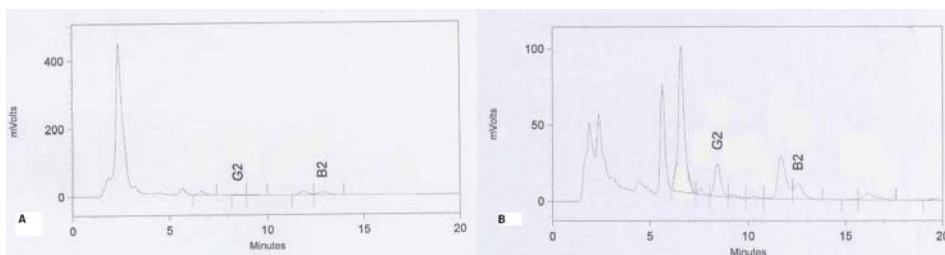
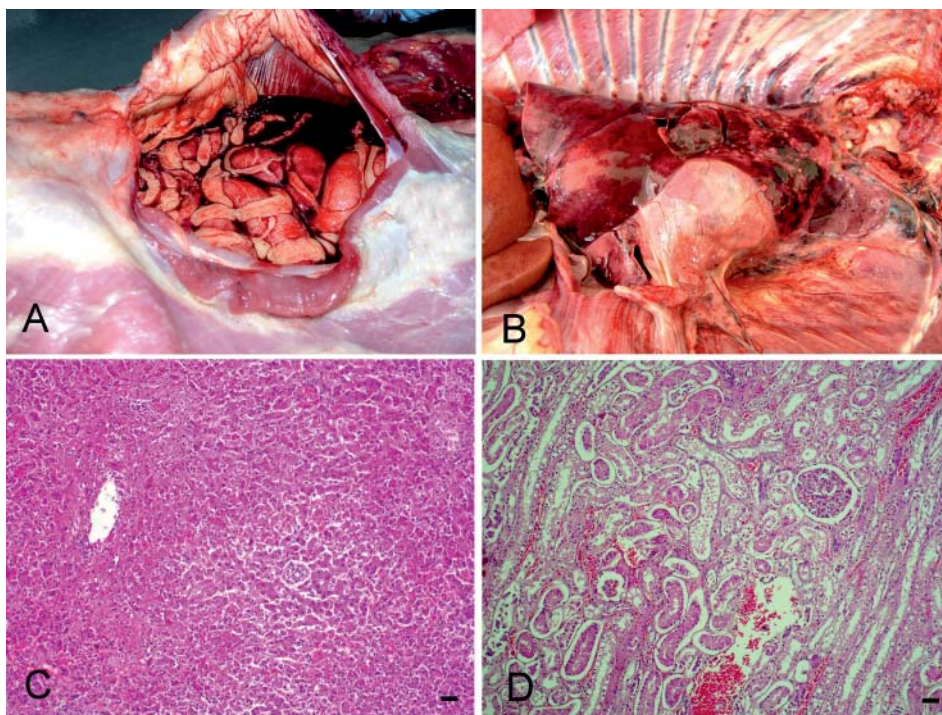Fig. 1. HPLC chromatograms of analyzed (A) gastric content and (B) liver extract

Fig. 2. Acute aflatoxicosis in dogs. (A) The abdominal cavitiy filled with a hemorrhagic fluid, (B) the petechial to ecchymotic hemorrhages were observed in the lungs and thymus, (C) focal necrotic areas, with extended from periacinar to centroacinar regions were seen in the liver, H&E, scale bar = 50 µm, (D) tubular epithelial degeneration and necrosis in the kidney, H&E, scale bar = 50 µm.

Pathological results. Necropsies were performed on all three dogs that died. The most common gross findings in those dogs were generalized icterus, mucosal or submucosal edema and petechial to ecchymotic hemorrhages in the organs. The abdomen was distended and a hemorrhagic fluid was present in the abdominal cavity (Fig. 2a). Major mucosal and subserosal hemorrhages were particularly recorded in the intestines, gall bladder, thymus and lungs (Fig. 2b). The liver was swollen and yellowish-brown in color and friable. Kidneys were swollen and showed yellowish-brown discoloration on the cut surface. The gastrointestinal lumen was filled with a hemorrhagic fluid without gastrointestinal ulceration.

Histopathological findings were marked in the liver and kidney of all dogs. Focal necrotic areas, extending from the periacinar to the centroacinar regions were seen in the liver (Fig. 2c). The majority of hepatocytes showed pyknosis and karyorhexis within the necrotic areas. All the other hepatocytes were swollen and eosinophilic, and some of them contained sharply defined cytoplasmic fatty vacuoles. Marked hepatocellular atypia, including megalocytosis and megakaryocytosis, were the other degenerative changes of the liver cells. Sinusoids were focally congested. Large numbers of macrophages containing hemosiderin pigment were usually present. The biliary epithelium was hyperplastic, and cholestasis and oval cell proliferation were also noted in the liver parenchyma.

In the kidneys, there were multifocal hemorrhages. Degeneration and necrosis of the proximal epithelium and, to a lesser extent, in the distal renal tubular epithelium were generally observed. The other histopathological changes of the kidney were characterized by cytoplasmic vacuolation, karyopyknosis and cellular enlargements as well as by abnormalities ranging from disappearance of the nucleus to necrosis and the presence of proteinaceous cylindrical casts in the tubular lumina (Fig. 2d). Additional lesions were noted in the lungs and gastrointestinal tisuues. In the lungs, alveolar septa were expanded by eosinophilic, proteinaceous fluid, and multiple foci of hemorrhage were frequent in the alveoli. Lesions of the gastrointestinal tract and other organs were characterized by vascular changes such as edema, hyperemia and focal or diffuse hemorrhages.

Discussion

Dogs are not frequently affected by aflatoxicosis, but they are highly prone to it and may present with clinical signs of hepatopathy (NEWMAN et al., 2007). Typical histopathologic changes, and especially determination of toxin content in feed (KETTERER et al., 1975), may help pathologists detect the precise toxicity of moldy feedstuffs (NEWMAN et al., 2007). Aflatoxin B_1 is the major toxin asoociated with aflatoxicosis, and to a lesser extent other relevant aflatoxins such as G_1, G_2 and B_2 (KETTERER et al., 1975; STENSKE et al., 2006; DERESZYNSKI et al., 2008). Liver specimens and gastric contents from the dead, untreated dogs, from the same household, were tested for aflatoxin concentrations

by HLPC. Aflatoxin levels were determined to be high for all samples (mean results of total aflatoxin analysis were 0.230 ppb and 0.051 ppb for liver and gastric content, respectively). Although it is not very easy to determine the exact duration the dogs were fed the contaminated feed, the owner determined it was more than several weeks. The moldy material that was fed to the animals was not available for analysis. The susceptibility of dogs individually depends on sex hormones, age, dose and degree of feed rejection (STENSKE et al., 2006). All these conditions may influence the severity of the disease. In the present study, it was mainly the puppies that lived and adults died. The fact that the damage apparently was stronger in the older animals that died, showed a discrepancy from the classical literature which suggests that younger animals are much more susceptible to poisoning with aflatoxins.

Aflatoxin B_1, one of the major toxins associated with aflatoxicosis, has the ability to induce hepatoxicity (KETTERER et al., 1975). The Food and Drug Administration suggests a zero tolerance for aflatoxin in food, and lists a legal limit of 20 μg/kg (ppb) in feed. For dogs, the toxic dose of aflatoxin is 60 μg/kg (ppb) and the lethal dose 50 % (LD_{50}) value is 500 to 1000 μg/kg (ppb) (AGAG, 2004; STENSKE et al., 2006; NEWMAN et al., 2007). In animal species, ratios of aflatoxins in feed and tissues range from 500: 1 to 14.000:1 (excluding the liver) (AGAG, 2004). It was concluded in the present study that the moldy bread contained 25.5-3220 ppb total aflatoxin, compared with other results. These results are above the allowed legal limit and toxic dose for dogs.

In a foodborne aflatoxin outbreak with hepatotoxicity (DERESZYNSKI et al., 2008) and in a previous experimental aflatoxicosis study in dogs (KING, 1963), markedly increased serum liver enzyme activities and hyperbilirubinemia were reported. In general, serum liver enzyme levels reflect cellular changes corresponding to the histopathological features of liver degeneration (CENTER, 2007). Indeed, regarding previous studies in dogs with aflatoxicosis, biochemical analysis showed variations in levels, depending on whether it was a acute or chronic disease (KING, 1963; BASTIANELLO et al., 1987). In acute high dose aflatoxicosis, death may occur from fulminant hepatic failure prior to marked changes in liver enzymes (KING, 1963; PACK et al., 1993). It must be mentioned that a discordance among circulating liver enzymes and histopathological signs in the liver were reported in dogs with chronic experimental aflatoxicosis (KING, 1963). Previous studies regarding aflatoxicosis in dogs reported minimally increased alanine aminotransferase activity (CENTER, 2007; DERESZYNSKI et al., 2008), similar to that found in many dogs involved in our study. The cellular liver enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), as well as total bilirubin, were elevated in some of the dogs reported herein.

All 3 dogs necropsied had high levels of aflatoxin confirmed in the liver and gastric content, accompanied by the typical histopathological changes supporting the diagnosis

of mycotoxicosis. The livers from affected dogs showed focal necrotic areas extending from the periacinar to centroacinar regions and swollen. There was hyperplasia in the biliary epithelium with cholestasis. Histological findings, similar to previous descriptions involving aflatoxicosis in dogs (KETTERER et al., 1975; NEWMAN et al., 2007), strongly suggested acute toxic hepatopathy, consistent with aflatoxicosis in these cases.

There is scarcity of information available in the literature regarding therapy for aflatoxicosis in dogs, most report solely supportive cure and unsuccessful results. Deg Nala disease, characterised by necrosis and gangrene of the limbs and other relevant dependent parts of the body (IRFAN and MAQBOOL, 1986; MAQBOOL et al., 1998), is associated with feeding with fungal infested straw mixed with rice straw. Fungi such as *Aspergillus* sp., *Fusarium* sp., *Mucor* sp., *Cladosporum* sp., and *Penicillium* sp., infesting rice straw produce mycotoxins (SHIRLAW, 1939; IRFAN and MAQBOOL, 1986). In cattle with Deg Nala disease, therapeutic trials, in an attempt to neutralise the mycotoxins, included a penta-sulphate mixture (an antidote involving ferrous sulphate, copper sulphate, zinc sulphate, cobalt sulphate and magnesium sulphate) accompanied with a vasodilator. This resulted in a 90% cure rate (IRFAN and MAQBOOL, 1986). Similarly, in this study, we adapted a tetrasulphate mixture, including ferrous sulphate, copper sulphate, zinc sulphate and magnesium sulphate for the dogs. At the time of therapy, commercially available cobalt sulphate was not available, therefore a tetrasulphate combination was applied. With this protocol we achieved complete cure and all dogs treated made an uneventful recovery.

Conclusion

The findings of the present study indicate that the tetrasulphate treatment protocol we used may have helped hasten clinical recovery and the rapid improvement of clinical signs attributable to aflatoxicosis in the dogs involved. Tetrasulphate solution might be an example of an inexpensive and available therapy and may have helped the survival of the dogs and reverse the adverse health effects of mycotoxins. However, more controlled studies are necessary for evaluating the effects of the latter drug. Apart from successful treatment, a better understanding of the clinical, hematological, toxicological and histopathological results involving canine aflatoxicosis was attained.

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SAŽETAK

Svrha je ovog rada iznijeti kliničke, hematološke, patoanatomske, patohistološke i toksikološke nalaze te izvijestiti o uspješnom liječenju pasa koji su jeli pljesnivu i vlažnu hranu što je sadržavala aflatoksin. U 10 pasa jednog vlasnika primijećeni su znakovi otrovanja nakon što su jeli pljesniv kruh namočen u vodu koji je prethodno bio čuvan neodređeno vrijeme, a u potpunosti je bio prekriven sivo-zelenom plijesni. Svi su psi povraćali uz obilno slinjenje i hiperesteziju. Od sedam preživjelih pasa, tri su imala proljev, bili su potišteni te su pokazivali bol u trbuhu, a dva su imala i žuticu. Jedan od uginulih pasa na početku je upućivao na toksikozu. Dva su uginula nakon postavljene dijagnoze, a prije početka liječenja. Patoanatomski je ustanovljena generalizirana žutica, edem sluznice ili submukoze te petehijalna do ekhimotična krvarenja po organima. Patohistološki su ustanovljena nekrotična žarišna područja pružajući se od periacinarnih do centroacinarnih područja u jetri, zatim bilijarna hiperplazija, kolestaza i multifokalna krvarenja po bubrezima i plućima. Na osnovi nagle pojave kliničkih znakova, nedostatka dokaza izloženosti drugim toksinima, potvrđenog dokaza uzimanja pljesnivog kruha, rezultata analize uzoraka jetre i patohistoloških nalaza postavljena je konačna dijagnoza mikotoksikoze. Za liječenje je peroralno bio primijenjen tetrasulfat (antidot) i uravnotežena otopina elektrolita, zatim antiemetici i antagonisti H_2 receptora. Svi liječeni psi (7/7) u potpunosti su se oporavili za 18 do 24 sata. Prikazani rezultati opisuju kliničke i patološke značajke aflatoksikoze u rotvajlera i upućuju na zaključak da se ona može liječiti otopinom tetrasulfata kao jeftinim i pristupačnim lijekom koji može pomoći u preživljavanju pasa te smanjiti štetne učinke mikotoksina na zdravlje.

Ključne riječi: aflatoksikoza, rotvajler, pljesniv kruh, mikotoksikoza, tetrasulfat, liječenje