

Amanita muscaria toxicosis in two dogs

John H. Rossmeisl, Jr., DVM, MS, DACVIM, (Internal Medicine and Neurology), Michael A. Higgins, DVM, Dennis J. Blodgett, DVM, PhD, DABVT, Matthew Ellis, DVM, Delbert E. Jones, MS

Abstract

Objective: To report the manifestations, history, and pathophysiologic basis of disease in 2 dogs with *Amanita muscaria* toxicosis.

Case summaries: Two dogs were evaluated for an acute onset of gastroenteritis and seizures. *A. muscaria* toxicosis was suspected in each dog after confirmation of environmental exposure and visualization of ingested mushrooms in vomitus. The diagnosis was confirmed following identification of toxic *Amanita* metabolites in the urine and serum of each dog. Administration of supportive and symptomatic therapies resulted in the complete recovery of each animal.

Unique information provided: Ingestion of the mushroom, *A. muscaria*, by dogs can result in acute gastrointestinal distress that precedes a potentially life-threatening central neurologic syndrome characterized by seizures, tremors, and somnolence. Central nervous system dysfunction results primarily from the actions of ibotenic acid and its decarboxylation product, muscimol, which are analogues of the neurotransmitters glutamate and γ -aminobutyric acid (GABA), respectively. Identification of these toxins in the urine and serum of affected dogs using high-performance liquid chromatography (HPLC) provides a definitive diagnosis.

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Introduction

Several species of *Amanita* mushrooms have been previously associated with clinically significant human and animal toxicoses.^{1–6} Veterinary publications concerning toxicoses associated with *Amanita* species are rare.^{2–5} Reports of canine ingestion of *A. ocreata* and *A. phalloides*, which contain cyclopeptide amatoxins, describe clinical signs of gastroenteritis and fulminant hepatic necrosis.^{1,2,4}

Amanita muscaria, commonly referred to as the fly agaric, and *A. pantherina* mushrooms are the 2 species most frequently implicated in the pantherina–muscaria poisoning syndrome in humans.^{6,7} This syndrome is primarily characterized by signs of central nervous system (CNS) dysfunction including hallucinations, auditory and visual hypersensitivity, tremors, convulsions, and somnolence. Clinical manifestations result

from the effects of 2 main toxins, ibotenic acid and muscimol, which interfere with normal glutamate (Glu) and γ -aminobutyric acid (GABA)-mediated neurotransmission.⁷ In humans, accidental ingestion of *A. pantherina* is the most common reason for toxicosis associated with this species. However, the psychoactive properties of *A. muscaria* have been recognized for centuries, and voluntary ingestion of fly agaric for both ritualistic and recreational purposes result in most human intoxications requiring medical treatment.^{7–9}

Presumptive cases of *A. pantherina* poisoning have been reported to cause muscle tremors, somnolence, delirium, and convulsions in cats and dogs,^{3,5} but to the authors' knowledge, descriptions of disease resulting from ingestion of *A. muscaria* have not appeared previously in the veterinary literature. The purpose of this case series is to describe the clinical signs, history, and pathophysiologic basis of disease in 2 dogs with *A. muscaria* poisoning.

From the Departments of Small Animal Clinical Sciences (Rossmeisl, Higgins), and Biomedical Sciences and Pathobiology (Blodgett, Jones), Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA and Emergency Veterinary Services of Roanoke, Roanoke, VA (Ellis).

Address correspondence and reprint requests to:

Dr. John H. Rossmeisl, Jr., Department of Small Animal Clinical Sciences, Virginia-Maryland Regional College of Veterinary Medicine, Mail Code 0442, Blacksburg, VA 24061.
E-mail: jrossmei@vt.edu

Case Summaries

Case 1

A 2-year-old, intact male, English setter (Dog 1) was referred for evaluation of cluster seizures and diarrhea.

The dog was reportedly normal the previous evening, but was discovered the next morning by the owner in its outdoor enclosure in lateral recumbency and covered in diarrhetic feces. Then, while transporting the dog to the referring veterinarian, the owner witnessed the first of 3 generalized, tonic-clonic seizures. Upon arrival, the referring veterinarian reported that the dog was hypersalivating, stuporous, and had profuse small bowel diarrhea. Results of a packed cell volume, total protein, and blood glucose concentration were within reference ranges. No abnormalities were found on fecal flotation. The veterinarian also observed a partial facial seizure that subsequently became generalized. Intravenous (IV) diazepam^a (0.23 mg/kg) temporarily abated the seizure. Approximately 20 minutes later, a second generalized tonic-clonic seizure occurred and a second dose of diazepam^a (0.23 mg/kg IV) was administered. Over the course of the next 2 hours, the dog was reported to have 2 additional episodes of diarrhea and several, intermittent bouts of facial twitching, of which 2 progressed to generalized seizure activity. A third, identical IV dose of diazepam^a was administered and the dog was referred for further diagnosis and treatment.

At the time of presentation, the dog was in status epilepticus. Additional physical examination abnormalities recorded at the time of admission included miotic pupils, fluid distended bowel loops, and a cardiac bradyarrhythmia associated with occasional peripheral pulse deficits. An IV bolus of diazepam^a (0.48 mg/kg) was administered and seizure activity ceased. Metabolic (i.e., hypoglycemia, hepatic insufficiency, hypoadrenocorticism), toxic (i.e., chlorinated hydrocarbons, lead, tremorigenic mycotoxins, organophosphates/carbamates, *Amanita* spp. mushrooms), and infectious diseases (i.e., canine distemper) were considered as the primary differential diagnoses for the clinical signs noted in this dog. The SpO₂ was 97% in room air, and an electrocardiogram (ECG) identified a high-grade, second-degree atrioventricular block, with the ratio of atrial and ventricular complexes being fixed at 3:1. A complete blood count (CBC) was consistent with a stress leukogram. Metabolic causes of disease were considered unlikely after results of a serum biochemical profile, urinalysis, activated partial thromboplastin time, and prothrombin time were all within reference ranges. The blood lead concentration in this dog was less than the detectable limit of the assay (<50 ppb). Atropine^b (0.05 mg/kg IV and 0.15 mg/kg SQ) was subsequently administered and abolished the atrioventricular block. The dog was then bathed with warm, soapy water to remove fecal soiling before the placement of an additional IV catheter. Crystalloid therapy^c with supplemental potassium chloride

(20 mEq/L) was administered at a rate of 4 mL/kg/h to maintain hydration and account for ongoing gastrointestinal fluid loss.

While being instrumented for continuous ECG telemetry and pulse oximetry, the dog appeared delirious and manifested several episodes of fly-biting behavioral stereotypy that preceded another generalized tonic-clonic seizure. The seizure activity abated after the administration of 2 IV doses of diazepam^a (0.48 mg/kg, followed immediately by 0.96 mg/kg). A loading dose of phenobarbital^d (8.6 mg/kg) was then given as a slow IV infusion, during which time the dog had 2 cluster seizures that prompted the initiation of a diazepam^a constant rate infusion (CRI) at a dosage of 1 mg/kg/h. Additionally, phenobarbital^d maintenance therapy (2.2 mg/kg q 12 h) was initiated intramuscularly (IM). The dog was maintained on the diazepam^a CRI for approximately 2 hours, after which time, 2 additional cluster seizures were observed that failed to completely respond to 2 IV boluses of diazepam (0.96 mg/kg). Propofol^e was then administered (3 mg/kg IV loading bolus, followed by 0.1 mg/kg/min IV CRI) which resulted in cessation of the seizures. After 6 hours of the propofol^e CRI, attempts were made to wean the propofol^e therapy. However, as soon as the infusion was discontinued, appendicular and facial muscular tremors were observed, and the propofol^e CRI was restarted at the same dosage.

Approximately 2 hours later, the dog vomited twice in rapid succession while being maintained on the propofol^e infusion. The vomitus contained partially digested commercial dog kibble and multiple fragments of organic material that resembled mushroom caps and stems. Oropharyngeal and esophageal suction was performed to remove residual vomitus. Venous blood gas analysis was within reference ranges.

The dog experienced an additional generalized seizure 1 hour later. An electroencephalogram^f (EEG) was obtained that revealed intermittent bursts of epileptiform activity that were associated with clinically apparent facial motor disturbances. The propofol^e CRI dosage was incrementally escalated to 0.25 mg/kg/min in an attempt to achieve burst suppression on the EEG, but continual interpretation of the EEG was complicated by a large amount of motion artifact associated with the frequently occurring facial muscular tremors and fasciculations. Therapy with methocarbamol^g (50 mg/kg IV q 8 h) was also initiated, which completely abolished the appendicular muscular tremors and markedly reduced the frequency and severity of the facial tremors.

Over the next 6 hours, the muscular tremors largely abated and the dog was gradually weaned from the propofol^e infusion. The dog remained recumbent and

somnolent for an additional 10 hours. Thoracic radiographs were obtained to examine for evidence of aspiration pneumonia; no abnormalities were observed. Approximately 40 hours after admission, the dog was able to assume a sternal position and was eating and drinking voluntarily, so was weaned from IV fluid therapy. The dog was switched from IM to oral maintenance phenobarbital^h therapy (2.9 mg/kg PO q 12 h). By the third day of hospitalization, the dog appeared clinically normal. The methocarbamol^g was discontinued and the dog was discharged to the owner with instructions to continue the phenobarbital^h therapy as prescribed.

The owners of the dog were questioned about possible environmental exposure to mushrooms, and subsequently, they provided intact and partially eaten mushrooms found in the dog's living area. Samples of partially eaten mushrooms provided by the owner were submitted to a botanist and veterinary toxicologist and confirmed to be *A. muscaria* var. *formosa* (Figure 1).

Materials and Methods for High-Performance Liquid Chromatographic (HPLC) Identification of Ibotenic Acid and Muscimol

The HPLC procedure utilized was a modification of the ion-interaction method previously described by Genaro et al.¹⁰ Briefly, the following chemical grade reagents were obtained from commercial sources: ibotenic acid,ⁱ muscimol,^j octylamine,^k phosphoric acid,^l methylene chloride,^m and methanol.ⁿ

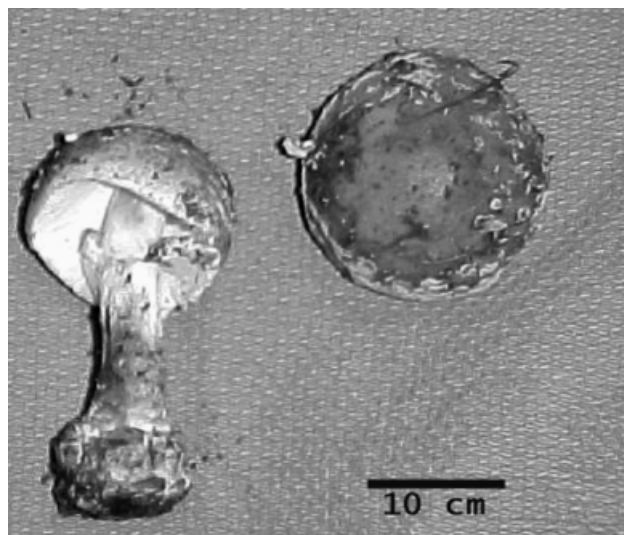


Figure 1: Photograph of *Amanita muscaria* var. *formosa*. Intact mushroom (left), and orange–yellow pigmented cap with white ‘warts’ (right) that is characteristic of mature mushrooms inhabiting the eastern United States.

Sample preparation of intact mushrooms involved cleansing of a 10 g mixed sample of previously identified *A. muscaria* stems and caps with ultrapure water,^o crushing the sample in a mortar, followed by extraction with 20 mL of methanol.ⁿ The resulting solution was filtered through a 0.22 µm micropore nylon membrane before injection into the HPLC apparatus. To prepare urine and serum samples, 1 mL aliquots were mixed with 3 mL of a 90%/10% vol/vol mixture of methylene chloride^m/methanol,ⁿ agitated for 10 minutes, and allowed to settle. The bottom layer was removed, evaporated,^p and subsequently reconstituted with 100 µL of methanolⁿ for injection into the HPLC apparatus.

The chromatographic apparatus utilized was a commercial HPLC system^q interfaced with proprietary analytical software.^r A 7 µm, 250 × 4.6 mm reverse phase column^s was used together with a guard pre-column.^t

The mobile phase was created by adding phosphoric acid to a 5 mM aqueous solution of octylamine,^k titrated to a pH of 6.4. The chromatographic system was conditioned until a stable baseline signal was obtained at a flow rate of 1.0 mL/min. Spectrophotometric detection was performed at 230 nm. Reference standards were made of ibotenic acid and muscimol. Lower detection limits of the HPLC assay were 0.006 µg/mL for ibotenic acid and 0.024 µg/mL for muscimol.

Urine and serum ibotenic acid concentrations determined by HPLC were 0.706 and 0.239 µg/mL, respectively, in Dog 1. Muscimol was also present in the serum and urine of Dog 1 at concentrations of 0.083 and 4.814 µg/mL, respectively. Serum and urine samples obtained from a healthy control dog were simultaneously assayed using HPLC for ibotenic acid and muscimol. Both substances were undetectable in test samples from the control dog. HPLC analysis of an extract prepared from partially eaten mushrooms from Dog 1 yielded concentrations of ibotenic acid and muscimol that exceeded the upper detection limits of the assay.

Two months following discharge, during follow-up telephone conversations with the owner and referring veterinarian, Dog 1 was reported to be clinically normal with no additional seizures or gastrointestinal signs observed. At the 2-month telephone contact, the serum phenobarbital concentration was reportedly 19.3 µg/mL (therapeutic range 15–40 µg/mL). Dog 1 is currently being weaned off of phenobarbital^h therapy.

Case 2

A 4-year-old, castrated male, mixed-breed dog (Dog 2) was evaluated for somnolence, ptialism, vomiting, and diarrhea of 12 hours duration. Upon examination, the dog was approximately 6% dehydrated, hypersalivating, and had miotic pupils; other physical parameters

were normal. A CBC, serum biochemical profile, and urinalysis were performed, and the dog was admitted for IV fluid therapy and continued observation. The results of the CBC and urinalysis were within reference ranges. Abnormalities present on the serum biochemical profile included an elevated alanine aminotransferase (ALT) (139 U/L; reference range 17–66 U/L) and hyperglycemia (130 mg/dL; reference range 84–118 mg/dL). During placement of a peripheral IV catheter, the dog was observed to have a generalized tonic-clonic seizure that abated following the administration of diazepam^a (0.55 mg/kg IV). A crystalloid solution^c was administered at a rate of 14 mL/kg/h for the subsequent 6 hours to replace the hydration deficit, after which time the fluid rate was decreased to 2.3 mL/kg/h.

The ptialism gradually subsided within 12 hours of admission, although the dog remained somnolent throughout this period. Approximately 18 hours after presentation, a second generalized tonic-clonic seizure, that lasted approximately for 1 minute, was witnessed. An IV bolus of phenobarbital^d (6.5 mg/kg) was administered, and maintenance therapy with phenobarbital^h (2.1 mg/kg PO q 12 h) was initiated. The dog appeared clinically normal approximately 12 hours later and was discharged with instructions to continue the phenobarbital^h therapy. The owners visually confirmed the presence of *A. muscaria* mushrooms growing in close proximity to the dog's outdoor enclosure. Serum concentrations of ibotenic acid and muscimol, determined using the HPLC method described for Dog 1, were 0.858 and 0.305 µg/mL, respectively. The urine concentration of muscimol was 6.909 µg/mL. The urine concentration of ibotenic acid was below the detection limit of the HPLC assay in Dog 2. The owner of Dog 2 was contacted for approximately 3 weeks after discharge and reported that the dog appeared clinically normal.

Discussion

The cases presented here demonstrate that *A. muscaria* toxicosis has the potential to cause an acute and severe clinical syndrome in dogs characterized by gastroenteritis and CNS dysfunction from which rapid and complete recovery can be expected with supportive and symptomatic therapies. *A. muscaria* mushrooms are common inhabitants of temperate coniferous and deciduous forests of many continents of the world, including North and South America, Asia, and Europe. There are 2 varieties of *A. muscaria* typically found in the United States. The characteristic brilliant scarlet red pileus with accompanying white surface 'warts' usually facilitates visual identification of mature *A. muscaria* var. *muscaria* that is found in the western United States. *A. muscaria* var. *formosa* is the eastern North American

variety and has a cap with a more yellow-orange hue. The growth season of *A. muscaria* typically lasts from early summer to late autumn.

A. muscaria was originally named following the isolation of muscarine from the mushroom and it was initially speculated that the clinical poisoning resulted from the toxic effects of muscarine. However, muscarine was later discredited as the primary toxin responsible for the clinical manifestations of fly agaric poisoning in humans.^{6–12} The concentrations of muscarine found in *A. muscaria*, although variable, are generally considered insufficient to cause the pantherina-muscaria syndrome, and explain the paucity of cholinergic clinical signs demonstrated by humans with *A. muscaria* toxicosis.^{9,13} The quaternary ammonium group present on muscarine prohibits entrance into the CNS, and thus muscarine functions as a competitive muscarinic receptor agonist in the peripheral nervous system, and is not subject to acetylcholinesterase-mediated degradation.¹¹ Muscarinic cholinergic receptors capable of binding muscarine are found in highest concentrations in the cardiac muscle and nodes, smooth muscle, and glands.⁹ Both dogs reported here displayed variably severe degrees of cholinergic signs, such as ptialism, miosis, vomiting, diarrhea, and second degree atrioventricular block. Cholinergic crises are rarely reported in adult humans with pantherina-muscaria poisoning which results from recreational or ritualistic consumption of fly agaric. Occasionally, however, nausea and vomiting have been observed in children with *A. muscaria* toxicosis that results from the ingestion of large quantities of mushrooms, as was suspected in both the dogs described here.^{7,13} Experimental peritoneal injections of *A. muscaria* extracts into rodents have been shown to decrease acetylcholinesterase activity.⁷ It is also possible that the gastrointestinal manifestations that accompany some cases of pantherina-muscaria poisoning are caused by an unidentified toxin.

The CNS derangements that are characteristic of pantherina-muscaria poisoning in humans and animals can be primarily attributed to the chemically related toxic isoxazole amino acid metabolites, ibotenic acid, and muscimol.^{7,10–12} Ibotenic acid is synthesized by the mushroom, whereas muscimol is formed by decarboxylation of ibotenic acid.^{7,11} The highest concentrations of toxic metabolites are found within the pileus of the mushroom, especially in the flesh immediately beneath the external cuticle. Both amino acids have been shown to be qualitatively present in *A. muscaria* species regardless of the geographic origin of the mushroom, but toxin content may quantitatively vary with both geographic site of origin, season harvested and, in instances of voluntary consumption, the method of

preparation.^{7,12} In addition, it has been previously reported that *A. muscaria* mushrooms consumed in September are more often associated with nausea and vomiting and have less potent hallucinogenic effects than those ingested earlier in the summer.¹⁵ In the dogs described here, intoxications occurred in late September and early October.

Both ibotenic acid and muscimol, although zwitterionic structures, are capable of crossing the blood–brain barrier by active transport, which explains their ability to produce CNS dysfunction.⁹ Ibotenic acid and muscimol are alkaloids that biochemically function *in vivo* as agonists of the neurotransmitters Glu and GABA, respectively.^{10–12} The amino acid Glu and its conformational analogs, which include ibotenic acid, mediate most of the excitatory synaptic neurotransmission in the mammalian brain through 2 distinct classes of Glu receptors, ligand-gated ionotropic receptors (iGlu) and G protein-coupled metaboreceptors (mGlu). Experimentally, ibotenic acid pharmacologically activates the *N*-methyl-D-aspartate subclass of iGlu, and acts as an agonist of variable potency at several distinct mGlu subfamilies.¹⁶ Its interaction with these Glu receptors is responsible for the signs of CNS excitation, including seizures, muscle tremors, and hallucinations that are the clinical hallmarks of acute *A. muscaria* toxicosis.¹⁶ Intraparenchymal delivery of ibotenic acid into rodent hippocampi has also been previously used as a clinical and electroencephalographic excitotoxin-induced seizure model.¹⁷ In most naturally occurring toxicoses associated with *A. muscaria* ingestion, the toxic effects of ibotenic acid are largely due to neuronal functional derangements, as morphologic brain damage is not a consistent feature of the pantherina–muscaria poisoning syndrome. However, repeated local and systemic experimental administration of ibotenic acid to laboratory animals induces degenerative morphologic neuronal and glial lesions in basal nuclei.^{7,17}

Muscimol exerts a primarily depressant effect on the CNS which occurs through multiple interactions of the metabolite with GABA-mediated inhibitory neurotransmission. Muscimol is a potent and biologically active conformational analog of GABA, capable of *in vivo* activation of postsynaptic GABA receptors.^{7,11} Experimental *in vitro* studies in rodent models suggest that muscimol has a high affinity for both postsynaptic GABA receptors as well as autoregulatory receptors that may function in release of GABA from presynaptic neurons.¹⁰ Muscimol also functions as a substrate for neuronal, and possibly glial, GABA uptake systems.^{7,11} Following ingestion of *A. muscaria*, the acidic gastric microenvironment causes ibotenic acid to be continually decarboxylated, which quantitatively yields muscimol. Thus, the clinical signs seen in the subacute

phase of pantherina–muscaria toxicosis are predominantly reflective of the depressant actions of muscimol, and primarily consist of sedation and somnolence, which may be associated with hallucinations and vivid dreams in humans.

Previous veterinary reports of mushroom poisoning are likely rare because of the difficulty associated with establishing a definitive diagnosis.⁹ An empirical diagnosis is usually made based on historical evidence of mushroom exposure and ingestion in combination with concurrent clinical signs. Visualization of ingested mushrooms obtained from vomitus or gastric lavage fluid further supports the diagnosis, but definitive taxonomic identification of mushroom samples obtained in this manner can be very difficult, and may require mycological expertise that is not readily available. Preservation of mushrooms retrieved from ingesta for future identification is best achieved by wrapping samples in paper towels and storing them in a paper bag.⁹

As demonstrated in this study and previous human reports of *Amanita* species toxicoses, metabolites of the incriminated mushroom species can be identified in a variety of body tissues and fluids using HPLC, which allows for the definitive diagnosis of mushroom poisoning.¹² Ion-interaction HPLC techniques, similar to the one described here, have several advantages over other methods, such as paper chromatography (PC) and gas chromatography-mass spectrometry (GC-MS), that have been previously employed to detect ibotenic acid and muscimol in suspected cases of pantherina–muscaria poisoning. Ion-interaction HPLC techniques, that have been demonstrated to be sensitive for the simultaneous detection of both metabolites, are readily and economically performed with equipment available in most toxicology laboratories and require minimal sample preparation when compared to PC and GC-MS.^{7,10–12} In humans, ibotenic acid and muscimol appear in urine in quantities detectable by ion-interaction HPLC within 1 hour of mushroom ingestion, which makes urine the preferred test substrate for toxic metabolite detection.^{7,12} Unfortunately, commercial veterinary diagnostic laboratories do not routinely offer diagnostic HPLC services for mycologic toxins.

As few antidotes are available, and antidote administration is recommended only when the specific toxic mushroom species has been identified,^{1,9} general treatment for suspected mushroom poisoning includes gastrointestinal decontamination. The principles of gastrointestinal decontamination have been previously reviewed in detail,¹⁴ but induction of emesis in animals with normal mentation and swallowing reflexes, performance of gastric lavage, and administration of activated charcoal to facilitate adsorption of remaining

toxin are advocated. Activated charcoal was not administered to the patients reported here because of the prolonged interval between toxin exposure and presentation, and the perceived risk of aspiration associated with the CNS signs displayed by both dogs.

Additional symptomatic therapies have been prescribed in humans with pantherina-muscaria poisoning including administration of a variety of anxiolytics, sedatives, and anti-convulsants in cases where seizures are observed. Diazepam, clonazepam, and phenobarbital have been used successfully to treat seizures associated with *A. muscaria* toxicosis in adults and children, as well as in dogs and cats with *A. pantherina* poisoning.⁹ Additional precautionary measures should be employed when benzodiazepines or barbiturates are administered, as both of these classes of drugs are GABA agonists and may act synergistically with muscimol to potentiate CNS depression.^{7,11} Patients receiving benzodiazepines or barbiturates should be monitored closely for respiratory depression which may necessitate mechanical ventilation in some cases. Administration of parenteral diazepam and phenobarbital did not result in any respiratory depression in the 2 dogs reported here, as determined by serial clinical observation and continual pulse oximetry (Dog 1). Administration of methocarbamol largely abated the muscle tremors seen in Dog 1, and was not associated with any significant adverse effects. However, it is possible that the CNS depressant effects of this drug may have acted synergistically with the administered anti-convulsant and delayed the patient's recovery.

The results of this report suggest that the natural history of canine poisoning resulting from ingestion of *A. muscaria* is chronologically and clinically similar to those caused by *A. pantherina* with the notable exception of the acute gastrointestinal distress that preceded the signs of CNS dysfunction in dogs ingesting *A. muscaria*.⁹ Variably severe signs of CNS excitation, caused by the agonistic effects of ibotenic acid on Glu receptors, including stereotypical behaviors, seizures, and muscle tremors, typically develop within 4–12 hours of apparent ingestion of fly agaric and last for 12–24 hours. The final phase of intoxication consists of CNS depression and somnolence, mediated by the GABAergic effects of muscimol. Full recoveries typically occur within 24–48 hours when treatments are administered. Based on the outcomes of the dogs reported here, and from previous publications appearing in both the human and veterinary literature, the prognosis for recovery from pantherina-muscaria syndrome should be considered excellent with prompt recognition and appropriate supportive therapy, even in cases with severe clinical signs resulting from ingestion of large numbers of mushrooms.^{3,5,7,9} The diagnosis of panther-

ina-muscaria syndrome can be confirmed by the demonstration of ibotenic acid and muscimol in the urine or serum using HPLC in animals with concurrent clinical signs, in which historical or physical identification of *A. muscaria* or *A. pantherina* species mushrooms is problematic or impossible.

Acknowledgement

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Footnotes

- ^a Diazepam, Abbott Laboratories, North Chicago, IL.
- ^b Atropine, Abbott Laboratories.
- ^c Lactated Ringer's solution, Baxter Animal Healthcare Co., Deerfield, IL.
- ^d Phenobarbital, Elkins-Sinn Inc., Cherry Hill, NJ.
- ^e Propofol, Abbott Laboratories.
- ^f Neurofax EEG 1100, Nihon-Kohden America Inc., Irvine, CA.
- ^g Robaxin, Fort Dodge Animal Health, Overland Park, KS.
- ^h Phenobarbital, Qualitest Pharmaceuticals Inc., Huntsville, AL.
- ⁱ Ibotenic acid, Sigma-Aldrich Co., St Louis, MO.
- ^j Muscimol, Sigma-Aldrich Co.
- ^k Octylamine, ACROS Organics, Morris Plains, NJ.
- ^l Phosphoric acid, J.T. Baker Division of Mallinckrodt Baker Inc., Phillipsburg, NJ.
- ^m Methylene chloride, J.T. Baker Division of Mallinckrodt Baker Inc.
- ⁿ Methanol, J.T. Baker Division of Mallinckrodt Baker Inc.
- ^o Millipore, MilliQ, Milford, MA.
- ^p Speed Vac Model SVC-100H Concentrator, SAVANT Instruments Inc., Farmingdale, NY.
- ^q Beckman Coulter System Gold HPLC, solvent module 126, Model 166 UV-VIS detector, Beckman Coulter Inc., Fullerton, CA.
- ^r 32 Karat, version 5.0, Beckman Coulter Inc.
- ^s Absorbosphere, Alltech, Nicholasville, KY.
- ^t RP-18, Phenomenex, Macclesfield, UK.

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